TABLE OF CONTENTS

WELCOME

Dear Meeting Attendee,

Welcome to the 13th annual meeting of the Association of Biomolecular Resource Facilities (ABRF)! The title and theme of the meeting is "ABRF 2009: Application and Optimization of Existing and Emerging Biotechnologies." The meeting will present the latest developments in life sciences technologies and the use of these technologies as shared research resources. We hope you enjoy this opportunity to network with colleagues, to learn about biotechnology advances and applications, and to discuss the challenges and results of implementing shared research resources.

There will be keynote presentations by outstanding leaders in genomics and proteomics. There will be a keynote presentation on applying advanced biotechnology in evolutionary biology, in recognition that February 2009 will mark the 200th anniversary of Charles Darwin's birth and the 150th anniversary of the publication of On the Origin of Species. Award presentations and lectures will recognize outstanding contributions in biotechnology research and applications.

The ABRF Research Groups will give presentations of their multi-institutional studies on a wide range of biotechnologies and methodologies. There will be Scientific Sessions and Technical Workshops on genomics, functional genomics, proteomics, imaging, and other technologies, including next generation sequencing, genotyping, microarrays, real-time PCR, proteomics and mass spectrometry, protein production and characterization, optical imaging, bioinformatics and bio-IT. There will be workshops and discussion forums on operational and management issues facing life sciences core facility and biotechnology research laboratories.

The meeting will include both Scientific Research Poster Sessions and a Core Facility Poster Session. The core facility session will be a forum for presenting and discussing the resources and services of shared resource laboratories.

Participants will be able to interact with a diverse array of vendors that will be exhibiting their products and services in the Exhibit Hall and presenting in Exhibitor Workshops.

Satellite workshops will be held before the main conference. Satellite Educational Workshops will cover genomics and proteomics technologies. There will be a Satellite Workshop of the National Network of IDeA-Funded Core Laboratories (NICL). There will also be tours of core facilities at nearby institutions.

There will be numerous opportunities to network with colleagues, including an opening reception and a closing social. The closing social will have good food, games, dancing, Memphis music, and maybe even Elvis will be in the house!

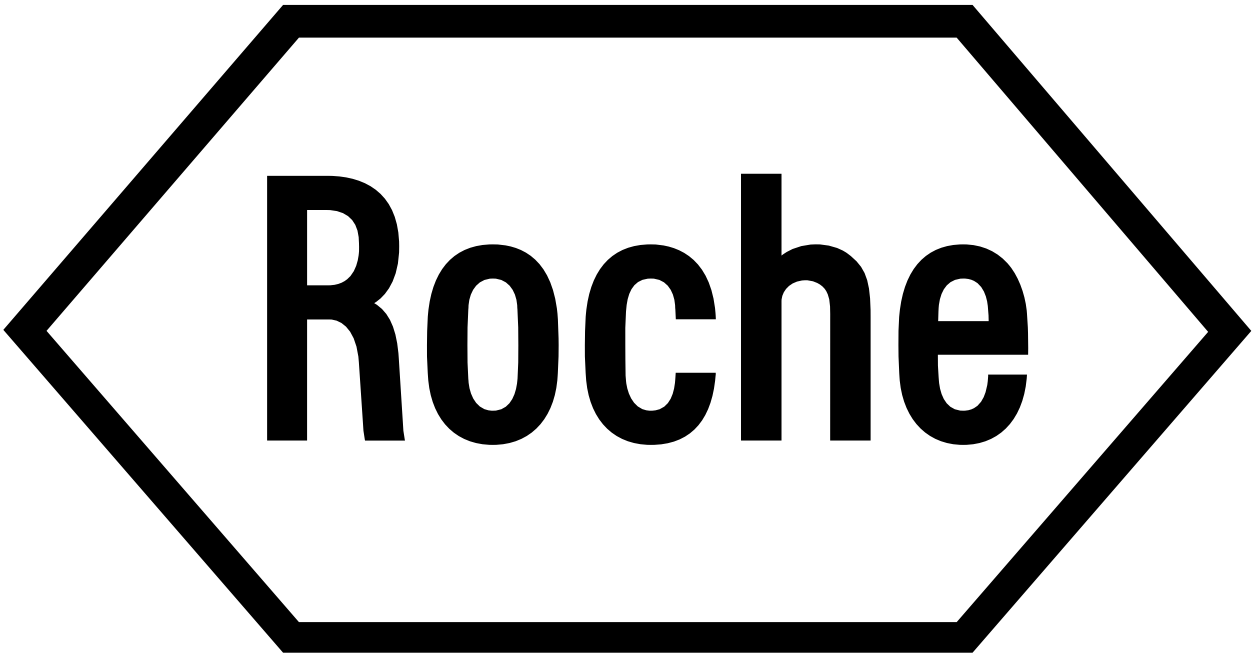
A survey will be made available on the web soon after the meeting. Your feedback through your response to the survey will help shape future ABRF meetings.

We thank the meeting sponsors for their generous support and the invited speakers and session organizers for their enthusiastic involvement. Moreover, we thank all the meeting attendees for their support and participation.

We look forward to seeing you at the meeting!

Sincerely,

The ABRF 2009 Organizing Committee, including George Grills (chair, Cornell University), Chris Turck (co-chair, Max Planck Institute), Margaret Robertson (*ad hoc*, UCSF), and Theodore Thannhauser (*ad hoc*, USDA-ARS).

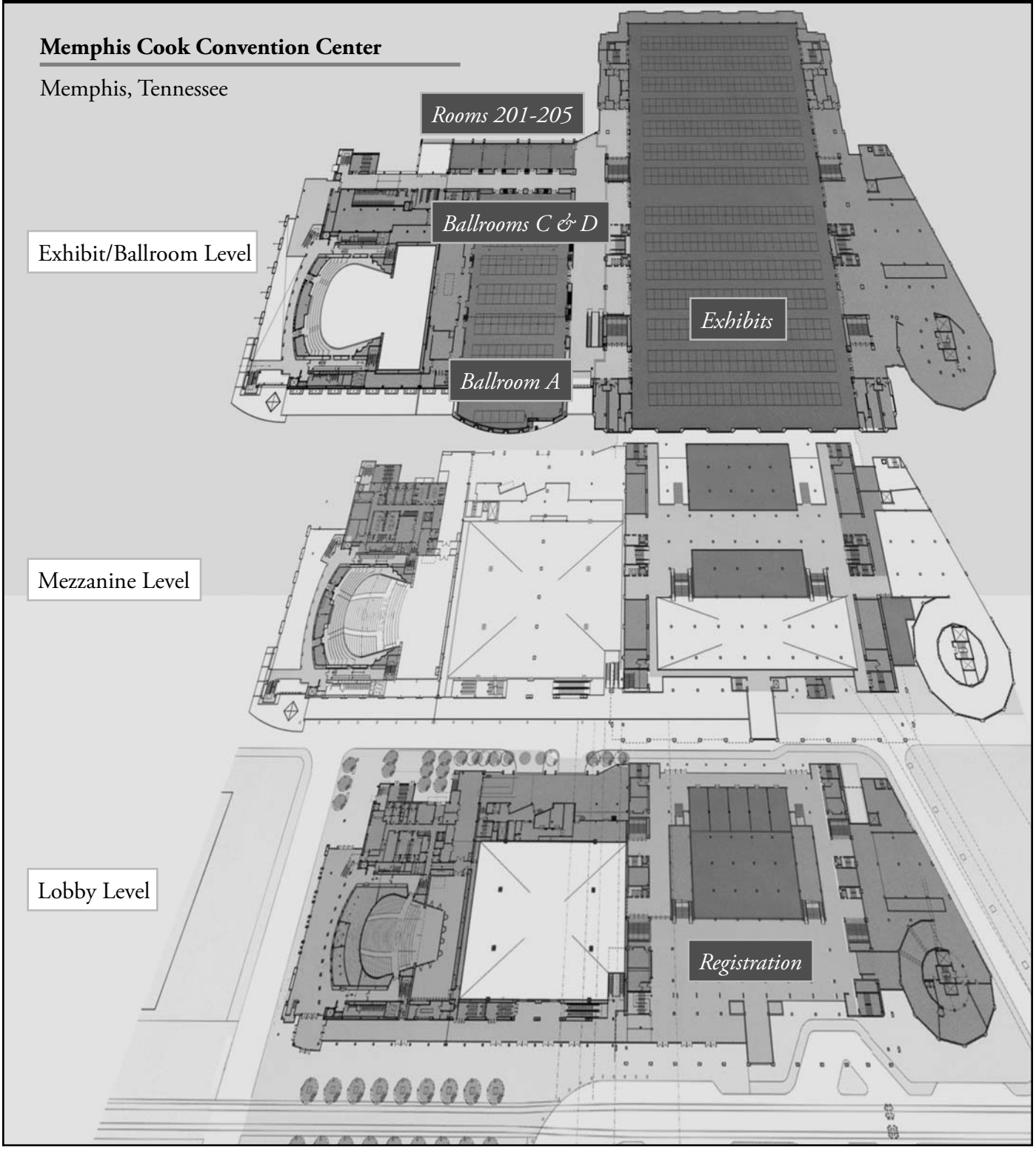


Booth 400

MAPS

Memphis Cook Convention Center

Memphis, Tennessee



Meeting Management

Cynthia Stubits, Meeting Manager
Catherine Portner, Exhibits & Sponsorship Manager
Chezka Solon, Program & Registration Manager

ABRF 2009 c/o Courtesy Associates
2025 M Street NW, Suite 800
Washington, D.C. 20036
Phone: +1 (202) 973-8670 • Fax: +1 (202) 331-0111
Email: abrf@courtesyassoc.com

PROGRAM-AT-A-GLANCE

Time	Saturday, February 7	Sunday, February 8	Monday, February 9	Tuesday, February 10
7:00 am – 7:45 am	Registration Open (7:00 am – 6:00 pm)	Continental Breakfast Registration Open (7:00 am – 6:00 pm)	Continental Breakfast Registration Open (7:00 am – 6:00 pm)	Continental Breakfast Registration Open (7:00 am – 6:00 pm)
7:45 am – 8:00 am		**Opening Remarks**	**Outstanding Scientist/Technologist Award** *(sponsored by Thermo Fisher Scientific)*	**Journal of Biomolecular Techniques Award**
8:00 am – 8:50 am	**Satellite Educational Workshops** (8:00 am – 4:00 pm) (sw1) Next Generation DNA Sequencing *(Workshop sponsor: Illumina; Session sponsors: Roche and Applied Biosystems; Speaker sponsors: Fluidigm, DNAStar, Genome Quest and Geospiza)* (sw2) Proteomics Instrumentation *(Session sponsor: Applied Biosystems)* (sw3) Recombinant Protein Laboratory (Fri., 2/6 - Sat. 2/7) (sw4) HPLC Theory and Practice (sw5) Proteome Informatics	Plenary Session on Genomics: *The Changing Landscape of Genomics* — **Richard Gibbs**, Baylor College of Medicine	Plenary Session on Proteomics: *Technologies for Comprehensive Proteome Quantitation* — **Matthias Mann**, Max Planck Institute	Plenary Session on Evolutionary Biology: *Genomics of Extinct and Endangered Species* — **Stephan Schuster**, Pennsylvania State University
9:00 am – 10:15 am		**Concurrent Scientific Sessions** (s1) Personalized Medicine (s2) Biofuels (s3) Optical Imaging	**Concurrent Scientific Sessions** (s7) Metagenomics (s8) Label Free Detection (s9) Stem Cell Applications	**Concurrent Scientific Sessions** (s13) Epigenomics (s14) Targeted Proteomics (s15) Bioinformatics
10:15 am – 10:45 am		**Exhibits & Poster Viewing** (10:00 am – 6:30 pm) Refreshment Break (Exhibit Hall)	**Exhibits & Poster Viewing** (10:00 am – 6:30 pm) Refreshment Break (Exhibit Hall)	**Exhibits & Poster Viewing** (10:00 am – 2:00 pm) Refreshment Break (Exhibit Hall)
10:45 am – 12:00 pm		**Concurrent Research Group Presentations** (r1) Joint Proteomics RG: Proteomics, Proteomics Standards, Proteome Informatics (r2) Nucleic Acids	**Research Group Presentation** (r3) Joint Genomics RG: Microarrays, DNA Sequencing, Genomic Variation	**Concurrent Research Group Presentations** (r4) Joint Protein RG: Edman Sequencing, Protein Expression (r5) Light Microscopy
12:00 pm – 1:00 pm		Lunch (Munch & Mingle) Exhibit Hall Expo	Lunch (Munch & Mingle) *(sponsored by Protea)* Exhibit Hall Expo	Lunch (Munch & Mingle) Exhibit Hall Expo
1:00 pm – 2:00 pm		**Poster Session I: Research Posters** Exhibit Hall Expo	**ABRF Award Presentation & Lecture** *The Creation of a Human Protein Atlas* *(sponsored by Agilent Technologies)* — **Mathias Uhlen**, Royal Institute of Technology	**Concurrent Workshops** (w7) Core Support & Instrumentation Funding (w8) Bio-Information Technology (Bio-IT) (w9) Protein Expression
2:00 pm – 3:15 pm		**Concurrent Scientific Sessions** (s4) Next Generation Nucleic Acid Quantitation (s5) Biotech in Forensics & Clinical Diagnostics (s6) Quantitative Proteomics	**Concurrent Scientific Sessions** (s10) Next Generation Sequencing *(sponsored by Applied Biosystems)* (s11) Phosphoproteomics (s12) microRNA	**Concurrent Workshops** (w10) Next Generation Sequencing Instruments (w11) Proteomics Data Publication (w12) Molecular Interactions Instruments **ABRF Poster Awards** *(sponsored by Waters)*
3:15 pm – 3:45 pm		Refreshment Break (Exhibit Hall)	Refreshment Break (Exhibit Hall)	Refreshment Break (Ballroom Foyer)
3:45 pm – 5:00 pm	**Tours of Core Facilities at St. Jude Children's Research Hospital** (4:30 – 6:30 pm) **Satellite Workshop of IDeA Core Laboratories** (4:30 – 6:30 pm)	**Concurrent Workshops** (w1) Core Facility Management Models (w2) Women in Science and Core Laboratories (w3) Educational Outreach	**Concurrent Workshops** (w4) Implementing Next Gen Sequencing (w5) Implementing Mass Spec Technologies (w6) Implementing Optical Imaging Technology	**Scientific Session** (s16) Disruptive Life Sciences Technologies
5:15 pm – 6:30pm		**Poster Session II: Research Posters** Student/Post Doc Poster Awards Exhibit Hall Expo Networking Reception	**Poster Session III: Core Facilities Posters** Networking Reception	**ABRF: Current Status and Future Plans** Research Group/Committee Member Award ABRF Lifetime Achievement Award Closing Remarks
6:30 pm – 7:30 pm		**Concurrent Exhibitor Presentations I**	**Concurrent Exhibitor Presentations III**	
7:40 pm – 8:40 pm	**Welcome Reception** (7:00 – 8:30 pm)	**Concurrent Exhibitor Presentations II**	**Concurrent Exhibitor Presentations IV**	**Closing Social** *(Brought to you by Intel and HP)* (7:00 – 10:00 pm)

REGISTRATION SERVICES

Meeting Registration

Full meeting registration includes food functions served by the ABRF 2009, access to the Exhibit Hall, Receptions, Scientific Sessions, Workshops, and Research Group Sessions. You must present a badge to enter all conference areas. Additional guest tickets for the Closing Social can only be purchased at the Onsite Registration Desk.

On-site Fees

ABRF Member	$500
Non-Member	$625
Student	$185
Membership + Meeting Registration	$585

Ticketed Events

Guest Ticket, Closing Social	$50

(Brought to you by Intel and HP)

Registration Hours

Saturday through Tuesday
Lower Level Lobby

On–site registration will be open during the following hours:

Saturday, February 7	7:00 am – 6:00 pm
Sunday, February 8	7:00 am – 6:00 pm
Monday, February 9	7:00 am – 6:00 pm
Tuesday, February 10	7:00 am – 6:00 pm

Receipts

All who register online will receive a receipt/confirmation via email. Participants who register onsite will receive a paper receipt if requested.

Certificate of Attendance

Certificates of Attendance *will not* be provided at ABRF 2009.

Badges

Badges should be worn at all official functions of the meeting. Badge checkers will be stationed throughout the convention center. Only those with full meeting registrations will be allowed into sessions.

If you forget or lose your badge, you may obtain a second badge at registration with proof of registration.

Ticketed Events

Activities for which you have purchased tickets or guest tickets require the actual ticket for entrance. Tickets are provided at the time of check-in. Please be sure to check that you received tickets for all purchased items.

Satellite Educational Workshops*

Saturday, February 7

Next Generation DNA Sequencing	$320
Proteomics Instrumentation	$320
HPLC Theory and Practice	$320
Proteome Informatics	$320

Friday, February 6 – Saturday, February 7 *(2 day workshop)*

Recombinant Protein Laboratory	$375

*Please note that Satellite Educational Workshops are not included in the regular meeting registration. You must purchase a separate registration to attend a specific Satellite Educational Workshop. Session Organizers will be checking badges for all Satellite Educational Workshops.

SPECIAL EVENTS

Opening Reception

Saturday, February 7
Ballroom Foyer
7:00 pm – 8:30 pm

Kick-off the conference week and join your colleagues for light refreshments and good company at the ABRF 2009 Opening Reception.

Closing Social *(Brought to you by Intel and HP)*

Tuesday, February 10
Offsite – Rum Boogie Café
7:00 pm – 10:00 pm

Good food and drinks with Memphis music, dancing, games, informal networking with colleagues, and maybe Elvis will be in the house! All full meeting registrants receive one ticket to the Closing Social. Tickets will be collected upon arrival. If you do not receive your ticket to the Closing Social, please visit the Registration Desk, located on Level 1, Lobby. Additional guest tickets may be purchased at the Registration Desk.

MEALS & REFRESHMENTS

Meeting registration includes all meals, receptions, and refreshments provided by the ABRF 2009, as well as one ticket to the Closing Social.

Opening Reception

Saturday, February 7
Ballroom Foyer
7:00 pm – 8:30 pm

Continental Breakfast

Sunday through Tuesday
Ballroom Foyer
7:00 am – 7:45 am

Morning Refreshment Break

Sunday through Tuesday
Exhibit Hall
10:15 am – 10:45 am

Lunch — Munch & Mingle

Sunday through Tuesday
Exhibit Hall
12:00 pm – 1:00 pm

Afternoon Refreshment Break

Sunday through Tuesday
Exhibit Hall
3:15 pm – 3:45 pm

Evening Networking Receptions

Sunday through Monday
Exhibit Hall
5:15 pm – 6:30 pm

Closing Social

Tuesday
Rum Boogie Café
7:00 pm – 10:00 pm

GENERAL INFORMATION

Accessibility for Registrants with Disabilities

The meeting staff will work with attendees to provide reasonable accommodations for those who require special needs. To request assistance on-site, please check in at the Registration Desk located on Level 1, Lobby.

Cameras, Recording Devices, and Cell Phones

The use of cameras, recorders and/or cell phones is strictly prohibited during the oral and poster sessions. Limited use is allowed for Exhibitors in their own booth area. Personal photography is allowed at social functions.

Cyber Café

Computer terminals with complimentary Internet access will be available in the Exhibit Hall during Exhibit Hall hours only. Access will be granted on a first come first served basis; however, as a courtesy to fellow meeting attendees, we ask that you limit your time at the computer to 10 minutes.

First Aid

The First Aid room is on the 2nd floor (Ballroom/Exhibit Hall level) on the south west side of the building. ABRF will have EMT's on hand during show hours for any attendee requiring First Aid. All calls for aid should be routed through extension 1214 or the Convention Manager on Duty.

Hotels

The ABRF 2009 has two participating hotels, each within easy walking distance to the Memphis Cook Convention Center.

Conference Hotels

Marriott Memphis Downtown
250 North Main Street
Phone: +1 (800) 228-9290

Crowne Plaza Memphis
300 North Second Street
Phone: +1 (901) 525-1800

Message and Information Center

Sunday through Tuesday
Level 1, Lobby
Phone: +1 (901) 576-1257

Anyone trying to contact meeting attendees should call the number listed above. Telephone messages will be posted on message boards. Please plan to check these boards regularly in case other attendees are trying to reach you. Outside of registration hours it is recommended that messages be left at the attendee's hotel. Attendees will not be paged.

Lost & Found

Lost & Found will be located at the Registration Desk, located on Level 1, Lobby.

Local Transportation & Parking

There are a number of transportation options to and from the airport and in and around the city of Memphis. The estimated one way fare to and from the airport is approximately $30.00. *Please contact individual transportation companies for actual fares.*

Taxis & Shuttles

Yellow Cab and Checker Cab
581 S. Second Street
Memphis, TN 38103
Phone: +1 (901) 577-7777
Fax: +1 (901) 577-7765
www.premiereofmemphis.com

Tennco Downtown Shuttle Service
22 N. Third
Memphis, TN 38103
Phone: +1 (901) 522-0001
Fax: +1 (901) 522-1019
www.tenncoexpress.com
alonzo@tenncoexpress.com

Premier Transportation Services
581 South Second Street
Memphis, TN 38126
Phone: +1 (901) 577-7700
Fax: +1 (901) 577-7765
www.premierofmemphis.com
info@premierofmemphis.com

Parking

Onsite parking at the Memphis Cook Convention Center will be available for conference attendees. Parking rates will vary. Please check with the convention center for updated parking fees.

ABRF Membership

Interested in joining ABRF as a member? Visit an ABRF representative at Booth 521 and learn more about the benefits of becoming part of one of the leading organizations dedicated to advancing core and research biotechnology laboratories. An ABRF Membership Application is available at the end of the conference program book.

Additional Program/Abstract Book

Each registrant will receive one copy of the Program/Abstract Book. Additional copies will be sold for $10 each. Please visit the Registration Desk for more information.

PRESENTER INFORMATION

Speaker Ready Room

Saturday through Tuesday
Room L4

ALL Oral Presenters must check in at the Speaker Ready Room at least 4 hours prior to their scheduled presentation. Even if you have submitted your presentation in advance and have no changes, you must check and confirm that the presentation is correct. The Speaker Ready Room may be accessed during the following hours:

Saturday, February 7..... 7:00 am – 6:00 pm
Sunday, February 8..... 7:00 am – 6:00 pm
Monday, February 9 ..... 7:00 am – 6:00 pm
Tuesday, February 10..... 7:00 am – 5:00 pm

You may also edit your presentation at this time. When you are finished reviewing your presentation and verify it is ready, the AV personnel will queue your presentation onto the networked conference computers. The file will then be transferred to the computer network for presentation in the scheduled room.

Staff in the Speaker Ready Room will be available during the following hours:

Saturday, February 7.....2:00 pm – 5:00 pm
Sunday, February 8* ..... 7:00 am – 6:00 pm
Monday, February 9*..... 7:00 am – 6:00 pm
Tuesday, February 10* ..... 7:00 am – 5:00 pm

**Staff will not be available between 11:30 am and 1:30 pm on Sunday, Monday and Tuesday.*

Poster Presentations

Sunday through Tuesday
Exhibit Hall

Over 150 posters will be on display all day Sunday through Tuesday. The poster presenters should be available for questions at their display boards during their assigned session times. Poster Session times are:

Poster Session I – Scientific Research Posters
Sunday, February 8
1:00 pm – 2:00 pm

Poster Session II – Scientific Research Posters
Sunday, February 8
5:15 pm – 6:30 pm

Poster Session III – Core Facility Posters
Monday, February 9
5:15 pm – 6:30 pm

All posters should be set up at least one hour prior to the opening of the Exhibit Hall on Sunday, February 8th. Poster presenters will be given a special pass to allow them access into the exhibit hall during these hours. If you are missing this pass, please visit the Registration Desk to receive the access pass. All posters must be removed from the exhibit hall between the hours of 2:00 pm – 3:00 pm on Tuesday, February 10th. Do not leave your presentation on the poster board. Posters remaining after 3:00 pm on Tuesday will be discarded.

ABRF MEETING ORGANIZERS

ABRF 2009 Organizing Committee

George Grills (chair)
Life Sciences Core Laboratories Center
Cornell University, Ithaca, NY

Chris Turck (co-chair)
Max Planck Institute of Psychiatry
Munich, Germany

Margaret Robertson (ad hoc)
Ernest Gallo Clinic and Research Center
University of California, San Francisco

Theodore Thannhauser (ad hoc)
USDA — Agricultural Research Station
Cornell University, Ithaca, NY

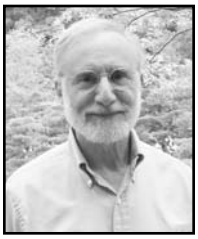
ABRF EXECUTIVE BOARD

Mike Doyle
President
Bristol – Myers Squibb Pharma
Princeton, NJ

Pamela "Scottie" Adams
Secretary/Treasurer
Trudeau Institute
Saranac Lake, NY

Nancy Denslow
University of Florida
Gainesville, FL

Michelle Detwiler
President-Elect
Roswell Park Cancer Institute
Buffalo, NY



Arnold Falick
Secretary/Treasurer-Elect
University of California
Berkeley, CA

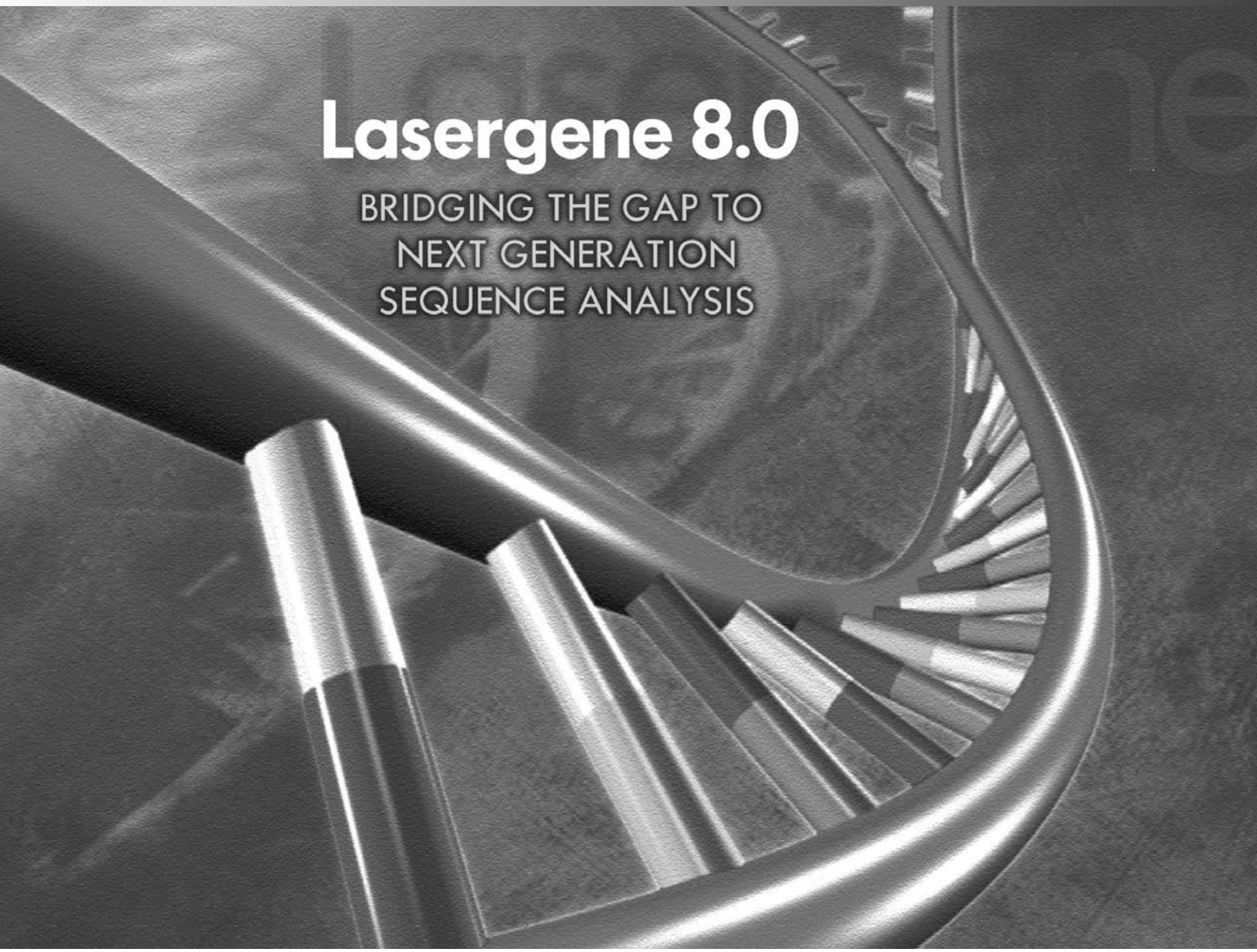
Karen Jonscher
Board-Elect
University of Colorado Denver
Denver, CO

Jeff Kowalak
NIMH
Bethesda, MD

Jack Simpson
Board-Elect
SAIC — Frederick
Cascade, MD

Chris Turck
Max Planck Institute of Psychiatry
Munich, Germany

Tony Yeung
Fox Chase Cancer Center
Philadelphia, PA



Meet the needs of today with a solution for tomorrow.

The Lasergene 8.0 suite of sequence analysis tools bridges the gap between conventional and next generation sequencing – allowing you to use the same software for analysis and visualization of both sequencing methods.

Featuring the highly effective functionality of previous versions, Lasergene 8.0 also includes many new capabilities.

New Lasergene 8.0 benefits your research with:

- Simplified primer design and virtual cloning, thanks to the added primer designing capabilities in SeqBuilder
- Faster and easier SNP detection and analysis for your specific project needs
- Ideal for Next Generation platforms; can be used with SeqMan NGen for enhanced analysis and visualization of data

Discover how Lasergene 8.0 is bridging the gap between the needs of today and the promise of tomorrow. Contact us for a free 30-day fully functional trial version.

DNASTAR, Inc • Madison, WI, USA • www.dnastar.com
Toll free: 866.511.5090 • 608.258.7420 • UK 0.808.234.1643

DNASTAR® Lasergene

MEETING SPONSORS

The Association of Biomolecular Resource Facilities (ABRF) and the ABRF 2009 Organizing Committee would like to extend their gratitude for the generous support received from the following organizations:

Closing Social

Meeting Backpacks & Water Bottles

Cyber Café

Munch & Mingle Lunch (Monday)

Scientific Session (s10)

Conference Pens

Conference Notepads

Award Sponsors

ABRF Award

ABRF Poster Awards

ABRF Outstanding Scientist/ Technologist Travel Awards

Academic Sponsors

Media Sponsors

CORPORATE SPONSORS

Platinum Corporate Sponsors

Affymetrix

Applied Biosystems

Dionex

DNASTAR, Inc.

Intel Corporation

Life Technologies

Roche Applied Science

Thermo Fisher Scientific

Gold Corporate Sponsors

Agilent Technologies

Silver Corporate Sponsors

AAA Laboratory

AnaSpec

Biosearch Technologies

Edge Bio

Fluidigm Corporation

Genisphere Inc.

Grizzly Analytical

Hewlett-Packard Company

Illumina

International Equipment Trading Ltd.

Princeton Separations

Promega Corporation

Protea Biosciences, Inc.

Waters Corporation

Academic Sponsors

Fox Chase Cancer Center

Interdisciplinary Center for Biotechnology Research (ICBR), University of Florida

ABRF Award Presentation & Lecture

Monday, February 9
Ballroom A
1:00 pm – 1:50 pm

The ABRF Award is sponsored by Agilent and is awarded annually by the ABRF in recognition of outstanding contributions in the field of biotechnology. The ABRF 2009 Award recipient is **Mathias Uhlen** of the Royal Institute of Technology.

Mathias Uhlen is Professor of Microbiology at the Royal Institute of Technology (KTH), Stockholm, Sweden. Dr. Uhlen is a member of the Royal Swedish Academy of Engineering Science (IVA), the Royal Swedish Academy of Science (KVA), the European Molecular Biology Organization (EMBO) and is a member of the Human Proteome Organization (HUPO) council. He was Vice-President of the Royal Institute of Technology (KTH), responsible for external relations, from 1999 to 2001. He was the chairman of the Swedish Biochemical and Molecular Biology Society (SFMB) from 1994 to 1999. Dr. Uhlen has more than 300 publications in bioscience with the focus on the development and use of affinity reagents in biotechnology and biomedicine. In the early eighties, Dr. Uhlen cloned and characterized staphylococcal protein A, which is now used extensively for purification of antibodies both in diagnostics and therapy. He also showed in 1983 that protein A could be used as an affinity tag for purification of other proteins. Affinity tags for purification of recombinant proteins are now widely used in bioscience.

In the late eighties, Uhlen published the use of magnetic micro spheres with streptavidin for automated solid phase applications. Such laboratory systems based on streptavidin beads are at present frequently used both in research and diagnostics. In the nineties, his group described a new principle for affinity reagents, called Affibodies, and showed their use as research tool and recently as potential cancer therapeutics. Uhlen and colleagues also developed a new strategy for DNA analysis called Pyrosequencing, a method that has recently been further developed by a US company into a highly parallelized sequencing instrument.

Past ABRF Award Winners

1994	Frederick Sanger
1995	Klaus Bieman
1996	David Lipman
1997	Lloyd Smith
1998	Bruce Merrifield
1999	Marvin Caruthers
2000	Leroy Hood
2001	Csaba Horváth
2002	John Fenn
2003	Franz Hillenkamp and Michael Karas
2004	Edwin Southern
2005	Stephen Fodor
2006	Roger Tsien
2007	Don Hunt
2008	Ruedi Abersold

Dr. Uhlen is currently working on the Human Protein Resource Project (HPR), with the aim to systematically map the human proteome. At present, the Human Protein Atlas portal (www.proteinatlas.org) contains more than 5 million high-resolution images representing 5000 human proteins. He has founded several companies, including Pyrosequencing AB (now Biotage AB), Affibody AB, SweTree Technologies AB, Magnetic Biosolutions AB (now Nordiag AS), Atlas Antibodies AB and Creative Peptides AB.

He has received numerous awards, including The Svedberg prize in 1992, the Göran Gustavsson prize in 1993, the gold medal of the Royal Swedish Academy of Engineering Sciences in 2004, the Most Noble Order of the Seraphim — the Order of His Majesty the King in 2004, the Jerker Porath award in 2005, the Akzo Noble Award in 2005, the HUPO Distinguished Award and KTH Great Prize both in 2006 and the Scheele prize in 2007.

ABRF Lifetime Membership Award

The ABRF Lifetime Membership Award is presented by the ABRF Executive Board to members with longstanding contributions to the success of ABRF and its missions. This year's recipient is **Ronald Niece** of Research Resources & Technologies.

Thermo Scientific ABRF Oustanding Scientist/Technologist Travel Award

Recipients for the ABRF Travel Awards are ABRF members who have made outstanding contributions in their institutional core facilities, have developed new biotechnologies with applications in core facilities, and/or have been active in ABRF activities.

The 2009 awardees are:

Doug Bintzler
DNA Analysis, LLC
Cincinnati, OH

Ronald Niece
Research Resources & Technologies
Tustin, CA

Sridar Chittur
Center for Functional Genomics
University at Albany, SUNY
Rensselaer, NY

Maria Person
University of Texas at Austin
Analytical Instrumentation Facility Core
Austin, TX

Jennifer Holbrook
Nemours/A.I. duPont Hospital
for Children
Wilmington, DE

Rakesh Rathore
Genome Research Institute
University of Cincinnati
Cincinatti, OH

Alexander Ivanov
Harvard Proteomics Resource,
Harvard School of Public Health
Boston, MA

Matthew Robinson
Program in Molecular Medicine
Fox Chase Cancer Center
Philadelphia, PA

Angen Liu
Fox Chase Cancer Center
Laser Capture Microdissection Facility
Philadelphia, PA

Christof Straub
Department of Biochemistry
and Molecular Biology
The University of Texas Medical Branch
Galveston, TX

ABRF 2009 Travel Award Selection Committee

Mike Doyle *(Chair), Bristol Myers-Squibb*
Lester Taylor, *ThermoFisher Scientific*
Richard Pon, *University of Calgary*
Satya Yadav, *Cleveland Clinic Lerner Research Institute*

ABRF Research Group / Committee Member Awards

The ABRF Research Group/ Committee Member Award is presented to individuals in recognition of their significant contributions as members of ABRF Research Groups and Committees. The 2009 award will be presented to **Caprice Rosato** of The Center for Genome Research and Biocomputing, Oregon State University.

The Journal of Biomolecular Techniques (JBT) Award

The JBT Award recognizes the most outstanding research article published during the last year in the journal. This year's JBT Award recipient is:

Tissue Fractionation by Hydrostatic Pressure Cycling Technology: The Unified Sample Preparation Technique for Systems Biology Studies

Vera Gross[1], Greta Carlson[1], Ada T. Kwan[1], Gary Smejkal[1], Emily Freeman[2], Alexander R. Ivanov[2], and Alexander Lazarev[1]

[1]Pressure BioSciences, Inc., Woburn, MA, United States; [2]Harvard School of Public Health, Boston, MA, United States

This award will be accepted by Vera Gross.

ABRF Poster Awards

Waters Corporation is sponsoring prizes for the four best posters presented at ABRF 2009. The ABRF Education Committee will review all poster abstracts submitted to the meeting and select a limited number of candidates for award consideration. Lead authors of the candidate posters will be invited to give a short (10 minute) presentation of their poster to the Education Committee on Sunday, February 8, 2009. The authors of the four posters judged to be the best will receive the Waters Corporation sponsored awards at the Poster Award Session on Tuesday, February 10, 2009.

ABRF Student/Post-Doc Poster Awards

The ABRF presents awards to students and post docs who present research made possible through collaboration with a core facility. The following students have been awarded this year's ABRF Student/Post Doc Award:

Ze-Qiang Ma
Department of Biomedical Informatics
Vanderbilt University Medical Center
Nashville, Tennessee

Rakesh Rathore
Proteomics Laboratory
Department of Cancer and Cell Biology
Genome Research Institute,
University of Cincinnati
Cincinnati, Ohio

Christof Straub
The University of Texas Medical Branch at Galveston
Galveston, Texas

Agnieszka Kendrick
Proteomics Division of the Clinical Nutrition Research Unit
Mass Spectrometry Core Facility
Department of Anesthesiology
University of Colorado Denver
Denver, Colorado

A Streamlined Solution for Quantifying Targeted Proteins

Qualitative studies can identify possible protein biomarkers, but targeted quantitative studies are essential to verify your candidates. The Thermo Scientific targeted protein quantitation solution provides a seamless transition from discovery to verification of putative biomarkers.

- Application-specific SRM Workflow software helps you leverage the invaluable information from your discovery experiments to optimize your quantitative methods.
- The TSQ Vantage™ LC-MS/MS offers the highest sensitivity and lowest noise for a virtually unlimited number of targeted peptides. Its superior selectivity eliminates interferences from complex matrices, lowering the limits of quantitation.
- HeavyPeptide™ stable isotope-labeled internal standards can be customized to exactly match your target peptides for accurate absolute quantitation.

To streamline the critical transition from biomarker discovery to targeted quantitative verification, contact us for the most complete, most productive solution.

Learn more at ABRF Booth 401

Attend a free workshop:

Quantification of Phosphoproteins

New experimental approaches for investigating cellular proliferation

Sunday, February 8, 2009
7:40 p.m. – 8:40 p.m.
Room 202
Memphis Cook Convention Center

Moving science forward

Thermo SCIENTIFIC

Part of Thermo Fisher Scientific

DAILY PROGRAM

Please note that this program is current as of 01/27/09. Refer to your Program Addendum for additional updates.
All events are located in the Memphis Cook Convention Center unless otherwise noted.

Friday, February 6, 2009

8:00 am - 4:00 pm **Satellite Educational Workshops**

(sw3) Recombinant Protein Laboratory (Day 1)
Offsite, St. Jude Children's Research Hospital

Instructors: John Hawes, *Miami University (organizer)*, Richard Heath, *St. Jude Children's Research Hospital (co-organizer)*, James Bryson, *Bristol-Myers Squibb*, Preston Hensley, *Pfizer*, Cynthia Kinsland, *Cornell University*, and Francis Rajamohan, *Pfizer*

Saturday, February 7, 2009

7:00 am - 6:00 pm **Registration Open** — *Lobby*

8:00 am - 4:00 pm **Satellite Educational Workshops**

(sw1) Next Generation DNA Sequencing
Room 205

Massively Parallel Sequencers in the Core Facility: Applications and Computation.
Instructors: Michael Zianni, *Ohio State University (organizer)*, Caprice Rosato, *Oregon State University (co-organizer)*, Michelle Detwiler, *Roswell Park Cancer Institute (co-organizer)*, Charles Nicolet, *University of California at Davis (co-organizer)*, Peter Schweitzer, *Cornell University (co-organizer)*, Alvaro Hernandez, *University of Illinois*, James Hadfield, *Cambridge Research Institute, UK*, Todd Smith, *Geospiza*, Scott Givan, *Oregon State University*, Faye Schilkey, *National Center for Genome Resources*, and Jer-Ming Chia, *USDA-ARS / Cold Spring Harbor Laboratory*

(sw2) Proteomics Instrumentation
Room 203

Introduction to Proteomics Platforms and Mass Spectrometry: Applications for Qualitative and Quantitative Studies.
Instructors: David Friedman, *Vanderbilt University (organizer)*, and Hayes McDonald, *Vanderbilt University (co-organizer)*

(sw3) Recombinant Protein Laboratory (Day 2)
Offsite, St. Jude Children's Research Hospital

Instructors: John Hawes, *Miami University (organizer)*, Richard Heath, *St. Jude Children's Research Hospital (co-organizer)*, James Bryson, *Bristol-Myers Squibb*, Preston Hensley, *Pfizer*, Cynthia Kinsland, *Cornell University*, and Francis Rajamohan, *Pfizer*

(sw4) HPLC Theory and Practice
Room 202

Practical Aspects of Protein and Peptide HPLC Separations for Proteomics.
Instructors: Andrew Alpert, *PolyLC Inc. (organizer)*, and Kerry Nugent, *Michrom BioResources (co-organizer)*

(sw5) Proteome Informatics
Room 204

Real-life Proteome Bioinformatics for Laboratories.
Instructors: Lennart Martens, *EMBL-European Bioinformatics Institute, UK (organizer)*, David Tabb, *Vanderbilt University (co-organizer)*, Brian Searle, *Proteome Software*, and Sean Seymour, *Applied Biosystems*

4:30 pm - 6:30 pm **Tours of Core Facilities**
Hartwell Center for Bioinformatics & Biotechnology, St. Jude Children's Research Hospital

4:30 pm - 6:30 pm **Satellite Workshop of National Network of IDeA-Funded Core Laboratories (NICL)**
Room 201

Building Resources: The National Network of IDeA-Funded Core Laboratories (NICL).
Instructors: Katia Sol-Church, *Center for Pediatric Research, NCC-Delaware (organizer)* and Timothy Hunter, *University of Vermont (co-organizer);* NICL Steering committee also includes Stephen Bobin, *Dartmouth Medical School*, Steven F. Jennings, *University of Arkansas at Little Rock*, and Faye Schilkey, *NM Genome Sequencing Center*

7:00 pm - 8:30 pm **Welcome Reception** — *Ballroom Foyer*

Sunday, February 8, 2009

7:00 am - 6:00 pm **Registration Open** — *Lobby*

7:00 am - 8:00 am **Continental Breakfast** — *Ballroom Foyer*

7:45 am - 8:00 am **Opening Remarks** — *Ballroom A*
George Grills, *Cornell University (Meeting Organizing Committee Chair)*

8:00 am - 8:50 am **Plenary Session on Genomics** — *Ballroom A*

The Changing Landscape of Genomics
Richard Gibbs, *Wofford Cain Professor, Department of Molecular and Human Genetics, Director, Human Genome Sequencing Center, Baylor College of Medicine*

9:00 am - 10:15 am **Concurrent Scientific Sessions**

(s1) Personalized Medicine — *Ballroom A*
Eric Wieben, *Mayo Clinic (session organizer)*

(s1-a) Moving Genomics from Research to Clinical Care in Childhood Leukemia
Mary Relling, *St. Jude Children's Research Hospital*

(s1-b)
W. Edward Highsmith, *Mayo Clinic*

(s1-c)
Ulrich Broeckel, *Medical College of Wisconsin*

(s2) Biofuels — *Ballroom C*
Towards a Biofuels Economy: Technologies and Infrastructure Associated with Developing New Bioenergy Crops
Jocelyn Rose, *Cornell University (session organizer)*

(s2-a) Instrumentation and Methods for the High Throughput Analysis of Plant Materials as a Resource for Biofuels
Markus Pauly, *Michigan State University*

(s2-b) From Single Molecules Spectroscopy to Process Batch Fermentation: Methods and Instrumentation Implemented in the Biofuel Research Laboratory at Cornell University
Stephane Corgie, *Cornell University*

(s2-c) Identify Molecular Features of Biomass Recalcitrance Using Non-Destructive Microscopy and Spectroscopy
Shi-You Ding, *National Renewable Energy Laboratory (NREL)*

(s3) Optical Imaging — *Ballroom D*

(s3-a) Developments in Microscopic Imaging of Intravital Dynamics
Sam Wells, *Vanderbilt University School of Medicine (session organizer)*

(s3-b) Intravital Multiphoton Microscopy in a Microscopy Core Resource
Kenneth Dunn, *Indiana University School of Medicine*

10:00 am - 6:30 pm **Exhibits Open** — *Exhibit Hall*

10:15 am - 10:45 am	**Refreshment Break** — *Exhibit Hall*
10:45 am - 12:00 pm	**Concurrent Research Group Presentations**

(r1) Joint Research Group Presentation on Proteomics — *Ballroom A*
Evaluating the State of the Art in Quantitative Proteomics

Proteomics Research Group (PRG)
(r1-a) PRG 2009 Study: Relative Protein Quantification in a Clinical Matrix
Michael MacCoss, *University of Washington (session organizer)*

Proteomics Standards Research Group (sPRG)
(r1-b) sPRG 2009 Study: Challenges Along the Way to a Quantitative Proteomics Standards
James Farmar, *Albert Einstein College of Medicine (session organizer)* and Jeffrey Kowalak, *National Institute of Mental Health (NIMH)*

Proteome Informatics Research Group (iPRG)
(r1-c) iPRG 2009 Study: Testing for Qualitative Differences Between Samples in MS/MS Proteomics Datasets
Brian Searle, *Proteome Software (session organizer)*

(r2) Nucleic Acids Research Group (NARG) — *Ballroom C*
NARG 2008-2009 Study: A Comparison of Different Priming Strategies for cDNA Synthesis by Reverse Transcriptase, as Evaluated by Real-Time qPCR
Kevin Knudtson, *University of Iowa (session organizer)* and Scott Tighe, *University of Vermont*

12:00 pm - 1:00 pm	**Lunch: Munch & Mingle** — *Exhibit Hall*
1:00 pm - 2:00 pm	**Poster Session I: Scientific Research Posters** — *Exhibit Hall*
2:00 pm - 3:15 pm	**Concurrent Scientific Sessions**

(s4) Next Generation Nucleic Acid Quantitation — *Ballroom C*
Kevin Knudtson, *University of Iowa (session organizer)*

(s4-a)
Roger Bumgarner, *University of Washington*

(s4-b)
Syed Hasham, *Michigan State University*

(s4-a) Profiling Circulating Tumor Cells as Prognostic Biomarkers in Patients with Metastatic Prostate Cancer
Daniel Danila, *Memorial Sloan-Kettering Cancer Center*

(s5) Biotech in Forensics and Clinical Diagnostics — *Ballroom D*
Lessons in Optimizing Specialty Facilities: Forensics, Systematics and Clinical
Howard Cash, *Genecodes (session organizer)*

(s5-a) Assay Development: From Proof-of-Concept to CLIA-validation of Assays for Patient Testing
Ellen Paxinos, *Monogram Biosciences*

(s5-b) Biodiversity Documentation via DNA Barcoding at the Smithsonian Institution's L.A.B.
Lee Weigt, *Smithsonian Institution, National Museum of Natural History, Laboratories of Analytical Biology*

(s5-c) Existing and Emerging Biotechnologies for Forensic DNA Applications
Mitch Holland, *Pennsylvania State University*

(s6) Quantitative Proteomics — *Ballroom A*
Reproducibility in Quantitative Proteomics
Kathryn Lilley, *University of Cambridge, UK (session organizer)*

(s6-a) Reproducibility of 2D Gel-based Proteomics Experiments
Jules Westbrook, *University College Dublin, Ireland*

(s6-b) Reproducibility of Protein MRM-Based Assays: Towards Verification of Candidate Biomarkers in Human Plasma
Steven Hall, *University of California at San Francisco*

3:15 pm - 3:45 pm	**Refreshment Break** — *Exhibit Hall*
3:45 pm - 5:00 pm	**Concurrent Technical Workshops**

(w1) Core Facility Management Models: Development and Culture — *Ballroom A*
Valerie Scott, *Jackson Laboratory (session organizer)*

(w1-a)
Julie Auger, *University of Chicago*

(w1-b)
Kelvin Lee, *University of Delaware*

(w1-c) Enhancing Core Research Facility Management, Strategy, and Investment
Rand Haley, *Huron Consulting Group*

(w2) Women in Science and Core Laboratories — *Ballroom C*
Michelle Cilia-Reeve, *USDA-ARS (session organizer)* and Michelle Detwiler, *Roswell Park Cancer Institute (session co-organizer)*

(w2-a) From Proteins to DNA and Back Again
Kathryn Lilley, *University of Cambridge, UK*

(w2-b) One Step at a Time: Directing a Core Facility
Nancy Denslow, *University of Florida*

(w2-c) Sustaining Women in Science, Lessons from the American Society for Cell Biology
Joan Goldberg, *American Society for Cell Biology*

(w3) Educational Outreach — *Ballroom D*
Panel: Janet Murray, *University of Vermont (session organizer)*, Cherilynn Shadding, *Washington University*, Laurel Southard, *Cornell University* and Virginia Shepherd, *Vanderbilt University*

5:15 pm - 6:30 pm	**Poster Session II: Research Posters** — *Exhibit Hall*
	Networking Reception
	ABRF Student/Post Doc Poster Awards
6:30 pm - 7:30 pm	**Concurrent Exhibitor Presentations I**

(e1) Part I: Enhanced Detection and Identification of Multiple Phosphorylated Peptides Using TiO2 Enrichment in Combination with MALDI TOF/TOF MS; Part II: Improved Methods for Phosphopeptide Analysis | Dionex Corporation — *Room 201*
(e2) Join HP — Discover HP Petabyte-Scale Storage Solutions for Life Sciences: "Mastering the Data Challenge in Life Sciences" | Hewlett-Packard — *Room 202*
(e3) Roche Applied Science (Part I) — *The location for this presentation has changed — please visit booth #400 for details.*
(e4) MALDI TDS: A Top-Down-Sequencing Approach for Biotherapeutics Characterization | Bruker Daltonics — *Room 204*
(e5) miRNA - Small Tools Enabling Big Discoveries — Recent Advances in miRNA Research and Validation with TaqMan® microRNA Assays | Applied Biosystems — *Room 205*

7:40 pm - 8:40 pm	**Concurrent Exhibitor Presentations II**

(e6) A Novel Multiplexed Digital Gene Expression Technology | NanoString Technologies — *Room 201*
(e7) Quantification of Phosphoproteins: New experimental approaches for investigating cellular proliferation | Thermo Scientific — *Room 202*
(e8) Roche Applied Science (Part II) — *The location for this presentation has changed — please visit booth #400 for details.*
(e9) New Epigenetics Technologies for Biomedical Research: microRNA, Chromatin IP, and DNA Methylation PCR Arrays | SABiosciences Corporation — *Room 204*
(e10) miRNA — Small Tools Enabling Big Discoveries — MicroRNA Discovery and Profiling Using Next Generation Sequencing | Applied Biosystems — *Room 205*

Monday, February 9, 2009

7:00 am - 6:00 pm	**Registration Open** — *Lobby*
7:00 am - 8:00 am	**Continental Breakfast** — *Ballroom Foyer*
7:45 am - 8:00 am	**ABRF Outstanding Scientist/Technologist Travel Awards** — *Ballroom A*

(Sponsored by Thermo Scientific)

8:00 am - 8:50 am **Plenary Session on Proteomics** — *Ballroom A*

Technologies for Comprehensive Proteome Quantitation
Matthias Mann, *Head of Proteomics and Signal Transduction, Max Planck Institute for Biochemistry, Germany*

9:00 am - 10:15 am **Concurrent Scientific Sessions**

(s7) Metagenomics — *Ballroom A*
Computational Approaches for Metagenomic and Supragenomic Data Sets

(s7-a) Development and Application of a Global Comparative Genomics Pipeline for Performing Inter-isolate Analyses within Microbial Species
Garth Ehrlich, *Allegheny Singer Research Institute and Drexel University College of Medicine (session organizer)*

(s7-b) Massively Parallel Barcoded Pyrosequencing Reveals Unexpected Diversity in the Human Microbiome
Rob Knight, *University of Colorado at Boulder*

(s7-c) MG-RAST: a Web-based Tool for the Analysis of Metagenomic Data Sets
Folker Meyer, *Argonne National Laboratory*

(s8) Label Free Detection — *Ballroom C*

(s8-a) Label-Free Characterization of Protein-Peptide and Protein-Drug Interactions
Gil Privé, *University of Toronto and Ontario Cancer Institute (session organizer)*

(s8-b) Application of Differential Static Light Scattering and Isothermal Denaturation to Investigate Thermostability and Binding Specificity of Protein Families
Masoud Vedadi, *Structural Genomics Consortium, University of Toronto*

(s8-c) Biochemical and Cell-based Detection by Epic System
Meng Wu, *Department of Neuroscience and High Throughput Biology Center, School of Medicine, Johns Hopkins University*

(s9) Stem Cell Applications — *Ballroom D*

(s9-a) Adult Stem Cell Cores and Regenerative Medicine
Jeffrey Spees, *University of Vermont (session organizer)*

(s9-b) Probing Human Embryonic Stem Cell Proliferation with Arrayed Cellular Microenvironment Technology
Karl Willert, *University of California at San Diego*

(s9-c) The Ellison Stem Cell Core
Carol Ware, *University of Washington*

10:00 am - 6:30 pm	**Exhibits Open** — *Exhibit Hall*
10:15 am - 10:45 am	**Refreshment Break** — *Exhibit Hall*

10:45 am - 12:00 pm **Concurrent Research Group Presentations**

(r3) Joint Research Group Presentation on Genomics — *Ballroom A*
Detection of Human microRNAs across miRNA Arrays and Next Generation DNA Sequencing Platforms

DNA Sequencing Research Group (DSRG)
(r3-a) DNA Sequencing
Peter Schweitzer, *Cornell University (session organizer)*

Microarrays Research Group (MARG)
(r3-b) 2008-09 Joint Research Group Project: Detection of Human microRNAs Across miRNA Array and Next Generation DNA Sequencing Platforms
Susan Hester, *U.S. Environmental Protection Agency (session organizer)*

Genomic Variation Research Group (GVRG)
(r3-c) GVRG 2009 Study
Christian Lytle, *Dartmouth Medical School (session organizer)* and Brewster Kingham, *University of Delaware*

12:00 pm - 1:00 pm **Lunch: Munch & Mingle** — *Exhibit Hall*

(Sponsored by Protea)
Protea Biosciences Introduces the GPR-800, an Advanced Gel Protein Recovery System
Dr. G. Reid Asbury, the Director of Marketing, will introduce the new GPR-800. The GPR-800 is an advanced microfluidic based system for the rapid and efficient recovery of proteins from polyacylamide gels. During the brief presentation, Dr. Asbury will discuss the proprietary de-coupled recovery process. He will also outline the features and discuss potential applications. Finally, he will present the latest data acquired at Protea's state of the art protein mass spectrometry facility.

1:00 pm - 1:50 pm **ABRF Award Presentation & Lecture** — *Ballroom A*

The Creation of a Human Protein Atlas
(Sponsored by Agilent Technologies)
Awardee and Speaker: Mathias Uhlen, *Royal Institute of Technology (KTH), Sweden*

2:00 pm - 3:15 pm **Concurrent Scientific Sessions**

(s10) Next Generation Sequencing — *Ballroom A*
(Session sponsored by Applied Biosystems)

(s10-a) Toward the $1000 Genome: Molecular Engineering Approaches for DNA Sequencing by Synthesis
Jingyue Ju, *Columbia University College of Physicians and Surgeons (session organizer)*

(s10-b) Nearly Complete Genomic Profiling of Individual Identified Neurons: SOLiD Approach
Leonid Moroz, *University of Florida*

(s10-c) Using the SOLiD System for Genomic Solutions
Robert C. Nutter, *Applied Biosystems*

(s11) Phosphoproteomics — *Ballroom C*

(s11-a) A New Acid Mix Enhances Phosphopeptide Enrichment on Titanium and Zirconium Dioxide for Mapping of Phosphorylation Sites on Protein Complexes
Karl Mechtler, *University of Vienna, Austria (session organizer)*

(s11-b) Current Methods in Phospho-Proteomics
Albert Sickmann, *University of Würzburg, Germany*

(s11-c) Analysis of the Yeast Protein Kinase - Substrate Networks by Quantitative Phosphoproteomics
Bernd Bodenmiller, *Swiss Federal Institute of Technology Zürich (ETH Zürich), Switzerland*

(s12) microRNA — *Ballroom D*
MicroRNA Profiling in Medical Practice
George Calin, *M.D. Anderson Cancer Center, Univ. of Texas (session organizer)*

(s12-a) Real-time PCR Expression Profiling of microRNA
Thomas Schmittgen, *Ohio State University*

(s12-b) microRNA: Regulation, Development and Disease
Michael Thomson, *University of North Carolina*

3:15 pm - 3:45 pm	**Refreshment Break** — *Exhibit Hall*
3:45 pm - 5:00 pm	Concurrent Technical Workshops

(w4) Implementing Next Generation Sequencing Technologies — *Ballroom A*
Hit the Ground Running with Next Gen!

(w4-a) Implementing and Running the Illumina GA and Roche 454 at a DNA Sequencing Core Lab
Robert H. Lyons, *University of Michigan*

(w4-b) Implementing and Running SOLiD Services at a Microarray Core Facility
Jeff Palatini, *Comprehensive Cancer Center*

(w4-c) Platform Cross-Comparison and Core Facility Next Gen Survey Data
Helaman Escobar, *University of Utah (session organizer)*

(w5) Implementing Mass Spectrometry Technologies — *Ballroom C*
Challenges of Implementing New Mass Spectrometry Technologies in Shared Resource Facilities
Joseph Loo, *University of California at Los Angeles (session organizer)*

(w5-a) Application of Advanced Mass Spectrometry Technologies at the University of Tennessee Health Science Center
Sarka Beranova-Giorgianni, *University of Tennessee Health Science Center*

(w5-b) Center for Mass Spectrometry and Proteomics at the University of Minnesota: Organization and Policies
Gary Nelsestuen, *University of Minnesota*

(w5-c) The NIH National Center for Research Resources Mass Spectrometry at Washington University
Michael Gross, *Washington University*

(w6) Implementing Optical Imaging Technologies — *Ballroom D*

(w6-a) Implementing Optical Imaging Technologies
Simon Watkins, *University of Pittsburgh School of Medicine (session organizer),* Scott Henderson, *Virginia Commonwealth University*, Victoria Frohlich, *University of Texas Health Science Center*

5:15 pm - 6:30 pm	**Poster Session III: Core Facilities Posters** — *Exhibit Hall*
5:15 pm - 6:30 pm	**Networking Reception —** *Exhibit Hall*
6:30 pm - 7:30 pm	**Concurrent Exhibitor Presentations III**

(e11) Improved Chromatography Solutions for the Analysis of Biopharmaceuticals | Dionex Corporation — *Room 201*
(e12) Identifying Functional Consequences of Molecular Profiles of Cancer through Pathway Analysis | Ingenuity Systems — *Room 202*
(e13) Illumina Technology Workshop | Illumina — *Room 203*
(e14) Development and Validation of the maXis UHR - TOF: A New High Performance Mass Spectrometer for Fast Chromatography | Bruker Daltonics — *Room 204*
(e15) Advion — *Room 205*
(e16) Core Strengthening — Beyond Your Average Routine (Part I) | Applied Biosystems — *Room L2*

7:40 pm - 8:40 pm	**Concurrent Exhibitor Presentations IV**

(e17) Room Temperature DNA Storage, in a Mineral Matrix | GenVault Corporation — *Room 201*
(e18) Fluidigm Integrated Fluidic Circuits: Enabling Core Labs to Meet Future Needs in High-Throughput Genetic Analysis | Fluidigm, Inc. — *Room 202*
(e19) The Apollo 100 System: An Automated Microfluidic System for Integrated Thermocycling and Preparation of 'Ready-to-Inject' Sanger Sequencing Samples | Microchip Biotechnologies, Inc. — *Room 203*
(e20) Fortebio's Octet Platform for Label-free, Real-time Biomolecular Interaction Analysis | ForteBio Inc. — *Room 204*
(e21) Core Strengthening — Beyond Your Average Routine (Part II) | Applied Biosystems — *Room L2*

Tuesday, February 10, 2009

7:00 am - 6:00 pm	**Registration Open** — *Lobby*
7:00 am - 8:00 am	**Continental Breakfast** — *Ballroom Foyer*
7:45 am - 8:00 am	**Journal of Biomolecular Techniques (JBT) Award** — *Ballroom A*

8:00 am - 8:50 am — Plenary Session on Evolutionary Biology — *Ballroom A*

(In recognition of the 200th anniversary of the birth of Charles Darwin and the 150th anniversary of the publication of On the Origin of Species)

Genomics of Extinct and Endangered Species
Stephan Schuster, *Professor of Biochemistry and Molecular Biology, Pennsylvania State University*

9:00 am - 10:15 am — Concurrent Scientific Sessions

(s13) Epigenomics — *Ballroom A*

(s13-a) Techniques for Cytosine Methylation Patterns
Masako Suzuki, *Albert Einstein College of Medicine*

(s13-b) Deciphering the Methylome
Bill Caldwell, *University of Missouri School of Medicine*

(s13-c) High Throughput Chromatin Immunoprecipitations, Integrative Epigenomics, and Personalized Medicine
Ari Melnick, *Weill Cornell Medical College (session organizer)*

(s14) Targeted Proteomics — *Ballroom C*
Practical Targeted Proteomics
Brett Phinney, *University of California at Davis (session organizer)*

(s14-a) Establishing SRM as a Robust and Reliable Technique for Targeted Proteomics
Daneila Tomazela, *University of Washington*

(s14-b) Targeted Proteomics as a Translational Tool in Drug Discovery
Weixun Wang, *Merck Research Laboratories (MRL)*

(s14-c) Practical Aspects of Quantitation with Triple-quadrupole Mass Spectrometers
Benjamin Moeller, *University of California at Davis*

(s15) Bioinformatics — *Ballroom D*
Gil Alterovitz, *Harvard Medical School and MIT (session organizer)* and Gail Rosen, *Drexel University (session co-organizer)*

(s15-a) Informatic Challenges in Metaproteomics
Patricia Carey, *University of Tennessee at Knoxville*

(s15-b) An Agua-Silico Algorithm for Genome Assembly Validation and DNA Biomarker Discovery, and its Potential as a Mass-Market Application for Microfluidic Platforms
Robert Boissy, *Allegheny Singer Research Institute, Center for Genomic Sciences*

(s15-c) Bioinformatics for Metagenomic Taxonomic Classification
Gail Rosen, *Drexel University*

10:00 am - 2:00 pm	**Exhibits Open** — *Exhibit Hall*
10:15 am - 10:45 am	**Refreshment Break** — *Exhibit Hall*

10:45 am - 12:00 pm — Concurrent Research Group Presentations

(r4) Joint Research Group Presentation on Proteins — *Ballroom A*

Edman Sequencing Research Group (ESRG)
(r4-a) ESRG Study 2009: Comparison of Edman and Mass Spectrometry Techniques for N-terminal Sequencing
Peter Hunziker, *University of Zürich, Switzerland (session organizer)* and Wendy Sandoval, *Genentech, Inc.*

Protein Expression Research Group (PERG)
(r4-b)
John Hawes, *Miami University (session organizer)*

(r5) Light Microscopy Research Group (LMRG) — *Ballroom C*
Performance Testing and Standard Good Operating Practice in Light Microscopy
Richard Cole, *Wadsworth Center / NY State Dept. of Health (session organizer)* and Carol Bayles, *Cornell University*

12:00 pm - 1:00 pm	**Lunch: Munch & Mingle** — *Exhibit Hall*
1:00 pm - 2:00 pm	**Concurrent Technical Workshops**

(w7) Core Support and Instrumentation Funding — *Ballroom A*
Structure and Function of Cores: New is Old and Old is New

(w7-a) Structure, Function and Funding of Shared Research Resources
Jay Fox, *University of Virginia (session organizer)*

(w7-b) Evolving Role of Shared Resource Cores and Their Support in the Future
Barbara Alving, *NIH National Center for Research Resources (NCRR)*

(w7-c) Instrumentation for Core Facilities: The NIH SIG and HEI Programs
Marjorie Tingle, *NIH National Center for Research Resources (NCRR)*

(w8) Bio-Information Technology (Bio-IT) — *Ballroom C*
Custom Software Development in Support of Core Facilities

(w8-a) Removing Data Silos with ISIS
Michael McFarland, *Jackson Laboratory*

(w8-b) Internal Software Development and Integration Experiences at the Cornell University Life Sciences Core Laboratories Center
James VanEe, *Cornell University*

(w8-c) SRM 2.0: Building the Next Generation of Core Facility Management Systems
Matthew Stine, *St. Jude Children's Research Hospital (session organizer)*

(w9) Protein Expression — *Ballroom D*
John Hawes, *Miami University (session organizer)*

2:10 pm - 3:15 pm	**Concurrent Technical Workshops**

(w10) Next Generation Sequencing Instruments — *Ballroom A*
Massively Parallel Sequencing Instrumentation Panel Session
Ken Dewar, *McGill University and Genome Innovation Centre (session organizer),* Robert Nutter, *Applied Biosystems,* and Avak Khavejian, *Helicos Biosciences*

(w11) Proteomics Data Publication — *Ballroom C*
Searching and Sorting: Preparing Protein Identification Data for Publication
Ralph Bradshaw, *University of California at San Francisco (session organizer)*

(w11-a) From Results to Publication: Journal Guidelines and Protein Prospector
Robert Chalkley, *University of California at San Francisco*

(w11-b) Providing Mascot Search Results in a Format Suitable for Submission as Supplementary Data
David Creasy, *Matrix Sciences*

(w11-c) Organizing MS/MS Proteomic Data for Publication
Brian Searle, *Proteome Software*

(w12) Molecular Interactions Instruments — *Ballroom D*

(w12-a) Technical Workshop on Real-Time Biophysical Technologies Used for Characterization of Biomolecular Interactions
Satya Yadav, *Cleveland Clinic Lerner Research Institute (session organizer)* and Aaron Yamniuk, *Bristol-Myers Squibb Co. (session co-organizer)* **Panel:** Yasmina N. Abdiche, *BBC Rinat Laboratories-Pfizer, Inc. (Bio-Rad)*, Anthony G. Frutos, *Corning*, Theres Jägerbrink, *Attana AB*, Sriram Kumarswamy, *ForteBio*, and Eric Rhous, *GE Healthcare, Advanced Systems – Biacore*

2:10 pm - 3:15 pm **ABRF Poster Award Presentations** — *Room 201*

(Sponsored by Waters Corporation)

3:15 pm - 3:45 pm **Refreshment Break** — *Ballroom Foyer*

3:45 pm - 5:00 pm **Scientific Session**

(s16) Disruptive Life Sciences Technologies — *Ballroom A*
New Integrated Analytical Systems Based on Miniaturized Optics and Fluidics
Harold Craighead, *Cornell University (session organizer)*

(s16-a) Optical Biosensors: Future Trends and Perspectives
Chris Taitt, *U.S. Naval Research Laboratory*

(s16-b) Lithographically Printed Optical, Fluidic, and Electronic Systems
Axel Scherer, *California Institute of Technology*

(s16-c) Real-Time DNA Sequencing from Single Polymerase Molecules
David Rank, *Pacific Biosciences*

5:10 pm - 6:30 pm **ABRF: Current Status and Future Plans** — *Ballroom A*

Overview of ABRF Progress for 2008 and a Look Toward the Future
Michael Doyle, *Bristol-Myers Squibb (ABRF President)*

Financial State of the ABRF
P. "Scottie" Adams, *Trudeau Institute (ABRF Secretary/Treasurer)*

ABRF Corporate Relations Committee Overview
Mark Lively, *Wake Forest Univ. School of Med. (Corporate Relations Committee)*

ABRF Education Committee Update
Karen Jonscher, *University of Colorado at Denver (Education Committee)*

ABRF Membership Committee Activity 2008
Stephen Bobin, *Dartmouth Medical School (Membership Committee)*

ABRF Research Group / Committee Member Award
Awardee: Caprice Rosato, *Oregon State University*

ABRF Lifetime Achievement Award
Awardee: Ronald Niece, *Research Resources & Technologies*

Closing Remarks

7:00 pm - 10:00 pm **Closing Social** — *Offsite, Rum Boogie Café*

(Brought to you by Intel and HP)

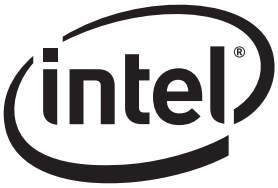
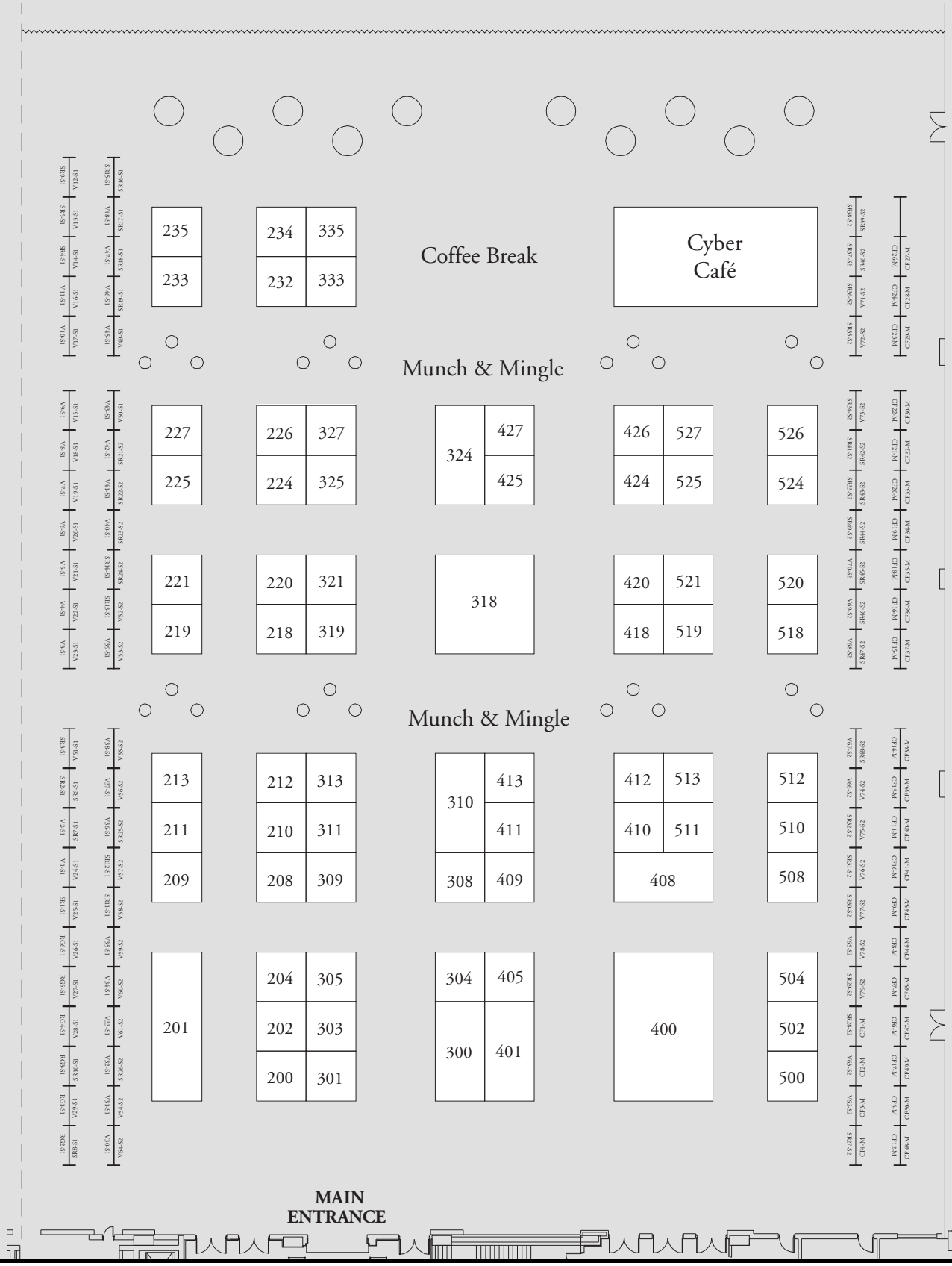


EXHIBIT HALL FLOORPLAN

Memphis Cook Convention Center
Main Exhibit Hall — Memphis, Tennessee



EXHIBITOR LIST *IN BOOTH ORDER*

Exhibitors confirmed as of January 12, 2009.

(M) = ABRF Annual Meeting Sponsor (C) = ABRF Corporate Sponsor

200 ..... Advion
201 ..... Applied Biosystems (M) (C)
204 ..... Genisphere, Inc.
208 ..... NanoString Technologies
209 ..... Bachem Americas Inc. (C)
210 ..... Morehouse School of Medicine — RCMI Program
211 ..... Shodex
212 ..... Geneva Bioinformatics (GeneBio) S.A.
213 ..... Nonlinear Dynamics
218 ..... CovalX
219 ..... New England Biolabs, Inc.
220 ..... Ambry Genetics
221 ..... Bio-Rad Laboratories (C)
224 ..... Enzo Life Sciences
225 ..... GenVault Corporation
226 ..... Michrom Bioresources
227 ..... SABiosciences Corporation
232 ..... Digilab Genomic Solutions
233 ..... New England Peptide, LLC
234 ..... Partek Incorporated
235 ..... Active Motif, Inc.
300 ..... Illumina, Inc. (C)
301 ..... Agilent Technologies (C)
303 ..... FASEB
304 ..... Proteome Software
305 ..... Protein Forest, inc
308 ..... Fluidigm Corporation (C)
309 ..... SeqGen, Inc.
310 ..... Sigma Life Science
311 ..... The Gel Company
313 ..... AnaSpec, Inc. (C)
318 ..... Hewlett-Packard (M) (C)
Closing Social Sponsor with Intel
319 ..... Matrix Science (M)
321 ..... DNAStar (C)
324 ..... Bruker Daltonics
325 ..... BIOINQUIRE
327 ..... CEM Corporation
333 ..... Bioinformatics Solutions, Inc.
400 ..... Roche Applied Science (M) (C)
401 ..... Thermo Scientific
405 ..... New Objective, Inc.
408 ..... Geospiza
409 ..... Affymetrix (C)
410 ..... MassTech, Inc.
411 ..... Cambridge Isotope Labs, Inc.
412 ..... Microchip Biotechnologies, Inc.
413 ..... Eksigent Technologies
418 ..... Dionex Corporation (C)
420 ..... ForteBio, Inc.
424 ..... EdgeBio (M) (C)
425 ..... AAPPTEC
426 ..... GenomeWeb LLC
427 ..... Promega Corporation (C)
500 ..... Agencourt Bioscience Corp., a Beckman Coulter Co. (C)
502 ..... Varian, Inc.
504 ..... GenomeQuest, Inc
508 ..... Waters Corporation (C)
510 ..... Phoenix S&T
511 ..... Protein Discovery
512 ..... SoftGenetics
513 ..... Bioteam, Inc.
518 ..... Ingenuity Systems
519 ..... caprotec bioanalytics gmbh
520 ..... Protea Biosciences, Inc. (M) (C)
521 ..... ABRF
524 ..... Caliper Life Sciences
525 ..... CLC bio
526 ..... AMPTEC
527 ..... PolyLC Inc.

ABRF 2009 EXHIBITS*

All ABRF 2009 Meeting attendees are invited to visit the Exhibit Hall featuring leading experts and commercial providers in life sciences research and biotechnology.

Exhibit Hours

Sunday through Tuesday
Exhibit Hall

Sunday, February 8, 2009..... 10:00 am – 6:30 pm
Monday, February 9, 2009 ..... 10:00 am – 6:30 pm
Tuesday, February 10, 2009..... 10:00 am – 2:00 pm

The exhibition will provide an opportunity to network with an outstanding array of vendors, and will showcase instruments, products and services in a wide range of fields of biotechnology.

*Disclaimer: Participation in the Exhibits Program, including attendance of Exhibitor Presentations, does not constitute an endorsement by the Association of Biomolecular Resource Facilities (ABRF) of the claims, products, or services offered.

EXHIBITOR LIST *IN ALPHABETICAL ORDER*

Exhibitors confirmed as of January 23, 2009.

AAPPTEC

6309 Shepherdsville Road
Louisville, KY 40228
P (502) 968-2223
f (502) 968-3338
www.aapptec.com
Booth 425

ABRF

9650 Rockville Pike
Bethesda, MD 20814-3998
P (301) 634-7306
f (301) 634-7420
abrf@abrf.org
www.abrf.org
Booth 521

The Association of Biomolecular Resource Facilities is an international society dedicated to advancing core and research biotechnology laboratories through research, communication, and education.

Active Motif, Inc.

1914 Palomar Oaks Way, Suite 150
Carlsbad, CA 92008
P (877) 222-9543
f (760) 431-1351
www.activemotif.com
Booth 235

Active Motif offers superior antibodies along with unique and innovative products to analyze nuclear function. Our high-quality reagents and assay kits provide simple, effective solutions to further research in transcriptional regulation, chromatin biology and epigenetics.

Advion

19 Brown Road
Ithaca, NY 14850
P (607) 266-0665
f (607) 257-5761
info@advion.com
www.advion.com
Booth 200

Advion is a scientific leader in microfluidics systems and mass spectrometry. TriVersa NanoMate and its chip-based nanoelectrospray technology generate higher quality information from complex samples than LC/MS alone. RePlay provides a second nanoLC/MS analysis with twice the sensitivity of a duplicate injection resulting in the identification fo 20% more proteins.

Affymetrix

3420 Central Expressway
Santa Clara, CA 95051
P (408) 731-5000
f (408) 731-5380
sales@affymetrix.com
www.affymetrix.com
Booth 409

Affymetrix is widening the reach of your services with the greatest expansion of genomic tools in its history. Our portfolio now encompasses platforms for microarrays, genotyping, and gene quantitation. Visit us and discover the power to offer multiple technologies for Gene Expression, Copy Number, Whole Genome Association Studies and Cytogenetics.

Agencourt Bioscience Corp., a Beckman Coulter Co.

500 Cummings Center
Suite 2450
Beverly, MA 01915
P (978) 867-2600
f (978) 867-2600
www.agencourt.com
Booth 500

Agencourt Bioscience Corporation, a Beckman Coulter company, is a trusted provider of genomic services and nucleic acid purification products. Built on the patented SPRI® (Solid Phase Reversible Immobilization) paramagnetic bead-based technology, Agencourt is dedicated to designing solutions for meeting all of our clients' current and future discovery needs.

Agilent Technologies

2850 Centerville Road
Wilmington, DE 19808
P (800) 227-9770
f (302) 633-8916
agilent_inquiries@agilent.com
www.agilent.com/chem
Booth 301

Agilent Technologies is a leading supplier of life science instrumentation and services; including solutions for the genomics, proteomics, metabolomics, drug development, QA/QC, pharmaceutical manufacturing, and regulatory compliance markets. Products include bioreagents, microarrays, real-time PCR, DNA scanner, microfluidic Lab-on-a-Chip, microscopy systems, HPLC, LC/MS Solutions, HPLC-Chip/MS, laboratory automation, and data analysis software.

Ambry Genetics

100 Columbia #200
Aliso Vielo, CA 92656
P (949) 900-5500
f (949) 900-5501
www.ambrygen.com
Booth 220

Ambry Genetics is a leader in providing genetic services focusing on clinical diagnostics, pharmacogenomics and research support services. We have next generation sequencing capabilities that can support a wide range of needs, from basic research to custom assay design in our CAP-accredited and CLIA-approved lab. Visit www.ambrygen.com for more information.

AMPTEC

Koenigstr 6A
Hamburg HH 22767
Germany
P +49 4392 408 257
www.amptec.com
Booth 526

AnaSpec, Inc.

2149 O'Toole Ave
San Jose, CA 95131
P (408) 452-5055
f (408) 452-5059
www.anaspec.com
Booth 313

Quality, Innovation, Ethical Service... Integrated Proteomics SolutionsTMAnaSpec, Inc. is a leading provider of integrated proteomics solutions™ for worldwide life science research. With a vision for innovation through synergy, AnaSpec offers expertise in three primary technologies: peptides, detection reagents, and combinatorial chemistry.

Applied Biosystems

850 Lincoln Centre Drive
Foster City, CA 94404
P (650) 570-6667
f (650) 638-5884
www.appliedbiosystems.com
Booth 201

Applied Biosystems Group (NYSE:ABI), an Applera Corporation business, develops and markets instrument-based systems, consumables, software, and services to the life science industry and research community. Applied Biosystems offers innovative technologies that enable biological discoveries in basic research, pharmaceutical research and development, and human and microbial identification.

Bachem Americas Inc.

3132 Kashiwa Street
Torrance, CA 90505
P (888) 422-2436
f (310) 539-9428
sales@usbachem.com
www.bachem.com
Booth 209

Bachem is an independent, technology-based, public biochemicals company providing full service to the pharma and biotech industry. Bachem is specialized in the process development and the manufacturing of peptides and complex organic molecules as active pharmaceutical ingredients (APIs), as well as innovative biochemicals for research purposes.

Bioinformatics Solutions, Inc.

470 Weber Street North
Suite 204
Waterloo, Ontario N2L 5B7
Canada
P (519) 885-8288
f (519) 885-9075
www.bioinfor.com
Booth 333

BIOINQUIRE

220 Riverbend Rd
Suite 103
Athens GA, 30602
P (706) 583-4000
f (706) 583-4037
sales@bioinquire.com
www.bioinquire.com
Booth 325

ProteoIQ is advanced software for comparative and quantitative proteomics. Built for a demanding research environment, ProteoIQ supports the entire proteomic data analysis pipeline, from statistical validation to quantification. Discover an intuitive, easy to use application that interprets your proteomics data faster and more accurately than you ever believed possible.

Bio-Rad Laboratories

2000 Alfred Nobel Drive
Hercules, CA 94547
P (510) 741-5052
f (510) 741-5630
olga_padilla@bio-rad.com
discover.bio-rad.com
Booth 221

For over 50 years, Bio-Rad has been a preferred provider of instrumentation and reagents to support life science research. Depend on Bio-Rad for tools, technologies and expertise to enable genomic and proteomic analysis: conventional and real-time PCR, SPR, transfection technology, RNAi, biomarker expression profiling, xMAP technology, expression proteomics, electrophoresis, blotting-systems, chromatography, imaging.

Bioteam, Inc.

7 DeRosier Drive
Middleton, MA 01949
P (978) 304-1222
f (978) 304-1225
www.bioteam.net
Booth 513

As a high-performance consulting practice, BioTeam delivers solutions to address the IT, storage, and data management challenges faced by scientists adopting next-generation sequencing technologies, mass spectrometry, confocal microscopes, and other instruments used by core facilities. We are dedicated to delivering objective, technology agnostic solutions to the life science researchers. From objective counsel to hands-on implementation, BioTeam enables science by bringing together technologies that turn research into results. www.bioteam.net

Bruker Daltonics

40 Manning Road
Billerica, MA 01821
P (978) 663-3660
f (978) 667-5993
ms-sales@bdal.com
www.bdal.com
Booth 324

Bruker Daltonics is a leading provider of Mass Spectrometry instruments for the Analytical Sciences. Our innovative and easy to use product families (TOF, Ion Trap, FTMS, MALDI TOF) provide the highest performance, highest value systems for a wide range of small molecule and protein analysis applications.

Caliper Life Sciences

68 Elm Street
Hopkinton, MA 01748
P (510) 435-9500
f (510) 435-3439
www.caliperls.com
Booth 524

Cambridge Isotope Labs, Inc.

50 Frontage Road
Andover, MA 01810
P (978) 749-8000
(800) 322-1174 *(Toll Free)*
f (978) 749-2768
cilsales@isotope.com
www.isotope.com
Booth 411

CIL is the world leader in the manufacture and separation of stable isotopes and stable isotope labeled compounds. CIL offers the largest inventory of stable isotope labeled compounds including amino acids, cell free protein synthesis products, peptide synthesis products, buffers, fatty acids, sugars, Bio-Express cell growth media, RNA/DNA products, recombinant protein expression products, and environmental contaminants standards.

caprotec Bioanalytics Gmbh

Volmerstr. 5
D-12489
Berlin, Germany
P +49 30 6392 3990
f +49 30 6392 3989
info@caprotec.com
www.caprotec.com
Booth 519

Capture Compound Mass Spectrometry (CCMS) enables gel-free LC-MS/MS-based analysis of small molecule - protein interactions through complexity reduction of biological samples via selective isolation of targeted protein families. The CCMS technology enables discovering, isolating and profiling members of functional protein families within a variety of biological samples.

CEM Corporation

PO Box 200
Matthews, NC 28106
P (704) 821-7015
(800) 726-3331 *(Toll Free — US & Canada)*
f (704) 821-8710
www.cem.com
Booth 327

Make higher purity peptides faster with the Liberty™ Microwave Peptide Synthesizer. This system has the fastest cycle times available for routine and difficult peptides, including peptides you can't access under conventional conditions. The Discover® System provides improved sequence coverage and higher efficiencies for your enzymatic digests in only 15 minutes.

CLC bio

245 First Street
Cambridge, MA 02142
P (800) 208-5981
f (617) 444-8754
www.clcbio.com
Booth 525

CovalX

Technoparkstrasse 1
Einstein G44
CH-8005
Zurich, Switzerland
P (617) 297-2263
f (857) 288-6348
infona@covalx.com
www.covalx.com
Booth 218

CovalX focuses on measuring intact protein complexes by MALDI mass spectrometry. CovalX provides the tools for measurements in your laboratories or using our laboratories with our CRO services. CovalX's objective is solving customer problems such as stoechiometry of complexes, antibody characterization (epitope mapping, multibinding assays) or quantitation of biopharmaceutical aggregates.

Digilab Genomic Solutions

84 October Hill Road
Holliston, MA 01746
P (508) 893-3130
f (508) 893-8011
www.genomicsolutions.com
Booth 232

Digilab's Genomic Solutions division designs, manufactures and markets analytical instruments, software and consumables for the global life sciences research, health care, manufacturing and safety & security markets. Digilab's differentiated products service the needs of researchers working in the fields of genomics, proteomics, spectroscopy and diagnostics.

Dionex Corporation

1228 Titan Way
PO Box 3603
Sunnyvale, CA 94088-3603
P (408) 737-0700
f (408) 730-9403
www.dionex.com
Booth 418

Dionex is a premier provider of HPLC and ion chromatography systems for the life science, pharmaceutical, and biotechnology industries. The UltiMate 3000 HPLC series include Rapid Separation LC (RSLC) for ultrafast high resolution separations; the biocompatible Titanium systems for biomolecules separation and analysis; and sensitive nanoflow LC/MS systems for proteomics.

DNAStar

3801 Regent Street
Madison, WI 53705
P (608) 258-7420
f (608) 258-7439
info@dnastar.com
www.dnastar.com
Booth 321

DNAStar develops and sells software for DNA sequence analysis (Lasergene), Next Generation sequencing assembly (SeqMan NGen) and gene expression analysis including RNA-Seq (ArrayStar with the QSeq module). The software can be networked and is designed for use on desktop computers.

EdgeBio

201 Perry Parkway
Suite 5
Gaithersburg, MD 20877
P (800) 326-2685
f (301) 990-0881
lurrego@edgebio.com
www.edgebio.com
Booth 424

EdgeBio is the sole provider of PERFORMA® DTR gel filtration products, the gold standard in DNA sequence reaction cleanup. Additional innovative products include plasmid preparation kits, PCR purification systems, and a line of ultra competent cells that includes Acella™ Competent Cells, enabling cloning and protein expression in one transformation.

Eksigent Technologies

5875 Arnold Road
P (925) 560-2600
f (925) 560-2700
info@eksigent.com
www.eksigent.com
Booth 413

Eksigent is creating new possibilities for life science research and drug discovery & development with innovative Microflow™ and Nanoflow™ fluid delivery systems. Delivering dramatic increases in analysis speed, throughput, and sensitivity, Eksigent's leading-edge liquid chromatography systems are being used by prominent research, pharmaceutical, and biotechnology firms around the world.

Enzo Life Sciences

5120 Butler Pike
Plymouth Meeting, PA 19462
P (800) 942-0430
f (610) 941-9252
info@biomol.com
www.biomol.com
Booth 224

Enzo Life Sciences, incorporating BIOMOL and Alexis Biochemicals, provides over 8000 products and services for genomics, molecular & cell biology, and biochemistry. Products include proprietary labeling and detection products for DNA sequencing, gene expression, and genetic & cellular analysis as well as specialty biochemicals for signal transduction, apoptosis, neuroscience, immunology, and drug discovery. Services include peptide and chemical synthesis, antibody production and custom-labeled FISH probes.

FASEB

9650 Rockville Pike
Bethesda, MD 20814
P (301) 634-7930
f (301) 634-7353
cadams@faseb.org
http://marc.faseb.org/
Booth 303

FASEB MARC Program provides a variety of activities to support the training of minority students, postdoctorates, faculty and scientists in the biomedical and behavioral sciences. We offer travel awards for scientific meetings, research conferences, and student summer research opportunities programs. We also sponsor Career Development Programs including grantsmanship training seminars.

Fluidigm Corporation

7000 Shoreline Court
Suite 100
South San Francisco, CA 94080
P (650) 266-6000
f (650) 871-7152
biomark@fluidigm.com
www.fluidigm.com
Booth 308

Fluidigm develops, manufactures and markets Integrated Fluidic Circuits and systems that significantly improve productivity in life science research. These "integrated circuits for biology" simultaneously perform thousands of sophisticated biochemical measurements in expremely minute volumes. Fluidigm products are not approved for use as a diagnostic and are available for research only.

ForteBio, Inc.

1360 Willow Road
Suite 201
Menlo Park, CA 94025
P (650) 322-1360
f (650) 322-1370
gmilan@ForteBio.com
www.fortebio.com
Booth 420

ForteBio markets a novel, label-free, dip and read, 96 well format technology platform that includes instrument hardware, biosensors and data analysis software to measure affinity, kinetics, and concentration in crude or purified samples. This method allows greater throughput and cost-effectiveness in many applications compared to existing methods such as SPR, ELISA and HPLC.

Gel Company (The)

665 Third Street
Suite 240
San Francisco, CA 94107
P (415) 247-8760
f (415) 247-8765
gelinfo@gelcompany.com
www.gelcompany.com
Booth 311

The Gel Company, based in San Francisco, offers innovative products for DNA sequencing, microarray, and liquid handling. Some new products include new tools for next-gen sequencing, capillary array regeneration, bandpickers with disposable tips for gel excision, slide imprinter/partitioner for microarray analysis, and genetic analyzer accessories.

Geneva Bioinformatics (GeneBio) S.A.

25 avenue de champel
CH-1206
Geneva, Switzerland
P +41 22 702 99 00
f +41 22 702 99 99
info@genebio.com
www.genebio.com
Booth 212

GeneBio is a leading bioinformatics company providing the life science community with world-class proteomics software that bridges the gap between information gathering and knowledge generation. We focus our expertise on protein identification and characterization as well as proteome imaging, offering a high degree of specialization essential to proteomics research.

Genisphere, Inc.

2801 Sterling Drive
Hatfield, PA 19440
P (215) 996-3000
f (215) 996-0370
www.genisphere.com
Booth 204

GenomeQuest, Inc.

1700 West Park Drive
Westborough, MA 01581
P (508) 616-0100
f (508) 616-0110
contact-us@genomequest.com
www.genomequest.com
Booth 504

GenomeQuest™, is a web-based sequence information platform that enables end-users to exploit genomic information resources including next-generation sequencing data and worldwide biological sequence reference databases. GenomeQuest's customers leverage high-speed algorithms and sophisticated analysis and reporting tools to transform data into functional knowledge, on-demand.

GenomeWeb LLC

125 Maiden Lane, 2nd Floor
New York, NY 10038
P (212) 651-5632
f (212) 269-3686
ganderson@genomeweb.com
www.genomeweb.com
Booth 426

GenVault Corporation

6190 Corte del Cedro
Carlsbad, CA 92011
P (760) 268-5200
f (760) 268-5201
mtran@genvault.com
www.genvault.com
Booth 225

GenVault is the leading global provider of next-generation technologies for room temperature storage and management of biosamples for genomic research. GenVault's products enable extraction, recovery and preservation of DNA and RNA. With newly launched GenTegra™ DNA, and scalable archiving solutions, GenVault is pioneering new standards for biosample management. Visit: www.genvault.com.

Geospiza

100 West Harrison North Tower
Suite 330
Seattle, WA 98119
P (206) 633-4403
f (206) 633-4415
info@geospiza.com
www.geospiza.com
Booth 408

Geospiza™ has redefined the genetic analysis software market with the sole solution to help customers manage genetic analysis from sample to results™. Designed by biologists, Geospiza's products are configured to reflect best practices and to work out-of-the-box, so researchers can focus on their science.

Hewlett-Packard Company

200 Forest Street
Marlborough, MA 01752
P (800) 752-0900
www.hp.com/go/lifesciences
Booth 318

HP innovations enable researchers to focus on what matters to them — science. HP provides expertise, innovative products, services and partnerships to help you achieve scientific breakthroughs. HP offers a choice of industry-standard, high-performance solutions to give you the flexibility and confidence to implement the technology you need to achieve success.

Illumina, Inc.

9885 Towne Centre Drive
San Diego, CA 92121
P (800) 809-4566
f (858) 202-4766
info@illumina.com
www.illumina.com
Booth 300

Illumina enables discovery by providing comprehensive DNA and RNA analysis solutions, including genotyping and copy number analysis, gene expression, sequencing, and epigenetics: the cornerstones of genetic research. Our portfolio of tools integrates our sequencing and microarray technologies, as well as powerful assay protocols to make possible a new scale of biological research.

Ingenuity Systems

1700 Seaport Boulevard
3rd Floor
Redwood City, CA 94063
P (650) 381-5100
f (650) 381-5190
www.ingenuity.com
Booth 518

IPA is a web-based software application that enables you to analyze data derived from expression and SNP microarrays, proteomics experiments, and small-scale experiments that generate gene lists. It also enables you to search for targeted information on genes, proteins, chemicals, and drugs, as well as build your own biological models.

MassTech, Inc.

6992 Columbia Gateway Drive
Columbia, MD, 21046
P (443) 539-1758
f (443) 539-1759
msms@apmaldi.com
www.apmaldi.com
Booth 410

Worldwide manufacturer of atmospheric pressure (AP) MALDI ion sources for all major commercial mass spectrometers, including Thermo Finnigan LTQ/FT/Orbitrap, Agilent QTOF/TOF/Trap, ABI/SCIEX Q-TRAP, Bruker Esquire, Shimadzu LCMS-IT-TOF, and JEOL Accu TOF. Our newest line of products includes Pulse Dynamic Focusing technology, which increases the sensitivity of AP/MALDI by more than an order of the magnitude. AP/MALDI PDF+ which includes a high repetition rate all-solid-state Nd:YAG laser used for high throughput applications, better ion signal and gives lower lifetime ownership cost. And AP/IRIS: Infrared AP/MALDI for analysis without solid matrix.

Matrix Science

64 Baker Street
London
W1U 7GB, UK
P (800) 716-6702
f (800) 716-6704
www.matrixscience.com
Booth 319

Take the guesswork out of protein identification with Mascot, the benchmark for high throughput protein identification and quantitation. Complementary tools include Mascot Distiller, a cross platform data browser that provides a uniform interface to all popular mass spectrometry file formats, and Mascot Integra, for proteomics data management.

Michrom Bioresources

1945 Industrial Drive
Auburn, CA 95603
p (530) 888-6498
f (530) 888-8295
knugent@michrom.com
www.michrom.com
Booth 226

Michrom Bioresources is celebrating 20 years of innovation in life science LC/MS products, applications and services. Michrom will be displaying its full line of Paradigm MDLC instruments, accessories and consumables including the ADVANCE "Plug and Play" source for robust, high sensitivity LCMS on most commercial MS platforms.

Microchip Biotechnologies, Inc.

6693 Sierra Lane
Suite F
Dublin, CA 94568
p (925) 574-7300
f (925) 574-7373
barney.saunders@microchipbiotech.com
www.microchipbiotech.com
Booth 412

Microchip Biotechnologies, Inc. (MBI), Dublin, California, is dedicated to providing integrated systems for the life sciences that enable reliable microsample preparation and analysis. The Apollo 100 System, based on MBI's MOVe™ microfluidics platform, integrates, automates and simplifies upstream sample preparation for Sanger sequencing, increasing efficiency and decreasing costs while maintaining data quality.

Morehouse School of Medicine - RCMI program

720 Westview Drive
Atlanta, GA 30310
p (404) 752-8669
f (404) 756-5047
palexander@msm.edu
www.msm.edu/rcmi
Booth 210

Morehouse School of Medicine is home to top notch, cutting edge research. Come by booth 210 and learn more about our outstanding RCMI core facilities: Analytical Chemistry/Protein Profiling, Cell and Tissue Imaging, Gene Profiling, Biomedical Technology Service Laboratory (BTSL) and the services, technology and equipment we have to offer.

NanoString Technologies

201 Elliott Avenue West
Suite 300
Seattle, WA 98119
p (206) 378-NANO
f (650) 570-6267
www.nanostring.com
Booth 208

NanoString Technologies has developed a novel technology for digital gene expression analysis by creating molecular barcodes for tagging individual molecules in a biological sample. This technology has been fully automated in the nCounter™ Analysis System and enables researchers to perform assays on hundreds of genes in a single tube with superior sensitivity and precision.

New England Biolabs, Inc.

240 County Road
Ipswich, MA 01938
p (800) 632-5227
f (978) 921-1350
info@neb.com
www.neb.com
Booth 219

NEB, the first choice for recombinant and native enzymes for genomic research, including for nucleic acid sample preparation. Products include Taq DNA Polymerase and Phusion DNA Polymerase for fast/robust PCR, competent cells and ladders. NEB's reagents set standards for quality and value, and are also available in large scale for bulk purchase.

New England Peptide, LLC

62 Zub Lane
Gardner, MA 01440
p (978) 630-0020
f (978) 630-0021
www.newenglandpeptide.com
Booth 233

New England Peptide (Gardner, MA) is a contract service company providing custom peptides, peptide arrays, and polyclonal antibodies for discovery and research applications. We believe in a strong commitment to customer-focused service and quality. NEP strives to provide a quick response to quotation requests and your satisfaction is guaranteed.

New Objective, Inc.

2 Constitution Way
Woburn, MA 01801
P (781) 933-9560
f (781) 933-9564
www.newobjective.com
Booth 405

New Objective brings high-performance to LC-MS with state-of-the-art systems for nano-ESI. The PicoClear™ Union for capillary connections provides visual validation of your capillary connection, yet reduces clogging and swept volume. PicoView® sources improve productivity by increasing throughput and maximizing control. PicoTip® Emitters and PicoFrit® Columns provide dependable, reliable high-performance analysis.

Nonlinear Dynamics

4819 Emperor Boulevard
Suite 44
Durham, NC 27703
P (919) 313-4556
(866) GELS-USA *(Toll Free)*
f (919) 313-4505
info@nonlinear.com
www.nonlinear.com
Booth 213

Nonlinear Dynamics' product range includes analysis software for 1D and 2D electrophoresis gels, label-free LC-MS, MALDI and SELDI data as well as tools for multivariate statistical analysis. Our unique analysis approach has transformed proteomics research — it provides faster more accurate quantitation, robust statistical analysis and has enabled cross-lab reproducibility.

Partek Incorporated

12747 Olive Blvd
Suite 205
St. Louis, MO 63141
P (314) 878-2329
f (314) 275-8453
www.partek.com
Booth 234

Partek Incorporated is the maker of Partek® Genomics Suite™ — software designed to easily analyze and compare gene expression, digital gene expression, exon expression, human gene ST, association studies/copy number analysis, and regulation/ChIP-chip, ChIP-Seq data. Partek GS is part of the illumina•Connect software program and is Affymetrix GeneChip-compatible™.

Phoenix S&T

1350 Edgmont Avenue
Suite 2580
Chester, PA 19013
P (610) 876-6081
f (610) 876-6084
www.phoenix-st.com
Booth 510

PolyLC Inc.

9151 Rumsey Road
Suite 175
Columbia, MD 21045
P (410) 992-5400
f (410) 730-8340
info@polylc.com
www.polylc.com
Booth 527

HPLC columns for life sciences.Introducing ERLIC! For: 1) Selective isolation and separation of tryptic phosphopeptides andsialylated glycopeptides; 2) Gradient separations of peptides - without a gradient.Products for proteomics: PolySULFOETHYL A for 2-D SCX-RPC separations; columns for predigest fractionation of intact proteins via mixed-bed IEX, HIC and HILIC, including membrane proteins.

Promega Corporation

2800 Woods Hollow Road
Madison, WI 53711
P (608) 274-4330
f (608) 277-2516
www.promega.com
Booth 427

Promega Corporation is a worldwide leader in applying biochemistry and molecular biology to the development of innovative, high-value products for the life sciences. Product focus includes genomics, cellular analysis and functional proteomics, including products for protein purification, expression, imaging, and protein interaction analysis.

Protea Biosciences, Inc.

955 Hartman Run Road
Suite 210
Morgantown, WV 26505
P (304) 292-2226
f (304) 292-7101
inquiry@proteabio.com
www.proteabio.com
Booth 520

Protea empowers Life Science Researchers with new products and services that improve the speed, quality and reproducibility of protein mass spectrometry data obtained from their biological samples. We improve the quality and reproducibility of data obtained by our customers in their laboratories. All our products are tested to assure that they will not interfere with mass spectrometric analytical techniques.

Protein Discovery

418 South Gay Street
Knoxville, TN 37902
P (865) 521-7400
f (865) 521-3548
kelly@proteindiscovery.com
www.proteindiscovery.com
Booth 511

Protein Forest, Inc.

128 Spring Street
Lexington, MA 02421
P (617) 926-4778
f (617) 926-4794
info@proteinforest.com
www.proteinforest.com
Booth 305

Protein Forest's digital ProteomeChip™ system fractionates and concentrates proteins and peptides by parallel isoelectric focusing for superior mass spec IDs or detection by isoelectric Western Blotting. This system not only improves sample prep reproducibility but enables you to discover biomarkers missed by current methods.

Proteome Software

1340 SW Bertha Boulevard
Portland, OR 97219
P (800) 944-6027 *(Toll Free)*
f (928) 244-6024
Jana.lee@proteomesoftware.com
www.proteomesoftware.com
Booth 304

Industry standard products for MS/MS meta-analysis; Scaffold Q+ Intelligently quantify MS/MS data with iTRAQ®, TMT and spectrum counting.;Scaffold 2 Compare results from Mascot, SEQUEST®, Waters, Spectrum Mill, Phenyx, and X!Tandem.

Roche Applied Science

9115 Hague Road
Indianapolis, IN 46256
P (800) 262-1640
www.roche-applied-science.com
Booth 400

Roche Applied Science provides complete solutions for life science research, including instruments, reagents, and software. Instruments include the next generation Genome Sequencer FLX System, the LightCycler® 480 Real-Time PCR System (96, 384 or 1536 well), NimbleGen microarrays with 2.1 million features and the xCELLigence Real-time, Label-free Cell Analyzer.

SABiosciences corporation

6951 Executive Way
Frederick, MD 21703
P (301) 682-9200; (888) 503-3187
f (301) 682-7300; (888) 465-9859
support@sabiosciences.com
www.SABiosciences.com
Booth 227

SuperArray Bioscience offers pathway-focused and genome-wide products and services for gene expression, epigenetic, and gene function analysis including real-time PCR Arrays and Assays for analysis of messenger and micro RNA expression, DNA methylation, and chromatin immunoprecipitation as well as RNA interference and cell-based GFP and luciferase reporter assays.

SeqGen, Inc.

12935 S. Budlong Avenue
Gardena, CA 90247
P (877) 377-3743
f (800) 790-4830
info@seqgen.net
www.seqgen.net
Booth 309

SeqGen is a full service provider of high quality, refurbished, DNA Sequencers, PCR Instrumentation, and Service Contracts. We maintain a large inventory of equipment/parts and have many years of experience supporting Biotech laboratories. Only expert SeqGen Engineers conduct installations, warranty calls, service contracts, and paid day service calls.

Shodex

489 5th Avenue
FL18
New York, NY
P (212) 370-0033
f (212) 370-4566
support@shodex.net
www.shodex.com
Booth 211

"Capture the Essence" with Shodex polymer-based HPLC columns. We provide an extensive selection of protein analysis columns in size-exclusion, ion-exchange, HIC, affinity, and reversed-phase modes. We have introduced highly effective solid-sphere ionic exchange columns for ultra fast protein analysis that can be used in conventional HPLC or UHPLC instruments.

Sigma Life Science

3050 Spruce Street
St. Louis, MO 63103
P (314) 286-7727
f (314) 286-7817
agant@sial.com
www.sigmaaldrich.com
Booth 310

SoftGenetics

200 Innovation Boulevard
State College, PA 16803
P (814) 237-9340
f (814) 237-9343
john@softgenetics.com
www.softgenetics.com
Booth 512

Thermo Scientific

355 River Oaks Parkway
San Jose, CA 95134
P (800) 532-4752
anayze@thermo.com
www.thermo.com/proteomics
Booth 401

Thermo Scientific is devoted to the creation of integrated solutions to help tackle the analytical challenges in proteomics. Our integrated solutions leverage the speed, sensitivity and accuracy of our market-leading mass spectrometers for definitive quantitation of protein mixtures, improving protein characterization, accelerating protein database searches and reducing false identification rates.

Varian, Inc.

Essex Road
Church Stretton
Shropshire
SY6 6AX, UK
P +44 1694 723581
f +44 1694 722171
eumktg@varianinc.com
www.varianinc.com
Booth 502

Varian, Inc. is a diversified, global technology leader solving critical challenges and creating breakthrough innovations for science and industry. Varian solutions for bioscience includes the highest-quality instrumentation, applications-based consumables for sample prep, synthesis, separations, and comprehensive services to meet your laboratory's needs from routine analysis to the most demanding applications.

Waters Corporation

34 Maple Street
Milford, MA 01757
P (508) 478-2000
f (508) 872-1990
info@waters.com
www.waters.com
Booth 508

Waters helps laboratory-dependent organizations by providing breakthrough technologies and solutions. Pioneering a connected portfolio of separation and analytical science, laboratory informatics and mass spectrometry, Waters provides the tools to improve the quality of today's science and explore the infinite possibilities of tomorrow. Waters, The Science of What's Possible.

NOTES

Sunday, February 8, 2008 — 6:30 pm - 7:30 pm

Room: 201

Dionex Corporation

1228 Titan Way PO Box 3603
Sunnyvale, CA 94088-3603 USA
P (408) 737-0700
f (408) 730-9403

(e1) Part I: Enhanced Detection and Identification of Multiple Phosphorylated Peptides Using TiO_2 Enrichment in Combination with MALDI TOF/TOF MS

Presenter: Karl Mechtler, IMP, Vienna, Austria

The analysis of Post-Translation Modifications (PTMs) such as phosphorylation has become an important field in MS because they can directly indicate protein states and interactions. Whereas the characterization of singly and doubly phosphorylated peptides has become almost routine, identifying phosphorylation events at multiple residues within a small region of a protein is still problematic. Here we present a strategy for the analysis of complex phosphopeptides that combines peptide enrichment by titanium dioxide, separation by RP separation on monolithic columns and MS using high energy HE-CAD in a MALDI TOF/TOF analyser.

Part II: Improved Methods for Phosphopeptide Analysis

Presenter: Albert Sickmann and Rene Zahedi, ISAS, Dortmund, Germany

The analysis of phosphopeptides is important in systems biology because of the high impact of signal transduction pathways on the regulation of cellular function. In particular, in the case of quantitative data, there is a need for highly reliable and reproducible methods that allow for the targeted analysis of protein phosphorylation. Here we present recent developments in automated enrichment and the resulting sequence analysis of phosphopeptides. These developments include coupling to nano LC-MS/MS and displaying a separation of non-phosphorylated and phosphorylated model peptides.

Room: 202

Hewlett-Packard Company

200 Forest Street
Marlborough, MA 01752
P (800) 752-0900
www.hp.com/go/lifesciences

(e2) Join HP — Discover HP Petabyte-Scale Storage Solutions for Life Sciences: "Mastering the Data Challenge in Life Sciences"

Presenter: Emmanuel Henri, PBM Enterprise NAS, StorageWorks HP

HP's innovative solutions for management and storage of petabyte scale data enables researchers to focus on what matters to them — science. Learn about HP innovations including the massively-scalable HP StorageWorks 9100 Extreme Data Storage system, which provides researchers with the storage volume required for instruments that generate terabytes of data per run. A breakthrough in price-per-GB that provides fast, affordable data archiving, the StorageWorks 9100 system offers up to 820TB of raw capacity, and 3.2 GB/sec raw throughput.

Room: *The location for this presentation has changed — please visit Booth #400 for details.*

(e3) Roche Applied Science

9115 Hague Road
Indianapolis, IN 46256 USA
P (317) 521-7435
f (317) 521-7317

Room: 204

Bruker Daltonics

40 Manning Road
Billerica, MA 1821 USA
P (978) 663-3660
f (978) 663-5993
dja@bdal.com

(e4) MALDI TDS: A Top-Down-Sequencing Approach for Biotherapeutics Characterization

Presenter: Darwin Asa, PhD

The methods used for establishing or confirming protein sequences with mass spectrometry are based on proteolytic digestion of protein followed by peptide fragmentation and database mapping. This "bottom-up" approach typically yields incomplete sequence coverage particularily due to variation of posttranslational modifications, N-Terminal modifications and ragged N- and C-termini further complicate the sequence characterization of the terminal peptides. To tackle the problem, Burker developed a technique based on matrix-assisted laser desorption/ionization in-source decay mass spectrometry (MALDI-ISD MS). This technique allows researchers to fetch sequence information on intact proteins (A top-down approach). Enabled by the unique pulsed ionization extraction mechanism and the Smartbeam laser, Bruker's UltraFlex and AutoFlex mass spectrometers produce efficient decomposition of proteins. Coupled with the LIFT TOF/TOF technology, ions represent N- or C-terimus can be further selected for MS/MS analysis. This terminus-specific TOF/TOF analysis, T3-sequencing, provides a powerful tool to cope with demanding tasks such as biotherapeutics analysis.

Room: 205

Applied Biosystems

850 Lincoln Centre Drive
Foster City, CA 94404 USA
P (650) 554-2337
f (650) 638-5884
Shari.Jongejan@appliedbiosystems.com

(e5) miRNA - Small Tools Enabling Big Discoveries — Recent Advances in miRNA Research and Validation with TaqMan® microRNA Assays

Presenter: Doug Raines, Senior Application Sales Specialist, Applied Biosystems

MicroRNAs are being studied more and more each day in our attempts to understand gene regulation, gene function and genetic diseases. We will present some of the most recent tools and advances in miRNA discovery and profiling, with practical examples of how they can impact your research today.

Sunday, February 8, 2008 — 7:40 pm - 8:40 pm

Room: 201

NanoString Technologies

201 Elliott Avenue West
San Mateo, CA 94403 USA
p (650) 570-6265
f (650) 570-6267
rdemarco@nanostring.com

(e6) A Novel Multiplexed Digital Gene Expression Technology

Presenter: Stephen Jackson, PhD, Field Applications Specialist, Nanostring Technologies

NanoString Technologies has developed a novel digital technology that can be used for non-enzymatic direct multiplexed measurement for gene expression that is ultra sensitive and has a high level of precision even at very low levels of gene expression. This technology has been developed into a fully automated system, the nCounter TM Analysis System, which enables researchers to examine or validate larger sets of transcripts with many fewer reactions while removing the risk of bias being introduced during enzymatic steps. In this study we examined the technical performance of the nCounter System and compared it with results generated with micorarrays, TaqMan® and SYBR® Green Real-Time PCR.

Room: 202

Thermo Scientific

355 River Oaks Parkway
San Jose, CA 95134 USA
p (800) 532-4752
f (408) 965-6113
analyze@thermo.com

(e7) Quantification of Phosphoproteins: New experimental approaches for investigating cellular proliferation

Presenter: Thermo Scientific

To better understand the complex regulation of phosphorylation in cellular proliferation, we describe a new integrated workflow for the simultaneous identification and quantification of phosphoproteins and phosphopeptides by mass spectrometry. Utilizing a multiplexed quantification strategy, isobaric mass labels called Tandem Mass Tags (TMT) and LTQ Orbitrap-based LC-MSn methodologies were used to resolve and quantify protein expression and phosphoprotein changes in various cell lines. This combination of isobaric labeling and high resolution mass spectrometry provides resolution, throughput, and accuracy for phosphoprotein variant quantification superior to traditional methods. Come enjoy refreshments while you learn about this workflow and all of Thermo Scientific's integrated solutions for quantitative biology and proteomics.

Room: *The location for this presentation has changed — please visit Booth #400 for details.*

(e8) Roche Applied Science

9115 Hague Road
Indianapolis, IN 46256 USA
p (317) 521-7435
f (317) 521-7317

Room: 204

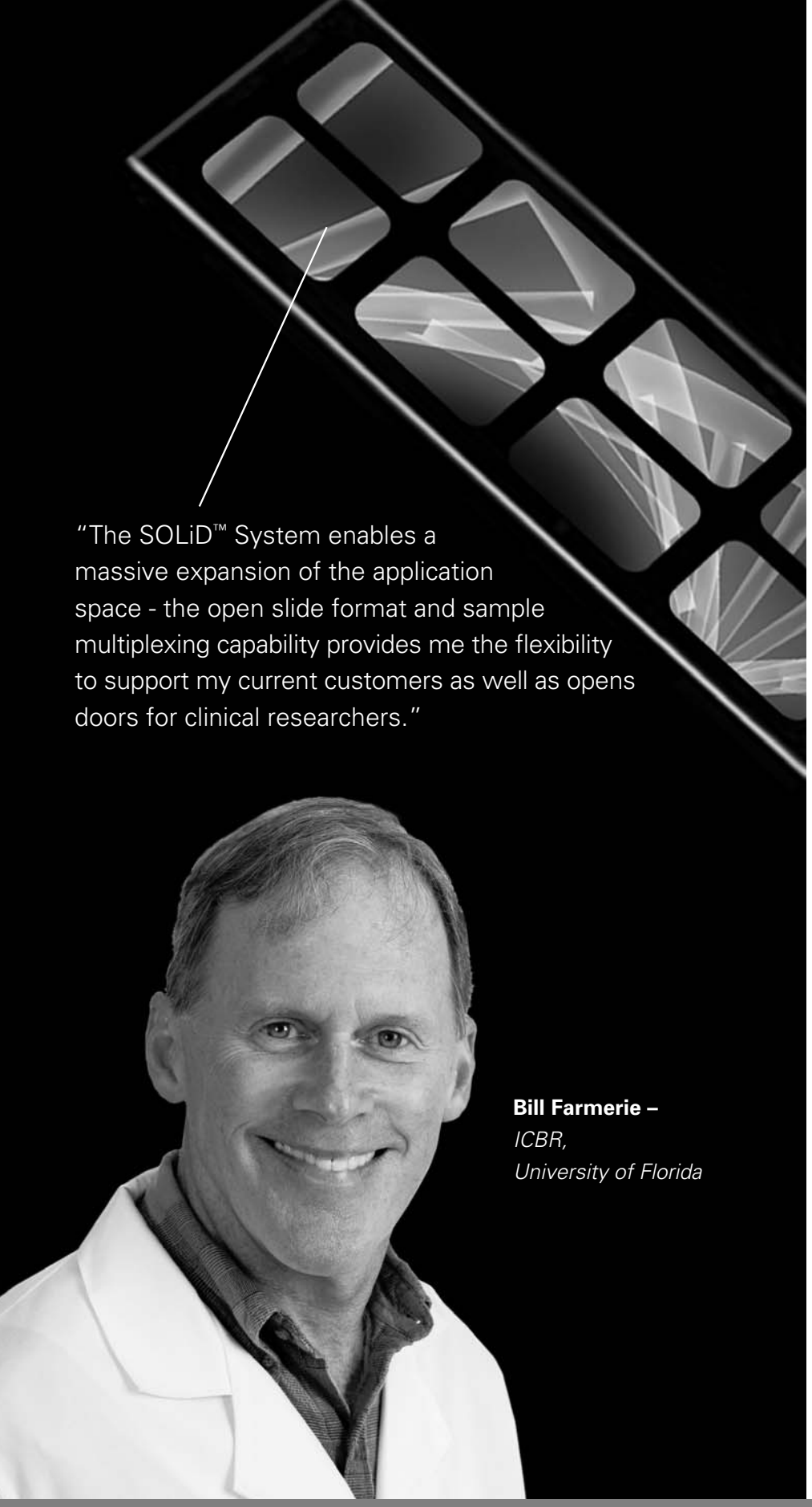
SABiosciences Corporation

6951 Executive Way
Frederick, MD 21703 USA
p (301) 682-9200
f (301) 682-7300
gdove@sabiosciences.com

(e9) New Epigenetics Technologies for Biomedical Research: microRNA, Chromatin IP, and DNA Methylation PCR Arrays

Presenter: George J. Quellhorst, Jr., Ph.D.; Product Manager

Epigentics has grabbed the attention of many researchers by providing new insights into cell differentiation and oncogenesis. These rapidly expanding studies examine how heritable factors not coded within genomic DNA sequences regulate gene expression. Such factors include DNY methylation, chromatin remodeling due to histone modification, and even, in some cases, microRNA. The experimental methods designed to discover and correlate these factors with biological phenotypes have evolved. However, may still suffer from poor sensitivity and reliability, low-throughput, and unnecessary complexity. This seminar introduces advanced yet easy-to-use real-time PCR Array technologies offered by SABiosciences that analyze microRNA as well as mRNA expression profiles, CpG island DNA methylation, and chromatin immunoprecipitation fractions. Find out how easy real-time PCR-based epigentics experiements can be performed in cancer and stem cell research to better understand the molecular epigentic mechanisms regulating key genes of interest.



"The SOLiD™ System enables a massive expansion of the application space - the open slide format and sample multiplexing capability provides me the flexibility to support my current customers as well as opens doors for clinical researchers."

Bill Farmerie –
ICBR,
University of Florida

NEED PROOF? HERE'S SOLID PROOF.

For more SOLID PROOF, visit solid.appliedbiosystems.com

Applied Biosystems

Room: 205

Applied Biosystems

850 Lincoln Centre Drive
Foster City, CA 94404 USA
P (650) 554-2337
f (650) 638-5884
Shari.Jongejan@appliedbiosystems.com

(e10) miRNA — Small Tools Enabling Big Discoveries — MicroRNA Discovery and Profiling Using Next Generation Sequencing

Presenter: Tony Dodge, Scientist, Applied Biosystems

MicroRNAs are being studied more and more each day in our attempts to understand gene regulation, gene function and genetic diseases. We will present some of the most recent tools and advances in miRNA discovery and profiling, with practical examples of how they can impact your research today.

Monday, February 9, 2008 — 6:30 pm - 7:30 pm

Room: 201

Dionex Corporation

1228 Titan Way PO Box 3603
Sunnyvale, CA 94088-3603 USA
P (408) 737-0700
f (408) 730-9403

(e11) Improved Chromatography Solutions for the Analysis of Biopharmaceuticals

Presenter: M. van Gils, W. Decrop, G. Gendeh, R. Swart, Dionex Corporation

One of the challenges in biopharmaceutical product development is the generation of analytical data for an increasing number of protein samples. In a later stage of the development, detailed characterization of a smaller number of samples is essential. Various liquid chromatographic techniques are employed to measure concentration, purity and stability of potential protein products. In this seminar we present a new biocompatible HPLC system comprising two gradient pumps, switching valves, and a sample fractionation option. Sample fractionation and automated re-injection is one of the main system features and can be used to couple multiple techniques, e.g. affinity purification coupled to size exclusion chromatography (SEC) or ion-exchange and reversed phase chromatography. Sample throughput can be increased by running the LC system in parallel or tandem mode. Finally, we will discuss various aspects of monolithic columns and how this technology can be applied to improve the separation of intact proteins.

Room: 202

Ingenuity Systems

1700 Seaport Boulevard, 3rd Floor
Redwood City, CA 94063 USA
P (650) 381-5100
f (650) 381-5190

(e12) Identifying Functional Consequences of Molecular Profiles of Cancer through Pathway Analysis

Presenter: Megan E. Laurance, Ph.D., Ingenuity Systems

The adoption of technologies that detect transcript and microRNA levels, as well as methylation patterns in cancer presents researchers with the challenge of translating those molecular profiles into clear understanding the core pathways and processes altered in cancer. Understanding the functional consequences of those molecular alterations ultimately provides a strategy for impacting the physiological processes affected by those pathways. In this session we will present a case study in which IPA has been used as a target and biomarker discovery tool to identify paths linking molecular profiles to cancer-specific phenotypes and physiological responses.

Room: 203

Illumina

9885 Towne Centre Drive
San Diego, CA 92121 USA
P (858) 202-4566
f (858) 202-4766
info@illumina.com

(e13) Illumina Technology Workshop

Presenter: TBD

Come learn about the very latest in Illumina® technology from leading experts in the industry.

Room: 204

Bruker Daltonics

40 Manning Road
Billerica, MA 1821 USA
P (978) 663-3660
f (978) 663-5585
pfa@bdal.com

(e14) Development and Validation of the maXis UHR - TOF: A New High Performance Mass Spectrometer for Fast Chromatography

Presenter: Darwin Asa, PhD

in may analytical laboratories, fast chromatography with its increased throughput and resolution is becomig or on the way to becoming the norm. However, the state of the art in Mass Spectrometry, especially with instruments designed to idenify unknowns or de-convolute complex mixtures, suffers from a decrease in instrument resolution as the speed of the separation increases. This problem has limited the utility of high performance Mass Spec in many key functions. To answer the challenge of retaining high performance, even at fast chromatography speeds, Bruker Daltonics has developed the maXisTM. This instrument is unique, and is a revolution in high-resolution tandem mass spectrometry and delivers exceptional accurate mass (< 1 ppm), high resolution (>50K), high sensitivity (attomole levels), and possesses a wide dynamic range (~5 orders of magnitude). Ideal for many analyses requiring high sensitivity and specificity, the maXis platform exceeds the performance of other hybrid mass spec instruments by a wide margin.

Room: 205

(e15) Advion

Room: L2

Applied Biosystems

850 Lincoln Centre Drive
Foster City, CA 94404 USA
P (650) 554-2337
f (650) 638-5884
Shari.Jongejan@appliedbiosystems.com

(e16) Part I: Core Strengthening — Beyond Your Average Routine

Presenter: Applied Biosystems Core Strengthening Experts

Discover What Products Will Enhance Your User's Experience. Meet the Experts and Learn How to Assemble the Ultimate Core Facility. Come join us for an interactive evening to learn how to both build up the muscle in your core and stretch your budget.

Monday, February 9, 2008 — 7:40 pm - 8:40 pm

Room: 201

GenVault Corporation

6190 Corte del Cedro
Carlsbad, CA 92011 USA
P (760) 268-5200
f (760) 268-5201

(e17) Room Temperature DNA Storage, in a Mineral Matrix

Presenter: Michael E. Hogan, Chief Scientific Officer, GenVault Corporation, Carlsbad CA

The oldest known DNA specimens have been obtained from ancient bone, where the mineralization process has encapsulated the DNA, freed from cellular contamination. That mineralization is nearly irreversible and requires grinding and extreme base treatment to liberate the DNA into solution. At GenVault, we have learned from that example, but have improved upon it, radically, to create GenTegra, an inert, rapidly reversible inorganic matrix for the storage, shipping and recovery of nucleic acids. We show representative data that validates GenTegra-DNA as a high quality substrate for whole genome microarray analysis, quantitative real time PCR, re-sequencing and whole genome amplification. Taken together, these data confirm what has been known, indirectly, for some time: that DNA in solution needs to be refrigerated, or frozen, but once dried and freed from water and access to oxygen, can be shipped at room temperature or preserved as an archive, for many years.

Room: 202

Fluidigm, Inc.

7000 Shoreline Court Suite 100
South San Francisco, CA 94080 USA
P (650) 266-6000
f (650) 871-7152

(e18) Fluidigm Integrated Fluidic Circuits: Enabling Core Labs To Meet Future Needs in High-Throughput Genetic Analysis

Presenter: Joseph Boland, National Cancer Institute, Gaithersburg, MD

Fluidigm systems significantly improve productivity in life science research through complete systems based on integrated fluidic circuits (IFCs). These "integrated circuits for biology" simultaneously perform thousands of sophisticated biochemical measurements in extremely minute volumes, enabling new capabilities for next generation sequencing as well as ultra high-throughput genotyping and gene expression studies.

Room: 203

Microchip Biotechnologies, Inc.

6693 Sierra Lane, Suite F
Dublin, CA 94568 USA
P (925) 574-7300
f (925) 574-7373
barney.saunders@microchipbiotech.com

(e19) The Apollo 100 System: An Automated Microfluidic System for Integrated Thermocycling and Preparation of 'Ready-to-Inject' Sanger Sequencing Samples

Presenter: Mary Trounstine and F.S. Pearson

MBI has developed the Apollo 100 System™ which integrates microfluidic chips with a standard laboratory robot to perform Sanger sequencing reactions. The system automates both dye-terminator cycle sequencing reactions at the sub-microliter scale and subsequence clean up on integrated microfluidic chips. This significantly reduces the cost of Sanger sequencing through reduction in the use of expensive reagents and minimizes 'hands on' time and the potential for human errors. The system is designed toe use either plasmid or PCR product input samples, loaded in a standard 96 well plate. The Apollo 100 outputs processed 'Ready-to-Inject'TM samples, dispensed in microtiter plate. These samples can then be placed in an AB Genetic Analyzer for separation analysis. The exhibitor presentation: Overview of the underlying microfluidic MOVeTM (Microscale On-chip Valves) technology, which is at the heart of the Apollo 100 System; Review the workflow, operation of the Apollo 100 System and protocol optimization; Compare sequencing and cleanup performance between the Apollo 100 System adn the standard methods used in Sequencing Core Labs.

Room: 204

ForteBio Inc.

1360 Willow Road, Suite 201
Menlo Park, CA 94025 USA
P (650) 322-1360
f (650) 322-1370
gmilan@fortebio.com

(e20) Fortebio's Octet Platform for Label-free, Real-time Biomolecular Interaction Analysis

Presenter: Sriram Kumaraswamy

Sample fractionation and automated re-injection is one of the main system features and can be used to couple multiple techniques, e.g. affinity purification coupled to size exclusion chromatography (SEC). The same system can also be used to couple ion-exchange and reversed phase chromatography with mass spectrometric detection. This technique can be used for the molecular weight determination of protein variants. Sample throughput can be increased by running the LC system in parallel or tandem mode. This technique will be demonstrated by SEC analysis of monoclonal antibodies. The last part of the seminar will discuss various aspects of monolithic columns and how this technology can be applied to improve the separation of intact proteins.

Room: L2

Applied Biosystems

850 Lincoln Centre Drive
Foster City, CA 94404 USA
P (650) 554-2337
f (650) 638-5884
Shari.Jongejan@appliedbiosystems.com

(e21) Part II: Core Strengthening — Beyond Your Average Routine

Presenter: Applied Biosystems Core Strengthening Experts

Discover What Products Will Enhance Your User's Experience. Meet the Experts and Learn How to Assemble the Ultimate Core Facility. Come join us for an interactive evening to learn how to build up the muscle in your core and learn how to stretch your budget.

Satellite Educational Workshop Sponsors

(SW1) Next Generation DNA Sequencing

illumina®

Workshop Sponsor

Roche

Session Sponsor

Applied Biosystems

Session Sponsor

fluidigm®

Speaker Sponsor

DNASTAR®

Speaker Sponsor

GenomeQuest INC.

Speaker Sponsor

geospiza™

Speaker Sponsor

(SW2) Proteomics Instrumentation

Applied Biosystems

Session Sponsor

(sw1) Next Generation DNA Sequencing

Saturday, February 7, 2009

8:00 am – 4:00 pm **Room 205, Memphis Cook Convention Center**

Massively Parallel Sequencers in the Core Facility: Applications and Computation

Organizer: Michael Zianni, *Ohio State University* Co-organizers: Caprice Rosato, *Oregon State University*, Michelle Detwiler, *Roswell Park Cancer Institute*, Charles Nicolet, *University of California at Davis*, and Peter Schweitzer, *Cornell University*

7:00 am - 12:00 pm **Registration Open** — *Lobby*

7:00 am - 8:00 am **Continental Breakfast** — *Ballroom Foyer*

Morning Session: Platforms and Applications

Peter Schweitzer, *Cornell University* (organizer)

8:00 am - 8:20 am **From Reads to Data Sets: Why Next Gen is not like Sanger Sequencing**
Todd Smith, *Geospiza*

8:20 am - 9:10 am **Sequencing with the Roche 454 FLX**
Alvaro Hernandez, *University of Illinois at Urbana-Champaign*

9:10 am - 10:00 am **Sequencing with the Applied Biosystems SOLiD**

10:00 am - 10:30 am **Refreshment Break** — *Ballroom Foyer*

10:30 am - 11:20 am **Sequencing with the Illumina Genetic Analyzer**
James Hadfield, *Cambridge Research Institute, United Kingdom*

11:20 am - 11:30 am **Sponsor Presentation**
Ilumina

11:30 am - 12:00 pm **Panel Discussion: Any Questions You Still Might Have**
Alvaro Hernandez, *University of Illinois at Urbana-Champaign*, James Hadfield, *Cambridge Research Institute*, Caprice Rosato, *Oregon State University*, Charles Nicolet, *University of California at Davis*, and Peter Schweitzer, *Cornell University*

12:00 pm - 1:00 pm **Lunch** — *Ballroom Foyer*

Afternoon Session: Computation and Analysis

Charles Nicolet, *University of California at Davis* (organizer)

1:00 pm – 1:20 pm **Subnet Architecture for MPS: a Core Manager's Computational Awakening**
Charles Nicolet, *University of California at Davis*

1:20 pm - 1:50 pm **Building a Core Infrastructure for High-Throughput DNA Sequencing**
Scott Givan, *Oregon State University*

1:50 pm - 2:30 pm **Next Generation Sequencing and Analysis Core: Case Study in Genetic and Functional Biomarker Discovery**
Faye Schilkey, *National Center for Genome Resources*

2:30 pm - 2:45 pm **Refreshment Break** — *Ballroom Foyer*

2:45 pm - 3:25 pm **A Pipeline for SNP Discovery and Genotyping Based on Short Read Plant Libraries**
Jer-Ming Chia, *USDA-ARS / Cold Spring Harbor Laboratory*

3:25 pm - 3:35 pm **Sponsor Presentation**
Roche Applied Science
Applied Biosystems

3:35 pm - 4:00 pm **Ask the Experts Session**
Informal discussion between speakers, attendees and sponsors

(sw2) Proteomics Instrumentation

Saturday, February 7, 2009

8:00 am – 4:00 pm **Room 203, Memphis Cook Convention Center**

Introduction to Proteomics Platforms and Mass Spectrometry: Applications for Qualitative and Quantitative Studies

David Friedman, *Vanderbilt University* (organizer), Hayes McDonald, *Vanderbilt University* (co-organizer)

7:00 am - 12:00 pm	**Registration Open** — *Lobby*
7:00 am - 8:00 am	**Continental Breakfast** — *Ballroom Foyer*
8:00 am - 8:15 am	**Introduction to Proteomics**
8:15 am - 9:00 am	**Major Technology Platforms Overview: LC/MS/MS, 2D-GEL/MS, Profiling/Imaging**
9:00 am – 10:00 am	**Mass Spectrometry Instrumentation in Proteomics (Part I): MALDI and ESI Sources; TOF, TOF/TOF, Triple-Quadrupole, Ion Traps (3D and Linear) Mass Analyzers**
10:00 am - 10:30 am	**Refreshment Break** — *Ballroom Foyer*
10:30 am – 11:30 am	**Mass Spectrometry Instrumentation in Proteomics (Part II): LC/MS/MS and MUDPIT; FTICR and Orbitrap Mass Analyzers and Hybrid Configurations**
11:30 am – 12:00 pm	**Qualitative and Quantitative LC/MS/MS Applications (PART I): Stable Isotope Labeling**
12:00 pm - 1:00 pm	**Lunch** — *Ballroom Foyer*
1:00 pm – 1:45 pm	**Qualitative and Quantitative LC/MS/MS Applications (PART II): Label-Free, MRM/SRM**
1:45 pm – 2:00 pm	**Qualitative and Quantitative Gel-Based Applications (PART I): 2DE and DIGE**
2:00 pm - 2:30 pm	**Refreshment Break** — *Ballroom Foyer*
2:30 pm – 3:30 pm	**Qualitative and Quantitative Gel-Based Applications (Part II): DIGE/MS, Multivariate Statistics, Variation, Power Analysis**
3:30 pm - 4:00 pm	**Ask the Experts Session** Informal discussions between presenters, organizers, and attendees

(sw3) Recombinant Protein Laboratory

February 6-7, 2009 *(Two-Day Workshop)*

8:00 am – 4:00 pm **St Jude Children's Research Hospital**

Practical Aspects of Recombinant Protein Expression and Purification

John Hawes, *Miami University* (organizer), Richard Heath, *St. Jude Children's Research Hospital* (co-organizer), James Bryson, *Bristol-Myers Squibb*, Preston Hensley, *Pfizer*, Cynthia Kinsland, *Cornell University*, and Francis Rajamohan, *Pfizer*

Friday, February 6, 2009 *(Day 1)*

7:00 am - 12:00 pm **Registration Open**

7:00 am - 8:00 am **Continental Breakfast** — *St. Jude, DTRC Room T4*

8:00 am - 9:00 am **Introduction: Basics of Protein Expression** — *St. Jude, E1004*

- A. Choice of a Host System
  1. Bacteria, yeast, insect and mammalian culture
  2. Equipment needed for core lab
- B. Biology of Heterologous Protein Overexpression in Bacteria
  1. Overview of the pET system
  2. The biggest failure in bacteria: insoluble protein
  3. Tips for shifting the balance to soluble expression
- C. Details of Lab Work
  1. Growth of E. coli in shake flasks
  2. Introduction to 10 L fermentors

9:15 am - 12:00 pm **Lab Work: Grow and Induce Recombinant Cultures** — *St. Jude, Lab E8066*

1. Hands on lab work in groups of 3
2. Demonstration of 10 L bench top fermentor

12:00 pm - 1:00 pm **Lunch** — *St. Jude, E1004*

1:00 pm – 1:30 pm **Solubility: Screening For Suitable Buffers** — *St. Jude, E1004*

- A. Use of Prefilled 96 Well Trays to Select Optimum Buffers for Purified Proteins
- B. Details of Lab Work

1:30 pm – 4:00 pm **Lab Work** — *St. Jude, Lab E8066*

- A. Solubility Screening
  1. Set up solubility screens using supplied purified protein
- B. Growth and Harvest of Cultures
  1. Continuation of morning growths
  2. Harvest by centrifugation

Saturday, February 7, 2009 *(Day 2)*

7:00 am - 12:00 pm **Registration Open**

7:00 am - 8:00 am **Continental Breakfast** — *St. Jude, E1004*

8:00 am - 9:00 am **Basics and Tips of Affinity Purification**

1. Introduction to metal chelation affinity chromatography
2. Other chromatography methods
3. Benchtop versus AKTA

9:15 am - 12:00 pm **Lab Work** — *St. Jude, Lab E8066*

- A. Cell Lysis
  1. Mechanical (demonstration)
  2. Detergent-based
- B. Protein Purification
  1. Running a HisTrap column on an AKTA (demonstration)
  2. Bench-top purification using MCAC resins

12:00 pm - 1:00 pm **Lunch** — *St. Jude, E1004*

1:00 pm - 1:30 pm **Ask the Experts Session** — *St. Jude, E1004*
Round-table discussion on protein expression/ purification as a shared resource

1:30 pm - 4:00 pm **Lab Work** — *St. Jude, Lab E8066*

- A. SDS-PAGE Analysis of Purified Protein
- B. Biochemical Assay of Activity

(sw4) HPLC Theory and Practice

Saturday, February 7, 2009

8:00 am – 4:00 pm **Room 202, Memphis Cook Convention Center**

Practical Aspects of Protein and Peptide HPLC Separations for Proteomics

Andrew Alpert, *PolyLC Inc.* (organizer), and Kerry Nugent, *Michrom BioResources* (co-organizer)

7:00 am - 12:00 pm **Registration Open** — *Lobby*

7:00 am - 8:00 am **Continental Breakfast** — *Ballroom Foyer*

8:00 am - 9:00 am **Introduction: Biochemical HPLC**

- A. Why bother? HPLC as a complement to MS for Proteomics
- B. HPLC Theory Applied to Biomolecules
  1. Efficiency, Resolution, Selectivity and Peak Capacity
  2. General requirements for HPLC of proteins and peptides
- C. Modes of HPLC for Proteins and Peptides
  1. Separations by size (SEC)
  2. Separations by charge (AX, CX, Mixed Bed)
  3. Separations by polarity (RPC, HIC, HILIC)
  4. Separations by functionality (Affinity)

9:00 am - 10:00 am **The Role of Separations in Proteomics**

- A. HPLC versus Electrophoresis and Other Separation Techniques
  1. Separation of intact proteins using gels, FFE or HPLC
  2. HPLC or solid phase extraction for sample prep
- B. HPLC as a Fractionation Tool for Comprehensive Proteomics
  1. The more you fractionate, the deeper you can dig
  2. Trade-offs: Degree of fractionation vs. sample throughput
  3. Advantages of separation of intact proteins
- C. HPLC as an Isolation Tool for Functional Proteomics
  1. Affinity separations for PTMs
  2. Non-affinity separations for PTM's
  3. Effects of peptide orientation and sequence on selectivity for PTM's
- D. Reversed Phase HPLC Coupled to MS for Proteomics
  1. Top down LCMS of intact proteins or large peptides
  2. Bottom up LCMS of protein digests

10:00 am - 10:30 am **Refreshment Break** — *Ballroom Foyer*

10:30 am - 12:00 pm **Using Only as Much Separation as Required**

- A. Fast Separations for Simple Samples
  1. High throughput LCMS of 1D or 2D gel digests
  2. Qualitative and quantitive analysis of specific proteins
- B. High Resolution Separations for More Complex Samples
  1. Simple proteome samples with wide range of abundances
  2. Analysis of low abundance proteins in sample preps
- C. Multidimensional (MD) Separations for Highly Complex Samples
  1. MDLC of intact proteins
  2. MDLC of complex proteome digests
- D. Optimizing Speed, Resolution, Capacity, Sensitivity and Recovery
  1. Choosing the proper HPLC modes for the sample
  2. Optimizing column parameters (ID, Pore Size, Particles, etc.)

12:00 pm - 1:00 pm **Lunch** — *Ballroom Foyer*

1:00 pm - 2:00 pm **Developing a Proteomics Separation Workflow**

- A. Defining the Application and Desired Results
  1. What do you know about the sample?
  2. What information do you want from the sample?
- B. Choosing the Tools that Best Fit the Problem
  1. HPLC vs. SPE
  2. Strategies to maximize throughput and resolution
- C. Integrating the Workflow to Maximize Results
  1. Choosing complimentary modes of separation
  2. Manual vs automated methods
- D. Setting Up Controls to Insure Integrity of Results
  1. Run standards to optimize methods and recoveries
  2. Run blanks to minimize errors

2:00 pm - 2:30 pm **Refreshment Break** — *Ballroom Foyer*

2:30 pm - 3:30 pm **Problems Encountered in Biochemical HPLC**

- A. Sample Solubility and Compatibility
  1. Minimize protein aggregation and precipitation
  2. Insure maximum recovery from HPLC columns
- B. Salts, Detergents and pH
  1. Useful for protein/peptide solubility
  2. May interfere with LC separation and/or MS detection
- C. Dynamic Range, Capacity and Recovery
  1. Choose HPLC column size to fit sample mass
  2. May require overload to improve dynamic range
- D. Playing "Twenty Questions" in Proteomics: Troubleshooting Separations
  1. Examples of proteomics HPLC separation problems
  2. Examples of proteomics LCMS instrumentation problems

3:30 pm - 4:00 pm **Ask the Experts Session**
Informal discussions between presenters, organizers, and attendees.

(sw5) Proteome Informatics

Saturday, February 7, 2009

8:00 am – 4:00 pm **Room 204, Memphis Cook Convention Center.**

Real-life Proteome Bioinformatics for Laboratories

Lennart Martens, *EMBL-European Bioinformatics Institute, UK* (organizer), David Tabb, *Vanderbilt University* (co-organizer), Brian Searle, *Proteome Software*, and Kathryn Lilley, *Cambridge University, UK*

7:00 am - 12:00 pm **Registration Open** — *Lobby*

7:00 am - 8:00 am **Continental Breakfast** — *Ballroom Foyer*

8:00 am - 9:00 am **MS and MS/MS Search Engines**
Brian Searle, *Proteome Software*
A variety of different algorithms to identify mass spectra, both commercial and free, are available today. This session will review the underlying mechanisms of the search algorithms and will examine the most common ones used today.

9:00 am - 10:00 am **Assessing the Reliability of Identifications**
Brian Searle, *Proteome Software*
This session will addresses the difficult problem of deciding whether a peptide identified from an MS/MS spectrum represents a correct identification or a false positive. We will explore how to estimate the false discovery rate from an experiment. We will discuss strategies aimed at minimizing the number of false positives in a dataset.

10:00 am - 10:30 am **Refreshment Break** — *Ballroom Foyer*

10:30 am - 11:00 am **Sequence Databases and Online Resources**
Lennart Martens, *EMBL-European Bioinformatics Institute, UK*
In the vast majority of cases, the sequence database that is used for peptide identification or protein inference plays an extremely important role in the overall workflow. However, the sequence database is typically the most neglected component in many workflows. This session will examine the differences between different databases and explore how their variation can be exploited in various circumstances. An overview of useful online resources will be presented.

11:00 am - 12:00 pm **Protein Inference**
David Tabb, *Vanderbilt University*
Identifying peptides from MS/MS spectra represents only half the work. Since the end goal of most experiments is the identification of proteins, peptide lists need to be processed into protein lists. This session will provide detailed information on the problems underlying protein inference. Tools and approaches that can facilitate protein inference will be presented and discussed.

12:00 pm - 1:00 pm **Lunch** — *Ballroom Foyer*

1:00 pm - 1:40 pm **Quantitation Strategies and Data Analysis**
Kathryn Lilley, *Cambridge University, UK*
A variety of proteomics quantitation strategies are currently available. This session will present an overview of these techniques. Issues related to processing and interpretation of data from these techniques will be discussed in detail.

1:40 pm - 2:20 pm **Identifying Modified or Mutated Proteins**
David Tabb, *Vanderbilt University*
This session will explore the various ways in which peptides or proteins can be identified when they are novel, if they carry complicated or unexpected posttranslational modifications, or if they contain mutations.

2:20 pm - 2:50 pm **Refreshment Break** — *Ballroom Foyer*

2:50 pm - 3:30 pm **Data Standards and Public Data Dissemination**
Lennart Martens, *EMBL-European Bioinformatics Institute, UK*
Funding agencies and journals are increasingly demanding that the results of funded or published experiments be readily available to the public. This session will discuss the state of standardization in the field and the means to disseminate data as easily and efficiently as possible.

3:30 pm - 4:00 pm **Ask the Experts Session**
Informal discussions between presenters, organizers, and attendees.

PLENARY SESSION ABSTRACTS

Plenary Session on Genomics

The Changing Landscape of Genomics

R. Gibbs

Baylor College of Medicine Human Genome Sequencing Center (HGSC), Baylor College of Medicine, Houston, TX

For more than two decades Genomics has grown as a discipline separately from Genetics. During this period the HGP was completed, other species were analyzed and the technology dramatically incremented. Methods for nucleic acids analysis now include the NexGen sequencing technologies for analysis of whole genomes and new DNA capture methods that allow sequencing of every human exon in a single sample. As a consequence, we are beginning to accrue the raw sequence of the whole genomes of multiple individuals and cell types. These methods are also being directed at finding specific disease alleles, and to characterize the full spectrum of variation in human populations as well as to study cancer. In combination these technologies provide unprecedented opportunity to study fundamental questions in biology. At long last the two disciplines of Genetics and Genomics are coming back together.

Plenary Session on Proteomics

Technologies for Comprehensive Proteome Quantitation

M. Mann, J.V. Olsen, L. de Godoy, M.L. Nielsen, N. Hubner, J. Cox

Proteomics and Signal Transduction; Max-Planck Institute for Biochemistry, Martinsried, Germany

Mass spectrometry (MS)-based proteomics has become a very powerful technology during the last decade. Despite tremendous and ongoing technological advances, identification and quantitation of entire proteomes had remained elusive and was even thought to be impossible with current approaches. Here we show that a combination of efficient sample preparation, high resolution LC MS/MS instrumentation, and novel computational proteomics techniques can now characterized essentially complete proteomes. We chose the yeast system because its proteome is independently characterized using tagging methods. The measured yeast proteome has a dynamic range of protein abundance of four to five orders of magnitude, with no bias against membrane or low level regulatory proteins. In each abundance class mass spectrometry identified as many or more proteins than the tagging methods. Quantitation of haploid vs. diploid yeast using SILAC revealed widespread, but not complete regulation of the pheromone signaling pathway. Comparison of proteome changes in this and in the Drosophila system revealed that the proteome is largely co-regulated with the transcriptome for long-term changes that are significant at the message level. We are now trying to achieve comprehensive proteome coverage in mammalian systems and report promising interim results. Important parameters are increased peptide sequencing speed as well as improved sample preparation. We further discuss extension of the SILAC method to in vivo situations in mice and in humans. The talk will also encompass practical issues of sample preparation using efficient proteome solubilization, OFFGEL peptide fractionation, StageTip clean up, computational proteomics analysis with the free MaxQuant family of algorithms as well as downstream bioinformatics.

Plenary Session on Evolutionary Biology

Genomics of Extinct and Endangered Species

S.C. Schuster[1], V.M. Hayes[2] and W. Miller[1]

[1] Center for Comparative Genomics and Bioinformatics, Penn State University, University Park, USA; [2] Children's Cancer Institute Australia for Medical Research, Sydney, Australia

Only 200 years ago the concept of static species was challenged by the first fledgling theories of evolution. Since then, ever-growing collections of fossils have allowed mankind to gain insight into the constant changes that have shaped fauna and flora for eons. While these studies initially were of strictly anatomical nature, it was discovered only in the last 25 years that in addition to petrified structural information, also biomolecules have survived the demise of individuals and species. With successful sequencing of DNA retrieved from the Quagga, an extinct species of zebra, the field of ancient DNA was invented in 1984 (1). The last three years have seen a rapid succession of improvements in sequencing of ancient DNA , driven by the onset of next-generation sequencing. This has now resulted in a draft version of the mammoth's nuclear genome (2), together with an extensive set of complete mitochondrial sequences of this extinct group of Proboscideans. The availability of a large set of genetic information from an extinct species allows for the assessment of the genetic diversity of animals that ceasedto exist several 10,000's of years before our time, and thereby allowing the investigation the contributions made by genetic factors to the extinction process. For these analyses, mitochondrial markers have historically been used, as the survival of nuclear DNA on a larger scale had not been documented in fossils until recently. Studies on mammoth populations have revealed a surprisingly small genetic diversity, as well as the existence of two previously undetected groups of animals (3). While this in itself does not explain the extinction process, it raises the question of a contributing factor in addition to population size. We have followed up on this lead from mammoth populations in an analysis on the recently extinct species of the Tasmanian Tiger (4), who's genetic analysis has unsuccessfully been attempted for the last decade. Our findings have lead us to believe that the observations made for the two extinct species come to play also in endangered species that are at the brink of extinction today. We are therefore sequencing the nuclear genome of the Tasmanian Devil, the largest remaining marsupial carnivore. This species is currently dramatically endangered through a form of infectious cancer. We will show how the lack of biological diversity in this species is relevant to the animal's failing immune response in the onset of the disease.

The lessons learned from past extinctions, documented through the efforts of today's paleogenomics, may therefore help to assess not only the status of endangerment of a species, but also help it from going extinct by directing breeding programs that are already underway.

ABRF: Current Status and Future Plans

Overview of ABRF Progress for 2008 and a Look Toward the Future

M.L. Doyle

Bristol-Myers Squibb Pharmaceuticals Research and Development, Princeton, NJ

A summary will be given of the accomplishments made by the ABRF Executive Board and other parts of the organization during 2008. A substantial amount of effort has been directed toward infrastructure and administrative matters, such as working with the new business and meeting management offices, optimization of our main web site, and the creation of our own annual meeting web site (which is now operational and integrated with our main web site). A report will also be given on the plans the ABRF Executive Board has for goals in 2009 and beyond.

Financial State of the ABRF

P.S. Adams

Trudeau Institute, Saranac Lake, NY

The ABRF remains a financially viable organization but faces the challenges associated with a stable membership/income, but escalating costs. The corporate Relations Committee (CRC) has done an exemplary job of recruiting corporate sponsors and newly added academic sponsors. Substantial savings in expenditures have been realized by having an electronic JBT, but have been offset by a loss in advertising revenue. Substantially less revenue is being generated by annual meetings due to static attendance, but rising costs. Stock market losses have affected ABRF investments somewhat, but a conservative portfolio has minimized our losses compared to the general market and greater than 2 years operating expenses are in reserve. 2009 will result in some "belt-tightening" measures to efficiently utilize reduced income.

The ABRF Education Committee Update

K. Jonscher[1], J.Neveu[2], K. Sol-Church[3], D. Needleman[4], M. Zianni[5], C. Rosato[6]

[1] University of Colorado Denver, [2] Harvard University, [3]AI duPont Hospital for Children, [4]USDA/ARS Eastern Regional Research Center, [5]Plant-Microbe Genomics Facility/Ohio State University, [6]Oregon State University

In 2008, the Education Committee spearheaded the development of five new Satellite Educational Workshops. We sent out an e-mail blast request for topics and developed a survey in which a number of topics were rated by the membership (136 respondents). Based on the survey results, we received approval for implementation of the following workshops: Next Generation DNA Sequencing, Proteomics Instrumentation, Recombinant Protein Laboratory, HPLC Theory and Practice and Proteome Informatics. We have worked with the meeting management company and the Executive Board to begin to develop an SOP and timeline for facilitating future workshop planning. Additionally, we have established objective rubrics for judging posters and secured funding for poster awards, a very important aspect of the Annual Meeting. We have represented the ABRF at the Annual Biomedical Research Conference for Minority Students and are investigating the potential of offering summer internships to undergraduate minority students. In the future, the EdComm hopes to help develop web-based educational resources to support ABRF members and core users.

ABRF Membership Committee Activity 2008

S.A. Bobin[1], G.S. Grills[2], K.S. Lilley[3], R.L. Niece[4], K.M. Schegg[5], P.A. Schweitzer[2], T.W. Thannhauser[6], M.M. Detwiler[7]

[1]Dartmouth Medical School, [2]Cornell University, [3]University of Cambridge, [4]Research Resources & Technologies, [5]University of Nevada, Reno, [6]United States Department of Agriculture/ Agricultural Research Service, [7]Roswell Park Cancer Institute

The ABRF membership represents a large international community of life sciences core facilities and biotechnology research laboratories that cover a wide range of research, technology platforms and life sciences applications. The organization?s membership trends reflect both the growth of biotechnology fields over time and the relevance of the organization to these fields. The ABRF Membership Committee tracks these trends with tools such as survey questionnaires, analyses their significance, and recommends policies to help the ABRF retain and add members and to help the ABRF to continue to be relevant to the evolving interests of it members. We will present both the past trends and current focus of the ABRF membership, including technology interests, institutional affiliation, and geographic distribution. We will also explore the goals and challenges of maintaining and possibly expanding the membership of the ABRF both now and in the future.

(s1) Personalized Medicine

The session will focus on the impact of microarray and NextGen sequencing technologies on the development of personalized medicine applications. Recent progress and future trajectories will be discussed, as well as regulatory issues and other challenges that must be met to successfully implement these technologies in a clinical setting. The session will feature talks from Dr. Mary Relling (St. Jude) on linking genome-wide genotype data with global gene expression data in patient samples, Dr. Ulrich Broeckel (Medical College of Wisconsin) on implementing microarray tests for copy number and pharmacogenomics applications in a CLIA/CAP environment, and Dr. Ed Highsmith (Mayo Clinic) on the development of NextGen Sequencing as a tool for clinical testing.

(s1-a) Moving Genomics from Research to Clinical Care in Childhood Leukemia

M.V. Relling

St. Jude Children's Research Hospital, Memphis, TN

Safety and efficacy of drug therapy are paramount in children with leukemia: individualization of therapy may increase the chance of cure of a life-threatening disease and decrease the chance of serious adverse effects of what can be toxic drug therapy. We and others have used candidate genotyping techniques, whole-genome interrogation of leukemic blasts and normal leukocytes, whole-genome SNP arrays, and whole-genome comparative genomic hybridization array techniques (for leukemic blasts vs germline) to discover genomic variation associated with interindividual differences in drug response in children with leukemia. Use of candidate gene genotyping is incorporated into the treatment of childhood leukemia at our institution to optimize use of a few medications. Additional genotyping is conducted on a research basis. At St. Jude Children's Research Hospital, drug therapy individualization is facilitated by a fully penetrant institutional mission that combines treatment and research, the effective maintenance of multidisciplinary disease-oriented research teams, and substantial financial support for clinical care, for generating and curating research data, and for shared research resources. The integration of clinical and research pharmacokinetics in a single department also facilitates utilization of pediatric biological specimens and provision of clinical pharmacogenetic consults. Examples of drug individualization in pediatric acute lymphoblastic leukemia, and their integration with pharmacokinetic, pharmacodynamic, and pharmacogenetic research, will be presented.

(s2) Biofuels — *Towards a Biofuels Economy: Technologies and Infrastructure Associated with Developing New Bioenergy Crops*

The combined issues of a dwindling oil supply, the impact of fossil fuel on global climate and concerns over national energy security are collectively driving an unprecedented search for alternative forms of energy: in particular transportation fuels. Public and private investment in biofuels and bioenergy crops research has escalated to the point where there is a growing demand for "biofuels" analytical facilities. This workshop will highlight come core technologies and techniques that are in growing demand for the profiling of plant biomass and the short to mid-term future of biofuels research will be discussed.

(s2-a) Instrumentation and Methods for the High Throughput Analysis of Plant Materials as a Resource for Biofuels

M. Pauly, C. Foster, N. Santoro, J. Walton

Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, MI

One bottleneck in generating economically sustainable biofuels from plant biomass is the recalcitrance of the biomass to degradation by chemical or enzymatic treatments. Plant biomass consists mainly of cell wall based lignocelluloses, composite materials consisting of polysaccharides such as cellulose and hemicelluloses but also polyphenols such as lignin. Various strategies can be employed to enhance degradation of wall materials, a process also known as saccharifiation. The feedstock, the plant biomass, can be selected for the identification of high biomass generating specialized energy-crops. In addition, lignocelluloses can be altered through e.g. breeding programs or genetical engineering, resulting in plants with more easily accessible wall polymers. Moreover, chemical treatments can be optimized to loosen wall architecture, and enzymes can be engineered to more efficiently degrade lignocelluloses. Here we present a high-throughput robotic platform that can evaluate and test all of the above mentioned parameters: assessment of plant lignocellulosic structure by mass spectrometry, and saccharification output by altering feedstock input, chemical pre-treatment steps and enzymes including mixtures thereof.

(s2-b) From Single Molecules Spectroscopy to Process Batch Fermentation: Methods and Instrumentation Implemented in the Biofuel Research Laboratory at Cornell University

S. Corgie[1], J. Moran-Mirabal[1], L. Walker[1]

[1]Biological and Environmental Engineering, Cornell University, Ithaca, NY

"The human command of inanimate energy grew from 0.9 megawatt-hour per year per person in 1860 to nearly 19 megawatt-hours per year per person in 1990." This increase has been achieved through the development of fossil fuels and advancements in energy technology which are now reaching their limits. Biomass, especially dedicated energy crops, can part of the renewable energy portfolio. However, strong research capacity and strategic partnerships in plant science, molecular biology, genetics, material science, nanobiotechnology, computational biology, and process engineering need to be integrated to explore biological phenomena and paradigms that are important for addressing environmental, biological, physical and chemical barriers to sustainable bioenergy production.

To help advance technologies that convert biomass to bioenergy and bioproducts, Cornell University has used a $10 million grant from the Empire State Development Corporation (ESDC) to develop a Biofuels Research Laboratory. These facilities have been designed to enhance our ability to carry out research to overcome the physical, chemical and biological barriers to liberating sugars from energy crops such as switchgrass, cold tolerant sorghum, and woody biomass, and to biologically convert these sugars into biofuels and bioproducts such as ethanol, butanol, hydrogen and methane. This facility houses laboratories for the following activities: 1) biomass pretreatment, 2) material size reduction

and handling, 3) solid-state fermentation, 4) biochemical conversion, 5) submerged fermentation, 6) state-of-the-art analytical systems.

To address the multiple fundamental research angles and integrate those in an engineering framework, we are developing and exploring methods such as TIRF microscopy for single molecule analysis of saccharification processes, integrated FT-NIR and chemometric modeling for biomass characterization and process analysis, integrated high-throughput systems (UV-vis, MIR, HPLC, RT-PCR) to carry microorganism screening, enzymatic synergism, metabolic activities in system biology framework, and bioconversion studies in heterogeneous frameworks.

(s2-c) Identify Molecular Structural Features of Biomass Recalcitrance Using Non-Destructive Microscopy and Spectroscopy

S. Ding

National Renewable Energy Laboratory, Golden, CO

Lignocellulosic biomass is a renewable material that can be converted to simple sugars and then fermented to transportation fuels. The most costly step of current technology used for biofuels production is the liberation of fermentable sugars from recalcitrant biomass, primarily the cell walls of higher plants, where cellulose forms the core of the relatively rigid microfibrils and other polymers are possibly randomly assembled or cross-linked with each other to form so called "liquid polymer crystal" nano-composites. The detailed molecular structure of cell walls, however, remains essentially unknown today, due to the limitation of traditional microscopy that do not allow direct observation of these biological entities at the nanometer scale. Our goal is to develop and apply non-destructive imaging tools that are suitable for characterizing biomass conversion processes at high spatial and chemical resolution. In this paper, I will summary new development of scanning probe and non-linear optical microscopy, and applications in imaging biomass and its conversion processes.

(s3) Optical Imaging

Applying technical advances in optical imaging to intravital microscopy in laboratory animals is challenging, yet highly rewarding. Fundamental hurdles include optical absorbance and scattering in dense, thick, pigmented tissues; working distances limited to relatively superficial anatomy; and monitoring and maintenance of complex physiological parameters. In addition, detection and measurement of highly resolved information in the context of macroscopic, living systems requires enormous amounts of data collection, all while the subject appears to be sleeping calmly, yet at the microscopic level behaves as a sustained, controlled explosion. In this session we will provide several examples of micro-imaging in live rodents, including point-scanning confocal microscopy to assess real-time visualization of tumor destruction by $CD8^+$ T cells; high-speed, line-scanning confocal microscopy to assess blood flow in transplanted pancreatic islets and muscle; and multiphoton-excited fluorescence microscopy to evaluate renal blood flow, clot formation, hematopoietic stem cell movement in bone marrow, biliary secretion in the liver, and tumor imaging. Remarkably, these applications have been implemented in heavily-used core resources.

(s3-a) Developments in Microscopic Imaging of Intravital Dynamics

S. Wells

Vanderbilt University School of Medicine, Nashville, TN

A large array of materials and equipment are available for imaging and measuring micro-anatomy and molecular dynamics in the context of isolated cells and tissues. However, the detailed roles and behaviors of biological molecules and cells are ideally studied in their natural environment, exposed to a host of highly evolved, complex feedback and support systems. Consequently, significant challenges and exciting scientific opportunities are emerging as investigators learn how to integrate the great advances in microscopy and probe development with techniques for imaging in live animals. The goal of this presentation is to provide a very brief overview of fundamental hurdles and current approaches to intravital microscopy of live mice using examples from point- and line-scanning confocal microscopy experiments conducted in a core resource environment. Following a simple definition of the imaging goal, essential experimental details are considered, including selection of probes, imaging platform, objective lenses, animal surgery, anesthesia, temperature control, monitoring vitals, and dealing with motion artifacts. These issues are briefly described in three examples: 1) real-time, direct visualization of tumor destruction by $CD8^+$ T cells conducted over a period of weeks, 2) rapid detection of blood flow patterns in transplanted pancreatic islets, and 3) insulin-regulated blood flow in leg muscle. The integration of materials, methods, equipment and expertise result in significant team efforts to reveal new information regarding the microscopic behavior of biological cells and molecules in their natural environment.

(s3-b) Intravital Multiphoton Microscopy in a Microscopy Core Resource

K. Dunn

Indiana University School of Medicine, Indianapolis, IN

Although intravital microscopy has been conducted for nearly 200 years, its capabilities have recently been extended with the development of multiphoton microscopy, which provides the capability to collect images with sub-micron resolution hundreds of microns into living tissues. Parallel developments in the field of molecular biology and fluorescent probe chemistry now make it possible for biomedical researchers to evaluate the functions of individual proteins in individual cells in living animals. Once the domain of specialized laboratories, multiphoton microscopy has been commercially developed to the point that it is becoming a standard tool of microscopy core resources. This development has been critical to the application of multiphoton microscopy to a broad range of new biomedical questions, and the realization of the potential of multiphoton microscopy as a research tool. Here we present a review of the diverse applications of multiphoton microscopy in biomedical research along with a summary of our experience in implementing multiphoton microscopy in a core resource for academic and industry research.

(s4) Next Generation Nucleic Acid Quantitation

Like DNA sequencing, next generation instrumentation used for nucleic acid quantification has emerged. These next generation technologies feature the ability to conduct nucleic acid quantification studies at a nano-scale. This scientific session encompasses three presentations that feature the use of these emerging technologies to quantify levels of nucleic acids.

(s4-a) Profiling Circulating Tumor Cells as Prognostic Biomarkers in Patients with Metastatic Prostate Cancer

D. Danila, M. Fleisher, H. Scher

Memorial Sloan-Kettering Cancer Centre, New York, NY

Tumor specific markers developed to select targeted therapies and to assess outcome in clinical trials and clinical practice remain significant unmet medical needs. Currently, assessing the disease progression in patients with metastatic prostate cancer relies on the modest association between changes in PSA, and the limitations of imaging distant metastases with actual disease prognosis. Profiling prostate tumor specimens demonstrated that patients with progression despite castrate levels of testosterone harbor changes in the androgen receptor (AR), such as gene amplification, rendering tumor cells more sensitive to lower levels of androgen, and promoting tumor proliferation. Antibody-based enumeration of circulating tumor cells (CTC) based on immunocytochemistry as cells staining positive for epithelial cellular adhesion molecule (EpCAM) and nuclear DAPI, and negative for CD45 white blood cell marker has been used to predict prognosis of patients with castrate metastatic prostate cancer. In patients with progressive castrate metastatic prostate cancer, a large number of patients (57%) had 5 or more circulating tumor cells, while only 21% patients had 1 or less CTC per sample of blood. CTC counts were higher in patients with bone involvement compared to soft tissue only metastasis, while only moderate correlations to BSI, and PSA were noted. CTC isolated from small volumes of peripheral blood are viable for molecular profiling at genomic and transcriptional levels. FISH analysis in CTC for AR showed more frequently gene amplification or gains in copy number in patients with higher CTC count. RT-PCR has been used to further characterize AR dependent gene expression in enriched CTC. These results suggest that shedding cells into the circulation and molecular profiles of CTC are intrinsic properties of the tumor, which can significantly impact patient management and clinical trial design. The profiles identified are prospectively evaluated in clinical trials currently evaluating novel agents acting on AR pathway.

(s5) Biotech in Forensics and Clinical Diagnostics — *Lessons in Optimizing Specialty Facilities: Forensics, Systematics and Clinical Diagnostics*

Some core DNA facilities need a great deal of flexibility to support a variety of constituents. The human genome race and the concentration of effort into a small number of dedicated centers showed us how much we can learn from the optimizations of core facilities that specialize in structured, repeated and sometimes regulated experiments. This session will introduce the audience to some applications that they may not ordinarily hear about and present three examples of core lab specialization so we can learn from their optimizations First, you will hear from a leading forensic biology facility that deals in human identification and criminalistics using a combination of extraction and typing techniques. Next, a commercial lab at the forefront of clinical decision making will present on the prediction of antiretroviral drug susceptibility from genotypic data in chronic infectious diseases like HIV and HCV. Finally, you will hear from the leader of Smithsonian Institute's DNA Barcoding project, contributing to an international effort to develop a DNA tag for every species on earth, beginning with every bird found in North America.

(s5-a) Assay Development: From Proof-of-Concept to CLIA-validation of Assays for Patient Testing

E. Paxinos

Monogram Biosciences, South San Francisco, CA

Developing assays suitable for diagnostic applications requires robust validation, and in several respects parallels the clinical development of drug candidates. Resourcing increases exponentially during the assay development process, and selection criteria at each step gate which assays are carried forward. Attrition rates are greatest earlier in the process and few assays that have been analytically validated undergo transitional development and validation for use in a regulated clinical setting. Our facility has generalized an assay development framework that allows us to systematically move from research and development to final validated assays. The process includes: (1) exploration of alternative assay formats, reagents, methodologies; (2) optimization, scale-up and automation of component processes (e.g. flow issues such as timing, workflow, hand-offs, layout, and ergonomics); (3) characterization of assay performance (e.g., guard-banding studies to determine dynamic ranges of various assay parameters and reagents); (4) validation for Research Use Only (RUO), including other pre-validation experiments (e.g., development of test reagents, positive and negative controls, and the definition of acceptance criteria for Accuracy Precision, Reproducibility, Linearity, Sensitivity and Specificity to be used in Assay Validation); (5) design and execution of rigorous validation studies; (6) documentation, reagent qualification, and proficiency testing of certified Clinical Laboratory Scientists; and (7) final assay validation. Final assay validation is done in accordance with regulations specified by the Clinical Laboratories Improvement Amendments (CLIA, 1988); IT systems are validated in compliance with the Health Insurance Portability and Accountability Act (HIPAA). As an interim step (4b), assays may be developed and validated to the Research-Use Only (RUO) stage until such time as an assay for patient testing is required. These principles will be illustrated using examples from our commercially available HIV-1 resistance tests.

(s5-b) Biodiversity Documentation via DNA Barcoding at the Smithsonian Institution's L.A.B.

L. Weigt

Smithsonian Institution, National Museum of Natural History, Laboratories of Analytical Biology, Washington, D.C.

Post genome technologies have enabled massive increases in throughput and reduced costs, but most of these advances have not been applied efficiently to global biodiversity documentation due to other constraints. I'll use a couple of examples including the DNA Barcoding/ Barcode of Life program and the BioCode Moorea project to illustrate what we've done, where we are, and where (we think) we need to go with our

technology development and IT infrastructure in order to facilitate "the 21st Century Museum". Birdstrikes, stream water quality assessments, seafood substitution, invasive species identifications, and rapid biotic inventories all rely on, or benefit from, a library of known organisms with genetic data aiding identifications. The building of that library is non-trivial, and metadata information flow is a major impediment. The DNA Barcoding effort is standardizing the types of information being captured, and increasing the quality of and confidence in the data and results by linking chromatograms and voucher specimens to sequence records. The Consortium for the Barcode of Life (CBOL) has established a Leading Labs Network (LLN) to gather and disseminate "best-practice" and how-to information on all steps in the process — from permitting and collecting, through identification and tissue sampling, and the basics of the lab workflow, including data quality control and analysis — and is working on ways to efficiently distribute that information. I'll report on progress on our species identification projects, our all-taxon biotic inventory projects, our comparison of technology studies, and our efforts to Wiki-fy the sharing of information on all our processes.

(s5-c) Existing & Emerging Biotechnologies for Forensic DNA Applications

M. Holland

Pennsylvania State University, University Park, PA

From the very first application of RFLP methods to identify perpetrators of crime back in the mid-1980's, the forensics community has continued to make use of the biotechnologies that have been developed since. By far, the most widespread and influential of these has been PCR; which, of course, has had an enormous impact on all molecular studies. PCR techniques are used daily by the thousands of forensic DNA facilities across the country, and around the world, primarily to determine STR profiles from 10-15 highly discriminating loci. This has resulted in the development of huge convicted offender databases (e.g., more than 6.4 million profiles in the U.S. database alone), and has allowed for the rapid processing of hundreds of thousands of sexual assault cases in just the last 5-10 years. From its roots, PCR has branch off into areas such as SNP genotyping, real time probing of molecular disease and now into the next generation DNA sequencing technologies that have revolutionized modern, 21st century approaches to genomics, and have paved the way for the era of the genomicist. With these recent, rapid and expansive developments in DNA sequencing technology, it is now possible to consider how widespread DNA sequencing approaches can be used in forensic cases.

Forensic investigations are often times quite difficult to predict, and forensic evidence comes in all sorts of sizes, shapes and sources. Therefore, while the instrumentation used by forensic DNA crime laboratories is highly advanced (robotics, real-time PCR, capillary array electrophoresis), the nature of forensic evidence has made it difficult to establish high volume, core facility environments. The core facility concept, past and present, and in relation to new generation sequencing approaches, will be discussed.

(s6) Quantitative Proteomics — *Reproducibility in Quantitative Proteomics*

As quantitative proteomics methods become more extensively used by multiple laboratories, inter-lab. reproducibility has become more of an issue. Using a common sample, comparable results ought to be achieved irrespective of the methodological approach or institution. Despite open studies from ABRF proteomics research group (PRG) over the last couple of years, and a closed study carried out by HUPO amongst others, cross-lab and cross approach studies are still few and far between. In this session we discuss the importance of experimental design, knowledge of maximum points of variance within an experimental protocol and appropriate data analysis within quantitative proteomics and their impact on reproducibility. We go onto to introduce two cross laboratory studies where 2D gels and MRM analyses respectively are carried on the same samples across multiple institutions.

(s6-a) Reproducibility of 2D Gel-based Proteomics Experiments

J.A. Westbrook[6], D. Bramwell[1], M. Caponite Hurley[2], A. Khan[3], G. Poschmann[4], K. Marcus[4], S. Hoving[5], H. Voshol[5]

[1]Nonlinear Dynamics, Newcastle upon Tyne NE1 2ET, UK; [2]Michigan Proteome Consortium, Ann Arbor, MI 48109-0404, USA; [3]Australian Proteome Analysis Facility (APAF), Macquarie University, Sydney NSW 2109 Australia; [4]Medical Proteom-Center, Ruhr-University of Bochum, D-44801 Bochum, Germany; [5]Novartis Institutes for BioMedical Research, WSJ-88.801, CH-4002 Basel, Switzerland; [6]Cancer Research UK Clinical Centre, Leeds Institute of Molecular Medicine, St James's University Hospital, Leeds, LS9; 7TF, UK

Large scale, in depth, proteomic studies of cells, tissues or body fluids, involving multiple laboratories, employing a variety of proteomics technologies have been executed in the past. Although the aim of these studies was not focused on achieving maximal reproducibility between the participating labs but rather to explore the deliverables of the individual technologies, it is apparent that different labs using different technologies on the same samples obtain rather different results. For any comparative study, i.e analysis of two or more different but related samples, such as healthy versus disease or different drug treatments, there is even more of a challenge to be investigated in such an experimental design.

The availability of internal or external standards in conjunction with comparative proteomics experiments are thought to be a major step forwards in obtaining data sets of such a quality that bioinformatics approaches can be applied productively. In order to take the next step forward it was deemed important by the HUPO-IAB (Industrial advisory board of the human proteome organization) to focus particular attention on analysis of the reproducibility of the various proteomics technologies.

A first study was initiated and was supported by 5 labs in a world-wide setting. The goal of this study was to demonstrate that cross lab experiments can be done, which would provide a very necessary leap forward in the ability to collect reliable data in proteomics. The study was of two different but related samples of H. Flu, one of which was treated with Actinonin. The study was a cross-lab 2-dimensional polyacrylamide gel electrophoresis analysis of the samples as it was believed by the organizers that that with the current technologies available and strict adherence to protocols, this would be possible, although a major challenge.

Ready to use samples were provided to the labs. Each participating lab ran two different but related samples on 2-D gels according to the supplied protocol, and then performed an analysis between the two samples using software provided to identify what are, in their opinion, the top 200 significantly changing spots. This of course includes newly appearing spots. The full analysis was uploaded to Nonlinear Dynamics (a HUPO-IAB member) for the comparison between labs and further analysis.

The presentation will provide an overview of the results achieved and demonstrate 2-D gel experiments can indeed be reproduced in different labs. This a major step forward for proteomics as a discipline.

(s6-b) Reproducibility of Protein MRM-Based Assays: Towards Verification of Candidate Biomarkers in Human Plasma

S.C. Hall[1], T. Addona[2], S. Abbatiello[2], D. Bunk[3], H. Keshishian[2], B. Schilling[4], S.J. Skates[5], C. Spiegelman[6], L. Zimmerman[7], S.A. Carr[2], The CPTAC Network

[1]University of California San Francisco, San Francisco, CA; [2]Broad Institute of MIT and Harvard, Cambridge, MA; [3]National Institute of Standards and Technology, Gaithersburg, MD; [4]The Buck Institute for Age Research, Novato, CA; [5]Massachusetts General Hospital, Boston, MA; [6]Texas A&M University, College Station, TX; [7]Vanderbilit University, Nashville, TN

Verification of candidate protein biomarkers requires high throughput analytical methodology that is quantitative, sensitive and specific. In addition, robust standard operating procedures (SOPs) for sample preparation, data acquisition and analysis must be strictly followed in order implement these types of studies across multiple sites with minimum variation in the results. Currently, validation of biomarkers is accomplished by employing immunoassays. However, development of immunoassays for protein biomarkers depends on suitable antibodies that, if not available, can result in considerable time and expense to produce. Recently, the utility of multiple reaction monitoring (MRM) coupled with stable-isotope dilution mass spectrometry (SID-MS) for quantification of proteins in human plasma and serum has been demonstrated. However, reproducibility and transferability of protein-based MRM assays across multiple laboratories has yet to be shown as feasible. Towards this goal, we describe a study that was implemented to assess intra- and inter-laboratory performance of an MRM/SID-MS assay for quantitating seven target proteins that were spiked into human plasma.

(s7) Metagenomics — *Computational Approaches for Metagenomic and Supragenomic Data Sets*

Next generation DNA sequencing technologies when applied to microbial communities provide vast amounts of data. Whether these data are produced by deep16S sequencing of a microbiome; result from metagenomic and metabolomic analyses of complex ecosystems; or from intraspecies supragenomic analyses they require user-friendly, robust, computational analysis methods. This session is designed to provide participants familiarity with state-of-the-art computational aids for the design, processing, and analysis of large and complex microbial DNA sequence data sets.

(s7-a) Development and Application of a Global Comparative Genomics Pipeline for Performing Inter-isolate Analyses within Microbial Species

G.D. Ehrlich, J.S. Hogg, B. Janto, R.A. Boissy, N.L. Hiller, A. Ahmed, and F.Z. Hu

Center for Genomic Sciences, Allegheny Singer Research Institute, Pittsburgh, PA

We have developed a comparative genomics pipeline for performing genome level comparisons among any number of isolates for any microbial species. This pipeline was developed to test the Distributed Genome Hypothesis which posits that chronic bacterial pathogens utilize a strategy of polyclonal infection and continual reassortment of genic characters to ensure persistence. The pipeline begins by performing gene clustering according to an empirically-derived single linkage algorithm. Genes are binned as either core or distributed. The core genome is composed of those gene clusters that are shared among all strains and the supragenome is composed of all core and distributed gene clusters. The system then plots the core and supragenome sizes as a function of the number of strains sequenced, and performs an exhaustive pair-wise genic comparison among all strains to determine the degree of gene sharing for each individual strain pair, as well as providing the average level of gene sharing/differences for the species as a whole. The percentage of distributed genes/genome and the rate of increase in the number of unique genes/genome sequenced is then used by the finite supragenome model to predict the number of genomes that need to be sequenced to obtain any desired level of coverage of the species supragenome. The core genome data serves as a excellent marker for determining if an assembled group of strains all belong within a species, or whether current taxonomy has lumped together multiple independent species; it can also be used to determine if two or more current species are actually a single species. We have used this pipeline to analyze >20 species and have determined that between 10-40% of the genome of all species is composed of distributed gene clusters. Comparisons within a species revealed average genic differences of between 100 and >1000 clusters.

(s7-b) Massively Parallel Barcoded Pyrosequencing Reveals Unexpected Diversity in the Human Microbiome

R. Knight

University of Colorado, Boulder, CO

Massively parallel pyrosequencing, such as that provided by the 454 GS FLX instrument, has revolutionized our view of the microbial world in general and the human microbiome in particular. The use of error-correcting barcodes allows hundreds of microbial samples to be sequenced in parallel with extremely low rates of sample misassignment, and powerful tools for community comparison such as UniFrac, which compares samples in terms of the evolutionary history they share, can allow us to understand the relationships among hundreds or thousands of communities simultaneously. There are two main uses of this technique: sequencing of the 16S rRNA gene provides a detailed view of which taxa are present in a sample, whereas metagenomic shotgun sequencing can provide a total view of the community. In this talk, I describe our studies of various human body habitats, focusing on the gut and the hand. The overlap at the species level between individual microbiomes is extremely

low, but radically different species assemblages converge on the same profile of gene functions at the whole-microbiome level. Thus, microbial ecosystems mirror macroscale ecosystems, in which e.g. grasslands and forests on different continents reach strikingly similar states while composed of different species: these analogies among ecosystems at different scales will be critical for understanding how changes in the human microbiome affect health and disease.

(s7-c) MG-RAST: a Web-based Tool for the Analysis of Metagenomic Data Sets

F. Meyer

Argonne National Laboratory, U.S. Department of Energy, Argonne, IL

Sequencing of random community shotgun data sets started a just few years back. Today there are more than 50 gigabases of metagenomic data, more than 200 data sets in available for comparison and it is clear that the field has left its infancy. The Metagenomics RAST server (http://metagenomics.nmpdr.org) provides a number of critical services to the metagenomics community. It acts as the *de-facto* repository and provides comprehensive analysis capabilities. Using data structures from the SEED (http://www.theseed.org) environment, the MG-RAST server provides reconstructions of both community composition and community metabolism. A user friendly web interface allows navigation of the data sets and supports various approaches for data mining and downloading subsets of relevant data.

(s8) Label Free Detection

This session will highlight selected technologies for label free detection. Biochemically, label-free methods can be used in to directly measure and characterize unperturbed intermolecular interactions. In cellular systems, they can be used to measure functional responses in primary and untransformed cells. Applications in screening and characterization will be discussed.

(s8-a) Label-Free Characterization of Protein-Peptide and Protein-Drug Interactions

G.G. Privé

University of Toronto and Ontario Cancer Institute, Canada

A wide variety of technologies are available for the measurement of intermolecular interactions through direct or indirect binding assays. Label-free methods can measure direct intermolecular associations and do not depend on many of the implicit assumptions inherent to labeled reporter systems. The methods vary greatly in terms of sample requirement, sensitivity, and adaptability to high-throughput formats, and several new technologies have recently become available to industrial and academic labs. This presentation will describe our work in developing small molecule inhibitors of a BCL6/corepressor protein-protein interaction interface.

(s8-b) Application of Differential Static Light Scattering and Isothermal Denaturation to Investigate Thermostability and Binding Specificity of Protein Families

M. Vedadi, G. Senisterra, G.A. Wasney, A. Allali-Hassani, P.J. Finerty, Jr., A.M. Edwards, C. Arrowsmith

Structural Genomics Consortium, University of Toronto, Toronto, Ontario, Canada

Structural genomics efforts have led to the expression of thousands of proteins, many of which have not been purified or characterized previously. Identification of small molecules that bind to and stabilize these proteins can promote their crystallization as well as provide valuable functional information. We have employed differential static light scattering (DSLS) and isothermal denaturation (ITD) to investigate the thermostability and ligand binding specificity of different families of human proteins. The screening results facilitated the comparison of substrate specificities and also identified compounds which appeared to be general inhibitors for some of these protein families. Moreover, other compounds were discovered that only bind to a subset of proteins in each family of proteins and thus appear to discriminate among different members of the family. Our preliminary results show that DSLS can also be used to screen and characterize membrane proteins.

(s8-c) Biochemical and Cell-based Detection by Epic System

M. Wu

Johns Hopkins University, Baltimore, MD

Label-free detection of molecular interactions and cellular signaling events are of great interest for isolation of bioactive molecules. We have applied Epicsystem to studies a variety of biochemical and cellular processes including kinase-mediated biomolecular interactions, virus infection and receptor-mediated signaling events. The results and utilities of these studies will be discussed.

(s9) Stem Cell Applications

Stem cells and their derivatives offer great hope for regenerative medicine. The potential of stem cells to ameliorate tissue injuries and acquired and heritable diseases is enormous. There are now many sources of stem cells available including embryonic stem cells (ES cells), induced pluripotent cells (iPS cells), umbilical cord stem cells, and adult stem cells from multiple tissues. To aid in the isolation, banking, and dissemination of the different stem/progenitor cell types, academic universities across the country have developed Stem Cell Cores. In addition to providing both cells and consultation, Stem Cell Cores often provide specialized training and reagents to investigators with interest in applying stem cell biology to their own research. The Stem Cell Applications session will discuss a variety of Stem Cell Core issues including: isolation, banking and quality control (standardization) of stem cells; student and investigator training; regulatory and safety issues; and moving stem cell technologies into the clinic. The featured speakers include: Jeffrey L. Spees, Ph.D., University of Vermont, Burlington; Karl Willert, Ph.D., University of California, San Diego; and Carol Ware, Ph.D., University of Washington, Seattle.

(s9-a) Adult Stem Cell Cores and Regenerative Medicine

J.L. Spees

University of Vermont, Burlington, VT

The Spees laboratory is broadly interested in the process of tissue repair after injury. We are currently developing therapeutics for stroke and myocardial infarction based on different subpopulations of adult human bone marrow progenitor cells and their secreted reparative factors. We are determining their abilities to rescue existing tissue and also to promote repair through the augmentation of resident tissue-specific stem/progenitor cells. In separate studies we are exploring the possible use of isolated human atrial cardiac progenitor cells for cell replacement strategies after myocardial infarction. As the UVM Stem Cell Core, we provide adult murine neural stem/progenitor cells, adult rodent bone marrow-derived stem/progenitor cells, and human umbilical cord stem/progenitor cells to investigators at UVM and nationally and internationally. We train students and investigators in how to isolate adult stem cells from their tissue of interest, how to expand them, and how to differentiate them.

(s9-b) Probing Human Embryonic Stem Cell Proliferation with Arrayed Cellular Microenvironment Technology

K. Willert[2,3], D. Brafman[1], K. Shah[1], T. Fellner[2], S. Chien[1]

[1]Department of Bioengineering, University of California, San Diego, CA, [2]Human Embryonic Stem Cell Core Facility, University of California, San Diego, CA, [3]Department of Cellular and Molecular Medicine, University of California, San Diego, CA

We have developed a multi-factorial cellular microarray technology platform to rapidly screen and optimize culture conditions of any cell type of interest. Application of this technology to define culture conditions of human embryonic stem cell (hESC) illustrates many of the challenges confronted by scientists who study and utilize hESCs in their laboratories. Previous research has primarily focused on developing defined media formulations for long-term culture of hESCs with little attention on the establishment of defined substrates for hESC proliferation and self-renewal. Using the cellular microarrays we identified fully defined and optimized culture conditions for the proliferation of hESCs. Through the systematic screening of extracellular matrix proteins and signaling molecules, we developed and characterized a completely defined culture system for the long-term self-renewal of hESCs. The efficacy of these conditions was confirmed over long-term culture using three independent hESC lines. In the future, the novel array platform and analysis procedure presented here will be applied towards the directed differentiation of hESCs and maintenance of other stem and progenitor cell populations.

(s9-c) The Ellison Stem Cell Core

C. Ware

University of Washington, Seattle, WA

The stem cell core at the University of Washington is housed in a new building, within a purpose-designed 3500 ft^2, 18-hood facility in the Institute for Stem Cell and Regenerative Medicine (ISCRM). The core was equipped with private donations while investigator hood rent recaptures some of the running expense and a program project grant from NIGMS supports the participating investigators. We focus on human embryonic stem cells (hESC), have 14 of the federally approved lines and are actively generating new hESC lines from donated embryos. The core trains in hESC and mESC culture and provides expertise and practical support for the 14+ laboratories that utilize the core. This represents a broad range of interests in regenerative medicine that fosters collaborations and exchange of information that encompasses the greater Seattle research community. The core extends resources on a collaborative basis for laboratories where it is impractical to take on hESC culture. We assist with induced pluripotent (iPS) cell production for ISCRM investigators. The core research focus is on maintenance of pluripotency, manipulation of the gradations of developmental stages associated with pluripotency and characterization and cryopreservation of hESC and iPS cells. The core has an abiding interest in comparative ESC studies, including mouse, non-human primate, dog and mink ESC. The future promises to include a focus on cancer stem cells and assistance in devising high throughput screening assays.

(s10) Next Generation Sequencing

Genome technology has become a central driving force for new discoveries and inventions in biomedical science and medicine. Recent discoveries from the international genome research consortium have reshaped our understanding of how the human genome functions. These findings indicate that the human genome of 3 billion base-pairs is a complex interwoven network and contains very little unused sequence. These new discoveries will drive the continued development of accurate, cost-effective and high-throughput DNA sequencing technologies to decipher the functions of the complex genome for applications in clinical medicine and healthcare. The new DNA sequencing technologies will also serve as fundamental discovery tools for investigations in comparative genomics, the functions of the nervous system and the Cancer Genome Project. This session will cover the development of new DNA sequencing technologies and harnessing the power of the new technologies to solve unique problems in biology.

(s10-a) Toward the $1000 Genome: Molecular Engineering Approaches for DNA Sequencing by Synthesis

Jingyue Ju

Center for Genome Technology and Biomolecular Engineering, Columbia Genome Center and Department of Chemical Engineering, Columbia University College of Physicians and Surgeons, New York, NY

DNA sequencing by synthesis (SBS) on a solid surface during polymerase reaction offers a new paradigm to decipher DNA sequences. We are pursuing the research and development of this novel DNA sequencing system using molecular engineering approaches. In one approach, four nucleotides (A, C, G, T) are modified as reversible terminators by attaching a cleavable fluorophore to the base and capping the 3'-OH group with a small reversible moiety so that they are still recognized by DNA polymerase as substrates. DNA templates consisting of homopolymer regions were accurately sequenced by using these new molecules on a DNA chip and a 4-color fluorescent scanner. This general strategy to rationally design cleavable fluorescent nucleotide reversible terminators for DNA sequencing by synthesis is the basis for a newly developed, next generation DNA sequencer that has already found wide applications in genome biology. In another approach, we have solved the homopolymer sequencing problem in pyrosequencing by using non-fluorescent nucleotide reversible terminators (NRT). We have also developed a new SBS approach using these NRTs in combination with the four cleavable fluorophore labeled dideoxynucleotides. DNA sequences are determined by the unique fluorescence emission of each fluorophore on the ddNTPs. Upon removing the 3'-OH capping group on the dNTPs and the fluorophore from the ddNTPs, the polymerase reaction reinitiates and the DNA sequence are continuously determined. Various DNA templates, including those with homopolymer regions were accurately sequenced by using this hybrid SBS method on a chip and with a four-color fluorescent scanner. Adapting our general strategy to rationally design cleavable fluorescent nucleotide reversible terminators for DNA sequencing by synthesis to a single molecule detection platform will have the potential to achieve the $1000 genome paradigm for personalized medicine.

(s10-b) Nearly Complete Genomic Profiling of Individual Identified Neurons: SOLiD Approach

L.L. Moroz[1,2,4], T. J. Ha[1], M. Citarella[1], E.V. Bobkova[1], C. Lee[3], F. Yu[4], B. Colman[3], C. Clouser[3], C. Barbacioru[3], M. Osentoski[3], A. Shah[3], W. Farmerie[4], A.B. Kohn[1]

[1]Whitney Laboratory for Marine Bioscience, University of Florida, St. Augustine, FL; [2]Department of Neuroscience, University of Florida, Gainesville, FL; [3]ABI, Foster City, CA; [4]ICBR, Genetics Institute University of Florida, Gainesville, FL

What makes a neuron a neuron? What are the genomic bases of unique neuronal phenotypes? How different is the transcriptional profile of one neuron from another? Here, we attempt to identify and quantify nearly all RNA species present in a given neuron. Therefore, we have provided the first unbiased view of the operation of an entire genome from a single characterized neuron. First, we developed protocols for digital expression profiling of identified *Aplysia* neurons representing interneuron, sensory and motor neuronal classes. The generated single-neuron cDNA libraries accommodate the emPCR for two complementary massive parallel sequencing technologies (stating from pyrosequencing to the-sequencing-by-ligation -SOLiD) and allow assembly of the shorter sequence reads. In summary, >9,000,000,000 bases from just three identified neurons were obtained (~80 million sequences from each neuron). It is estimated that such coverage represents >99% of all RNA species in a single neuron. Using absolute real-time PCR we demonstrated that our method is fully quantitative with the dynamic range covering the entire neuronal transcriptome (from the rarest transcripts with only a few copies per cell to the most abundant RNAs with many thousands of copies). Quantitative Real-time PCR and *in situ hybridization* further validated this method of digital profiling. As a result of our initial analysis we propose that >70% of a genome is expressed in a single neuron with a significant fraction being non-coding RNA including antisense RNAs. Many TxFrags, a fragment of a transcript defined as a genomic region, are also found to be specific to neuron subtypes, strongly suggesting a role in the generation of neuronal identity. Furthermore, the numerous classes of non-coding RNAs revealed here likely represent both enormous complexity and crucial contributions of epigenetic RNA signaling mechanisms in the regulation of multiple neural functions, including establishing and maintaining diverse neuronal phenotypes in neural circuits. Support Contributed By: NIH, NSF, & McKnight Brain Research Foundation & ABI.

(s10-c) Using the SOLiD System for Genomic Solutions

R. Nutter

Applied Biosystems, Foster City, CA

Since its introduction just over one year ago, the SOLiD™ System has rapidly developed longer readlengths, higher throughputs and a wide range of applications. The first portion of my presentation will focus on the latest technical advances that are part of the SOLiD3 System. This system permits the generation of more than 400 million mappable 50 bp sequence reads with each run. This number of sequence tags is ideally suited for many genomic, transcriptomic and epigenomic applications. The second portion of my presentation will focus on the wide range of end-to-end application solutions that have been developed for SOLiD users. Examples of these are miRNA discovery, whole transcriptome analysis and ChIP Seq studies. For the first time, the combination of molecular barcodes with the ultra high throughput of the system makes these applications much more cost effective for use in a core environment and allows core lab managers to provide services to a broader range of clients.

(s11) Phosphoproteomics

New Developments in Analysis of Phosphoproteomics has drawn great attention all over the world. In this session we will present new enrichment techniques like ERLIC, improved methods for phosphopeptide fragmentation in combination with different quantification techniques (label free quantification, iTRAQ). Different model organism for systems biology will be discussed. Further we will discuss relevant bioinformatics questions (e.g. phospho-database, software).

(s11-a) A New Acid Mix Enhances Phosphopeptide Enrichment on Titanium- and Zirconium Dioxide for Mapping of Phosphorylation Sites on Protein Complexes

K. Mechtler[1,2], G. Mitulović[1], O. Hudecz[1], J. R. A. Hutchins[1], B. Hegemann[1], E. Roitinger[1], T. Taus[1], C. Stingl[2], J. Peters[1], M. Mazanek[2]

[1]Research Institute of Molecular Pathology (IMP), Vienna, Austria, [2]Institute of Molecular Biotechnology (IMBA), Vienna, Austria

Reversible protein phosphorylation plays a major role in regulating many complex biological processes such as cellular growth, division, and signaling. Also, the phosphorylation and the regulation of cell signalisation are taught to have important role in human diseases, primarily for cancer. The high importance of protein phosphorylation makes the proper identification of phosphorylation sites an important task. Compared to other proteins in biological system, phosphorylated peptides are present only in low abundance and this presents a problem for their detection and identification. The selective enrichment of phosphorylated peptides prior to reversed phase separation and mass spectrometric detection significantly improves the analytical results in terms of higher number of detected phosphorylation sites and spectra of higher quality. Recently, there have been several publications describing offline and online chromatographic approaches for selective enrichment of phosphorylated peptides using Metal Oxide Chromatography (MOC) with titanium dioxide and zirconium dioxide media. We have established a rapid method based on titanium dioxide chromatography, suitable for the enrichment of phosphopeptides from digested purified protein complexes that is compatible with subsequent analysis by nano-reversed phase HPLC-MS/MS. In the present work we have tested the effect of a modified loading solvent and new wash conditions on the efficiency of the enrichment of phosphopeptides from a test mixture of synthetic and BSA-derived peptides and from digested protein complexes and have shown this to improve the performance of the previously-published method. For the test mixture, the new method showed improved selectivity for phosphopeptides, whilst retaining a high recovery rate. Application of the new enrichment method to the digested protein complexes resulted in the identification of a significantly higher number of phosphopeptides as compared to the previous method. Additionally, we have compared the performance of TiO_2 and ZrO_2 columns for the isolation and identification of phosphopeptides from purified protein complexes and found that for our test set, both media performed comparably well for the selective enrichment of phosphopeptides. In summary, our improved method is highly effective for the enrichment of phosphopeptides from purified protein complexes prior to mass spectrometry, and is suitable for large-scale phosphoproteomic projects that aim to elucidate phosphorylation-dependent cellular processes.

(s11-b) Current Methods in Phospho-proteomics

A. Sickmann and R. Zahedi

ISAS – Institute for Analytical Sciences Dortmund, Germany

Substantial progress has been made over the last years regarding the analysis of phosphorylation in mass spectrometry-based proteomics. New methodologies have been introduced not only on the MS level but also for the enrichment of phosphorylated species and for the data interpretation and correct annotation of phosphopeptide MS/MS spectra. Thus, revealing Ã¢€œtheÃ¢€¡½ phosphoproteooteome seems to be an accomplishable task and what is even more relevant for biomedical research: uncovering dynamic changes in phosphorylation patterns due to specific stimuli is possible when applying quantitative mass spectrometry. The latter, however, still poses a major challenge, since generating reliable results rather than just loads of data strongly depends on highest repdroducibility in sample preparation/processing, phosphopeptide enrichment as well as LC-MS analysis. In this context, there is an exceptional need for automation in order to minimize sample-to-sample variations upon slightly different treatment resulting in wrong interpretation of cellular signaling.

(s11-c) Analysis of the Yeast Protein Kinase — Substrate Networks by Quantitative Phosphoproteomics

B. Bodenmiller[1,2], S. Wanka[3], C. Kraft[4], B. Gerrits[5], D. Campbell[6], M.Y. Brusniak[6], P. Picotti[1], O. Vitek[7], R. Schlapbach[5], M. Peter[3], C. von Mering[4] and R. Aebersold[1,6,8]

[1]Institute of Molecular Systems Biology, ETH Zurich, 8093 Zurich, Switzerland, [2]Zurich PhD Program in Molecular Life Sciences, 8057 Zurich, Switzerland, [3]Institut of Molecular Biology, 8057 Zurich, Switzerland, [4]Institute of Biochemistry, ETH Zurich, 8093 Zurich, Switzerland, [5]Functional Genomics Center Zurich, UZH | ETH Zurich, 8057 Zurich, Switzerland, [6]Institute for Systems Biology, Seattle, WA 98103, USA, [7]Purdue University, Departments of Statistics and Computer Science, West Lafayette, Indiana 47107, USA, [8]Faculty of Science, University of Zurich, 8057 Zurich, Switzerland

Protein phosphorylation plays a pivotal role in the regulation of cells and is controlled by kinases and phosphatases. So far it has been difficult to identify substrates of a particular kinase *in vivo* and it was impossible to understand kinase-substrate relationships on a system-wide scale. This is because kinases, phosphatases and their substrates form complex networks that hitherto could not be comprehensively analyzed. Here we describe a novel method based on quantitative phosphoproteomics allowing such analyses and present a nearly complete network of kinase-substrate relationships in *S. cerevisiae*.

The method consists of five steps: (1) Phosphopeptides are reproducibly isolated from a digest of a *S. cerevisiae* wild-type and a kinase gene deletion mutant proteome (2) Of each isolate LC-MS(/MS) maps are generated (3) These maps are analyzed using the algorithms *SuperHirn* and *Corra*, determining regulated phosphopeptide features (4) These features are identified using targeted LC-MS/MS (5) The kinase-substrate networks

are generated and further analyzed. To validate our approach we first studied the TOR kinase. We identified both known targets and novel candidates to be regulated. As yet several candidates were confirmed to be direct targets or dependant on TOR. As these results underlined the reliability of our approach we applied it to all viable yeast kinase and phosphatase gene deletion mutants. So far over 2,000 phosphoproteins were confidently identified and quantified in our kinase-substrate networks, allowing for a system-wide analysis. Besides identifying many known *in vitro*, *in vivo* and novel targets of a given kinase in the generated networks, we were also able to show that we first, can determine the biological function of a particular kinase and second, can derive target phosphorylation motifs. From our data it can be concluded that the presented approach allowed for the first system-wide analysis of most kinase- and phosphatase-substrate networks in yeast.

(s12) microRNA — *microRNA Profiling in Medical Practice*

MicroRNA alterations are involved in the initiation, progression and metastases of human cancer. The main molecular alterations are represented by variations in gene expression, usually mild and with consequences for a vast number of target protein coding genes. The causes of the widespread differential expression of miRNA genes in malignant compared with normal cells can be explained by the location of these genes in cancer-associated genomic regions, by epigenetic mechanisms and by alterations in the microRNA processing machinery. MicroRNA expression profiling of human tumors has identified signatures associated with diagnosis, staging, progression, prognosis and response to treatment. In addition, profiling has been exploited to identify microRNAs genes that may represent downstream targets of activated oncogenic pathways or that are targeting protein coding genes involved in cancer. Recent studies proved that miRNAs are main candidates for the elusive class of cancer predisposing genes and that other types of non-codingRNAs participate in the genetic puzzle giving rise to the malignant phenotype. These discoveries could be exploited for the development of useful markers for diagnosis and prognosis, as well as for the development of new RNA-based cancer therapies.

(s12-a) Real-time PCR expression profiling of microRNA

T.Schmittgen

Ohio State University, Columbus, Ohio

microRNAs (miRNAs) are short noncoding regulatory RNAs. miRNAs are initially transcribed as long miRNA precursors (pri-miRNA) which are then subsequently processed into the precursor miRNA (pre-miRNA) and then the active mature miRNA. Altered miRNA expression has been implicated in a number of human diseases including cancer. miRNAs are challenging molecules to amplify and quantity by PCR because the miRNA precursors exist as a stable hairpin and the mature miRNA is roughly the size of a standard PCR primer. Despite these challenges successful approaches have been developed to amplify and quantify both the precursor and mature miRNA by qRT-PCR. In a systematic evaluation of several hundreds of precursor and mature miRNA by real-time qRT-PCR we demonstrated that many of the miRNAs are initially transcribed as precursors but are not processed into mature miRNA. This observation occurs more frequently in human cancer cell lines and in liver tumors as compared to normal tissues and pancreatic tumors. The expression of miR-31 was of particular interest in that it was processed to mature miRNA in some cell lines but not in others. In the cell lines that did not process mature miR-31, the precursor was shown by in situ RT-PCR to be localized to the nucleolus. Numerous mature miRNAs including miR-21, miR-221, miR-301 and miR-212 were increased

in pancreatic cancer tissues compared to normal and adjacent benign pancreas. By quantifying the pri-, pre- and mature miRNA by qRT-PCR, we were able to demonstrate that the increase in mature miRNA resulted from an increase in processing of the pri-miRNA and was not due to new transcription. In summary, real-time PCR may be used to identify examples of post-transcription regulation of miRNA biogenesis.

(s12-b) MicroRNAs: Regulation, Development, and Disease

M. Thomson

Vanderbilt University, Nashville, TN

MicroRNAs are short non-coding RNA transcripts that post-transcriptionally regulate gene expression. Several thousand microRNA genes have been identified in a multitude of organisms including Caenorhabditis elegans, Drosophila, fish, plants, mammals and viruses. MicroRNAs have been linked to developmental processes in C. elegans, fish, Humans, and plants, and cancer, cell growth and apoptosis in Humans, mice, C. elegans and Drosophila. A major impediment in the study of microRNA function was the lack of quantitative expression profiling methods. To close this technological gap we developed custom microarrays that monitored expression levels for over 900 microRNAs encompassing human, mouse, rat, and fish. Here we show how these arrays have revealed distinct patterns of expression in mammalian development and how these expression patterns are altered in cancer.

(s13) Epigenomics

(s13-a) Techniques for Cytosine Methylation Patterns

M. Suzuki, J.M. Greally

Albert Einstein College of Medicine of Yeshiva University, Bronx, NY

Methylation of DNA is believed to be involved in various biological phenomena, causing gene silencing, stabilizing chromosomal structure and suppressing the mobility of retrotransposons. However, very few studies have been published that studied methylation patterns genome-wide, despite recent advances in techniques based on microarrays or massively-parallel sequencing assays. We developed a technique, the HpaII-tiny fragment Enrichment by Ligation-mediated PCR (HELP) assay, that is based on microarray hybridization following restriction enzyme digestion and ligation-mediated PCR. Using this method, we can analyze the methylation patterns at the genome-wide level in many cells or tissue types. The assay allows us to gain insight into cytosine methylation differences between normal tissues and cancer, or stem cells and differentiated cells. The assay reveals that methylation in promoter region and also in gene bodies is correlated with gene expression status. In addition, we can use the data generated for HELP assays to analyze copy number variability simultaneously. We developed the assay further to use massively-parallel sequencing (HELP-seq). We find the two techniques are comparable for most genomic regions, but HELP-seq can analyze short fragments that are not detectable in microarray-based HELP, allowing greater insights into CG-rich regions. In this presentation, we will discuss the application of the HELP assay for simultaneous copy number analysis, HELP-seqandthe supporting bioinformatic analyses.

(s13-b) Deciphering the Methylome

B. Caldwell

University of Missouri School of Medicine

Sequencing the human genome was a huge accomplishment, but still does not answer most of our questions regarding health and disease. DNA mutations can cause disease, but so can changes in gene activity that are independent of mutations. Addition of methyl groups to cytosines within the regulatory regions of genes, an epigenetic process known as DNA methylation, typically alters the gene's activity. Similarly, the methylation, acetylation or ubiquitination of certain amino acids on histone proteins can change the way DNA is wrapped around the histone cores and can affect activation of genes in conjunction with DNA methylation. Many cancers and other diseases have aberrant DNA methylation at particular sites in the genome and are key to biological events such as embryonic development, ageing and cellular regulation. Researchers have access to a collection of tools to decipher the epigenome, which could lead to new ways to treat diseases. These tools mainly involve a set of preparative techniques including modification of DNA using sodium bisulfite, which converts unmethylated cytosine into uracil. The sample can then be analyzed in several ways, including DNA sequencing to identify sites of C to T transitions following PCR. Other methods rely on restriction enzymes that are sensitive or insensitive to cytosine methylation or precipitation with antibodies or methyl binding proteins that recognize 5' methylcytosine. Detection systems can include PCR-based methods, microarray hybridizations, mass spectrometry, and direct high-throughput DNA sequencing. The next five years will see a boom in epigenomic research thanks to advances in these and other technologies. One major issue in these genome-scale studies is the bioinformatics data analyses. Several groups are taking advantage of these developments to characterize the entire human epigenome in both healthy and disease states. The NIH has recognized the importance of epigenomics by making this one of their Roadmap Initiatives.

(s13-c) High Throughput Chromatin Immunoprecipitations, Integrative Epigenomics, and Personalized Medicine

A. Melnick, M. Figueroa, Y. Li

Weill Cornell Medical College, Ithaca, NY

In recent years technical advances allowing the combination of chromatin immunoprecipitations (ChIPs) with genomic microarray and deep sequencing technology has contributed fundamental observations concerning the nature of gene regulation in many different biological contexts. The different variations of ChIP allow the location of specific transcription factors, chromatin modifying proteins, and histones to be determined throughout the genome. The most frequently applied versions of ChIP involve a chemical crosslinking step in order to stabilize the interaction of proteins with nearby DNA followed by one ore more affinity purification steps to isolate specific complexes of interest. DNA fragments enriched through this procedure can then be amplified using protocols such as LMPCR, whole genome amplification, or linear amplification, each with their pros and cons, and then hybridized to genomic microarrays or directly prepared for analysis using deep sequencing platforms. Enrichment of specific DNA fragments should be indicative of binding by the proteins of interest. However, Interpretation

of ChIP-on-chip and ChIP-seq can be complicated by challenges related to assay reproducibility, particular design features of the many different types of microarrays or sequencing devices, and the inherent difficulty in ascertaining the biological relevance of the data. High throughout ChIP methods have been used to map the location of previously un-identified transcription start sites, to determine the epigenetic regulatory state of different cell types, alterations in epigenomic programming typical to disease states such as cancer, to identify target genes of transcription factors, etc. In spite of the technical challenges, the reliability of the assay is such that it can be readily applied to low abundance clinical specimens and used to identify robust chromatin regulatory signatures. Integrative analysis combining ChIP-on-chip with other high throughput methods allow biological differences between patient biopsies to be captured with far greater depth that single platform analysis.

(s14) Proteomics Sample Prep — *Practical Targeted Proteomics*

This session will cover the practical aspects of targeted proteomics including, preparing samples, designing experiments, analyzing data, and the practical aspects of using triple quadrupole mass spectrometers.

(s14-a) Establishing SRM as a Robust and Reliable Technique for Targeted Proteomics

D.M. Tomazela[1], B. MacLean[1], B. Frewen[1], G. Merrihew[1], A. Prakash[2], S. Peterman[2], and M.J. MacCoss[1]

[1]University of Washington, Seattle, WA; [2]Thermo Fisher Scientific, San Jose, CA

Selected Reaction Monitoring (SRM) is a quantitative technique widely employed for the quantification of targeted compounds in complex mixtures. The ability to select and monitor specific pairs of precursor-/fragment-ions confers high selectivity and multiplexing capacity to the technique making it of particular promise for hypothesis driven proteomics. The concept of monitoring specific peptides from proteins of interest is becoming well established. These methods have high specificity within a complex mixture and, thus, can be performed in a fraction of the instrument time relative to discovery based methods. Ultimately, targeted studies are intended to complement discovery based analysis and facilitate hypothesis-driven proteomic experiments.

One of the challenges in the application of SRM for protein analysis and assay development is the streamlining of method development. This process involves the (i) selection of a proteotypic set of peptides that best represent the targeted proteins, (ii) prediction of their most sensitive and selective SRM transitions, and (iii) selection of a narrow time window in which to measure the respective transitions during the chromatographic gradient. In the case where no protein/peptide standards are available, these steps become particularly challenging.

In this presentation, we will discuss the techniques and informatics tools developed in our laboratory to simplify and streamline the development and application of SRM methods. In particular, we will present the use of discovery based MS/MS spectral libraries to improve the development of SRM methods and the interpretation of the results. Furthermore, an approach will be presented to establish scheduled SRM methods for peptides that have never been detected previously.

(s14-b) Targeted Proteomics as A Translational Tool in Drug Discovery

W. Wang, F. Meng, K. Southwick, J. Man, Y. Du, K. Zhou, N. Yates, R. Hendrickson;

Department of Proteomics, Merck & Co., Inc., Rahway, NJ

Proteins as biomarkers are increasingly being used in pharmaceutical research. Proteomics discoveries in areas such as target engagement, pharmacodynamics, toxicity and disease progression could play important roles in crucial decision makings at every stage of drug development. A sustainable biomarker workflow normally begins with discovery based proteomics where candidate markers are discovered with unbiased measurements of thousands of proteins. Immediately after the discovery phase is the biomarker verification stage where potential protein biomarkers are tested with greater number of subjects than in the discovery phase using hypothesis based methods. LC-MS based targeted proteomics with triple quadrupole (QQQ) mass spectrometer enables the translational strategy with prompt method development, high sample throughput and multiplexing capacity. In situations where antibody based assays are not available, targeted proteomics is the only path forward to protein biomarker verification. We will present two case studies in which targeted proteomics played a translational role in verifying biomarkers. In the first example, we will describe the development of a translational QQQ method that quantifies a panel of 20 Alzheimer's disease (AD) progression markers in cerebral spinal fluid (CSF). Specifically, we will discuss how we transformed a rather complicated biochemical workflow in the discovery phase to a relatively simple, fit for purpose one for the targeted QQQ method. The simplified workflow not only reduced the variation introduced by biochemical sample processing but also increased the throughput significantly—two requirements in biomarker verification phase. Selection and evolution of internal standards as well as comparison of analytical characteristics between the two platforms will also be discussed. The second case study is an example of QQQ as a translational strategy for genomics studies when antibody based assays are not readily available. We will describe the development of a QQQ method that quantifies a low-abundance protein in plasma as drug efficacy marker. The challenges were to develop a targeted QQQ method without any mass spectrometry data from the discovery phase and to handle the complexity of plasma proteome. We will present how the method evolved to a stage with practical utilities.

(s14-c) Practical Aspects of Quantitation with Triple-quadrupole Mass Spectrometers

B. Moeller, S.D. Stanley

University of California, Davis, CA

Targeted proteomics is a developing field in need of standardized methodologies to properly implement quantitative analysis. The field can incorporate strategies developed for small molecule quantitation and adapt them for use in determination of low level proteins from complex matrices. Triple-quadrupole mass spectrometers play a large role in small molecule quantitation in both academic and industrial settings. The use of multiple reaction monitoring (MRM) allows for the combination of fast scan rates, specificity and sensitivity required to analyze low abundance compounds. Standardized method validation strategies are necessary to produce high quality reproducible data. This presentation will detail small molecule method validation strategies and possible applications in targeted proteomics. Real world examples of validated small molecule quantitative methods will be presented with an overview on the determination of limits of detection, limits of quantitation, assay linearity, precision, accuracy,

robustness and matrix effects. Practical aspects of triple-quadrupole mass spectrometer use in quantitative analysis will be discussed including the design of methods that incorporate the collection of MRM spectra and use of stable isotope standards.

(s15) Bioinformatics

The Bioinformatics session will highlight the emerging need of bioinformatics tools for handling mass amounts of data from high-throughput DNA sequencers and metagenomics/metaproteomics projects. Speakers will discuss database resources and analysis tools needed for the large datasets from such projects.

(s15-a) Informatic Challenges in Metaproteomics

P. Carey, B. Dill, M. Shah, D. Hyatt, C. Pan, R.L. Hettich, N.C. VerBerkmoes

Oak Ridge National Laboratories, Oak Ridge, TN

Microbial communities play key roles in the earth's biogeochemical cycles. Our knowledge of the structure and function of these communities is limited because analyses of microbial physiology and genetics have been largely confined to isolates grown in laboratories. Recent acquisition of genomic data directly from natural samples has begun to reveal the genetic potential of communities (Tyson, Nature 2004) and environments (Venter, Science 2004) and spawned the field of metagenomics. The ability to obtain whole or partial genome sequences from microbial community samples has opened up the door for microbial community proteomics. We have developed and applied a combined proteogenomic approach using genomics and mass spectrometry-based proteomic methods (Ram, Science 2005). The key to this technology is the combination of robust multidimensional nano liquid chromatography with rapid scanning tandem mass spectrometry and a variety of informatic tools. Concurrent with developments in analytical technologies, such as liquid chromatography and mass spectrometry, are advances in proteome informatics. From the start of metaproteomics of microbial communities, it was clear challenges existed not present in simple microbial isolate measurements. We developed and implemented many changes to our informatics pipeline to address these needs but it's a continual evolving process. Prime examples include the need to clearly classify between unique, semi-unique and non-unique peptides in complex microbial mixtures across many strains of microbes. The issue of false positives and the need for high mass accuracy was recognized from the start. Quantitation methodologies are seriously challenged, with differences not only in protein concentrations, but species concentrations. Very often in metaproteomics there is not a one to one correlation with the metagenome and metaproteome being measured. It has been widely recognized that metaproteomics desperately needs advanced de-novo/sequence tagging approaches that can be used in a high throughput mode. These are only a few of the many informatic challenges associated with the developing field of microbial metaproteomics.

(s15-b) An aqua-silico algorithm for genome assembly validation and DNA biomarker discovery, and its potential as a mass-market application for microfluidics platforms

R. Boissy

Allegheny-Singer Research Institute, Pittsburgh, PA

Personal genomics typically promises affordable re-sequencing, not de novo sequencing. This is one of the largest "blue ocean" markets on the horizon. However, every genome assembly is a hypothesis, and the supporting evidence for that hypothesis should be commensurate with its implications. Thus, an equally large blue ocean market awaits the developers of affordable genome-scale assembly validation procedures. Given the potentially enormous implications of a personal genome sequence assembly, it is unreasonable to expect regulatory agencies to refrain from requiring such a procedure in the personal genomics era. I describe the computational implementation of a scalable, combinatorial aqua-silico algorithm for genome assembly validation (and its corollary, DNA biomarker discovery). The full implementation of an aqua-silico algorithm combines computation and a related wet-lab protocol, and though I also fully describe this protocol, it has not been attempted yet. Microfluidics platforms offer the potential for an affordable wet-lab implementation of this aqua-silico algorithm, which in turn has the potential to offer microfluidics platform developers one of their first mass-market applications.

(s15-c) Bioinformatics for Metagenomic Taxonomic Classification

G. Rosen

Drexel University, Philadelphia, PA

Metagenomics is an emerging field of genomic analysis applied to entire communities of microbes alleviating the need to isolate and culture individual microbial species. The field opens up study of the 99% of species that cannot be cultured in the lab, and now the challenge lies in classifying their placement in the evolutionary tree of life. Current taxonomic annotation relies on extracting 16S sequences which exploits highly conserved and hypervariable regions for evolutionary distance. I survey the advantages and pitfalls of using 16S-only techniques and propose machine learning classifiers which can use ANY genome fragment. Indiscriminate annotation on a population of fragments will enable us to answer not only "Who is there?" but "How much is there?". Also, high-throughput sequencing technologies are enabling a deep sampling of a community's population but at a price of short-read lengths which limits the resolution of annotation. We benchmark the performance of homology-based BLAST to a composition-based Bayesian classifier for taxonomic classification. Our study demonstrates that a naive Bayesian classifier has similar and reasonable performance to BLAST for fine-resolution (strain and species classification), and I will discuss the potential of machine learning to conquer the "annotation" problem.

(s16) Disruptive Life Sciences Technologies — *New Integrated Analytical Systems Based on Miniaturized Optics and Fluidics*

Techniques of micro and nanofabrication allow the formation of a variety of optical devices and structures that can be employed in ultra-sensitive chemical and biochemical analysis. Optical methods provide highly sensitive detection of molecular binding, reaching to single molecule sensitivity. Combined with chemically specific reactions, these approaches can lead to highly sensitive immunoassays, spectroscopic chemical analysis and high-throughput DNA sequencing. The technologies that create the optical devices can also be integrated with electronics and fluid handling structures to form integrated systems. The fabrication approaches also allow creating highly parallel systems for drastically increasing the analytical throughput by carrying out many simultaneous analyses. These approaches that resemble the integration and parallelism of electronic integrated circuits are producing increasingly functional analytical systems and labs-on-a-chip. This session will consider some of the advancing technologies and breakthrough applications in life science technologies.

(s16-a) Optical Biosensors: Future Trends and Perspectives

C. Taitt and F. Ligler

U.S. Naval Research Laboratory, Washington, D.C.

Optical biosensors originated as large, cumbersome laboratory curiosities with limited capabilities for sample analysis. Since that time, these systems have developed into analytical instruments with much broader applications and capabilities. However, these instruments have largely not yet made the leap from the lab to on-site testing. Sensitivity and selectivity are perhaps the most important factors affecting commercial success of lab instruments. However, other critical issues required for transition for on-site use include: ease of use, short response time, reduced size and cost (per instrument, per sample), stability/robustness (instrument, biological components), overall versatility (different sample types, different targets). At the Naval Research Laboratory (NRL), we are addressing many of these issues through research in nanotechnology, microfluidics, biological recognition, and systems integration. Several optical biosensor systems — in various stages of development at NRL — will be highlighted. Also described will be research on new chemical and biological materials for target recognition and signal generation/transduction.

(s16-b) Lithographically Printed Optical, Fluidic, and Electronic Systems

A. Scherer

California Institute of Technology, Pasadena, CA

We have integrated electronic, optical, magnetic, thermal and fluidic devices into systems to construct useful analysis tools. Over the past several years, we have developed soft lithography approaches to define microfluidic systems in which pico-Liter volumes can be manipulated. These fluidic delivery systems have more recently been integrated with optical and electronic devices. We have also developed thermal control systems with fast (>50oC/s) cooling and heating ramp speeds and excellent accuracy. Moreover, the sizes of microfabricated fluidic elements now match those of electronic, optical and magnetic measurement devices, and lithographically assembled systems can be constructed. These integrated chips can be applied to address medical analysis needs, and to construct compact and efficient immuno-assay chips, cell and bacterial sorters, cell culturing chips, and hand-held reverse-transcriptase polymerase chain reactors (RT-PCR) for pathogen identification.

By integrating fluidic and photonic systems, picoliter sample delivery and spectroscopic measurements can be realized. In general, such device miniaturization results in the opportunity to reduce the original analyte volumes and leads to new opportunities, such as single cell analysis. Systems based on silicon on insulator technology, photonic crystal sensors, the introduction of gain into spectroscopic systems and magnetic observation of nanoliter samples through nuclear magnetic resonance is expected to ultimately result in realistic "laboratory on chip" capabilities — rather than "chips in laboratories" with many biological and medical applications.

Here, we show our latest results and capabilities, as well as microlithography techniques optimized from the microelectronic industry for integrating optics with fluidics and magnetics to build integrated micro-chips. We show the opportunities of silicon photonics to generate inexpensive optical systems for data communications, as well as optofluidic and electromagnetic system for antibody analysis chips.

(s16-c) Real-Time DNA Sequencing from Single Polymerase Molecules

D. Rank

Pacific Biosciences, Menlo Park, CA

SMRT (single molecule real time) DNA sequencing is a high-throughput method for eavesdropping on template-directed synthesis by DNA polymerase in real time. Pacific Biosciences has developed two critical technology components which enable this process: The first is phospholinked nucleotides where, in contrast to other sequencing approaches, the fluorescent label is attached to the terminal phosphate rather than the base. The enzyme cleaves away the fluorophore as part of the incorporation process, leaving behind completely natural double-stranded DNA. The second critical component is zero-mode waveguide (ZMW) confinement technology that allows single-molecule detection at concentrations of labeled nucleotides relevant to the enzyme. Through the combination of these innovations, our technology allows sequencing at speeds of multiple bases per second with a read length distribution competitive with Sanger sequencing on average and extending out to thousands of bases in length. We apply this technology to shotgun sequencing using a fast and simple sample preparation concept that facilitates whole-genome sequencing directly from genomic DNA. Using a strand-displacing DNA polymerase, we will also demonstrate sequencing multiple times around a circular strand of DNA, allowing consensus sequencing on an individual molecule. This enables high confidence detection of low frequency variants even in heterogeneous samples, enabling new research and diagnostic applications.

(w1) Core Facility Management Models: Development and Culture

Over time academic, medical and independent research communities have become increasingly reliant upon core facility operations for cost-effective access to current technologies. As such successful core operations are essential partners in the conduct of basic research, critical to sustaining research competitiveness and serve as effective faculty recruitment and retention tools. In a perfect world alignment of institutional objectives, with research faculty needs should serve to drive the development, management and operation of core facilities. Influenced largely by institutional culture, vision and objectives the organizational structures and management approaches of these facilities differ from institution to institution. Nonetheless common challenges exist for all cores which transcend the boundaries technical specialty.

This session will review findings from a recent assessment of existing core facility management models which considered the various architectures across research sectors. Current crosscutting core issues including the translation of institutional vision into operational practices and decision making through core leadership will be explored. Panelists will share different institutional approaches, experiences, actions and outcomes relative to hot operational topics as well as facility evaluation and accountability.

(w1-c) Enhancing Core Research Facility Management, Strategy, and Investment

R. Haley

Huron Consulting Group, Roswell, GA

Research institutions — including research universities, academic medical centers, and independent research institutes — are increasingly realizing the important role that core research facilities play in their ability to conduct cutting-edge research and their competitiveness for recruiting and retaining strong faculty members and for securing external research funding, especially in biological science and engineering (S&E) disciplines. With this realization comes an understanding that more attention needs to be placed on effective management and strategy of these important components of institutions' overall research enterprises. For example, how should core facility investment decisions be made, how should facilities be governed and evaluated, how can sharing and usage be enhanced, and how can related compliance risks be best managed? Huron Consulting Group's Higher Education practice was engaged by a major research university to review the state of its core research facilities in the biological S&E area, with the goal of enhancing the institution's investments in these facilities via recommendations related to core research facility organizational structures and business models. As part of the process, we interviewed approximately 40 individuals at the university (including unit leadership, facility managers and administrators, senior and junior faculty members, and university administrators), reviewed facilities data, conducted interviews with peer institutions to discuss their related opportunities and challenges, and reviewed the most relevant literature related to regulatory compliance.

Our recommendations focus on the creation of enhanced organizational structures and business models 'tuned' to the history and culture of the university and units involved. During this presentation, generalized findings from our work will be presented. Observed challenges and opportunities will be presented and selected recommendations related to enhancing core research facility management, strategy, and investment will be shared.

(w2) Women in Science and Core Laboratories

This workshop will focus on issues pertaining to women making the transition to a career as a core laboratory director. Approximately 50% of the membership in the Association of Biomolecular Resource Facilities (ABRF) includes scientists working in core facilities. In an ABRF survey study published in *Nature Biotechnology* in 2000 [1], across all core facility sectors, the percentage of male employees holding MDs or PhDs was significantly greater than the percentage of female employees (24% to 9% respectively). This discrepancy raises the important question as to whether women with PhDs are represented in the job applicant pool in the expected ratio and whether women are selected for core facility director positions in numbers that are reflective of their overall numbers within the field. What role can the ABRF play in helping to sustain women in core facility careers? During a panel discussion, Kathryn Lilley, Director of the Cambridge Center for Proteomics, and Nancy Denslow, Associate Professor in the Department of Biochemistry and Molecular Biology at the University of Florida, will focus on practical tips that helped them successfully make the transition to a core director position, obstacles they encountered along their career paths and how they overcame the obstacles, as well as their ideas on how the ABRF can help women desiring to advance into core director positions. Also during the panel discussion, Joan Goldberg, the Executive Director of the American Society for Cell Biology (ASCB), will be describing the ASCB's successful programs aimed at promoting women in their society and field, and perhaps ignite interest in similar initiatives for the ABRF. Following the panel discussion, workshop participants will be encouraged to share their own experiences, ask the speakers questions, and submit ideas for a survey to address issues pertaining to women in core laboratories.

(w3) Educational Outreach

J.M. Murray[1], C. Shadding[2], L. Southard[3] and V. Shepherd[4]

[1]Vermont Genetics Network, University of Vermont, Burlington, VT. [2]Genome Sequence Center, Washington University School of Medicine, St. Louis, MI. [3]Cornell Institute for Biology Teachers, Cornell University, Ithica, NY. [4]Office for Science Outreach, Vanderbilt University School of Medicine, Nashville, TN

Core facilities are charged with bringing education about their services and technology to their users. Networking through outreach endeavors with other higher educational institutes can lead to collaborations and an increase in the number of users in a facility. Outreach to K-12 institutions can be very rewarding and can help to make connections with the entire community. There are many different ways that outreach can be delivered to primary and secondary educational institutions. The focus of this panel is to discuss different types of outreach resources and the potential for outreach through core facilities. We will address questions and concerns in developing such activities. Including but not limited to, 1. How do you implement outreach within your core facility? 2. What are the risks and rewards in doing these activities? 3. What are examples of outreach activities? 4. What are the costs of such activities? 5. How do you fund such activities? 6. What are some of the obstacles? We encourage an active discussion with audience members participating and offering individual insight and perspective.

(w4) Implementing Next Gen Sequencing Technologies — *Hit the Ground Running with Next Gen!*

The new generation of sequencing technology such as AB SOLiD, Illumina GA Analyzer and Roche 454 FLX brings to Core labs the ability to generate unparalleled amounts of data used for a myriad of research applications. But such technology requires a level of sample preparation, ancillary equipment, data handling and fixed cost structure that calls for careful planning before implementation. In this session we will discuss the most important aspects of human and financial resources, technical skills, physical space and IT infrastructure necessary to implement and manage successful next generation sequencing services by institutional Core labs. We will also present survey data on labs that have already adopted those technologies. We will attempt to address issues such as choice of instrument, types of services, costs and fee structures, trends in demand and plans for the future.

(w4-a) Implementing and Running the Illumina GA and Roche 454 at a DNA Sequencing Core Lab

R.H. Lyons

University of Michigan, Ann Arbor, MI

This talk will address the implementation process of the Illumina GA and Roche 454 in the DNA Sequencing Core of The University of Michigan and will attempt to answer some of the following questions: Why where those instruments chosen? What preparations were necessary and what are the necessary resources? Can the instruments pay for themselves? How is the upfront sample preparation set up between the Core lab and the users? What are the pitfalls to avoid (the things they didn't tell you)? What QC checks need to be in plate?

(w4-b) Implementing and Running SOLiD services at a Microarray Core Facility

J. Palatini

Ohio State University, Columbus, OH

This talk will address some of the same questions of the first talk in this session but from a Microarray Core Lab point of view using the Applied Biosystem SOLiD platform. Additionally issues involving the data handling and downstream analysis pipeline will be addressed: How much bioinformatics support should the Core provide? What resources are needed for data management and IT infrastructure?

(w4-c) Platform Cross-Comparison and Core Facility Next-Gen Survey Data

H. Escobar

University of Utah, Salt Lake City, UT

This talk will present the results from an online and call survey on next-gen services offered by Core labs. The survey data will attempt to provide the audience with a broad picture of how current Core labs are handling next-gen technology, how have they structure their services, the costs behind them, and what is the demand for services now, with some forecasting for the future. Finally, a comparison summary will be presented of the specs for the Applied Biosystems, Illumina and Roche next generation sequencing instruments, and the necessary planning for successfully implementation in an institutional Core facility.

(w5) Implementing Mass Spectrometry Technologies — *Challenges of Implementing New Mass Spectrometry Technologies in Shared Resource Facilities*

The aim of the workshop is to enable mass spectrometry and proteomics core directors who have experience with implementing both conventional and new mass spectrometry technologies to share and discuss their practical experiences in using these platforms for multiple user applications. The range of topics that will be discussed include the challenges of securing resources and funding for new instrumentation and maintenance of existing equipment, deciding what types and sizes of projects to support, how conventional and new mass spectrometry technologies factor in the decision of the types of projects to support in a shared resource, and training personnel to use/apply conventional and new MS technologies. The experience from Directors of different sized academic core facilities, from small to very large, will be featured in the workshop.

(w5-a) Application of Advanced Mass Spectrometry Technologies at the University of Tennessee Health Science Center

S. Beranova-Giorgianni

University of Tennessee Health Science Center, Memphis, TN

This presentation will discuss selected aspects related to the activities of the Mass Spectrometry Core Facility at the University of Tennessee Health Science Center (UTHSC). Topics to be discussed include: how the facility developed over the past years in reaction to the expansion of mass spectrometry and the introduction of improved technologies; how the shared resources are maintained and upgraded; and what strategies are being used to meet the evolving needs of the scientific community at UTHSC. The UTHSC mass spectrometry center is an established laboratory that conducts independent and collaborative research, and provides mass spectrometry services to investigators at multiple colleges at UTHSC, and at neighboring institutions. The facility is headed by a Director and two Associate Directors; additional personnel include a Research Associate responsible for day-to-day management of the facility. The instruments available at the facility encompass matrix-assisted laser desorption/ionization (MALDI) and electrospray (ESI) ionization modes, in combination with different types of analyzers (ion trap; time-of-flight; and quadrupole-time-of-flight). Three nanoflow LC systems are interfaced with the mass spectrometers. Instrument access (trained-user, or facility-personnel-only) depends on the instrument type and on the nature of the analysis. The facility currently supports research projects of 14 Principal Investigators. Majority of the applications are in the area of peptide and protein analysis, in particular identification of proteins by MS/MS-based approaches. Recent additions to the services of the facility include characterization of phosphopeptides and phosphoproteins, and accurate mass measurement (for support of medicinal chemistry projects). Facility personnel also conduct instrument demonstrations and training of new users (faculty, students, postdoctoral fellows). Directors of the facility spearhead funding applications for new equipment, direct graduate courses with emphasis on mass spectrometry, and promote mass spectrometry through discussions and seminars.

(w5-b) Center for Mass Spectrometry and Proteomics at the University of Minnesota: Organization and Policies

G. Nelsestuen, T. Griffin, L. Higgins, T. Krick, L. Anderson, B. Witthuhn, T. McGowan, S. Harvey, and M. Stone

University of Minnesota, St. Paul and Minneapolis, MN

The Center for Mass Spectrometry and Proteomics (CMSP) grew from a long-standing mass spectrometry facility under Thomas Krick. Major expansion occurred since 1998. Numerous funding sources included 5 federal instrumentation grants and local opportunities that ranged from the cigarette settlement to support from individual colleges and units, to funds made available when ear-mark items were dropped from the federal budget. CMSP operates 15 mass spectrometers for proteomic and metabolomic research, including the following (dates of purchase): Orbitrap with ETD (2008), ESI-TOF (2008), Leco GC/GC-TOF (2008), Agilent single quad with mass-triggered sample collection (2008), ABI 4800 (2007), ABI QTrap 4000 (2006) and 2000 (2004), an LTQ (2005), ABI Pulsar and XL QStars plus four older instruments. The University recently pledged 5-year continuation of funding sufficient to cover personnel in the facility (three PhD-, one MS- and one BS-level laboratory scientist, a computer scientist and half-time appointments for postdoc, research associate and BS-level technician). Properties of the facility include at least the following: A) A history of providing mass spectrometry to the biological sciences. B) Strong technical expertise to maintain instruments in operating condition. C) Active participation of a faculty director with access to University officials. D) Active recruitment of science collaborators in areas such as the medical School. E) Extensive support from the Minnesota Supercomputing Institute for software and hardware purchase and maintenance. F) A strong educational mission with open door policies that educate and train users of the facility, including bimonthly 3-day workshops. G) Employment of CMSP personnel as available collaborators for biological scientists. H) Recruitment of new faculty with interest in biological mass spectrometry and who require expansion of a central core facility as part of their start-up package. Overall, the CMSP is seen as a strong recruitment tool for new faculty in the biological sciences.

(w5-c) The NIH National Center for Research Resources Mass Spectrometry at Washington University

M.L. Gross

Washington University, St. Louis, MO

Washington University in St. Louis has been the host on an NIH NCRR Mass Spectrometry Facility for over 30 years. In 1994, M.L. Gross became PI and J.W. Turk, co PI. In 2003, Reid Townsend joined as a second coPI to help expand the foci of the resource in proteomics. The resource exists in three different laboratories, two in the School of Medicine and one in the Department of Chemistry. The laboratory in Chemistry has over ten mass spectrometers, ranging from ion-trap/FT ICR and ion-trap/orbitrap, to MALDI TOF/TOF and MALDI FT-ICR, a QToF, and a number of ion traps. Soon to be installed is a 12-Tesla FT-ICR for top-down proteomics and a high performance QToF (Bruker MaXis). One of the medical school labs (J. Turk, director) has lipidomics as its theme and utilizes ion traps, a QToF, and triple quadrupoles for this research. Isotope ratio measurements are also available in this lab. The second medical school lab (R. Townsend, director) specializes in proteomics and makes use of TOF/TOF and orbitrap and FT-ICR technologies. The three labs collaborate on projects of mutual interest, share instrumentations, sponsor dissemination activities from local seminars to regional workshops, and train graduate students and medical scientists in mass spectrometry.

(w6) Implementing Optical Imaging Technologies

(w6-a) Implementing Optical Imaging Technologies

S. Watkins[1], S. Henderson[2], V. Frohlich[3]

[1]University of Pittsburgh School of Medicine, Pittsburgh, PA; [2]Virginia Commonwealth University, Richmond, VA; [3]University of Texas Health Science Center, San Antonio, TX

In the post-genomic era of biomedical research understanding the functionality of molecules at the cellular and subcellular level in living systems will become predominant. In this era we must move beyond static "snapshots" of the cellular state to an understanding of the biology of cells over time and in 3-dimensional- space. Within the cellular environment it is expected that we will be able to study the expression, the functional role(s) and interactions of multiple unique molecules concurrently. Furthermore, it will be desirable to determine the effects of these molecules on cell development, organization and fate over extended periods of time. To perform these types of studies it is necessary to develop new methodologies that will allow multiparametric analysis of cells while maintaining their functional viability. In the past this goal would have been extraordinarily difficult to achieve. However, developments in optical and computational technology and the development of spectrally discrete, extremely efficient fluorescent dyes have empowered modern microscopists to undertake these previously forbidding tasks. However to perform these tasks is both highly technical and extremely expensive. Thus the high end multicapability imaging resource has taken a central stage allowing unskilled researchers access to these extremely powerful and multiplexed imaging technologies.

This workshop will consist of three sections and for the large part will be a group forum presentation with extensive questions and answers. The three sections will be "What can I do with optical imaging today" — which will discuss the limits of the current technologies, "How and what do I need to organize a high end optical facility" and "How do I get money to pay for all this stuff." The session organizer, Dr. Frolich and Dr. Henderson all direct very large well-equipped imaging centers, and have extensive experience attracting, maintaining and improving the resources for the centers. Therefore following a brief introduction to each topic will work as a round table to help attendees plan and implement strategies for building or expanding pre-existing imaging centers.

(w7) Instrumentation Funding Opportunities — *Structure, Function and Funding of Cores: New is Old and Old is New*

The speakers will review the history of shared research resource core structure, function and funding followed by the nature and role of cores in the context of integrative biology and translational sciences such as advocated by the NIH Clinical and Translational Sciences Award as well

as funding mechanisms for the purchase of shared instrumentation. The session will end with a panel discussion with the audience on the topics.

(w7-a) Structure, Function and Funding of Shared Research Resources

J.W. Fox

University of Virginia School of Medicine, Charlottesville, VA

Shared Research Resources or as commonly called "cores" began appearing in a very rudimentary form in the late 1960's. Initially these were focal points of faculty expertise and technology/instrumentation housed within an investigator's own laboratory and developed primarily for the faculty member's own use and that of selected colleagues. As biomedical research became more dependent on sophisticated instrumentation and the demand by investigators for access to such technology and expertise became widespread a more formalized structure and operation for such cores began to be developed. Currently, a variety of models are observed for shared research resource cores with equally variable mechanisms for operation and funding, most of which are somewhat specifically tailored for the host institution. In this presentation I will discuss the range of core models and well as the various operational and funding mechanisms utilized by most cores. The expectation is that one will see that often one size does not fit all and flexibility and dynamic positioning of the cores within an institution is critical for both fiscal and scientific success. Factors which influence such success will also be discussed.

(w7-c) Instrumentation for Core Facilities: the NIH SIG and HEI Programs

M.Tingle

NIH National Center for Research Resources (NCRR), Bethesda, MD

For many years the NIH has provided expensive state of the art equipment to the biomedical community through the NCRR Shared Instrumentation Grant (SIG) and more recently, the High-End Instrumentation (HEI) programs. In many cases, these instruments are located in institutional core facilities which provide access and the high level technical expertise in cutting edge technologies and complex analytical procedures required for both basic and translational studies. Although the SIG and HEI programs fund instrumentation that span the technology spectrum, some instruments are placed in DNA sequencing, microarray and mass spectrometry cores managed by facility directors who are members of the ABRF. This talk will summarize the funding levels and trends in equipment for both the SIG and HEI programs.

(w8) Bio-Information Technology (Bio-IT) — Custom Software Development in Support of Core Facilities

Core facilities are faced with numerous information management issues, ranging from daily operations support to complex year-end queries needed for budgeting and grant renewals. Commercial off-the-shelf (COTS) software that can effectively address these issues is rare, and even when available, tends to create more issues that it solves. Often COTS solutions only address a small portion of the information management needs for a particular core, leading to an amalgamation of several different tools addressing different areas. These tools are then often "glued together" by an ad-hoc, tangled web of custom scripting and manual processes. Each core at an institution ends up with its own combination of homegrown solutions, leading to tremendous information integration and consistency issues at the institutional level. This session will examine the need for software development groups "in context" with the core facilities themselves and their ability to develop consistent, integrated solutions to core facility information management issues. It will also highlight the solutions being developed at the panelists' institutions.

(w8-a) Removing Data Silos with ISIS

M.M. McFarland and C. Donnelly

The Jackson Laboratory, Bar Harbor, MA

This paper discusses the ongoing process at The Jackson Laboratory (JAX) for the integration of scientific and administrative data under a unified data management plan called Integrated Services Information System, or ISIS. This effort is in line with, albeit on a smaller scale, the goals of larger national and international efforts such as caBIG. Indeed, caBIG is an increasingly important resource for ISIS. The drivers for implementing ISIS have scientific, technological, and economic roots. The Scientific Services can be considered JAX's biggest scientific data generator. But until recently, the services have generally treated data products as individual research results generated by a single service at the specific request of one or more investigators. Each data product (e.g. a sequencing run result) was generated and delivered to research clients with little or no consideration of its integrated scientific value with other scientific data generated in other services or even other data generated within a the same service. As the number of requests has grown and the complexity of the assays has increased, this thinking has begun to change. Data generated for one investigator may be of interest to another investigator or experiment. This data silo concept is wasteful and, from an IT perspective, costly. To overcome the problems of developing expensive LIMS and disparate databases at JAX, we are standardizing controlled vocabularies, creating common underlying data models, implementing upon standard system architectures, and providing open application programming interfaces. As more services utilize relational database management systems to track data and workflow, the potential for adding value to data products through integration grows rapidly. In addition, as services expand and contract and new services are added, it is increasingly important yet more difficult to gather productivity and use metrics. ISIS is a significant step towards solving that problem.

(w8-b) Internal Software Development and Integration Experiences at the Cornell University Life Sciences Core Laboratories Center

J. VanEe

Cornell University, Ithaca, NY

The Core Laboratories Center at Cornell University provides a wide variety of technologies, platforms and expertise to internal and external investigators. We have a long history of supporting the operations of our laboratories through internal development efforts, while integrating vendor software and commercial LIMS offerings. Rapidly changing technologies and the accelerated adoption of new platforms present unique challenges to an internal development effort. A creative combination of software development, software acquisition, deployment and integration along with planning and communication with laboratory managers is necessary to successfully support laboratory operations. I will offer a historical perspective of our software development and deployment from our earliest efforts in supporting slab gel DNA Sequencing to invoicing, equipment scheduling, complex proteomics workflow and massively parallel DNA Sequencing while describing our successes as well as unresolved problems and challenges.

(w8-c) SRM 2.0: Building the Next Generation of Core Facility Management Systems

M. Stine

St. Jude Children's Research Hospital, Memphis, TN

St. Jude's Shared Resource Management (SRM) system is a laboratory management system designed to support core facility activities. It was originally designed to support the laboratories in the Hartwell Center for Bioinformatics and Biotechnology at St. Jude, including DNA synthesis, Peptide synthesis, DNA sequencing, Functional Genomics (spotted microarray), and Affymetrix laboratories. An original goal for SRM was for it to be sufficiently modular and scalable to support additional laboratories and potentially support all core facilities within an institution, providing a single portal for investigators to requisition services, retrieve data and invoices for services, and generate reports. The original implementation of SRM allowed new facilities to be deployed within 3-6 months, assuming 2-3 developers concurrently dedicated to developing modules for a new facility. Little did we know that the rate of desired SRM adoption and introduction of new facilities to St. Jude would far outstrip our ability to deliver the required modules. Clearly a different approach was necessary. Work is now underway to deliver SRM 2.0. SRM 2.0 will largely be driven by domain-specific metadata specified at runtime, allowing new facilities to be deployed without writing any additional code modules. According to our estimates, this will allow us to deliver at least 75-80% of the required functionality to support any one facility. The remaining functionality will be delivered by utilizing an innovative plug-in system, whereby various pre-defined extension points throughout the system can be enhanced by developing domain specific plug-in modules that will be deployed independently of the core system. We believe these two fundamental concepts will allow us to shorten deployment time to 3-6 weeks or possibly less. It is our vision that SRM 2.0 will represent the next generation of core facility management systems and be the best available software in the world for managing core facilities.

(w10) Next Generation Sequencing Instruments — *Massively Parallel Sequencing Instrumentation Panel Session*

This panel session will focus on descriptions of commercially available massively parallel DNA sequencing systems, and provide updates on protocol improvements and new applications. Representatives from commercial vendors (last year's representatives included ABI SOLiD, Illumina, and Roche) will present overviews of the technologies and summaries of new developments. The session will provide an opportunity to compare and contrast systems in terms of output, applications, and pricing. The session will conclude with an interactive discussion period between the vendors and the audience.

(w11) Proteomics Data Publication — *Searching and Sorting: Preparing Protein Identification Data for Publication*

The identification of proteins in complex mixtures as well as characterizing the myriad of post-translational modifications that they undergo remains a major part of proteomic research. The vast majority of such data are generated by mass spectrometry and analyzing, interpreting and refining the output of experiments utilizing this technology in preparation for their publication is of great significance as these manipulations directly affect the reliability of the reported results. There are many search engines and other programs for refining protein identification data including some that assess the confidence in the findings and the variability inherent in these programs poses problems for the editors and reviewers of journals. In this workshop, several general aspects of this issue will be discussed illustrated by three major software packages: Protein Prospector, Mascot and Scaffold.

(w11-a) From Results to Publication: Journal Guidelines and Protein Prospector

R. Chalkley

University of California, San Francisco, CA

As amounts of proteomic data acquired in a given experiment have exploded over the last few years, analysis of mass spectrometry data by software has moved from a semi-supervised process to an essentially complete reliance on automated search engine results. This has lead to more pressure on search engines by both researchers and journals to report results of measurable reliability. In this presentation the changes that have been made to Protein Prospector to adjust to these demands will be presented, including use of expectation values, target-decoy database searching strategies and reporting of quantitation statistics. Strengths and weaknesses of these approaches will be highlighted.

(w11-b) Providing Mascot Search Results in a Format Suitable for Submission as Supplementary Data

D. Creasy

Matrix Sciences, London,United Kingdom

The different journals publication guidelines for the analysis and documentation of peptide and protein identifications have proved to be a challenge to many authors when submitting manuscripts for publication. In this talk, three requirements and potential solutions for the Mascot search engine will be described: a) «For large scale experiments, provide the results of any additional statistical analyses that indicate or establish a measure of identification certainty, or allow a determination of the false-positive rate.» While this requirement has certainly improved the quality of data being provided, generating this data has caused difficulties for laboratories with limited bio-informatic resources; b) For all experiments, provide reports with information about proteins identified, for example sequence coverage, «score(s) and any associated statistical information obtained for searches conducted». Standard Mascot reports don't always provide all the required information in a format suitable for the different journals. It is also often unclear what statistical data should be provided; c) For 'one hit wonders', the additional requirement of «annotated spectra with masses observed as well as fragment assignments». When the MCP

guidelines were developed, it was probably assumed that this would entail producing at most a handful of spectra. What about cases where 100s of such spectra are required?

The talk will conclude with a description of the AnalysisXML format developed by the HUPO Proteomics Standard Initiative. As further tools are developed, this format will greatly ease the burden of producing supplementary data for publication and enable reviewers to more readily make judgements about the reliability of submitted data.

(w11-c) Organizing MS/MS Proteomic Data for Publication

B.C. Searle

Proteome Software, Portland, OR

Large-scale proteomics studies generate enormous amounts of data that are impossible to curate by hand. As a result, all of the major proteomics journals have adopted guidelines to insure high quality standards for publication. However, meeting these guidelines can be difficult when studies span years, multiple instruments and tens or hundreds of result files. Scaffold was designed to help researchers organize and manipulate these data as well as automatically collect the necessary search parameters for publication. Scaffold stays instrument, methodology and search engine agnostic by reinterpreting all search engine results on an even playing field using generic statistical techniques. These techniques let scientists and journal reviewers filter data in expected ways to separate trusted results from the portion of data that requires hand curation. Finally, Scaffold implicitly acknowledges that the assumptions it and other software make about MS/MS data can be wrong and gives scientists and journal reviewers the tools to independently audit the accuracy of the analysis.

(w12-a) Molecular Interactions — *Technical Workshop on Real-Time Biophysical Technologies Used for Characterization of Biomolecular Interactions*

S.P. Yadav[1] and A.Yamniuk[2]

[1]Cleveland Clinic Foundation, Cleveland, OH; [2]Bristol-Myers Squibb Co., Lawrenceville, NJ

There are many existing techniques, such as ELISA, analytical ultracentrifugation, ITC, NMR for studying biomolecular interactions. New technologies based on Surface Plasmon Resonance (SPR), Quartz Crystal Microbalance (QCM), Fluorescence Fluctuation Spectroscopy (FFS) are emerging to complement the existing ones to offer a label-free, real time biomolecular interaction analysis. Recently a wide spectrum of biosensor platforms has come on the market from several manufacturers. These biosensors offer a comprehensive analysis of binding kinetics, affinity, specificity, and concentration measurements of a wide range of biomolecules including small molecules, antibodies, lipids, nucleic acid and proteins. Comprehensive characterization of biomolecular interactions is central to understanding of molecular mechanisms, structure-function relationships of biomolecules for research and development, antibody characterization, drug discovery, biotheurapeutic development, and proteomics. The technology workshop will focus on the new generation of instruments based SPR and QCM technologies developed by GE health sciences, Fortebio Inc., Bio Rad, Corning and Attana and their applications to quantify biomolecular interactions. A 10-12 minute presentation by each speaker; Eric Rhous from GE Health Sciences, Sriram Kumaraswamy from Fortebio Inc., Yasmina N. Abdiche of BBC Rinat Laboratories-Pfizer Inc. on behalf of Bio Rad, Anthony G. Frutos from Corning and Theres Jägerbrink from Attana will be made followed by a round table discussion.

RESEARCH GROUP SESSION ABSTRACTS

(r1) Joint Research Group Presentation on Proteomics (Level 3, Ballroom A) — *Evaluating the State of the Art in Quantitative Proteomics*

Comparative proteomics and absolute protein quantitation continue to be difficult challenges that face proteomics researchers and core facilities from both an analytic and an informatics perspective. For ABRF 2009, the three Proteomics Research Groups have prepared studies tackling a broad range of quantitative proteomics techniques: relative quantitation, absolute quantitation, and difference testing. The main Proteomics Research Group (PRG) study deals with assessing the relative quantities of known protein targets in a complex mixture. This targeted approach is extended in the Proteomics Standards Research Group (sPRG) study, which uses isotopic labeled peptides in known concentrations to enable researchers to assess the absolute quantities of proteins. Finally, the Proteome Informatics Research Group (iPRG) study considers the shotgun informatics challenge of accurately identifying a large number of changes between samples despite sampling variation across multiple technical replicates. By combining participant results and survey answers, the PRG studies attempt to assess the state of the art concerning these three aspects of quantitative proteomics.

Proteomics Research Group (PRG)

(r1-a) PRG 2009 Study: Relative Protein Quantification in a Clinical Matrix

M.J. MacCoss[1], A.S. Chien[2], B. Friedman[3], D.Hawke[4], J.Krijgsveld[5], K.S. Lilley[6], R.E. Settlage[7], N.E. Sherman[8], C.W. Turck[9]

[1]University of Washington, Seattle, WA; [2]Stanford University, Stanford, CA [3]Vanderbilt University, Nashville, TN; [4]University of Texas M.D. Anderson Cancer Center, Houston, TX, [5]EMBL Heidelberg, Heidelberg, Germany; [6]University of Cambridge, Cambridge, United Kingdom; [7]Virginia Bioinformatics Institute, Blacksburg, VA; [8]University of Virginia, Charlottesville, VA; [9]Max Planck Institute of Psychiatry, Munich, Germany

The Proteomics Research Group (PRG) of the ABRF developed the 2009 study to assess approaches that individual laboratories would use to determine the relative abundance of target proteins in a complex mixture. An increasingly common request for proteomics laboratories is the detection of a specific target protein of interest in a complex mixture. Likewise, most of these requests are also interested in knowing the abundance of the target protein relative to that in a control sample. While this type of analysis has traditionally been addressed using Western blots or other immunoaffinity assay, recent advances in targeted mass spectrometry-based analyses are beginning to be reported in the literature as an alternative.

For this year's study, four different proteins were spiked into a plasma background matrix at three different levels. Two of these proteins are commonly measured plasma protein biomarkers, and the remaining two had identical primary structure and differed by only a single phosphorylation site. The participants were shipped six samples in total (three samples in blinded duplicate) and asked to report the relative abundances of the four target proteins in the six samples. Results from analysis of the samples and survey responses will be used to assess the different approaches that are used by the proteomics community to determine the relative abundance of a target protein of interest.

Proteomics Standards Research Group (sPRG)

(r1-b) sPRG 2009 Study: Challenges Along the Way to a Quantitative Proteomics Standards

J.G. Farmar[1], D. Arnott[2], A.R. Ivanov[3], J.A. Kowalak[4], W.S. Lane[5], K. Mechtler[6], B.S. Phinney[7], M.R. Raida[8], and S.T. Weintraub[9]

[1]A. Einstein College of Medicine, Bronx, NY; [2]Genentech, Inc., So. San Francisco, CA; [3]Harvard School of Public Health, Boston, MA; [4]National Institute of Mental Health, Bethesda, MD; [5]Harvard University FAS Center for Systems Biology, Cambridge, MA; [6]Research Institute of Molecular Pathology, Vienna, Austria; [7]Proteomics Core UC Davis Genome Center, Davis, CA; [8]Experimental Therapeutics Centre, Singapore; and [9]University of Texas Health Science Center, San Antonio, TX

A standard for quantitation of proteins with stable isotope labeled peptides would be a valuable tool for core laboratories to assess techniques and instrumentation. Designing and testing such a standard has been an educational tool for the members of the Proteomics Standards Research Group (sPRG). This presentation will detail how the challenges of design, synthesis, purification, quantification and analysis were overcome and should be valuable to others who want to design a protocol for quantitation of proteins.

Proteome Informatics Research Group (iPRG)

(r1-c) iPRG 2009 Study: Testing for Qualitative Differences Between Samples in MS/MS Proteomics Datasets

B.C. Searle[1], D.L. Tabb[2], J. Falkner[3], J.A. Kowalak[4], L. Martens[5], M. Askenazi[6], P.A. Rudnick[7], S.L. Seymour[8], W.S. Lane[9], K. Meyer-Arendt[10]

[1]Proteome Software, Inc., Portland, OR; [2]Vanderbilt University Medical Center, Nashville, TN; [3]University of Michigan, Ann Arbor, MI; [4]National Institute of Mental Health, Bethesda, MD; [5]EMBL-EBI, Hinxton, United Kingdom; [6]Dana-Farber Cancer Institute, Boston, MA; [7]NIST, Gaithersburg, MD; [8]Applied Biosystems, Foster City, CA; [9]Harvard University, Cambridge, MA; [10]University of Colorado, Boulder, CO

Determining significant differences between mass spectrometry datasets from biological samples is one of the major challenges for proteome informatics. Accurate and reproducible protein quantitation in complex samples in the face of biological and technical variability has long been a desired goal for proteomics. The ability to apply qualitative difference testing is a first step towards that goal, and is routinely used in tasks such

as biomarker discovery. In this work the Proteome Informatics Research Group (iPRG) of the ABRF presents the results of a collaborative study focusing on the determination of significantly different proteins between two complex samples. In this study, datasets representing five technical replicates of each sample were provided to volunteer participants and their ability to evaluate reproducible differences was tested. A survey was used to determine the relative merits of spectrum counting versus MS intensity-based differentiation, whether sophisticated statistical methods are necessary, and if computer software must be augmented by scientific expertise and intuition. Results and survey responses were used to assess the present status of the field and to provide a benchmark for qualitative difference testing on a realistically complex dataset.

(r2) Nucleic Acids Research Group (NARG)

NARG 2008-2009 Study: A Comparison of Different Priming Strategies for cDNA Synthesis by Reverse Transcriptase, as Evaluated by Real-Time qPCR

K.L. Knudtson[1], S.V. Chittur[2], D.S. Grove[3], D.J. Hollingshead[4], T.C. Hunter[5], G.L. Shipley[6], K.Sol-Church[7], and W.L. Taylor[8], **S.Tighe**[5], and A.T. Yeung[9]

[1]University of Iowa, Iowa City, IA; [2]University at Albany, Albany, NY; [3]Pennsylvania State University, University Park, PA; [4]University of Pittsburgh, Pittsburgh, PA; [5]University of Vermont, Burlington, VT; [6]UTHSC-Houston, TX; [7]A.I. duPont Hospital for Children, Wilmington, DE; [8]UTHSC-Memphis, TN; [9]Fox Chase Cancer Center, Philadelphia, PA

Real-Time Reverse Transcriptase Quantitative PCR (RT-qPCR) has become the method of choice to quantify transcript levels. Efficient priming, a highly processive enzyme, and quality RNA are key elements of a successful reverse transcription reaction to produce cDNA for qPCR. The Nucleic Acid Research Group (NARG) designed a study to evaluate RT priming strategies and enzymes. The NARG 2008-09 study was an extension of the 2007-08 study in which we evaluated the effect of reverse transcription priming strategies on RT-qPCR results. The previous study suggested a relationship between the assay sensitivity using cDNA generated with oligo(dT)20 primers and qPCR assay placement relative to the 3-prime end of the transcript. This year's study was designed specifically to compare oligo(dT)20 and random priming strategies as the assay target site varied. Because the previous study identified random hexamers or nonamers as most efficient of those tested, this years study was designed specifically to compare oligo(dT)20, random 6-mers and 9-mers or gene specific primers and combinations. Four reverse transcriptases; Superscript II, Superscript III, Transcriptor and MultiScribe, were employed to determine the effect of enzyme. In addition, the qPCR assays looked at three genes of varying abundance, b-actin (high copy), b-glucuronidase (medium copy) and TATA binding protein (low copy) as well as varying distance from the 3-prime end for each transcript. An unexpected challenge to the current study was ability to successfully transport and store the RNA. As a result, RNA handling workflows, reagents, consumables, quantification techniques, integrity analysis with the Bioanalyzer 2100, and preservation techniques were examined. Protocols for routine RNA isolation, isolation of RNA from FACS sorted and laser-capture micro-dissected cells, and working with partially degraded RNA will also be addressed.

(r3) Joint Research Group Presentation on Genomics — *Detection of Human microRNAs across miRNA Arrays and Next Generation DNA Sequencing Platforms*

DNA Sequencing Research Group (DSRG)

(r3-a) DNA Sequencing — The DNA Sequencing Research Group general survey, 2009: Second Generation Sequencing Instruments and Services in Core Facilities

P. Schweitzer[1], D. Bintzler[2], K. Dewar[3], J. Kieleczawa[4], A. Perera[5], S. Singh[6], R. Steen[7], M. Zianni[8], and M.M. Detwiler[9]

[1]Cornell University, Ithaca, NY; [2]DNA Analysis, Inc., Cincinnati, OH; [3]McGill University, Montreal, Quebec, Canada; [4]Wyeth Research, Cambridge, MA; [5]Stowers Institute for Medical Research, Kansas City, MO; [6]University of Minnesota, St. Paul, MN; [7]Harvard Medical School, Boston, MA; [8]Ohio State University, Columbus, Ohio; [9]Roswell Park Cancer Institute, Buffalo, NY

The ABRF DNA Sequencing Research Group (DSRG) has conducted a general survey to collect data on the current state of second generation sequencing instrumentation (often termed "massively-parallel" or "next generation" sequencers) and services offered by core facilities. The DSRG has monitored trends in sequencing platforms in core facilities by conducting surveys in years 2000, 2003, and 2006. This survey was the first to focus on second generation sequencers since their introduction. The information gathered this year provided data about the widespread availability of this equipment and these services in core facilities. The future acquisition and expectations for such instruments were also assessed. For comparison, the survey gathered information on Sanger (first generation) sequencing operations to determine the impact of the second generation technologies on conventional sequencing. The importance of this survey lies in the fact that it serves as an initial "snapshot" of the status of second generation sequencing services in core facilities while they are in their infancy, and therefore is a baseline for surveys in years to come. The results from this survey will be presented; some information may be presented in the "Implementing Next Generation Sequencing Technologies" concurrent technical workshop.

Microarrays Research Group (MARG)

(r3-b) 2008-09 Joint Research Group Project: Detection of Human microRNAs Across miRNA Array and Next Generation DNA Sequencing Platforms

S. Hester[1], C. Harrington[2], H. Auer[3], W. Wang[4], S. Potter[5], D. Baldwin[6], J. Tiesman[7], N. Jafari[8], N. Denslow[9], P.A. Schweitzer[4], K. Dewar[10], J. Kieleczawa[11], R. Steen[12], M. Zianni[13], D. Bintzler[14], A. Perera[15], S. Singh[16], and M.M. Detwiler[17]

[1]Environmental Protection Agency, Research Triangle Park, NC; [2]Oregon Health & Science University, Portland, OR; [3]Institute for Research in Biomedicine, Parc Cientific de Barcelona, Barcelona, Spain; [4]Cornell University, Ithaca, NY; [5]Cincinnati Children's Hospital, Cincinnati, OH; [6]University of Pennsylvania, Philadelphia, PA; [7]Procter & Gamble, Cincinnati, OH; [8]Nortwestern University, Chicago, IL; [9]University of Florida, Gainesville, FL; [10]McGill University, Montreal, Quebec, Canada; [11]Wyeth Research, Cambridge, MA;[12]Harvard Medical School, Boston, MA; [13]Ohio State University, Columbus, OH; [14]DNA Analysis, Inc., Cincinnati, OH; [15]Stowers Institute for Medical Research, Kansas City, MO; [16]University of Minnesota, St. Paul, MN; [17]Roswell Park Cancer Institute, Buffalo, NY

microRNA (miRNAs) are non-coding RNA molecules between 19 and 30 nucleotides in length that are believed to regulate approximately 30 per cent of all human genes. They act as negative regulators of their gene targets in many biological processes. Recent developments in microarray options and the introduction of high throughput DNA sequencing (HT DNA Seq) technologies now make it possible to use these advanced platforms for miRNA-expression profiling. To determine the effectiveness of each of these platforms in measuring miRNA expression and to compare the accuracy of the microarray and HT DNA Seq profiles with quantitative RT-PCR analysis, the Microarray Research Group (MARG) and the DNA Sequencing Group (DSRG) developed a joint research project. The goal of the MARG component of the research project was to evaluate miRNA platforms for their ability to detect miRNAs from complex total RNA samples. In addition to 3 DNA microarray platforms, two PCR-based platforms were included in the study for performance comparison. Each of the 5 miRNA platforms was tested with total RNA to evaluate the ability of each platform to detect and measure individual miRNAs in a complex biological sample. Aliquots from single pools of two different tissue RNAs (Ambion First Choice Human Total RNA) were analyzed at separate test sites for each of the following miRNA platforms: Agilent miRNA microarray, Illumina miRNA expression panel, Exiqon miRCURY LNA arrays, Applied Biosystems TaqMan miRNA assay, and FlexmiR by Luminex. The second component of the study was conducted in collaboration with the DSRG. miRNA expression profiles of the total RNAs used in the microarray phase of this study were determined using the "Next Generation" HT DNA sequencer, Illumina Genome Analyzer (Solexa). The miRNA profiles produced by the HT DNA sequencer were compared to the miRNA array platform results to determine correspondence of the two technologies, with a common reference to RT-PCR data. The results of miRNA profiling from both components of this study will be presented. *This abstract does not necessarily reflect EPA policy.*

Genomic Variation Research Group (GVRG)

(r3-c) GVRG 2009 Study

C. Lytle[1], A. Brown[2], J. Forrester[3], N. Bivens[3], B. Sanderson[4], A. Hutchinson[5], H. Escobar[6], M. Detwiler[7], B. Kingham[8]

[1]Dartmouth College, Hanover, NH; [2]Harvard Partners Center for Genetics and Genomics, Cambridge, MA; [3]University of Missouri, Columbia, MO; [4]Stowers Institute for Medical Research, Kansas City, MO; [5]CGF, NCI, SAIC-Frederick, Gaithersburg, MD; [6]University of Utah, Salt Lake City, UT; [7]Roswell Park Cancer Institute, Buffalo, NY; [8]University of Delaware, Newark, DE

Sequencing of Jim Watson's genome revealed 3.3 million single nucleotide polymorphisms (SNPs) of which approximately 10,500 caused amino-acid substitutions, with the potential to alter protein function. Also reported were over 200,000 other small genomic variations. Just how common or unique these genomic differences are should become clearer with the completion of the ongoing 1000 Genomes Project. While these genomic differences are small in number compared to the 6-gigabase human genome, they do present a real challenge for determining whether or not a base call is real or artifact when using next-generation sequencing platforms. Sequencing methods used by the current commercial next-generation sequencing platforms produce inherently lower quality base calls than Sanger Sequencing. This can make discrimination of genotypic variation difficult when dealing with heterozygous sites in a diploid organism. The Genomic Variation Research Group (GVRG) 2009 study investigated the current base calling accuracy of two commercial next-generation sequencing platforms and compared the base calls collected to other high, and low throughput genotyping platforms. A diploid strain of Yeast (Saccharomyces cerevisiae) sequenced by the Stowers Institute (Cell, 2008) was the subject of our study. GVRG collected genotyping data using two next-gen sequencers, the ABI SOLiD and Illumina Solexa GA II. The Sequenom MassARRAY system represents our high throughput genotyping platform and was used to assay 900+ previously identified SNP's discovered in the Stowers yeast strain. The low throughput platforms, ABI 3730, 3730XL DNA Analyzers and ABI 7900HT, interrogated regions in or near repetitive sequence that appear to contain a SNP. We will report on coverage levels achieved with the next-generation sequencing platforms, as well as the number of false positives and negatives generated by these systems. We will review the general procedures, time, and cost of running this sample on all the platforms used.

(r4) *Joint Research Group Presentation on Proteins*

Edman Sequencing Research Group (ESRG)

(r4-a) ESRG Study 2009: Comparison of Edman and Mass Spectrometry Techniques for N-terminal Sequencing

W. Sandoval[1], P. Hunziker[2], N.D. Denslow[3], B. Hampton[4], K. Maddox[5], J.S. Smith[6], R.S. Thoma[7]

[1]Genentech, Inc., [2]University of Zürich, Switzerland, [6]University of Texas Medical School, [7]Monsanto

For decades, automated Edman sequencing has been the method of choice for determining the N-terminal amino acid sequence of proteins. However, the advantages of mass spectrometric techniques have in recent years driven investigators to look beyond Edman chemistry to find alternative technologies to obtain N-terminal sequence. Several mass spectrometric methodologies have been published, primarily for proteomics analyses, which may be quicker, less costly and more sensitive than Edman sequencing. Because such techniques involve a range of biochemical and instrumental methodologies having different advantages and limitations the ESRG has created a study to ascertain how reliably they can produce N-terminal amino acid sequence information and to compare those results to those obtained by automated Edman sequencing.

The ESRG 2009 oral presentation will cover current methodologies used to extrapolate terminal information from a protein. The study was designed to allow the participants freedom to use their analytical technique of choice to obtain as much N-terminal amino acid sequence information as possible from two test proteins. Results of the multiple techniques and experiments produced by participating laboratories will be shown and success of the methods utilized will be evaluated. Finally the future directions of terminal sequence analysis, by Edman or other means, will be discussed.

(r5) Light Microscopy Research Group (LMRG)

Performance Testing and Standard Good Operating Practice in Light Microscopy

R. Cole [1,2]

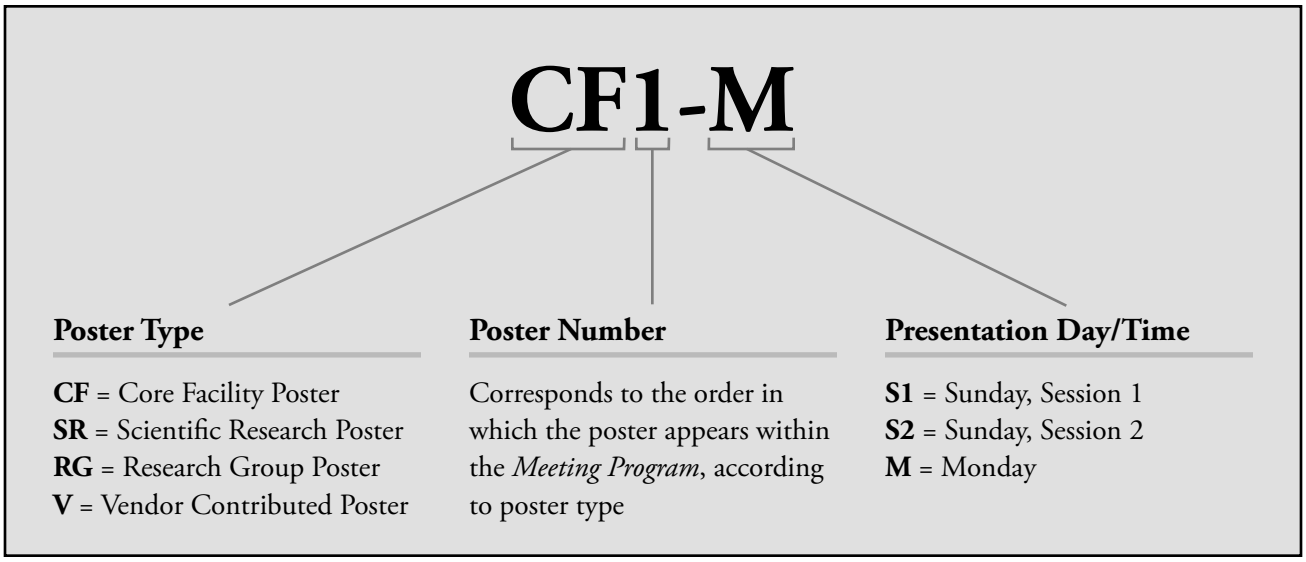
[1]Wadsworth Center, Albany, NY; [2]School of Public Health State University of New York, Albany, NY

Light microscopes have had a seminal influence on science for more than 300 years. The past three decades have seen a dramatic resurgence in the use of the light microscope, as well as very substantial technical advances in the field of light microscopy. This has in turn led to an increase in the use of the light microscope as a research tool. The most important advance has been the development of the confocal microscope, which combines the detection efficiency of fluorescence with the high resolution of the light microscope.

Improvements in design of optical components include, for example, aberration-corrected objectives (correction of both chromatic and spherical aberrations), more efficient filters (glass & AOBS), and improved detection (PMTs, CCD cameras and single photon avalanche diodes). As a result of these improvements, as well as improved performance and functionality of the systems, there has been a dramatic increase in costs of these types of instruments. The increase in cost coupled with decreasing grant support for research has resulted in many of these new instruments to be placed in multi-user facilities, i.e. imaging "cores".

Establishment of imaging cores has led to a shift in responsibility for instrument acquisition, maintenance and training, from an individual PI to the director of the core and core personnel. Among the myriad of functions of the core is performance testing of the instrumentation. Users need to be confident that data collected will be uniform over time and between specimens. There is a need to develop standard Good Operating Practice (GOP) procedures for the imaging instrumentation.

KEY TO ABSTRACT NUMBERING



An example of a paper listing is shown below. Bold represents the primary author.

SR5-S1 **Identification of a Novel Pathway in Stimulated Human T-cells that drives Macrophage Activation and Cytokine Production**

K. Jonscher, L.Li, and C.K. Edwards

University of Colorado Denver, Department of Dermatology

Affymetrix®

Revolutionize life

The world's first hands-free, automated microarray instrument.

To learn more, visit us at **booth #409** or www.affymetrix.com/genetitan

GeneTitan™ System

Less time acquiring data, more time for science.

Genotyping and Genomic Variation

SR1-S1 HRAS mutations and 11p allelic Imbalance in a Rare Case of Agminated Spitz Nevus: Bridging the Gap between Bench and Bedside through Collaborative Research

K. Sol-Church[1], S. Catalano[1], J. Holbrook[1], D. Stabley[1], K. Conard[3], D. Shurman[2]

[1]COBRE-Center for Pediatric Research, duPont Hospital for Children, Wilmington, DE, [2]Department of Dermatology and Dermatopathology, Thomas Jefferson University, Philadelphia, PA, [3]Department of Pathology, duPont Hospital for Children, Wilmington, DE

One of the major roles of the Nemours Biomolecular Core is to provide one-on-one mentoring for clinicians engaging for the first time in molecular genetics projects. This core function has successfully grown to include unique inter-institutional research collaborations. In this poster, we will present the detailed molecular analysis that was performed in collaboration with clinicians in DE and PA, on a rare and fascinating case of agminated Spitz nevus, and the methodologies developed for this project. Differentiating a Spitz nevus from a Spitzoid melanoma is often a challenging task faced by dermatopathologists. While the dysplastic nevus theory supports the notion that a Spitz nevus can progress to a melanoma, there is molecular evidence that refutes this possibility. Namely, Spitz nevi can carry unique mutations found in the mitogen-activated protein kinase (MAPK) pathway that are not found in melanomas. We used DNA sequencing as well as STR analysis for genotyping and copy number evaluation. The analysis revealed two HRAS "gain of function mutations" resulting in A11S and G13R amino acid substitutions in the ras protein and hyper activation of the MAPK signaling pathway. Allelic specific amplification PCR assays were developed to elucidate the allelic provenance of each mutation. We discovered that the maternally inherited allele carried both mutations in cis. The HRAS mutations observed in the patient's Spitz nevi, HRAS copy number increase, as well as allelic imbalance at 11p with gain of the maternal allele, was also observed in tissue containing the mutated gene.

V1-S1 Improvements for Applying Single-Stranded Conformation Polymorphism (SSCP) Analysis On An Automated Medium-Throughput Capillary Electrophoresis System

E. Schreiber, M Wenz, E. Currie Fraser, S. Jankowski

Applied Biosystems

Single-stranded conformation polymorphism (SSCP) analysis is an established and proven method for screening and detection of single nucleotide polymorphisms (SNPs) and small deletions and insertions. The SSCP analysis detects sequence variations through differences in electrophoretic mobility in a native gel system. A subtle sequence variant can cause a conformational change in the single-stranded DNA molecule that can result in detectable differences in mobility. Here we describe a formulation for a non-denaturing gel composition prepared from commercially available Conformation Analysis Polymer (CAP) in a glycerol and TBE buffer system. The polymer is used for capillary electrophoresis with the Applied Biosystems 3130/3130xl Genetic Analyzer along with size standard GeneScan ™-600 LIZ®). New run modules were designed and are described that support the application on the instrument. We also present data that show how changes in temperature affect the outcome for screening for the H63D mutation in codon position 282 of the HFE gene. The described conditions could be useful for SSCP-based applications like cost-effective screening for unknown SNPs in large number of samples.

V2-S1 The JAX Mouse Diversity Array: A New Very High-Density Genotyping Array

D. Hinerfeld[1], E. Antoniou[1], B. King[1], Y. Ding, T. Sterns[1], J. Peng Szatkiewicz[1], S. Kamdar[1], J. Pereira[1], F. Pardo-Manuel de Villena[2], G. Churchill[1]

[1]The Jackson Laborarory, [2]University of North Carolina, Chapel Hill

The Mouse Diversity Genotyping Array is the most advanced high-density mouse genotyping microarray available. The custom Affymetrix array can assay over 600,000 single nucleotide polymorphisms (SNPs) and over 900,000 invariant genomic probes (IGPs). IGPs may be used to assay copy number variation. SNPs were carefully selected from publicly available SNPs to be highly polymorphic among characterized strains. There are approximately one SNP every 4.4kb across the genome. 93% of all exons annotated by Ensembl have at least one IGP. All 238 mouse/human/rat ultra-conserved genomic regions have at least one IGP. All SNPs on the array have been validated with a prototype array. All SNPs and IGPs are annotated by position in the genome and by reference SNP cluster identifiers from dbSNP.

Example applications that can be performed by this array include: Characterizing novel or existing mouse strains or lines — for applications such as association or QTL studies; Genetic quality control of strains; Characterizing or comparing any set of mouse DNA samples (e.g., tumor tissues or cell lines) — for applications such as array CGH.

The technical performance of the array and computational methods have been evaluated by analysis of male and female DNA from a single inbred strain. A reference dataset of over 200 mouse genomes has been established which, as expected, increases the accuracy of genotype calls. Using these priors and optimized BRLMM-P parameters, the heterozygous SNP calls of inbred mice is significantly reduced.

Genomic Analysis

SR2-S1 Methylation and Pathway Focused Gene Expression Analysis: New Core Services for Translational Research

J. Holbrook, D.L. Stabley, M. Kendall, K. Sol-Church

A.I. duPont Hospital for Children/Nemours' Children's Clinic, Wilmington DE, United States.

The mission of the Biomolecular Core lab is to facilitate discoveries that begin at a molecular level and move rapidly from research to the bedside. Methylation analysis and micro RNA studies are rapidly growing areas of interest in translational research. This poster will review the tools and approaches we used to set-up services for site specific and global methylation analysis as well as pathway focused gene expression and micro RNA studies. We will discuss the challenges and opportunities of establishing these new services in a clinical setting. We will describe how to design a site specific methylation assay in the study of mitochondrial DNA. Global methylation analysis, which is based on the use of methylation sensitive (HpaII) and methylation insensitive (MspI) enzymes, will also be presented. Pathway focused gene expression analysis was evaluated using SA Biosciences Human MAP Kinase Signaling Pathway RT^2 Profiler PCR Array. These results were validated by both the ABI TaqMan assays and semi quantitative gel analysis. The micro RNA studies were performed using the Human miFinder RT^2 miRNA PCR array kit with patient samples. We saw similar results in expression levels using both total RNA and enriched miRNA. Both the pathway and miRNA focused arrays perform consistently well in our hands and we highly recommend them as part of our Real-Time PCR service.

DNA Sequencing — Capillary Electrophoresis

SR3-S1 Implementation of Fluidigm's SlingShot Absolute Quantitation Product Offers the Dual Advantage of Replacing the Costly and Time-Consuming Titration Step as Well as Recovery of Sub-Optimal DNA Samples for 454 Next Generation Sequencing

J. Boland[2], C. Matthews[2], L. Amundadottir[1], Z. Deng[2], S.J. Chanock[1]

[1]The National Cancer Institute, Gaithersburg, MD, United States; [2]SAIC-Frederick, Gaithersburg, MD, United States

We have successfully implemented Fluidigm's SlingShot technology into our 454 sequencing process at two key junctions: sub-optimal sample quantitation and replacement of the library titration. This implementation has allowed us to process previously failed samples (Roche recommends samples be between 3ug and 5ug) and replace the costly titration step saving us processing time (up to 2 days) plus the cost of a sequencing reaction.

Our initial experiment utilizing SlingShot consisted of 12 samples ranging in amounts from 990ng to 2.5ug. We processed these 12 samples using the approved Roche 454 protocol through library prep. After completion of the library prep, each sample was quantitated using the SlingShot product to accurately assess the amount of sample to go forward into bulk emulsion prep. Due to limitations of the existing quantitation methods available at the core genotyping facility (Ribogreen and Agilent), these samples would have never made it to the sequencer. We will present data highlighting the quality coverage attained (each sample had at least 20X coverage) from these samples using the quantitation obtained through SlingShot. We will also discuss future experimentation as well as future updates we would like to see with this platform.

V3-S1 Enhanced Workflow for Sequencing PCR Products by Capillary Electrophoresis

H. Le, E. Currie-Fraser, P. Kotturi, M. Bozzini

Applied Biosystems, Foster City, CA United States

Since the introduction of Sanger dideoxy sequencing, significant efforts have been directed toward increasing throughput by streamlining workflow. We describe here further enhancements for a PCR product resequencing workflow capable of reducing the total time, from beginning PCR reactions through completion of basecalling, to 6 hours or less. The workflow shown employs a new AmpliTaq Gold® Fast PCR Master Mix in conjunction with modified thermal cycler conditions to substantially reduce the time required for PCR amplification. Process time is further reduced through optimization of cycle sequencing conditions. We have coupled these improvements with an efficient sequencing reaction cleanup protocol and decreased Capillary Electrophoresis (CE) run time using MicroAmp® Fast 96-Well Reaction Plates on an Applied Biosystems 3130*xl* Genetic Analyzer. Overall data quality compares favorably with data obtained from previously documented methods. The increased efficiency and generation of high quality results will reduce time to discovery in both clinical and research applications.

V4-S1 An Automated Microfluidic System for Integrated Thermocycling and Preparation of Sanger Sequencing Samples

M. Trounstine, F.S. Pearson

Microchip Biotechnologies Inc., Dublin, CA United States

The poster describes the development of a sample preparation system for Sanger sequencing. The work covers the design of four integrated microfluidic chips, mated with a standard laboratory robot, to automate both dye-terminator sequencing reactions at the sub-1 µl scale and subsequent bead-based clean-up in glass microfluidic chips using SPRI beads. Data on the optimization of reactions for plasmid and PCR products are presented, including performance data for controls, and PCR and plasmid templates from core facilities. Microfluidic-scale reactions are contrasted to full volume control reactions for read length and signal-to-noise. Read lengths generated for plasmid samples range from 800 to 1,000 bp, using 125 to 600 ng/ul plasmid input concentration, and 0.2 to 1 ul of dye-terminator reagents. The poster also describes the basic function of the underlying microfluidic technology, which uses diaphragm pumps, actuated by pressure and vacuum, to distribute, mix and direct the flow of fluids and beads. This approach offers the prospect of lowering the cost of standard Sanger sequencing through reagent reduction and reduced 'hands on' time.

V5-S1 Optimizing Bisulfite DNA Conversion Method for Methylated CpG Island Discovery and Screening

H. Le, E. Currie-Fraser, B. Finkelnburg, V. Boyd, M. Barker, M. Bozzini, S. Jankowski, L. Xu

Applied Biosystems, Foster City, CA United States

DNA methylation at the 5' position of cytosine in CpG islands plays a critical role in the epigenetic regulation of gene expression. Typically methylation is inversely correlated with the transcription status of the gene.

Bisulfite DNA conversion is one of the most widely used techniques for methylation studies because of its relative simplicity, whereas other methods are frequently cumbersome and require significant optimization. The bisulfite conversion method allows precise analysis of methylation in a target region by converting all nonmethylated cytosines into uracils, while methylated cytosines remain unchanged. The workflows described here provide an effective solution for methylation analysis with straightforward protocols. We describe three different options: 1) Clone based sequencing, 2)Direct PCR sequencing and 3) Methylation sensitive mobility shift assay. We also present a methylation workflow decision tree that helps to direct to the appropriate method to choose for assessing the methylation status in the region of interest. These workflows are particularly useful for the analysis of samples where the amount of material is limited, and when analysis time is an important factor. Exemplary data from model gene systems are presented which validate the proposed workflows.

V6-S1 Applications of A New And Improved Size Standard For Fragment Analysis On Capillary Electrophoresis Instruments

R.A. Padilla, S. Santhanam, C. Davidson, S.C. Hung, S.M. Chen, S.M. Koepf, K.D. Jacobson, M.C. White, S. Lim, N. Patel, S. Berosik, S. Pistacchi, A.B. Shah, L.K. Joe, E.S. Vennemeyer, J. Walker

Applied Biosystems, Foster City, CA United States

A good size standard is an essential tool for researchers in the field of plant and animal genomics for fragment analysis applications such as AFLP®, T-RFLP, VNTR, mutation screening, MLST, and BAC fingerprinting. We have developed a new high density size standard using Applied Biosystems' 5th dye technology with a larger sizing range up to 1200 basepairs and no peak migration anomalies. Here we describe the Applied Biosystems GeneScan™ 1200 LIZ® Size Standard and illustrate its use with a variety of samples.

V7-S1 AutoDTR: Automatable, Reliable and Economic Sequencing Reaction Purification

J.O. Adigun, O. Zimerman

EdgeBio, Gaithersburg, MD, United States

Most Sequencing Reaction Purification (SRP) methods require centrifugation which hinders the ability to effectively automate. Magnetic technologies offer a relatively lower cost and are automatable, but results are often variable with signal intensity fading along the electropherogram. Recently introduced ion exchange technology, requires extensive vortexing, use of special plate sealers, centrifugation and is expensive. We have developed a surface-modified micro-plate to perform SRP without the need for centrifugation or magnets. All manipulations are performed on a robotic platform making this technology very amenable to automation. The extension products are selectively adsorbed to the surface-modified microplate, washed, and eluted in the desired buffer. This new technology offers not only a robust, high-capacity, and fully automatable SRP platform, but also works efficiently with reduced volumes of BigDye® Terminators. Testing was done with a 96-well head on both a Biomek® FX and Hamilton MICROLAB® STAR Workstation in less than 30 minutes. A variety of conditions were interrogated, using high-copy and low-copy plasmid, PCR, Templiphi template and DNA Libraries. Typical Phred20 scores of 800 and complete elimination of dye-blobs was achieved with ABI BigDye® Terminator v3.1 volumes ranging from 250-1000 nl. With no adjustment to the protocol, the technology was very robust to variations in template and BigDye®. Thousand of sequencing reactions were purified with extremely reproducible results (CV 0.02 – 0.06) over a wide range of reaction conditions. This new technology offers a robust, high-capacity, and fully automatable dye terminator removal platform to work efficiently with reduced volumes of dye terminators.

V8-S1 Fast and Cheap DNA Sequencing Plasmid Template Preparation with A 'All in One Well' Microplate System

Y. Yang, H.R. Hebron, J. Hang

EdgeBio, Gaithersburg, MD, United States

The purification is based on a set of solutions and a simple centrifugation procedure. Protocols are designed for the easy extraction and purification of genomic DNA from blood samples and tissue cells, including whole blood, buffy coat, bone marrow, body fluids, buccal cells, tissues, mouse tails, etc. Red blood cells are lysed by dilution into a hypotonic solution. Tissues are broken down and treated with protease K to release cells. An anion detergent solution is used for further cell lysis and DNA extraction. After precipitation of the detergent and proteins, a unique bead that bind proteins, lipids, and RNAs are added to achieve the supreme purity. Genomic DNA is then separated by alcohol precipitation. A proprietary nucleic acid precipitation reagent is used to enhance DNA recovery from low cell number samples. No DNA binding beads or column is used in the method, eliminated the problem of low yield and the risk of shearing genomic DNA. The purified samples are free of proteins, lipids, salts, and RNAs contamination, are stable for storage and suitable for all downstream applications.

V9-S1 Multiplex Sequencing on the SOLiD™ Platform

J. Bodeau[1], H. Breu[1], C. Hendrickson[2], J. Stuart[2], K. McKernan[2], L. Zhang[2], T. Sokolsky[2], E. Dimalanta[2], J. Manning[2], H. Peckham[2], A. Blanchard[2], G. Costa[2], K. Li[1], S. Kuersten[3]

[1]Molecular Cell Biology Division, Applied Biosystems, Foster City, CA, United States; [2]Applied Biosystems, Beverly, MA, United States; [3]Ambion, Austin, TX, United States

The SOLiD™ DNA sequencing system utilizes stepwise ligation of oligonucleotide probes and enables high fidelity, high throughput sequencing. Increases in throughput since release of the SOLiD™ system have been dramatic, yielding more DNA sequence in a single instrument run than is required for certain applications. In order to better match experimental needs to sequencing capacity, we have developed and commercialized a multiplexing method for the SOLiD™ system that enables pooling of 16 unique samples per sequencing spot, or up to 256 samples per 2-slide run. Multiplexing of 96 samples has been demonstrated, indicating that the method is scalable to even higher throughput. Multiplexing is achieved by making sequence fragment libraries using a set of oligonucleotide adapters that contain short unique sequences, or barcodes, which are sequenced as a short (5-10 base) read separate from the sample sequence. The barcode sequences are analyzed to parse the sample sequences by their library of origin. Parameters used to design effective barcode sequences include uniqueness, degrees of separation from other barcodes, color balance during SOLiD™ sequencing, and optimization with respect to observed performance characteristics of the platform. Data is presented from experiments using up to 96 barcodes. False assignment rates below 0.01% have been obtained, with over 96% of matching sequenced tags containing a barcode. Applications suited to SOliD™ multiplexing include, but are not limited to, small RNA expression analysis (for which a multiplexing kit has been commercially released), targeted resequencing, bacterial resequencing, and whole transcriptome gene expression.

V10-S1 Sample Quality Diagnosis in a Commercial Resource Company: An Effective Strategy for Sample Assessment

D. Bintzler, M. Jordan

DNA Analysis, LLC, Cincinnati, OH, United States

DNA Analysis, LLC is a new company located in Cincinnati, Ohio. We provide services related to automated DNA sequencing, fragment analysis and DNA fingerprinting to the academic and corporate research community. A primary goal of ours is to provide quality services while maintaining a close working relationship with our clients. Our company operates more as a commercial resource facility, not as a large high-throughput company. This requires providing customer services rarely found in commercial laboratories. Samples can be analyzed prior to or after processing for sequencing or genotyping. Potential problems that can interfere with achieving a quality result can be diagnosed. We have incorporated a testing strategy that includes: scanning to determine sample concentration and to identify contaminants using the Nanodrop 1000 spectrophotometer, testing for nicked DNA or DNA contamination using the Lonza Flashgel system, and sequencing with universal primers designed to sequence any plasmid template with the ampicillin or kanamycin resistance genes to help assess primer-template related problems. In combination, these testing methods provide information about sample submissions that are communicated to the clients, and are applied in determining a troubleshooting chemistry or procedure. This poster outlines our general process, from the time a researcher submits a sample, to diagnosing sample quality, to relaying the results and relative information to the customer.

V11-S1 SOLiD System Sample Preparation Amenable to High-Throughput Sample Multiplexing.

V. Bashkirov[1], U. Ulmanella[1], V. Aivazachvili[1], G. Costa[2], K. Guegler[1], C. Lee[2], Z. Liu[1], K. McKernan[2], D. Perez[2], G. Spier[1], S. Yang[1]

[1] Applied Biosystems, part of Life Technologies, Foster City, CA, United States; [2] Applied Biosystems, part of Life Technologies, Beverly, MA, United States

The sequencing throughput of the next generation sequencing platforms can be significantly increased by sample multiplexing, for example by using barcoding. The SOLiD System provides up to 256 barcodes to analyze as many samples in a single run. This new multiplexing technology is expected to dramatically reduce both labor and reagent costs for users. However, barcoding requires parallel processing of numerous samples during library construction until they receive distinct barcodes. The implementation of the conventional sample preparation workflow for treating tens or hundreds of samples at a time would be impractical due to the prohibitive increase of preparation time and labor. This would greatly limit the applicability and acceptance of barcode multiplexing. Moreover, not all of 14-15 major steps of SOLiD fragment library preparation are amenable to automation. Additionally, there are no high-throughput DNA fragmentation instruments on the market which can shear in parallel multiple samples to the size-range required by SOLiD. Here we present a high-throughput sample preparation solution for the SOLiD System. This includes a new DNA fragmentation instrument and a simplified 5-step library construction workflow. The new low-volume parallel DNA shearing protocol generates DNA fragments with such a tight size distribution to render size-selection unnecessary. Furthermore, the enzymatic steps of the new library preparation are directly coupled with the DNA shearing process, feature a simple one-tube workflow, and make possible the omission of the large-scale PCR step. Compared to the conventional method, the new sample preparation process is much shorter (less than 3 hours) and results in a greater library yield and quality. It also eliminates any base compositional bias resulting from PCR and over-shearing of DNA, and improves the coverage of otherwise "difficult-to-cover" regions. This method therefore enables sample multiplexing with the SOLiD System by providing the solution for automated sample preparation.

DNA Sequencing — Next Generation Sequencing Technologies

SR4-S1 Human Genome Sequencing Using the AB/SOLiD Platform

D. Muzny[1], J. Reid[1], S. Mclaughlin[2], F. Hyland[3], H. Peckham[2], E. Tsung[2], H. Dinh[1], C. Kovar-Smith[1], L. Nazareth[1], A. Sabo[1], A. Hawes[1], Y. Ding[1], M. Morgan[1], M. Holder[1], G. Costa[2], F. De La Vega[3], K. McKernan[2], R.A. Gibbs[1]

[1]Baylor College of Medicine, Houston, TX, United States; [2]Applied Biosystem, Beverly, MA, United States, [3]Foster City, CA, United States

NextGen platforms are now becoming widely used for human genome sequencing to characterize whole-genome polymorphism events and for functional mutation discovery. As the cost of producing whole genome data approaches $10K/Genome, thousands of human samples could be sequenced, requiring methods for quick data analysis and contamination screening such as eGenotyping. As a participant in the 1000 Genomes and TCGA (The Cancer Genome Atlas) projects we have developed a high throughput pipeline using the AB/SOLiD platform for whole genome sequencing. At the BCM-HGSC, over 320 GB of mapped data have been completed on the AB/SOLiD for whole genome sequencing activities in both mate pair and fragment libraries. Current runs have achieved up to 16GB generated per mate pair run with 58-60% matching reads. New procedures and methods were developed for the SOLiD pipeline to ensure library complexity (90+% unique reads), bead load density (100-120K/per panel), run transfers, off line analysis and submissions at the sequence generation level. The AB software tool Corona_lite has been used to determine SNPs and small indels for SOLiD data. Initial data on SOLiD 25X coverage data has shown that 100% of HapMap SNPs are found at 12-15X sequence coverage. Building on these pipeline advancements we are now determining the appropriate depth of coverage and analysis needed for whole genome sequencing to identify SNPs and structural variants for human disease related samples including patients with Ataxia, Pancreatic Cancer and Glioblastoma.

SR5-S1 Simplifying Illumina GA Sequencing and Multiplexing Samples as a Result

J. Hadfield

Cancer Research UK (CRUK), London, England, United Kingdom

We have developed a simplification to the multi-primer hyb protocols for sequencing DNA and smallRNA libraries on the same Illumina GA flow cell. This simplification requires minimal modifications to current protocols but also allows a degree of sample multiplexing with no change to library prep protocols. A doubling of sample throughput is possible with this approach.

V12-S1 Deep Sequencing-Based Whole Transcriptome Analysis of a Single Cell

K.Q. Lao[1], F. Tang[2], C. Barbacioru[1], Y. Wang[1], E. Nordman[1], C. Lee[1], N. Xu[1], X. Wang[1], J. Bodeau[1], M. Azim Surani[2]

[1]Molecular Cell Biology Division, Applied Biosystems, Foster City, CA, United States; [2]University of Cambridge, Cambridge, United Kingdom

The next generation sequencing technology is a new powerful tool for transcriptome analysis. However, under certain conditions, only small amount of material is available for analysis, which requires more sensitive techniques that can preferably work on a single cell. Here we developed a digital gene expression profiling assay at single cell resolution by combining a modified single cell whole transcriptome amplification method with the next generation sequencing technique, SOLiD™ System. Using this assay, we showed that blastomeres in a four-cell stage embryo have similar gene expression, which is compatible with the fact that they have similar developmental potential. We proved that, compared to cDNA microarray technique, our single cell cDNA deep sequencing assay can detect expression of thousands of more genes. Moreover, for the genes detected by both microarray and deep sequencing, our assay detect new transcript variants for a large proportion of them, which unambiguously confirms at single cell resolution that the transcriptome complexity is more than traditionally expected. Finally, by using our assay to Dicer knockout and Ago2 knockout oocytes, we showed that a significant amount of transposons were abnormally upregulated in Dicer/Ago2 knockout mature oocytes compared with wildtype controls. Development of this technique will greatly facilitate discovering new genes and understanding transcriptome complexity when material available is very limited, such as during early embryonic development or for stem cells, which are usually rare cell population in vivo.

V13-S1 Low Cost, off the Shelf, Automation of SOLiD™ System Template Bead Preparation with Freedom EVO® and Te-MagS™ from Tecan®

K.A. Poulter, J.P. Chang, R. Brady, D.P. Ching, G.P. Amparo, L. Pham, J.A. Hoshizaki, R.K. Tan, K.M. Perry

Applied Biosystems, Foster City, CA, United States

Preparation of the template beads for the SOLiD™ System is a tedious manual process with 230 steps per sample needed to prepare the beads for sequencing after emulsion PCR (ePCR). With the ability to sequence up to 16 samples per slide, this translates to 3680 steps. Full preparation of the beads for sequencing can take 3½ days and 4 weeks of training. We have taken two sections from this process that have the most repetitive wash steps and that do not require centrifugation or a hood and automated them with "low cost," off the shelf liquid handling equipment, Freedom EVO® and Te-MagS™ from Tecan®, for a quick and straightforward solution for AB customers. Of particular concern to researchers is: 1) template bead loss leading to lost sequence; and 2) incomplete washing leaving residual chemicals that could interfere with subsequent reactions. We will present data that will compare bead loss for manual vs. robot handling. We will also present data that compares P2-

enriched bead recovery, Noise/Signal Ratio and Cy3 intensity between manual vs. robotic handling. The purpose of this experiment is to verify the consistent performance of the robot over time, and show that no difference is observed between the robot and manual processes.

V14-S1 Modified cDNA Library Preparation Method for Transcriptome Profiling by 454 Sequencing

P. Bouffard, N. Levenkova, Z. Markovic, G. Irzyk

454 Life Sciences, Branford, CT, United States

454 Sequencing is well suited for transcriptome profiling because readlengths in the 400-600bp range can contribute significantly to the discovery and characterization of new splice variants in previously uncharacterized transcriptomes. Many standard cDNA library preparation methods have been used for 454 Sequencing applications with mixed success. Most notably, the classic approach of making oligo d(T) primed full length cDNA, fragmentation by nebulization, followed by ligation to standard 454 shotgun library adapters does not always yield optimal results. This is due primarily to the poor fragmentation of <1 kb cDNA fragments and the potential problems caused by excessive poly A/T signal. We have adapted the existing cDNA library preparation protocol recently described by Noonan et al.1 for use with 454 Sequencing. The protocol can be used with as low as 200ng of mRNA input and does not require any adapter ligation. We have used the method to sequence the transcriptome of Saccharomyces cerevisiae using standard GS FLX conditions. Two standard FLX runs generated 193 MB of data. Reads were assembled and mapped to > 94% of all predicted ORFs and 58% of ORFs had > 90% coverage. Positional coverage of ORFs is slightly lower in 5' and 3' as compared to the middle. Coverage of the 5' and 3' ends appears to be equivalent. We believe this method will offer an additional option to users of 454 Sequencing interested in transcription profiling.

V15-S1 Multiplex Sequencing on the SOLiD™ Platform

J. Bodeau[1], H. Breu[1], C. Hendrickson[2], J. Stuart[2], K. McKernan[2], L. Zhang[2], T. Sokolsky[2], E. Dimalanta[2], J. Manning[2], H. Peckham[2], A. Blanchard[2], G. Costa[2], K. Li[1], S. Kuersten[3]

[1] Applied Biosystems, part of LIFE Technologies, Foster City, CA, USA; [2] Applied Biosystems, part of LIFE Technologies, Beverly, MA, USA; [3]Ambion, , part of LIFE Technologies, Austin, TX, USA

The SOLiD™ DNA sequencing system utilizes stepwise ligation of oligonucleotide probes and enables high fidelity, high throughput sequencing. Increases in throughput since release of the SOLiD™ system have been dramatic, yielding more DNA sequence in a single instrument run than is required for certain applications. In order to better match experimental needs to sequencing capacity, we have developed and commercialized a multiplexing method for the SOLiD™ system that enables pooling of 16 unique samples per sequencing spot, or up to 256 samples per 2-slide run. Multiplexing of 96 samples has been demonstrated, indicating that the method is scalable to even higher throughput. Multiplexing is achieved by making sequence fragment libraries using a set of oligonucleotide adapters that contain short unique sequences, or barcodes, which are sequenced as a short (5-10 base) read separate from the sample sequence. The barcode sequences are analyzed to parse the sample sequences by their library of origin. Parameters used to design effective barcode sequences include uniqueness, degrees of separation from other barcodes, color balance during SOLiD™ sequencing, and optimization with respect to observed performance characteristics of the platform. Data is presented from experiments using up to 96 barcodes. False assignment rates below 0.01% have been obtained, with over 96% of matching sequenced tags containing a barcode. Applications suited to SOliD™ multiplexing include, but are not limited to, small RNA expression analysis (for which a multiplexing kit has been commercially released), targeted resequencing, bacterial resequencing, and whole transcriptome gene expression.

V16-S1 Polymorphism Discovery in High-throughput Resequenced Microarray-enriched Human Genomic Loci

A.A. Antipova, T.D. Sokolsky, C.L. Hendrickson, C.R. Clouser, E.T. Dimalanta, C.Duncan, C.C. Lee, S.S. Ranade, L. Zhang, A.P. Blanchard, K.J. McKernan

LIFE Technologies, Beverly, MA, United States

Identifying genetic variants and mutations that underlie human diseases requires development of robust, cost-effective tools for routine resequencing of the regions of interest in the human genome. Here we demonstrate that coupling Applied Biosystems SOLiDTM System sequencing platform with microarray capture of targeted regions provides an efficient and robust method for polymorphism discovery in human. Utilizing high-density Agilent microarrays with a custom probe design to pull down 4.3 Mb target DNA sequence from a HapMap Yoruba sample, we obtained sequencing coverage averaging 138-fold, with median coverage equal 59. This level of coverage enabled highly accurate and sensitive SNP detection, with 99.5% of identified HapMap SNPs called correctly. In addition, the enrichment/resequencing strategy allowed precise localization of insertion/deletion and inversion polymorphisms, as well as detailed characterization of fusion breakpoints in chromosomal translocations, in fragment libraries. These results demonstrate that the combination of SOLiD resequencing with microarray capture of the selected genomic regions provides a powerful tool for genetic analysis and will expedite the search for genes contributing to inherited common diseases and the diseases in which somatic mutations play a role, such as atherosclerosis and cancer.

V17-S1 Improving the Quality of DNA Libraries in the Solexa Sequencing Workflow by Implementing a High Sensitivity DNA Quantification Method

R. Salowsky, M. Gassmann

Agilent Technologies, Waldbronn, Germany

Next-generation sequencing technologies play an important role in investigating complete cancer genomes and transcriptomes. To further increase productivity of this compelling technique, the quality of DNA libraries plays an important role. One important step in the Solexa workflow is the amplification of the generated libraries for determining the exact sequencing cluster concentration. The drawback of this step is that amplification artefacts and errors will be introduced into the target sequence. An on-chip electrophoresis instrument has become a standard tool for implementing DNA library quality control and quantification in the Solexa workflow. The microfluidic device monitors the size and quantification of the amplified libraries and also helps to detect contaminating artefacts. With an optimized protocol and newly developed electrophoresis chemistry, the sensitivity could be increased by

a factor of 20-30, down to the pg/µl concentration range. This improved detection sensitivity allows for the significant reduction of amplification cycles thereby reducing the target sequence error rate.

V18-S1 The Utility of Capillary Electrophoresis and Next Generation Sequencing Platforms for Scientific Discovery

J. Walker, A. Pradhan, M. Bozzini. A. Shah, A. Tam

Applied Biosystems, Foster City, CA United States

With its long read lengths and high accuracy, capillary electrophoresis-based sequencing is the gold standard technology for *de novo* projects. Typically in de novo projects for genetic analysis of any organism CE is considered ideal for creating high quality scaffolds. Now with the availability of sequencing by short-read next generation sequencing technologies system, this process is complemented through finishing with high coverage. Alternatively, short-read sequencing technologies offer the throughput requirement for assaying large numbers of candidate regions or when resequencing pooled or heterogeneous samples. Next-generation sequencing is suited for large-scale discovery experiments, while CE with its high accuracy and unmatched data quality can be used to validate structural genetic variations. We will to look at de novo and targeted medical sequencing as two applications where each technology could be applied. In our analysis we consider sample preparation, and reagents, as well as key criteria that researchers consistently demand, including accuracy, coverage, read length, quality values and ease of use.

V19-S1 Next Generation Sequencing of the *Escherichia coli* O55:H7 Genome and Comparison with the Closely Related Enterohemorrhagic *E. coli* O157:H7

L. Degoricija [2], P. Vatta [1*], C. Cummings [2*], L. Wong[1], M. Barker [2], J. Ziegle [2], L. Jones [2], J. Chin [2], P. Brzoska[2], M. Rhodes[2], M. Furtado[1], O. Petrauskene[1]

*[1]Applied Markets R&D, Microbiology group, [2] Molecular & Cellular Biology Division, Applied Biosystems, Foster City, CA, USA *These authors contributed equally*

Detection in the food supply of pathogenic *E. coli*, particularly strains that cause hemorrhagic colitis (HC), has become a public health priority. The O157:H7 serotype of *E. coli* has been responsible for most HC outbreaks to date, so detection of this type is critically important. The ideal assay must detect O157:H7, but not any other serotypes, including the vast majority of commensal *E. coli* that are not pathogenic. *E. coli* O157:H7 is very closely related to the O55:H7 serotype, which does not frequently cause HC outbreaks. Numerous lines of evidence indicate that O55:H7 is the nearest phylogenetic neighbor of O157:H7, making the design of O157:H7-specific assays challenging. The *E. coli* O55:H7 genome sequence would be a valuable tool for identification of assay target sequences unique to O157:H7, but no such sequence was available. To this end, the genome of one *E. coli* O55:H7 strain, and another O157:H7 strain, were sequenced by oligonucleotide ligation and detection using the next-generation AB SOLiD™ platform. Comparison of the O55:H7 and O157:H7 genomes identified 500 kb of sequence that is present on the O157:H7 chromosome and absent or divergent in O55:H7. Comparison of these putative O157:H7-specific sequences against the publicly available genome sequences of other pathogenic and non-pathogenic *E. coli* and *Shigella* strains identified regions that are conserved beyond the O157:H7 lineage, further narrowing the list of putative assay design targets. The short time requirement (two to three weeks from library construction to sequence) and deep coverage obtained (>60X), makes the SOLiD™ system ideally suited for microbial genome sequencing when a closely related reference genome sequence is available. In particular, this method can be sufficiently robust to permit genome sequencing of a reference organism's nearest phylogenetic neighbors. Importantly, short-read mapping methods can define regions of difference between the query and reference genomic sequences, which is fundamental to the definition of specific target sequences for differential assay design.

V20-S1 Automation of the GS FLX Titanium Shotgun Library Preparation and Implementation at the 454 Sequencing Center

C.L. Turcotte, D.H. Birr, P.Bouffard, G.P. Irzyk

454 Life Sciences, Branford, CT, United States

Preparation of a single stranded DNA library is the initial step of the 454 Sequencing™ sample preparation process for many applications. Current manual batch size in the 454 Sequencing Center is 8 libraries/FTE/8 hour day. We have increased production capacity 12-fold by automating the GS FLX Titanium shotgun DNA library protocol to allow the parallel processing of up to 96 libraries/FTE/8 hour day using a commercially available liquid handler. Nebulization has been replaced by fragmentation on a Covaris™ E210 instrument. This allows for the unattended fragmentation of up to 96 samples in 3.5 hours (including set up and break down times). Because there is no DNA loss during fragmentation with the Covaris™ instrument we have been able to reduce the DNA input requirement from 5 to 3 ug of double stranded DNA. All post-fragmentation steps are carried out in a 96-well plate format on a Hamilton MICROLAB® STAR liquid handler. All Qiagen® MinElute® purification steps have been eliminated and replaced by purification using a combination of Agencourt AMPure® SPRI® beads and Qiagen® QIAquick® 96 well plate. The processing time from sizing SPRI® to single stranded library takes approximately 5 hours. The automated processing is robust and reproducible. We find library quality to be equivalent to the manual method. The method is currently in production at the 454 Sequencing Center.

V21-S1 *de novo* Assembly of SOLiD™ Colorspace Reads into Large Genomic Scaffolds Using a Velvet-Based Pipeline

S. Yang[1], C. Cummings[1], V. Sheth[1], Z. Zhang[1], M. Rhodes[1], D. Zerbino[2], E. Birney[2]

[1]Applied Biosystems (part of Life Technologies)., Foster City, CA, USA; [2]EMBL-European Bioinformatics Institute, Hinxton, Cambridge, UK

Next-generation sequencing technologies, such as the SOLiD™ system, provide the ability to sequence entire genomes quickly and cheaply. Although these platforms are well-suited for genome resequencing, many research projects seek to analyze previously uncharacterized genomes for which a reference sequence is not available. The feasibility of *de novo* genome assembly from short reads has recently been demonstrated, but to date, no tool has been available for the assembly of colorspace reads from the SOLiD™ platform, which offers the highest throughput of any commercially available sequencing platform. To accomplish this task, the Velvet *de novo* assembly tool* was adapted to handle SOLiD™ colorspace data. Specifically, all reverse complement operations in the Velvet source code were changed to reverse (not complement) in order to accommodate the properties of colorspace reads. In addition, an analysis pipeline was developed to facilitate the preparation of colorspace reads for input to Velvet, and convert the resulting contigs into nucleotide sequences. The latter step is accomplished by a colorspace-to-nucleotide adaptor that corrects colorspace contigs by aligning their component reads, and maximizing concordance of first base calls with the colorspace translation. Using this pipeline to assemble 50-mers from an *E. coli* DH10B mate-paired library with 147X coverage, we obtained a contig N50 of 2.2 kb and a scaffold N50 of 68.2 kb. This assembly covered 95% of the 4.6 Mb *E. coli* DH10 chromosome with high accuracy. Notably, the adaptor rescued over 78 kb of sequence that failed to align in colorspace. This computational pipeline, available at the SOLiD Software Development Community (http://solidsoftwaretools.com), provides researchers with an end-to-end solution for *de novo* assembly of previously uncharacterized genomes. These tools may also be useful for the reconstruction of insertions in resequencing projects, and clustering of expressed sequence tags.

V22-S1 Expression Profiles of small RNAs from Various Tissues Generated by SOLiD™ Sequencing

B. Nutter[1], S. Kuersten[2], J. Gu[2], C. Barbacioru[1], D. Wang[1], B. Gardiner[3], K. Lea[2], S. Heater[2], M. Barker[1], L. Chapman[2], T. Gulham[1], L. Wong[1], S. Grimmond[3]

[1]Life Technologies, Foster City, CA; [2] Life Technologies, Austin, TX; [3]Institute for Molecular Bioscience, The University of Queensland, St Lucia, Australia.

The combination of the SOLiD™ Small RNA Expression Kit (SREK) with the SOLiD Sequencing System presents a unique opportunity to study miRNA expression in a way not previously possible. To demonstrate the power of this approach, we barcoded and sequenced small RNA libraries from ten different human tissues to saturating levels of detection; generating up to 200 million total mappable tags of data. Comparing both independent sequencing runs and libraries indicates the system is highly reproducible and capable of up to 6 logs of dynamic range of detection. To analyze the quantitative ability of both the library method and the sequencing platform we compared tag count data to real-time PCR assays generated using TaqMan miRNA low density arrays. Fold-change comparisons between platforms show Pearson correlation values of up to 0.95, indicating the system is a valid and accurate profiling tool. Detailed analysis indicates a far greater repertoire of miRNA variants, or 'isomirs', than previously observed suggesting a much broader range of mRNA targets for miRNA-mediated regulation. We have also developed a Support Vector Machine to predict novel miRNAs contained within the SOLiD datasets. Using this approach we have identified hundreds of potentially novel sequence tags. We chose a subset of these novel transcripts and designed custom TaqMan miRNA assays to validate them by real-time PCR analysis. We are able to demonstrate both the presence and expression profile of >50% of the novel sequences, most of which are present at relatively low levels in the ten tissues. Interestingly, we failed to detect nearly all of these novel targets using conventional northern blotting highlighting the need for qPCR sensitivity for validation purposes. This human miRNA expression atlas provides a unique opportunity to understand the sequence complexity and identity of small noncoding RNAs present in a variety of human tissues.

V23-S1 Broad Genomics Services Offered by Complementary Next-Generation Sequencing Platforms

X. Tan, C.M. Morrison, D. Buck, F. Lu

SeqWright, Houston, TX, United States

The success of commercial sequencing is largely dependent on the capability of a service company to provide broad and cost effective solutions to meet the various needs of its customers. For providers of Next-Generation (Next-Gen) sequencing services, it's critical to evaluate and practice the synergy created by the combination of different platforms in order to make genomics projects of any size and scope accessible to researchers and hereby, increase the pace of genomics research. At SeqWright, two complementary Next-Gen platforms, the GS FLX system from Roche and the SOLiD system from ABI, were chosen to provide cost-effective genomic solutions for virtually any genomics project. The GS FLX system produces ten-fold longer sequencing reads than other next generation platforms, making this system ideal for de novo genome sequencing as well as targeted resequencing projects. The latest upgrade to GS FLX, known as Titanium, generates more than one million reads and up to 600 megabases of sequence per run in a matter of several hours. For projects requiring larger data sets, the SOLiD system is the system of choice. A full SOLiD run can currently generate more than 100 million reads and nearly 5 gigabases of sequence, and the newest upgrade is purported to produce over 40 gigabases per run. The experience at SeqWright and data generated demonstrate that the dual-system approach allows service provider to offer a variety of genomics services for a broad array of projects, including whole-genome sequencing/resequencing, SNP detection, transcriptome sequencing, non-coding RNA discovery, and ChIP sequencing, etc.

Nucleic Acid Extraction/Amplification

SR6-S1 Evaluation of Two Whole Genome Amplification Strategies for the Application of Buccal Cell DNA to High-Density SNP Array Analysis

K. Kopp[1], V. Pankratz [2], J. Carnahan[1], W. Lingle[1], J. Cunningham[1], W. Highsmith[1]

[1]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, United States; [2]Health Sciences Research, Mayo Clinic, Rochester, MN, United States

Mouthwash (MW) buccal cell collection and DNA purification is an economical and convenient option for gene association studies, though it typically results in lower DNA yields. Whole genome amplification (WGA) will increase the DNA mass of these samples, but reportedly produces allele dropout. We evaluated the concordance of SNP results between two strategies of WGA DNA from eight buccal cell samples and DNA from eight peripheral blood leukocytes (PBL) samples on Illumina Inifinium HumanLinkage-12 Arrays. WGA was performed on MW DNA per manufacturer's directions using the QIAgen Repli-g Midi kit (1x) and in four half volume WGA reactions pooled after amplification (4xP). Of the 6090 SNP's on the array, 71 did not yield results for any sample and were excluded from further analysis. For the remaining 6019 SNPs, call rates were 99.77% for PBL DNA, 90.44% for 1x WGA, and 92.03% for 4xP WGA. PBL DNA heterozygotes were called homozygotes (allele dropout) in 0.12% of both the 1x and 4xP WGAs. PBL DNA homozygotes were called heterozygotes in 0.04% 1x WGAs and 0.03% 4xP WGAs. The kappa value for overall concordance was 0.9975. Using the 36,144 SNP's with 100% call rates across all samples, allele dropout rates were 0.10% and 0.07% for 1X and 4xP WGAs, respectively. Homozygote to heterozygote miscalls were 0.03% for both WGA strategies. Kappa values increased to 0.9985 for 1x WGAs and to 0.9989 for 4xP WGAs. Overall SNP result concordance between high quality peripheral blood leukocyte DNA and whole genome amplified material from buccal cell DNA was excellent (>99.7%). The 4xP strategy showed some improvements in call rate and accuracy. Despite a reduction in overall SNP success with WGA buccal cell DNA, the high concordance suggests SNPs called should be expected to agree with calls on PBL DNA.

SR7-S1 Advances in SCODA Electrophoretic Biomolecule Concentration

D. Broemeling[1,2], D. Gunn[2], P. Eugster[2], M. Isbasescu[2], J. Pel[1], J. Perkins[1], H. Poon[1], J. Thompson[1], T. Willis[2], A. Marziali[1].

[1] University of British Columbia, Vancouver, BC, Canada; [2] Boreal Genomics Inc., Vancouver, BC, Canada

Continued development of SCODA, an electrophoretic biomolecule concentration method developed at the University of British Columbia, has led to substantial performance improvements which will be presented. This technology has been commercialized through Boreal Genomics who have developed an instrument for nucleic acid and protein purification with greatly improved performance over early proof-of-concept demonstrations. The SCODA technology offers unique advantages including exceptional rejection of PCR inhibitors and other contaminants, an unparalleled ability to enrich for low abundance nucleic acids, and ability to length-select nucleic acid fragments. Recent advances have reduced sample processing time from ~ 1hr to under 10 minutes, for samples up to 1 ml in volume. Nucleic acid purification from samples ranging from Athabasca tar sands, various soils and other environmental and food matrices have been demonstrated including high molecular weight DNA recovery when desired. The instrument's ability to reject contaminants and proteins has been benchmarked against competing column technologies, and has been demonstrated to be 100 – 1000 fold more effective at protein and contaminant rejection, while maintaining high DNA recovery efficiency even in highly contaminated samples. We have also demonstrated the ability to set up instrument conditions such that proteins will focus instead of being rejected, opening the way to a number of protein purification applications. The new alpha instrument will be presented, as will its performance specifications and recent advances in sequence and length specific DNA concentration.

V24-S1 Purification of Genomic DNA with Minimal Contamination of Proteins

H.R. Hebron, Y. Yang, J. Hang

EdgeBio, Gaithersburg, MD, United States

The purification is based on a set of solutions and a simple centrifugation procedure. Protocols are designed for the easy extraction and purification of genomic DNA from blood samples and tissue cells, including whole blood, buffy coat, bone marrow, body fluids, buccal cells, tissues, mouse tails, etc. Red blood cells are lysed by dilution into a hypotonic solution. Tissues are broken down and treated with protease K to release cells. An anion detergent solution is used for further cell lysis and DNA extraction. After precipitation of the detergent and proteins, a unique bead that bind proteins, lipids, and RNAs are added to achieve the supreme purity. Genomic DNA is then separated by alcohol precipitation. A proprietary nucleic acid precipitation reagent is used to enhance DNA recovery from low cell number samples. No DNA binding beads or column is used in the method, eliminated the problem of low yield and the risk of shearing genomic DNA. The purified samples are free of proteins, lipids, salts, and RNAs contamination, are stable for storage and suitable for all downstream applications.

V25-S1 TaqMan® Express Plates: A pre-plated format for easy mRNA quantification

P. Hegerich, M. Mouanoutoua, F. Chan, R.D. Canales, D.N. Keys, K.Y. Lee, J. Sherlock, S. Koepf, C. Chen

Applied Biosystems, Foster City, CA, United States

TaqMan® Express Plates contain up to 96 pre-spotted and dried TaqMan Gene Expression Assays on an optical 96-well plate. Pre-plated assays simplify, accelerate and help error-proof mRNA quantification experiments. This is especially important when there is more than one operator in a lab, when a study is being done across multiple labs, or when a study is extended over a period of time. The assays in TaqMan®

Express Plates are user-selected from a catalogue of > 50,000 TaqMan assays targeting human, mouse, rat, Rhesus and dog genes. Here, we show that TaqMan® assays which are aliquoted and dried perform comparably to standard wet assays aliquoted immediately before use (dynamic range, linearity, and limit of detection). The difference between the normalized Ct values of wet vs. dry assays was < 0.3. The data also showed strong reproducibility for replicates within and across plates and across manufacturing lots. In addition, excellent reproducibility was observed for plates run by different operators across different laboratories. Finally, we have generated a set of 32 human genes with low expression variability among biological samples. The set includes genes commonly used as endogenous controls in relative quantification experiments, plus genes found in a transcriptome-wide screen of multiple human tissues. The performance of assays targeting these genes was tested using total RNA from 20 different tissues. Analysis of expression stability using the geNorm and NormFinder algorithms shows the newly identified genes have expression stabilities equivalent to, and in some cases exceeding, genes historically used for endogenous controls. The full set of 32 assays is available on the TaqMan® Express Human Endogenous Control Plate, enabling rapid experimental identification of optimal control assays for individual user samples.

Bio-IT / LIMS

V26-S1 WikiLIMS as a Platform for Next Generation Sequencing and Bioinformatics Analysis

B. Osborne, M. Cariaso

Bioteam, Inc., Nyack, NY, United States

WikiLIMS is customizable software based on MediaWiki and Semantic MediaWiki that captures data from Next Generation sequencers. It is being used at a number of laboratories to facilitate lab workflows and display data. WikiLIMS users can use WikiLIMS to integrate expression data and sample data with their sequence studies. This poster describes WikiLIMS extensions that read data from caGRID using its WSRF-based services and Perl, and demonstrates that the WikiLIMS extensions can compare sequence data from caBIG with data from the Next Generation sequencer using an SGE computing cluster. WikiLIMS is also being used as a research desktop, an application to launch a variety of command-line applications, retrieve their output, and create useful, shared bioinformatic workspaces. This work shows the utility of MediaWiki and WikiLIMS within the framework of publicly available biomedical data.

V27-S1 Next Gen Laboratory Software Systems for Core Facilities

T.M. Smith, E. Olson

Geospiza, Seattle, WA, United States

Throughout the past year, as next generation sequencing (NGS) technologies have emerged in the marketplace, their promise of what can be done with massive amounts of sequence data has been tempered with the reality that performing experiments and working with the data is extremely challenging. As core labs contemplate acquiring NGS technologies, they must consider how the new technologies will affect their current and future operations. The old model of collecting and delivering data is likely to change to one where the core lab becomes an active participant in advising and helping clients set up experiments and analyze the data. However, while many labs want to utilize NGS, few have the Information Technology (IT) infrastructures and procedures in place to successfully make use of these systems. In the case of gene expression, NGS technologies are being evaluated as complementary or replacement technologies for microarrays. Assays like RNA-Seq and tag profiling that focus on measuring relative gene expression require that researchers and core labs must puzzle through a diverse collection of early version algorithms that are combined into complicated workflows with many steps producing complicated file formats. Command line tools such as MAQ, SOAP, MapReads, and BWA, have specialized requirements for formatted input and output and leave researchers with large data files that still require additional processing and formatting for other analyses. Moreover, once reads are aligned, datasets need to be visualized and further refined for additional comparative analysis. We present solutions to these challenges by showing results from a complete workflow system that includes data collection, processing, and analysis for RNA-seq suited for the core laboratory.

Microarrays – RNA

SR8-S1 Application of RIP-Chip to Study the Human Regulatory Code

S.V. Chittur, M. Kuentzel, D. Frank, T. Devine, R. Jain, A. George, S.A. Tenenbaum

*Center for Functional Genomics and Gen*NY*Sis Center for Excellence in Cancer Genomics, University at Albany-SUNY, Rensselaer, NY USA*

Eukaryotic organisms depend on the actions of RNA-binding proteins (RBPs) for successful post-transcriptional control of gene expression. RBPs provide the link between transcriptional and translational regulation and play essential roles in many regulatory processes including transcription, splicing, export, stability and translation. Previously we developed methods for purifying endogenous RBP-RNA complexes and identifying the associated RNA targets using whole-genome expression array technologies (termed ribonomic profiling or RIP-Chip). This advance enabled the large-scale identification of many mRNA targets of RBPs and provided new insight into the principles governing post-transcriptional gene regulation. Our studies demonstrated that, analogous to transcriptional regulation, groups of functionally related RNAs are coordinately regulated in a combinatorial manner by distinct classes of RBPs targeting related cis-regulatory elements located in the transcripts. In the process of developing this profiling methodology, we have evaluated different array formats. These studies compare the effectiveness of these platforms for RIP-Chip based on their probe design, amplification strategies, signal to noise and overall cost. Also discussed will be the quality control metrics developed specifically for immunoprecipitated mRNA samples and potential pitfalls. In conjunction with the NIH/NHGRI ENCODE project we have adapted ribonomic profiling to tiled-microarray platforms to determine the associations of both coding and non-coding RNAs for several RBPs. By combining RIP-Chip profiling with tiling-arrays to explore the post-transcriptional network, our studies indicate that in addition to targeting predicted mRNAs, many of the non-coding RNAs expressed from the genome also appear to be associated with RBPs in a specific and selective manner that is involved in reading our regulatory code.

SR9-S1 Choosing a Microarray for miRNA Analysis

J. Hadfield, A. Git

Cancer Research UK (CRUK), London, England, United Kingdom

There are several microarrays available for miRNA analysis. We present a comparison of six platforms and highlight some of the issues that users should consider for miRNA analysis. The different platforms have varying input RNA requirements, probe design and protocols. We discuss the choice of a platform for use at CRUK's Cambridge Research Institute.

SR10-S1 Multiplex Amplification and Microarray-Based Normalization for HT Cloning of Microbial Genes

J. Hasseman, E. Snesrud, K. Ong, S. Pradhan, B. Bishop, P. Venepally, C. Sun, M. Holmes, R. Fleischmann, S. Peterson

Gateway® Cloning & Protein Expression Group, Pathogen Functional Genomics Resource Center (PFGRC), Bethesda, MD, United States

The technology to rapidly clone hundreds or thousands of genes in parallel has been developed and refined over the last several years. The Pathogen Functional Genomics Resource Center (PFGRC) has established a robust high-throughput Gateway® cloning platform and now proposes a multiplex strategy to streamline HT cloning. By miniaturizing primer synthesis and multiplexing PCR and cloning reactions we can reduce cost and labor significantly. PCR primers are synthesized on Agilent custom arrays, cleaved, selectively pooled (1250 ORFs/ pool) and used in a multiplex PCR reaction. The diverse PCR product pool is then hybridized to a capture array to normalize relative abundance of each product in the pool. The normalized pool then enters a batch recombination reaction using BP clonase; unique clones are sorted and sequence verified downstream using Sanger and 454 sequencing methods. Preliminary results have confirmed the inherent bias in multiplex PCR and our proof-of-concept experiments explore the possibilities of a highly multiplexed cloning platform. Project funded by the National Institute of Allergy and Infectious Diseases (NIAID) under contract no. N01-AI15447.

V28-S1 A novel Digital Technology for Non-Enzymatic Direct Multiplexed Measurement of Gene Expression

G.K. Geiss[1,3], R. Bumgarner[2], B. Birditt[1], T. Dahl[1], N. Dowidar[1], D.L. Dunaway[1], H.P. Fell[1], S. Ferree[1], R.D. George[1], T. Grogan[1], J.J. James[1], M. Maysuria[1], J.D. Mitton[1], P. Oliveri[4], J.L. Osborn[3], T. Peng[2], A.L. Ratcliffe[1], P.J. Webster[1], E.H. Davidson[4], L. Hood[5]

[1]NanoString Technologies Inc., Seattle, WA, United States; [2]The Department of Microbiology, University of Washington, Seattle, WA, United States; [3]Department of Bioengineering, University of Washington, Seattle, WA, United States; [4]Division of Biology, California Institute of Technology, Pasadena, CA, United States; [5]The Institute of Systems Biology, Seattle, WA, United States

We have developed a novel digital technology that can be used for non-enzymatic direct multiplexed measurement for gene expression that is ultra sensitive and has a high level of precision even at very low levels of gene expression. This technology has been developed into a fully automated system, the nCounter TM Analysis System, which enables researchers to examine or validate larger sets of transcripts with many fewer reactions while removing the risk of bias being introduced during enzymatic steps. In this study we examined the technical performance of the nCounter System and compared it with results generated with micorarrays, TaqMan® and SYBR® Green Real-Time PCR.

Materials & Methods: nCounter hybridization reactions were performed in triplicate with total RNA samples isolated from mock and polio virus infected human A549 cells. nCounter reactions were set up as follows: 100ng of total RNA; Reporter and capture probes for 509 human mRNAs and controls made to non-human sequences (6 positive, 2 negative); DNA control targets spiked in at 0.1, 0.5, 1, 5, 10 and 50 fM; Hybridizations were carried out for 20h at 65°C. Excess reporters were then removed by using magnetic bead based purification. The same samples and amount of RNA were also analyzed with Affymetrix® U133Plus2 arrays, using the two-cycle amplification/labeling protocol recommended by the manufacturer. We selected a subset of 14 genes in which the measured log2 fold-change was significant in one platform but not the other for further analysis by TaqMan Real-Time PCR.

In a second experiment, nCounter hybridization reactions were performed in triplicate as described above with total RNA samples isolated from sea urchin embryos collected at seven different development time points. A set of 21 genes were selected for comparison with existing SYBR Green Real-Time PCR data generated in the Davidson Lab.

V29-S1 Development of New 1mm I.D. Dvinyl Bnzene Bsed Mnolithic Rversed Phase Column and Its Applications

G. Gendeh, S. Rao, C. Saini, K. Flook, Y. Agroskin, C. Pohl

Dionex Corporation, Sunnyvale, CA, United States

Monolith columns offer several advantages over conventional porous columns. They include fast mass transfer, high loading capacity, improved resolution even at elevated flow rates and wide pH stability. These characteristics support versatile performance in a wide range of bio-molecule separations. Earlier, we introduced reversed phase and ion exchange phases of ProSwift monoliths in 4.6x50 mm format. Ion-exchange phases include weak and strong anion and cation exchange phases. Subsequently, we introduced weak and strong anion exchange phases and a weak cation exchanger in 1x50 mm format. 1 mm format provides improved sensitivity and reduced solvent consumption as compared to 4.6 mm I.D. format. Another useful feature is that these 1mm monoliths could be run at flow rates up to 0.3 mL/min and therefore can be used on analytical HPLC instruments with minor plumbing modifications using a nano flow cell detector. Currently, the development of ProSwift RP 1 mm reversed phase columns is in progress. They have been tested in 2 different lengths. While ProSwift RP 1x50 mm is planned for high throughput fast chromatography separations, 1x250 mm is intended for complex protein sample analysis and purification. However, either of these columns could be used for protein chromatography and to combine with mass spectrometry applications. In this poster we present various applications including separation of complex proteins, monoclonal antibody (MAb) and other bio-molecules using both ion-exchange and reverse phased 1 mm columns. We show fast protein and MAb separations that are done under one minute using RP 1x50 mm columns. Relevant results including dynamic capacity, ruggedness and reproducibility of ProSwift monolithic 1 mm columns are presented.

V30-S1 Highly Reproducible Data from a Microfluidic Separation Platform for Application in Discovery Proteomics

J.B. Young, N. Hebert, D. Wyrick, E. Lin, R. Van Soest

Eksigent Technologies, Dublin, CA, United States

A microfluidic column and connector system has been developed for high performance chromatography for protein and peptide separations. The microfluidic system was used in conjunction with an Eksigent NanoLC-Ultra and Thermo Scientific LTQ linear ion trap equipped with a New Objectives Nanospray interface. Separation performance for a proteomic standard mixture was compared against a conventionally packed capillary column. For the analysis of complex proteomic mixtures the variability in the observed MS data and protein identification is a result of a combination of diverse factors. The enormous complexity of many proteomic samples can have thousands of peptides in abundances over many orders of magnitude. Online reverse phase separations have peak capacities in the hundreds, and the finite dynamic range of the mass spectrometer (both in terms of electrospray performance and detector considerations) often makes it difficult to achieve reproducible results. To compound this issue, inter-column reproducibility is difficult to achieve for capillary columns that operate at nanoliter flow rates. Inter-column reproducibility is primarily dependent on the column manufacturing process and the installation of these columns into the analytical system. The process of making connections for these nanoliter-per-minute separations is highly skill dependent, and method robustness very difficult to achieve. Microfluidic devices have inherent appeal as a chromatographic medium as the microchannels are defined lithographically and are highly reproducible. We reported recently on a microconnector which facilitates reproducible microfluidic connections at nanoliter flow rates with a very high degree of consistency. In this poster, we will be reporting on our progress on a new microfluidic platform based on this microconnector for nanoliter separations on microchips and demonstrate that robust, high quality separations can enhance the reproducibility of proteomic experiments. High run-to-run reproducibility, and low inter-column variation can be achieved, with retention times within 1% in each case.

V31-S1 Method for Heart-Cut Analysis Using 2D RP/RP nanoLC for Proteomic Samples

M.D. Stapels, K.E. Fadgen, J.I. Langridge

Waters Corporation, Milford, MA, United States

Two-dimensional chromatography is often used to separate peptides from proteomic samples in a biomarker discovery workflow. In order to validate a biomarker, many samples need to be analyzed to prove that the same peptides are reproducibly identified and are changing in a statistically significant manner due to a biological perturbation. Rather than running an entire 2D experiment, which is too time-consuming during validation, a better approach is to elute the targeted peptides in one fraction in a heart-cut manner. A highly reproducible method for performing on-line two-dimensional chromatography with mass spectrometry was developed. Peptides were separated by RP chromatography at high pH in the first dimension, followed by an orthogonal separation at low pH in the second dimension. An online dilution of the effluent was performed after the first dimension so that no hydrophilic peptides were lost in the second dimension. As peptides eluted from the second dimension, a hybrid quadruple time-of-flight mass spectrometer was used to detect the peptides and their fragments by alternating collision cell energy between a low and elevated energy state. All peptides were fragmented in this method, which takes out the irreproducible nature of typical MS/MS experiments. This fragmentation allowed for the identification of the peptides with a novel database searching algorithm that uses 14 physiochemical properties to score identifications and minimize false positives. Proteins had to be identified in two out of three replicate injections, which narrowed the list to the most confident identifications. Comparing the third fraction of a complete 2D experiment to the targeted run in the heart-cut analysis resulted in over 90% of the same proteins and peptides being identified. Comparisons of measured retention times and peak areas between the two methods will be made.

V32-S1 1 mm I.D. Poly(Styrene-Co-Divinylbenzene) Monolithic Columns for Fast and High Efficiency Protein Separations

S. Eeltink, R. Swart

Dionex, Abberdaan, Amsterdam, The Netherlands

Due to the unique properties of monolithic stationary phases, monolithic columns have become an attractive alternative for packed columns, especially for the separation of complex protein samples. The porous monolith is covalently anchored to the capillary wall. This attachment

increases the robustness of the column. The control that can be exerted over the preparation process facilitates optimization of the porous properties of the monolith, and consequently the chromatographic performance of the entire system. Furthermore, virtually no carryover effects are observed on monolithic stationary phases compared to frequent observation on silica materials, resulting in a more reliable identification and quantification of proteins, especially for the low abundant species.

In this study, the LC performance of a 1 mm I.D. monolithic column is demonstrated for the separation of proteins. The effects of gradient time and volumetric flow rate on peak capacity are demonstrated and loadability of the 1 mm I.D. monolithic column was determined for proteins. Furthermore, high efficiency separations of a complex E. Coli protein mixture are demonstrated. For these separations a volumetric flow rate < 100 µL/min was applied to ensure high detection sensitivity and maximum compatibility with ESI interfacing and MALDI spotting. Finally, the application of the 1 mm I.D. monolithic column used as a 2nd dimension RP column is demonstrated after an 1D ion-exchange separation.

V33-S1 Separation of Complex Peptide Samples Using Optimized Column Technology and 1D-LC Tonditions

S. Eeltink, B. Dolman, R. Swart

Dionex, Abberdaan, Amsterdam, The Netherlands

Determination of the proteome and identification of biomarkers is required to monitor dynamic changes in living organisms and predict the onset of an illness. Different approaches are available for the identification of proteins. One method is called shotgun proteomics, in which proteins are digested, the resulting peptides are separated by high-performance liquid chromatography (HPLC), and identification is performed with tandem mass-spectrometric detection. Digestion of proteins may lead to a very large number of peptides. For example, it has been estimated that digestion of a cell lysate may produce up to 500,000 peptides. The separation of highly complex peptide samples is one of the major challenges of analytical chemistry.

The effects of LC conditions (gradient time and temperature) and column length on peak capacity for the separation of tryptic peptides was studied in reversed-phase gradient elution nano LC. To perform the study, 75 µm I.D. columns up to 50 cm in length were packed with 3 µm silica particulate materials and tested. Increasing the gradient time and column temperature helped to increase peak capacity. A maximum peak capacity of 443 was obtained with a 50 cm long columns operating with a 120 min gradient time and a column temperature of 60°C.

V34-S1 Development of an Automated Method for Antibodies Purification and Analysis

W. Decrop, R. Swart

Dionex Corporation, The Netherlands

One of the major problems in biotherapeutics is aggregation of the active pharmaceutical ingredient. These product-related substances can have different efficacy than the main product and may cause serious side effects e.g. anti-drug-antibody formation. Protein aggregates are mostly the consequence of suboptimal production, purification or handling conditions (e.g. temperature, pH). In the purification of antibodies, a protein affinity separation is generally the first step. Affinity chromatography on protein A or G columns typically yields a purity of more than 95% in a single step. To verify the purification efficiency (or sample purity or antibody quality) a technique such as ion exchange or size-exclusion chromatography is needed. Ion exchange stationary phases provide good selectivity for separation of charge variants of the protein biopharmaceutical. The variations may be very subtle or small and finding the optimal chromatographic conditions requires optimization. This work discusses the development of an automated solution for purification and separation of antibodies using a single Ultimate 3000 HPLC system. In this process the autosampler performs the injection, high volume fraction collection, and reinjection.

V35-S1 Plug and Re-Play: How to Divide a Nano LC Flow Post-Column and Conquer the Information Content of Complex Proteomic Samples

D. Eikel[1], M. Baumert[2], R. Almeida[2], S. Prosser[1]

[1]Advion BioSystems Inc., Ithaca NY, United States; [2]Advion BioSciences Ltd., Hethersett, United Kingdom

Here we present a novel low volume splitting T integrated into a 6-port valve to split an analytical nano LC flow post-column. One portion of the analyte flow is directed towards the mass spectrometer immediately, whereas the other portion takes a defined detour and is directed to the mass spectrometer with a time delay, such that a first and second pass of all analytes towards the MS is generated. This brings about two analysis opportunities based upon a single sample injection, a valuable gain in mass spectrometer duty cycle and sensitivity compared to a multiple injection strategy of precious proteomic samples since the mass spectrometer is a concentration dependant detector. This presentation will demonstrate multiple ways to utilize these two analytical opportunities in order to increase the information obtained from complex proteomic samples (e.g. F9 cell lysate). In a non data dependant fashion, we can show an increase in protein identification by 20 % using either the same instrument setting for both analyte passes (exploiting random information gains), or using CID fragmentation of +2 charge state analytes during the first pass only and ETD fragmentation of +3 charge state analytes during the RePlay run only (exploiting the increased duty cycle of the MS). Furthermore, an information-dependant approach can utilize the first analyte pass, by providing either an immediate database search with a semi-automatic generation of an exclusion list for the second pass of analytes (30 % protein identification increase observed), or providing timed SRM settings for the quantification of the identified peptides during the RePlay run, thus allowing for identification and quantification based on the same initial sample injection. This report employed multiple MS systems and their software (LTQ-XL, HCT and QTrap 4000) since, to date, no single system has the combined hardware/software abilities for all modes described here. Flow splitting of nano LC systems post column enables twice the analytical possibilities from the same injection of a complex proteomic sample.

SR11-S1 Surface Plasmon Resonance (SPR) based Sensing of Transfer across Biological Membranes

M. Brändén[1], S. Tabaei[1], T. Pettersson[2], F. Höök[1]

[1]Chalmers University of Technology, Göteborg, Sweden; [2]Layerlab AB, Göteborg, Sweden

The cell is critically dependent on molecules, ions and signals being continuously transferred across its membrane. Water and other small non-electrolyte molecules can cross the lipid-bilayer membrane by passive diffusion, whereas transport across the membrane of ions and larger molecules are controlled by specialized membrane-spanning proteins. Here, we present, what to our knowledge is the first time-resolved method for direct monitoring of non-electrolyte transfer across biological membranes. The method is based on resolving the temporal change in refractive index upon a permeation-dependent change in the solute concentration inside liposomes attached to a surface plasmon resonance (SPR) active surface. As illustrated for the biologically important molecules glycerol, urea and hydroxyurea, this method, being biosensor-based, enables screening of multiple permeation events on a very same set of immobilized liposome, and a possibility to study selective alterations in lipid bilayer permeability, through in situ injection of effector molecules. Compared to previously available methods, which are based on averring several indirect measurements of solute-transfer induced size changes of liposomes or cells, this method provides a more sensitive as well as time and sample saving mean for studying permeability of non-electrolytes.

SR12-S1 A Robotic Assay for Antibody/Antigen Characterization with MALDI-TOF Mass Spectrometric Detection

J. Simpson[1], S. Colantonio[1,3], , H. Rodriguez[2], G. Whiteley[1], R. Fisher[1]

[1]Protein Chemistry Laboratory, SAIC-Frederick, Inc., NCI-Frederick, Frederick, Maryland, United States; [2]Office of Technology and Industrial Relations, Office of the Director, National Cancer Institute, Bethesda, Maryland, United States; [3]Regina Elena National Cancer Institute, Rome, Italy

The National Cancer Institute (NCI) Clinical Proteomic Technologies for Cancer (CPTC) Initiative is a five-year initiative to build a foundation of technologies, data, reagents, reference materials, analysis systems, and infrastructure needed to systematically advance protein biology for the diagnosis, treatment and prevention of cancer. As part of the CPTC initiative, monoclonal antibodies have been raised to potential protein biomarkers to provide a series of renewable and highly characterized affinity binding reagents to the research community.

Our laboratory has utilized a robotic assay based on functionalized magnetic beads to capture individual monoclonal antibodies (mAbs), followed by reaction of the beads with specific antigens to characterize their binding. Following antigen binding, the beads are treated with an acidic MALDI matrix solution to release the antigens and then analyzed by MALDI-TOF mass spectrometry to confirm antibody / antigen specificity. A robotic method capable of the analysis of 96 different mAbs and their potential antigens was written, along with design and manufacture of a MALDI plate holder compatible with the robot and a typical Applied Biosystems Voyager MALDI plate. MALDI-TOF analysis was automated to allow unattended data acquisition.

Several types of modified magnetic beads were evaluated with regard to binding characteristics, background binding, specificity and cost. Among the beads evaluated were carboxylic acid beads chemically modified with polyclonal rabbit anti-mouse Fc antibodies (RAMFc), protein A modified beads, goat anti-mouse IgG modified, and an Fc specific pan-mouse IgG modified surface. Optimal results were obtained using the pan-mouse IgG beads and minimal loading of the target mAb. Antigen concentrations were in the micromolar range with ample signal-to-noise.

V36-S1 Direct Monitoring of Therapeutic Protein Aggregation by MALDI Mass Spectrometry

R. Wenzel, A. Nazabal

CovalX AG, Zürich, Switzerland

Therapeutic protein aggregates were separated using Size Exclusion Chromatography (SEC) and fractions collected were submitted to a novel High-Mass MALDI (Matrix Assisted Laser-Desorption /Ionization) mass spectrometry (MS) protocol. In order to analyze the biopharmaceutical aggregates intact via MALDI two novel tools were utilized. Firstly, the fractions were cross-linked (K100 Stabilization Kits, CovalX, Zürich, Switzerland) followed by intact detection using High-Mass MALDI mass spectrometry (HM1, CovalX, Zürich, Switzerland). A drug-antibody candidate was analyzed which makes tumor cells more vulnerable to chemotherapy and radiation and slows tumor cell growth. Soluble and aggregated SEC fractions were prepared and submitted to cross-linking/high-mass MALDI showing various aggregation states. Candidates exhibiting multimeric aggregation under pressure and temperature stress were analyzed. Aggregated samples were fractionated using SEC and analyzed as the monomeric form and stressed fraction (showing a dimer (306.7 kDa), a trimer (462.9 kDa) and a tetramer (612.4 kDa)). A pharmaceutical formulation sample containing aggregates was directly analyzed (10 µL containing 4µM) without SEC purification. In the mass spectrum obtained, a large number of proteins are detected due to the absence of pre-fractionation in addition to the monomer and dimer peaks. In order to evaluate the ability to determine the percent of aggregation, a therapeutic protein that is forming a dimer when concentrated was analyzed. A sample containing a dimer was submitted to SEC. The collected fraction corresponding to the monomer and the dimer were submitted to the crosslinking protocol and directly analyzed by high-mass MALDI. The sample corresponding to the monomer was considered as 0% dimer. The one corresponding to the dimer fraction was considered as 100% dimer. After stabilization, the samples collected were mixed and plotted the percentage of dimer as the function of the area of the monomer and dimer peaks (RM and RD). Finally, known samples containing dimmers where analyzed. The described technique provided a rapid tool of measuring therapeutic pharmaceutical aggregates by MALDI mass spectrometry allowing the semi-quantitative measurement of aggregation. These results showed good agreement with the complementary techniques however required a fraction of the sample volume and can be calculated in a fraction of the time.

V37-S1 Using Ion Mobility to Measure Changes in Protein Structure upon Substrate-Ligand Binding

K. Compson[1], I. Campuzano[1], K. Compson[1], T. Mckenna[1], J. Langridge[1], Rachel Garlish[2]

[1]Waters Corporation MS Technologies Centre, Manchester, United Kingdom; [2]UCB-Celltech Ltd, Slough, United Kingdom

Over the past 10 years interest in macromolecule protein mass spectral analysis has increased due to the ability of the current mass spectrometers and electrospray sources to preserve the non-covalent protein/protein subunit interactions, allowing one to analyse proteins in their native conformation and stoichiometry. By coupling mass spectrometry with ion mobility, not only can the intact quaternary mass of biological macromolecules be accurately mass measured, but also the shape (collisional cross section) of the biological macromolecule inferred. All data presented here were acquired on a Synapt HDMS system (quadrupole orthogonal acceleration time of flight mass spectrometer enabled with T-wave ion mobility, Waters, Manchester, UK). Ion mobility calibration was carried out using a mixture of charge-reduced horse heart myoglobin and bovine cytochrome-C. Theoretical collision cross-sections were calculated using the open source-code MOBCAL. PDB files were downloaded from the RSCB Protein Data Bank. We demonstrate that not only can one monitor the change in mass of protein BCL-XL upon binding of a substrate, but also it's change in collisional cross-section (gas phase shape). We also demonstrate that the gas-phase collisional cross-section of the protein BCL-XL and BCL-XL bound to two different peptide substrates, are consistent with solution-phase NMR data, and that there is a marked increase in collisional cross-section of the BCL-XL protein upon binding of the peptide substrates. We also demonstrate that in the gas phase, we can clearly differentiate and measure the collisional cross sections of the folded protein and unfolded protein BCL-XL, and upon ligand binding, BCL-XL becomes significantly more structured, which is consistent with solution phase NMR data.

MALDI Imaging

V38-S1 Enhanced In-Situ Identification of Peptides Using High-Efficiency Ion Mobility Coupled with Direct MALDI Mass Spectrometry Imaging

R. Martin[1], E. Claude [2], J. Stauber[3], M.F. Snel [2], R. M.A. Heeren[3], T. McKenna[2], J. Langridge[2]

[1]Waters Corporation, Beverley MA USA; [2]Waters Corporation, Manchester, United Kingdom; [3]FOM Inst. Atomic/molecular Physics, Amsterdam, Netherlands

Imaging Mass spectrometry is an emerging tool in proteomics, lipidomics and metabolomics. Biomolecules are analysed directly from tissue sections, providing spatial information. Further development of this technique is the identification of diagnostic biomarkers in situ using on-tissue tryptic digestion of proteins followed by MS/MS analysis of tryptic peptides. However a limitation of MALDI imaging MS is the complexity of the data collected, which can hinder localization and/or identification. In this case a further dimension of separation is required. High efficiency ion mobility separation (IMS), which is based upon separating ions based on differences in mobility passing through a dense buffer gas under the influence of an electric field. Rat brain and human cerebellum (frozen tissue or formalin-fixed-paraffin embedded, FFPE tissue) are sectioned in 10µm thick sections with a cryo-microtome at –20°C, and digested with trypsin using a micro-spotting system (CHIP, Shimadzu) at 34°C during approximately 1 hour of matrix deposition: The alpha-cyano-4-hydroxycinnamic acid matrix in Methanol/ TFA 0.1% (50:50) is deposited by a vibrational spraying system (Imageprep, Bruker Daltonics) to cover the brain tissue. All data were acquired on a MALDI hybrid orthogonal acceleration time-of-flight mass spectrometer, (MALDI Synapt HDMS, Waters Corp.). MALDI imaging data from frozen sections demonstrating separation of endogenous lipids and tryptic peptides with nominally isobaric masses using IMS-MS was acquired.

We will show that distinct images can be produced for each of the separated compounds. IMS/MS/MS experiments of several isobaric species will be presented. In addition in FFPE section samples, where mainly peptides are present, we illustrate the different localization observed for different peptides. Identification of several peptides will be shown where direct tissue IMS/MS/MS experiments were performed and whereby nominally isobaric peptides of different intensities could be visualized after IMS.

Optical Imaging

V39-S1 A Comparison of Manual and Automated Cell Counting Instrumentation

K.L. Remple, L.R. Stone, D.R. Gray, M.P. Beaudet

Invitrogen, Eugene, OR, United States

Cell counting is a very routine, necessary task in many cell-based laboratory experiments and tissue culture facilities. In order to standardize starting material and to reduce experimental error and variation, researchers should count cells at the beginning of experiments. The hemocytometer is the most widely used device for determining cell concentrations, requiring consistent criteria and tenacity to obtain accurate and reproducible measurements. Regrettably, because the manual method is so tedious, researchers often skip the critical step of cell counting. To facilitate this necessary step, a number of instruments and methods have become available to researchers to facilitate cell counting in routine cell culture maintenance and experimentation. We examined nine different automated cell counting instruments and compared the ease-of-use, through-put capability, cost, accessories and consumables, and performance of these instruments to each other and to glass and disposable hemocytometers. All instruments were operated according to the manufacturers' instructions, first using a standardized bead solution, then observing relative cell counting and viability data. Several instruments were easy-to-use but the counting and viability data varied significantly from the standard bead solutions as well as from other instrument results. Through-put capabilities were represented by instruments with rapid analysis time (~30 seconds) to 12-sample, hands-off processes. In terms of cost, one instrument was under $5,000, five

were under $20,000, and three were over $40,000, and all required the use of consumable accessories. For any given laboratory or core facility, choosing the right instrument will predominately depend upon ease-of-use, needed through-put, and cost.

Mass Spectrometry

SR13-S1 Extending MALDI-QqQ-MS Enzyme Screening Assays to Targets with Small Molecule Substrates

R. Rathore[1]; Jay J. Corr[2]; Kenneth D. Greis[1]

[1]University of Cincinnati, Genome Research Institute, Cincinnati, OH, United States, [2]MDS Analytical Technologies, Concord, ON, Canada

Mass spectrometry-based high throughput screening has tremendous future potential as an alternative to current screening methods due to its speed, sensitivity, reproducibility and label-free readout. In addition, this method offers a direct readout of the substrate and product, thus minimizing the potential for false positive or false negative hits. We recently reported that a new generation matrixassisted laser desorption ionization-triple quadrupole mass spectrometer (MALDI-QqQ-MS) is ideally suited for a variety of enzyme assays and screening protocols. This instrument provides comparable speeds (at greater than a sample per second) with superior signal-to-background, better reproducibility and a reagent cost savings of greater than 90% as compared to typical fluorescence-coupled assays. Thus far the MALDI-based readout has been validated for a variety of enzyme classes (kinases, phosphatases, proteases, hydroxylases), however all these targets have peptide substrates that are readily monitored without interference from the MALDI matrix. To further extend the application of the MALDI-QqQ readout to enzymes with small molecule, non-peptide substrates, we evaluated this method for measuring enzyme activity and inhibition of acetylcholinesterase. Due to matrix interference in measuring these small molecules during the MALDI process, multiple reaction monitoring (MRM), available on the QqQ instruments, was used to generate a selective MS/MS transition and accurately measure both the substrate (acetylcholine) and the product (choline) of acetylcholinesterase. Importantly, accurate dose-dependant inhibition measurements were also demonstrated thus validating the MRM readout for enzymes with small molecule substrates and products. Collectively, these data demonstrate that a MALDI-QqQ-MS based readout platform is amenable for small molecule substrates and products and offers significant advantages over current HTS methods in terms of speed, sensitivity, reproducibility and reagent costs.

SR14-S1 Linear Epitope Mapping by Native Mass Spectrometry

X. Lu, M. De Felippis, L. Huang

Bioproduct Research & Development, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, United States

The identification of antigenic epitopes is critical to elucidating the molecular basis of immune responses. An understanding of binding interactions between antibodies and target antigens is also important for the optimization of monoclonal antibodies intended as therapeutic agents. Mass spectrometry has proven to be a powerful tool for the study of noncovalent molecular interactions such as those involved in antigen-antibody binding and the technique has seen broad application in this field. In this work, we describe a novel methodology for mapping a linear epitope based on direct mass spectrometric detection of antibody (Fab)-antigen complexes under native conditions. To demonstrate the utility of our methodology, we utilized a model system consisting of a Fab with specificity towards Peptide A. Two approaches, epitope excision and epitope extraction, were used in the method. In epitope excision, the Fab and Peptide A complex was treated with a variety of enzymes including: Lys-C, Glu-C, carboxypeptidase B, trypsin and chemotrypsin, and the digested complexes were fully desalted and directly monitored by mass spectrometry. Mass differences between the Fab-Peptide A complex and Fab revealed the size of epitope peptides that were protected by the binding interactions. Using the epitope extraction approach, Peptide A was first digested by Lys-C, and the fragment containing the epitope was selected by Fab binding. Data from both techniques allowed mapping of the epitope to amino acid residues 16-27 in Peptide A which is in good agreement with previously reported results. Combining the method with nanospray MS yielded a detection limit in the sub-picomolar range.

V40-S1 Separation of Peptide Precursors from Background Ions and Species with Different Charges by Automated Ion Mobility and Tandem MS Experiments

J. Langridge, C. Hughes, T. McKenna, R. Tyldesley-Worster

Waters Corporation MS Technologies Centre, Manchester, United Kingdom

An established technique for characterising proteins involves tryptic digestion followed by data dependent LC-MS/MS. Complex biological mixtures exhibit a wide dynamic range and the majority of peptides are in the lowest order of magnitude detectable. Despite tandem MS experiments, singly charged chemical noise in the MS spectrum can hinder precursor identification. Cross-linked peptides containing >2 charges exhibit low stoichiometry compared with tryptic peptides, and as such it may be difficult for the mass spectrometer to identify these in the MS survey as candidate precursors. Ion Mobility Spectrometry (IMS) separates by Drift Time and m/z giving increases in signal-to-noise ratio of low level species as they separate from the noise, allowing the mass spectrometer to more clearly identify them as candidates for MSMS.

All data was generated using a hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer incorporating a travelling wave ion mobility separation stage. Species from protein tryptic digests were introduced to the MS by separation on a nanoscale UPLC system or by direct infusion, both coupled to a nanoelectrospray emitter. The system processes and displays a specific band of the m/z versus drift time plot and species within this region are possible candidates. A further criterion of ion intensity was used to select precursor ions for tandem MS interrogation. These selected precursors were isolated by the quadrupole and, whilst maintaining ion mobility separation, were subjected to CID fragmentation by elevating the collision energy either before or after the IMS device. Examination of the data contained in a m/z versus drift time plot shows that clear separation of tryptic peptides based upon charge state can be achieved using an ion mobility device. By programming the mass spectrometer to only consider the selection of species in the area containing species of charge state 2+ or greater, we have analysed a dilution series of a standard four protein tryptic digest (Bovine Serum Albumin, Phosphorylase B, Alcohol Dehydrogenase and Enolase) to show how the enhanced signal to noise of low level species detected by the mass spectrometer in survey scans can lower the limits of detection of the tandem MS/MS approach. In addition, the subsequent separation of precursor ions from background ions by IMS prior to CID leads to high

quality MS/MS data of these species. We will show data from the injection of less than 100amol on column of the four protein digest mixture - these amounts being at a level where a TOF only survey identification is hindered by the presence of the background ions. In the case of cross-linked peptides, a tryptic digest of Bovine Serum Albumin was prepared without reduction and alkylation. This preserves the disulphide bonds and produces numerous large peptides similar in size and structure to chemically cross linked species. We will show that the disulphide linked peptides are ion mobility separated from the more intense doubly charged species present in the digest. Then, by selecting the region of the m/z versus drift time plot containing species with 3 or more charges, the mass spectrometer is programmed to generate MSMS spectra specific to the disulphide intact peptides contained within this region. For example, data dependent MSMS spectra have been generated on large peptides at approx m/z 1038 (3+), 1298 (3+) and 1500 (4+).

V41-S1 Minimization of Atmospheric Background Contaminants in Off- and On-Line Nanoelectrospray: Identification and Optimization

G. Valaskovic, B. Ngo

New Objective, Inc., Woburn, MA, United States

The high surface area of the (sub)micrometer droplets generated by low flow nanoelectrospray ionization results in the potential ionization of contaminants present in laboratory air (Volkmer-Engert R.,Schlosser A. J. Mass Spectrom. 2003; 38: 523-525). Neveu and co-workers recently reported (Proceedings of the 56th ASMS Conference, June 1-5, 2008, Denver, Colorado) an active background ion reduction (ABIRD) "bath gas" system designed to reduce these background levels. Here we report on the implementation of a similar system in combination with both off-line nanospray and on-line Nano-LC/MS. The bath gas system was implemented on a modified digital control nanospray source (PicoView, New Objective, Inc.) mounted to a conventional 3-D ion trap (LCQ Deca, Thermo Scientific Inc.). Mobile phase (water, acetonitirle gradient, 0.1% formic acid) was delivered by a gradient nanoflow LC (Eksigent). Samples for LC analysis (75 µm PicoFrit column, New Objective) were injected with a high performance autosampler (Nano PAL, Leap Technologies). System effectiveness at the suppression of these background contaminants along with other commonly observed background ions, arising from commonly encountered industrial, laboratory, and personal care products, has been studied. The commonly observed background ions (371, 445 m/z) were positively identified as cyclo-siloxane compounds through the use of reference standards. The reduction of ion current from siloxane contaminants of 10-fold or greater was commonly observed with a highly aqueous mobile phase composition (< 10% ACN). Background levels are readily reduced to a level suitable for removal of these ions from the typical data dependent mass exclusion list. A systematic study of operational parameters (gas flow rate, composition, etc.) were determined to minimize background ion current without compromising analyte ionization. Air was found to be superior to nitrogen for use with gradient elution LC since air exhibited a higher breakdown voltage and subsequently less chance of corona discharge at the nanospray emitter.

V42-S1 Image Mapping of NanoESI Ion Current with an Automated Digital Control Positioning System

M.S. Lee[1], G. Valaskovic[2]

[1]Milestone Development Services, Inc., Newtown, PA, United States, [2]New Objective, Inc., Woburn, MA, United States

Determination of nanospray emitter position for optimal signal is a trial-and-error process that involves adjustment of both emitter position and ESI voltage. We previously reported a feedback controlled source where voltage or emitter position is under feedback control (Valaskovic, et. al. JASMS, 2004, 15, 1201). Here we investigate a similar digital control system to map the spray current of the plume for differing experimental conditions (flow rate, applied voltage, distance). Total and selected ion currents are generated via the systematic three-dimensional raster scanning of the nanospray emitter relative to the spectrometer inlet. Digital PicoView Acquire software (New Objective, Inc.) was modified with a scanning module generating a step motor driven, scan pattern of the emitter with respect to the spectrometer (LCQ Deca, Thermo Scientific) inlet. Each movement to an emitter (X, Y, Z) position triggered MS data file acquisition. After conversion of RAW-formatted files to mzXML, a parsing and data visualization program (LabView, National Instruments) reconstructed data into a mass filtered ion current image map. Images were typically 2 x 2 mm with a pixel step size of 200 um. The acquisition of a 100 point (10 x 10) data set was on the order of 30 minutes. Signal from a sample containing a singly-charged low molecular weight drug (Buspirone) and a multiply charged peptide (Angiotensin) in aqueous-organic mobile phase, delivered by continuous infusion (340 nL/min) was acquired. Surprising differences in ion map intensity between the singly charged drug molecule (386 m/z), the triply charged peptide (433 m/z), and the doubly charged (649 m/z) ions were observed. Sampling at the edge of the plume showed a distinct relative increase in the population of the +3 ion. The data suggests that emitter position might be exploited in parameter control to selectively favor higher charged peptide ions.

V43-S1 Localization and Identification of Lipids by MALDI Tissue Imaging Using Ultra-High Resolution Mass Spectrometry

F. Salinas, J. Fuchser, M. Witt

Bruker Daltonics, Billerica, MA, United States

MALDI tissue imaging is a technique used extensively in the protein biomarker field. , However, in the low mass range up to m/z 1000 matrix clusters dominate MALDI spectra rendering specific analysis of small molecules difficult. Ultra-high resolution MALDI-Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometers can overcome that lack of specificity of lower resolution/mass accuracy technologies that is mandatory for small molecule imaging and identification. , FT-ICR (resolving power > 100,000) enables the identification of compounds directly based on high accuracy molecular weight determination providing a global analysis approach eliminating the need for morerestricted MS/MS analyses. Differentiating tissues by small molecules only a few mDa apart gives rise to tracking lipids directly in the tissue. Tissue slices were covered by MALDI matrix and mass spectra of different tissues

were acquired with a 9.4 T FT-ICR mass spectrometer equipped with switcheable ESI/MALDI ion source. MALDI images were acquired in positive and negative ion mode depending on tissue type and analytes of interest The 200 Hz smartbeam laser was focused to 40 µm spot size for image acquisition. The classification of different lipids with regard to headgroup and fatty acid composition was achieved based on high resolution high mass accuracy MS analysis alone. Some obtained lipid structures were additionally confirmed by in situ MS/MS analysis. This approach provides simultaneous high resolution images from a larger number of small molecules in a single dataset.

V45-S1 Automated Optimization of Triple Quadrupole Parameters for Nanoflow LC/MS of Peptides

S. Curtis

Agilent Technologies, Wilmington, DE, United States

Existing automated method development tools for triple quad MRM parameters optimization are based primarily on direct infusion of samples into a mass spectrometer ion source. For more complex samples and standard mixtures, ion suppression and adduct formation often accompany direct infusion methods and complicate the optimization routine. The optimization may fail for many compounds and results for successful ones are often far from optimum. This greatly limits the applicability and reliability of the results. This work explores an optimization routine designed specifically for peptides that allows for chromatographic separation, thus reducing these limitations and obtaining acquisition parameters very close to the optimum values that would be obtained by manual operation by an experienced user. The chromatography-based MRM parameters optimization was tested on synthetic peptides and on tryptic peptides from digestion of standard proteins. Acquisition parameter optimization focused on collision energy values as ion optic settings are generally the same for peptides. For each peptide tested, 3 transitions were preferred for each precursor with all transitions generally at a higher m/z than the precursor for optimal selectivity. For the synthetic peptides, 30 LC/MS analyses were done to examine collision energy in one-volt steps. Based on this, an automated routine was developed to optimize the predicted transitions for each peptide without operator intervention. A comparison of the automated and manual obtained optimization values will be presented for the selected peptides.

V47-S1 Specific Detection of Proteins by Immunoprecipitation Combined with High Sensitivity Protein Sizing on Microchips

C. Wenz, A. Ruefer

Agilent Technologies, Waldbronn, Germany

Today, immunoaffinity is a crucial tool for the targeted analysis of proteins in complex samples. Techniques like Enzyme Linked Immunosorbent Assay (ELISA) and Western Blotting are widely used for a wide range of applications such as biomarker candidate verification in body fluids or clone selection for recombinant protein expression. Here we present a new method that combines the specificity of an immunoprecipitation approach with the high sensitivity of protein detection on microchips using the High Sensitivity Protein 250 assay for the Agilent 2100 bioanalyzer. Initially, sample proteins are derivatized with a fluorescent dye. After incubation with the specific target antibody, the immunocomplexes are captured with Protein A/G coated magnetic beads, washed and eluted by heat denaturation in the presence of SDS. Samples are then directly loaded on microchips and analyzed automatically with the bioanalyzer for protein size and quantity. The final on-chip analysis takes about 30 min for 10 samples and yields digital data. Together with the sample preparation steps, the total assay time is about 3 hours. Results are compared to Western Blotting and pros and cons' of both methods are discussed.

V48-S1 Improved Peptide Identification with an Ultra High Resolution Quadrupole Time of Flight MS

M. Lubeck, C. Baessmann, M. Behrens, W. Jabs

Bruker Daltonik, Bremen, Germany

Q-TOFs are widely used working horses for proteomics due to their superior mass accuracy in MS as well as in MSMS, making them ideal instruments for de novo sequencing and PTM characterization. For peptide identification at very low concentrations or for highly complex samples, MSMS sensitivity and duty cycle become especially critical. A new designed ultra high resolution Q-TOF MS shows significant improvements for these types of samples. In contrast to small molecule applications where MSMS sensitivity is usually defined via signal to noise of a single fragment, peptide identification has additional requirements: Completeness of a fragmentation pattern is more crucial than optimized intensities for single masses. Thus generation of fragment ions covering a broad mass range and their efficient transfer from the collision cell into the TOF part of the instrument were optimized. Using a higher order multipole as collision cell and an additional ion cooler for guiding ions into the orthogonal acceleration showed a dramatic improvement for proteomics applications. As a model system for samples of high complexity, tryptic digests of E. coli, separated using a 90 min nanoLC gradient were used, leading to 7000-10000 fragment spectra and more than 600 significant protein identifications from 100ng sample. For single protein digests, analyzed with nanoLC, high sequence coverages could be obtained from sub fmol amounts.

Post-Translational Modifications

SR15-S1 Quantitative Phosphoproteomic Dissection of Signaling Pathways Applied to T Cell and Mast Cell Signaling

A.R. Salomon, V. Nguyen, L. Cao, K. Yu, N. Hung

Brown University, MCB and Chemistry Department, Providence, RI, United States

Here we introduce and evaluate a new quantitative approach for phosphoproteomic analysis of signaling pathway structure. Our approach combines genetic analysis of isogenic signaling pathway mutants with a quantitative phosphoproteomic method that examines disruption of downstream phosphorylation events through a time course of receptor activation using recently developed visual pathway analysis tools. This new approach is evaluated in the context of the T cell signaling pathway and a T cell clone lacking the upstream Zap-70 tyrosine kinase and its reconstituted counterpart. In our approach, label free quantitation using normalization to copurified phosphopeptide standards is applied to assemble high density temporal data within a single cell type, either Zap-70 null or reconstituted cells, providing a list of candidate phosphorylation sites that change in abundance after T cell stimulation. Metabolic labeling

of proteins using the SILAC method allows for the calculation of ratios used to compare Zap-70 null and reconstituted cells across a timecourse of receptor stimulation, providing direct information about the placement of newly observed phosphorylation sites relative to the critical T cell signaling protein Zap- 70. Quantitative phosphoproteomic signatures indicative of downstream inhibition, downstream activation, and mutant compensation are revealed and validated with proteomic pathway visual analysis tools using the established T cell signaling pathway structure as a scaffold. New predictions of the structure of T cell signaling pathway are provided at the phosphorylation site level. The approach described here seeks to transcend the publication of phosphoproteomic data with minimal biological analysis in favor of a targeted approach that positions the deluge of newly discovered phosphorylation sites relative to canonical signaling landmarks. Our methods are adaptable to any cell culture signaling system in which isogenic wild type and mutant cells have been or can be derived using any available phosphopeptide enrichment strategy.

SR16-S1 Microwave-Assisted Dephosphorylation (Beta-Elimination & Michael Addition) for Phosphoserine/threonine site mapping

P.S. Liu, W. Sandoval

Genentech, Inc., South San Francisco, CA, United States

Phosphorylation is a common post-translational modification (PTM), which can direct the activity and functions of proteins. The progression of many oncological pathways are dictated by kinase/phosphatase activity and phospho-specific events, hence phosphorylation mapping is currently one of the most active areas of proteomics research. Here, we expand on an alternative method for precise mapping of phosphorylation sites involving microwave assisted ß-elimination and Michael addition. Selective modification of these residues to more stable S-ethylcysteine (phosphoserine) and ß-methyl-Sethylcysteine (phosphothreonine) derivatives is a useful tool for determining the exact location of a phosphorylation site by Edman degradation. The nucleophile of choice was 1-propanethiol due to the fact that it was most compatible with our routine chromatographic separation on an ABI 494 Procise Sequencer. 1-propanethiol (76 Da) shows a net change of -22 Da (-98 Da from neutral loss of phosphoric acid and addition of 76 Da) after successful nucleophile incorporation. Traditionally the nucleophile derivatization reaction is performed in the water bath for 1 to 3 h at 60ºC. We have found that equivalent results may be obtained by microwave incubation for 2 min at 100ºC for protein samples in liquid or blotted on PVDF membrane. We demonstrate that derivatized protein on PVDF membrane can be subsequently Edman sequenced for mapping of specific phosphorylation sites, offering the investigator yet another option for elucidation of phosphorylation sites either near the N-terminus or internally after limited on-membrane enzymatic digestion.

Post-Translational Modifications

SR17-S1 TiOx Nanostructured Film MALDI Plate for Capture of Phospho- and Glyco-Peptides

G. Grigorean, R. Carbone, S. Vinati, P. Soffientini

Tethis s.r.l., Milan, Italy

Nanostructured TiO2 films were grown by depositing under high vacuum a supersonic seeded beam of ¬TiOx clusters produced by a pulsed microplasma cluster source (1), providing a porous specific substrate which enables selective capture of phosphorylated and glycosylated peptides. Tryptic digests of a 6-protein mixture containing phosphoprotein ?-casein and glycoprotein fetuin were used as analytes. Phospho-peptides were found to ionize best with alpha-cyano hydrocinnamic acid as a matrix, while the glycopeptide matrix was 2,5-dihydroxy benzoic acid. Several matrix solvents, wash and surface-elution solutions were tested.

Signal for the phospho- and glyco-peptides is increased as a result of steps of: deposition, capture, wash of non-specific peptides - all done in-situ on the TiO2 MALDI coated plate. For ?-casein, we observe the singly-charged 2061 Da phosphopeptide. For the fetuin, we observe sialylated peptides in the >3000 Da region. Ideal solution for matrix dissolving is one with lowest pH. Best wash solution for non-specific peptide and contaminant removal is one with highest acetonitrile content. Elution of TiO2 captured peptides by depositiong ammonia solution is not necessary. Film thickness range tested were from 50-200nm, with 200nm being the ideal. The TiO2 film was also tested for MALDI analysis of standard peptides used to calibrate the TOF/TOF. Performance was exactly as that of the stainless steel plate, showing that both the calibration and enrichment can be done from the same TiO2 coated plate. Results clearly demonstrate that the TiO2 surface concentrates both phosphopeptides and glycopeptides containing sialic acid from enzymatically digested proteins, along with allowing the same level of MALDI ionization for any peptide needed in calibrating the mass spectrometer. This eliminates need for (a) on-column separation/desalting prior to MALDI analysis (b) a separate MALDI plate for calibration.

SR18-S1 Application of In-gel IEF-LC-MS/MS to Human Phosphoproteomics

S. Beranova-Giorgianni, L. Chen, F. Giorgianni

University of Tennessee Health Science Center, Memphis, Tennessee, United States

This presentation will describe application of an in-gel IEF-LC-MS/MS analytical strategy for the characterization of human phosphoproteomes. Large-scale analysis of protein phosphorylation in vivo is a highly challenging undertaking that requires powerful analytical and bioinformatics tools. In our phosphoproteome research, we have recently adopted a new methodology that combines in-gel IEF, immobilized metal ion affinity chromatography (IMAC), and LC-MS/MS. The strategy encompasses seven steps: (1) extraction of proteins from the biological system under study; (2) separation of the protein mixture by IEF in an immobilized pH gradient (IPG) strip; (3) sectioning of the entire IPG strip; (4) digestion of the proteins in each gel section; (5) enrichment of

phosphopeptides by IMAC; (6) analysis of the enriched digests by LC-MS/MS; and (7) identification of the phosphopeptides/proteins through database searches, and assignment of the sites of phosphorylation in these proteins. The methodology relies on a proven IEF technology with commercially available IPG strips. Because the information about the apparent pI of the identified phosphoprotein is preserved, data from in-gel IEF-LC-MS/MS can be linked to 2D-PAGE data. In our work, we have applied in-gel IEF-LC-MS/MS to the study of the phosphoproteomes in two biological systems: 1. human pituitary tissue; and 2. LNCaP human prostate cancer cell line. In the analysis of the human pituitary, we have characterized a total of 50 phosphorylation sites in 26 proteins. The findings include discovery of novel phosphorylation sites in important pituitary hormones, including the growth hormone, proopiomelanocortin, and proteins of the secretogranin family. In the study of the LNCaP phosphoproteome, we have characterized over 600 different sites in 296 proteins. The characterized phosphoproteins are functionally diverse and include kinases, co-regulators of nuclear receptors, and proteins relevant to cancer. In summary, in-gel IEF-LC-MS/MS is a powerful analytical platform suitable for large-scale characterization of phosphoproteins in complex systems.

SR19-S1 Using Targeted Proteomics to Assess the Impact of Sirtuins on Liver Metabolism in Obesity

A. Kendrick[1], M. Choudhury[2], M. Rahman[2], J. Friedman[2], K. R. Jonscher[1]

[1]University of Colorado Denver, Department of Anesthesiology, Denver, CO, United States; [2]University of Colorado Denver, Deparment of Pediatrics, Denver, CO, United States

The NAD-dependent deacetylase SirT1 regulates lipid and glucose metabolism in liver and increased SirT1 activity in caloric-restricted models has been linked to extended life span in several species. Deacetylation of transcription factors and co-modulators, such as p53, NF-κB, C/EPBβ and PGC-1, allows SirT1 to sense and regulate energy levels. The SirT family can also deacetylate mitochondrial proteins, suggesting that posttranslational modification by sirtuins may have global effects on energy metabolism especially gluconeogenesis and lipogenesis. Livers from mice fed either control or a high fat (HF) diet (45 kcal % as fat, 12 wks) were harvested and homogenized. SirT1 levels were measured in whole cell lysates by Western blot. Proteins modified by lysine acetylation were immunoprecipitated with anti-acetyllysine antibody and subsequently separated by one dimensional gel electrophoresis. Bands showing differential staining between the control and HF fed mice were excised and proteins were digested with trypsin. Tandem mass spectrometry using an Agilent Ultra quadrupole ion trap generated product ion spectra that were searched with SpectrumMill against the SwissProt database. The levels and acetylation of identified proteins were validated by immunoprecipitation and Western blotting. Mice on the HF diet were obese, with fatty livers and reduced SirT1 activity as assessed by the NAD+/NADH ratio. SirT1 protein expression levels did not significantly change, however. Acetylation of a subset of the proteins identified, such as carbamoyl-phosphate synthase, uricase, pyruvate carboxylase and ATP synthase, has been previously reported. Interestingly, peroxiredoxin, catalase, and Hsp70, proteins involved in redox and the stress response, were hyperacetylated in the livers of obese mice. We postulate that *modification of those proteins could influence the ability of obese mice to modulate oxidative stress, gluconeogenesis and lipogenesis.* Globally surveying lysine acetylated proteins using immunoprecipitation and gel electrophoresis/tandem mass spectrometry provides insights into how obesity impacts liver metabolism.

V49-S1 Mapping of Disulfide Bonds in Salmon Egg Lectin 24K Using MALDI MS and MS/MS

F. Xiang[1], L. Zhao[2], A. Franz[2]

[1]Shimadzu Biotech, Pleasanton, CA, United States; [2]Department of Chemistry, Stockton, CA, United States

The carbohydrate-binding properties of lectins and their lack of enzymatic activity make this class of compounds "biological adhesives" of great importance. Lectins are invaluable for cell-cell and cell-matrix interactions. Lectins also play significant roles in the fertilization of eggs in higher animal species. There is mounting evidence that egg lectins provide a block to polyspermy via binding to glycoprotein ligands. Block to polyspermy is vital to ascertain proper procreation of species. Because of its superior sensitivity, mass spectrometric detection of peptides has become one of the most popular analytical methods in proteomics. Mass spectrometry allows not only the detection of peptides in minute quantities, but also the structural analysis of post-translational modifications such as phosphorylation, glycosylation, and oxidative disulfide bond formation. The disulfide bond pattern in the salmon egg lectin (SEL24K) from the Chinook salmon *Onchorhynchus tshawytscha* is presented. The disulfide bond pattern was established with a multi-enzyme digestion strategy in combination with MALDI-MS & MS/MS mass spectrometry. The disulfide bond pattern was found to be symmetrical in the tandem repeat sequence of SEL24K and is fully consistent with symmetrical bond patterns found in egg lectins from closely related fish species with highly conserved locations of cysteines. All cysteine residues were disulfide linked.

V50-S1 Chip-Based Enrichment and Identification of Phosphopeptides

N. Tang, M. Vollmer, K. Kraiczek, C. Miller, K. Waddell

Agilent Technologies, Santa Clara, CA, United States

Comprehensive identification of protein phosphorylation is a challenging application in the proteomics field due to the complexity and low abundance of phosphorylation events. Researchers have tried various enrichment approaches to simplify and enrich the phosphopeptides before analyzing with LC/MS/MS. Recent advances in microfluidic technology have provided an opportunity to allow easy automation of phosphopeptide enrichment and subsequent analysis of phosphopeptides. We have developed a re-usable microfluidic chip with an enrichment column consisting of titanium dioxide particles sandwiched between reverse-phase materials for on-line selective phosphopeptide enrichment. This approach allows robust, easy-to-use and reproducible enrichment of phosphopeptides from complex matrices. The chip also gives the user options of analyzing the unbound peptides. Specific enrichment of phosphopeptides from Casein, MAP kinases and complex biological samples will be demonstrated.

V51-S1 N- and O-Deglycosylation of Glycoproteins with Subsequent Proteomic Analysis

J. Boland, B. Xia, J. Irungu, G.R. Nicol, J.G. Dapron

Sigma-Aldrich Biotechnology, St. Louis, MO, United States

Glycoproteomics is an expanding field with many challenges. There are several techniques available for deglycosylation to allow the study of the glycan and protein separately. Unfortunately, these methods don't always result in both glycan and protein that are suitable for analysis. It is possible, using PNGase F, to release and analyze N-linked glycans, then do further proteomic analysis of the protein. However, if one is interested in O-glycosylated proteins and their glycans, the most common O-glycan release technology, alkaline beta-elimination, usually destroys the parent protein rendering further proteomic analysis impossible.

We present a method that allows for the separate release of N- and O-glycans, leaving the parent protein relatively undamaged. The fully deglycosylated protein can then be tryptically digested for peptide analysis by LC-MS/MS. This method is unique in that it leaves the glycans and the protein intact for analysis after beta-elimination. The control proteins fetuin and glycophorin A were deglycosylated with PNGase F to release the N-glycans. After the N-glycans were isolated, the proteins were O-deglycosylated using a proprietary, non-reducing beta-elimination reagent. The O-linked glycans were then separated from the proteins using standard techniques. The N- and O-linked glycans were permethylated using a solid phase permethylation protocol and analyzed by MALDI-MS. The deglycosylated proteins were tryptically digested and the peptides analyzed by LC-MS/MS. Glycans were analyzed and identified as the known structures from the proteins. Sequence coverage of the deglycosylated protein was as good as or better than the analysis performed on the intact glycoprotein, proving preservation of the proteomic value of the sample. This analytical workflow can be reversed, such that the O-glycans may be released first, followed by the N-glycans with subsequent proteomic analysis. We will illustrate the analysis of both the N- and O-glycans as well as the peptides generated from the deglycosylated proteins.

Protein Production and Characterization

SR21-S2 Qualification of a Sensitive Assay for Determination of Norleucine Using Post-Column Amino Acid Separation and Ninhydrin Detection

S. Chamberlain, J. Kwong, A. Cordoba, P. Motchnik

Genentech, Inc., South San Francisco, CA, United States

Norleucine (Nle) misincorporation for methionine, which occurs in E. coli expression systems, requires an assay which is sensitive, robust and specific. *De novo* biosynthesis of leucine in bacterial expression systems leads to a minor amount of norleucine production, particularly in leucine-depleted fermentations (1). Since the side chain of Nle is similar enough to methionine (Met), some of the $tRNA^{Met}$ will be acylated by norleucine, leading to Nle incorporation at Met positions (2). Amino acid analysis is one of the few easily adapted methods which can detect norleucine at trace levels. We have developed an amino acid analysis method which detects norleucine at approximately the 10 pmol level and is capable of quantification at the 30 pmol level. A detailed description of the method qualification including controls, system suitability, accuracy, and precision will be provided.

SR22-S2 Vertebrate Courtship Pheromones: Assembly PCR for Codon-Optimized Expression of a Highly Disulfide-Bonded 7 kDa Protein in Pichia Pastoris

D.B. Wilburn, K.E. Bowen, K.A. Leichty, P.W. Feldhoff, R.C. Feldhoff

Department of Biochemistry, School of Medicine, University of Louisville, Louisville, KY

Plethodon shermani is a species of salamander native to the mountains of western North Carolina that exhibits a complex courtship ritual known as "Tail Straddling Walk." The courtship enables the male to deliver non-volatile proteinaceous pheromones to a female's nares that bind to receptors in the vomeronasal organ, thus increasing her receptivity to mate and reducing courtship time. The pheromone extract is composed of two major components: a 22-kDa family called "Plethodontid Receptivity Factor" related to IL-6 cytokines and a 7-kDa hypervariable family known as "Plethodontid Modulating Factor" (PMF) that contains 4 disulfide bonds and is related to snake venom cytotoxins and other three-finger proteins. Three-finger proteins represent a complex group of highly disulfide-bonded proteins serving a plethora of biological functions; however, all reported attempts at recombinant bacterial expression have resulted in disulfide bond scrambling and dimerization. The aim of this study was to produce and characterize a biologically functional, recombinant form of PMF using the methylotrophic yeast *Pichia pastoris*. A two-step assembly PCR was performed to produce a codon-optimized PMF gene for *Pichia*. The gene was cloned into a vector containing the *Saccharomyces* α-mating type secretion signal and transformed into chemically competent *Pichia pastoris* cells. Expression was performed in shaking culture at 28° C using 1% methanol for induction. Recombinant proteins were purified by AX- and RP-HPLC and characterized by mass spectroscopy. Of the multiple conformations of rPMF that were produced, one was identical to the native PMF. These inexpensive and straightforward protocols may be appropriate for a variety of disulfide-bonded proteins and peptides.

SR23-S2 Protocol Development for Protein Extraction from Sediment Bound Microbes for Bioremediation Purposes

C. Nicora, M. Lipton, J. Turse, A. Norbeck, R. Smith

Pacific Northwest National Laboratory, West Richland, WA, United States

Sediments contaminated with heavy metals pose a significant risk to human health and the environment. Remediating these contaminated sediments has historically been difficult due to the cost and labor involved. Bioremediation offers an approach for cleaning up pollutants by enhancing the same biodegradation processes that occur naturally. For example, certain sediment microbes have the capacity to reduce heavy metals into a non-soluble form which prevents them from leaching into waterways. Characterizing these sediment microbes in terms of protein expression provides information needed to enhance microbial nutrient factors for reducing heavy metals and to better understand the unique biological pathways that make these remediation processes possible. Rifle, Colorado is home to a Uranium Mill Tailings Remedial Action (UMTRA) site where these processes are being studied. A significant challenge in studying microbe proteins from the UMTRA site is their initial removal from sediment particles. Aspects such as the isoelectric point (pI) of the proteins, surface properties of the highly variable sediment particles, biofilms, electrostatic and hydrophobic interactions, and van der Walls and entropic forces among others all play a major role in achieving effective protein removal for proteomic analysis. We will be presenting results from ongoing investigations of various methods for removing microbes specifically from Rifle sediment with special emphasis on methods that are compatible with tryptic digestion and mass spectrometric analysis. These methods include treatments with amino acids and metal oxides to block binding sites of the protein prior to lysis, salts to weaken cross-linking electrostatic interactions, chelating agents to break cross-linking multivalent cations, surfactants to disrupt hydrophobic interactions, and chaotropic agents to desorb proteins from sediment by breaking hydrogen bonds.

SR24-S2 Analysis of the C-terminal Amino Acid Sequence, Glycosylation Site and Disulfide Bond Pattern of Recombinant ISU302 by MALDI-TOF MS and Edman Sequencing

M. Hee Nam[1], J. Hee Kim[1], S. Ho Kim[2], H. Hee Park[2], S. Hyun Kim[3]

[1]Korea Basic Science Institute, Metabolome Analysis Team, Seoul, Korea; [2]ISUABXIS, Seoul, Korea; [3]Korea Basic Science Institute, Life Science Research Group, Daejeon, Korea

Glucocerebrosidase is an enzyme that catalyze the cleavage of the ?-glycosidic linkage of glucosylceramide. The deficiency of the enzyme in patients with Gaucher disease results in the accumulation of excessive quantities of glucosylceramide in tissues of patients. Enzyme replacement therapy with recombinant glucocerebrosidase is the mainstay of treatment for Gaucher disease. Here we described the analysis of the disulfide bond pattern, glycosylation site and C-terminal amino acid sequence analysis of recombinant glucocerebrosidase, ISU302. The analysis of the N-glycosylation site was performed by tryptic digestion of the protein and mass measurement before and after enzymatic deglycosylation. We identified four glycosylation sites. It was reported that human acid ?-glucocerebrosidase has two disulfides in N-terminal position. We used endoproteinase Glu-C digestion to analyze the N-terminal disulfide bonds. N-terminal and C-terminal fragments were isolated by HPLC and MALDI-TOF MS analysis from endoproteinase Glu-C digests. The isolated N-terminal peptide was chemically modified with or without addition of DTT. This analysis revealed that the first four cysteins located in N-terminal of the protein form two disulfide bonds. C-terminal amino acid sequence was analyzed by Edman sequencing from isolated C-terminal peptide.

V52-S2 A New Algorithm for the Analysis of Intact Proteins

K. Waddell, D. M. Horn, C. A. Miller, X. Li

Agilent Technologies, Santa Clara, CA, United States

Currently, the most common algorithm for intact protein mass determination is maximum entropy deconvolution. This method transforms a mass spectrum in m/z units into a mass spectrum containing the zero-charge representation of the intact protein (in Dalton units). Complex data produces false positive "overtone" peaks, which correspond to masses calculated from randomly dispersed peaks from the raw data. Also, if proteins do not perfectly coelute, it is difficult to choose one or more averaged spectra from the chromatogram that produces high quality results for these multiple proteins. Here we describe a new algorithm called "Large Molecule Feature Extraction" (LMFE) for the determination of the masses of large molecules in complex mixtures. This approach first produces extracted ion chromatograms for all peaks in the raw LC/MS data and subsequently groups the peaks with the same retention time and elution profile into "coelution groups". The peaks within a given coelution group will contain the different charge states of the same protein, which are subsequently grouped together by algebraic charge state deconvolution. While algebraic deconvolution is generally untenable for very complex spectra, the coelution grouping greatly simplifies the peak spectra that are used during algebraic deconvolution making the process much more robust. For a mixture of all E. coli cytosolic proteins, the base peak chromatogram was integrated and peak spectra extracted from each integrated peak. Subsequently, maximum entropy deconvolution was performed on the 90 averaged spectra using a mass range of 6 to 70 kDa. This required 90 minutes of analysis time and resulted in 140 protein compounds. Using LMFE, 682 protein masses were found in 15 minutes, which is almost 5 times as many compounds in 1/6th the amount of time. Thus, for complex mixtures, LMFE greatly outperforms maximum entropy deconvolution.

V53-S2 Protein N-terminal Sequencing by Mass Spectrometry for Antibody Characterization

J. Walters, K. Ray

Sigma-Aldrich, Fenton, MO, United States

To develop a mass spectrometry based method which can reliably characterize the N-terminus of antibodies (and proteins in general) in a simple mixture, including determining endogenous N-terminal modifications, and distinguishing minor N-terminal variants. We blocked all primary amines with D_3-acetylation in gel separated proteins. This was followed by enzymatic digestion, peptide extraction, primary amine capture (when necessary), LC-MS/MS, data base search, and manual verification of data to identify any free and modified N-termini in the gel band. We started with two standard proteins, one known to have a free, unmodified N-terminus (Bovine serum albumin, BSA) and one that is known to have an acetylated N-terminus (Bovine Carbonic Anhydrase II, CAII) to optimize the labeling and digesting chemistries. The goal of the method is to have every protein N-terminal peptide labeled and that this peptide be the only labeled peptide in the post-digestion mixture. By labeling with heavy (D_3)-acetylation we can tell the difference between endogenously and chemically labeled N-termini. We successfully identified both the D_3-acetylated N-terminal peptide of BSA and the endogenously H_3-acetylated N-terminus of CAII. This method was then applied to the light and heavy chains of a gel separated antibody and data compared to Edman degradation of both chains. Edman degradation identified a single N-terminal species for both the light chain (pyroQ/E removed then SALTQPRSV) and heavy chain (EVQLVESGGG). These results corresponded to the LC-MS/MS analysis of the tryptic peptides of the light chain (pyro-QSALTQPR) and heavy chain (D_3-acetylated-EVQLVESGGGLVQPGGSLR). In addition, MS analysis identified other lower level N-termini in both samples. In the light chain, two other termini were identified, D_3-acetyl-SWAQSALTQPR and D_3-acetyl-SALTQPR. In the heavy chain, low levels of pyro-EVQLVESGGGLVQPGGSLR were observed. This method readily confirms the N-termini of gel purified antibodies/proteins and can distinguish endogenously modified N-termini from chemically modified N-termini. In addition, he can detect lower abundant N-termini, a clear advantage over Edman sequencing.

V54-S2 Rapid Screening of Protein Aggregates and Protein Impurities

H. Hedlund, I. Salomonson, J. Zou, M. Carlsson, A. Bergh, F. Lundström

GE Healthcare Bio-Sciences AB, Uppsala, Sweden

Gel filtration (GF) is an excellent tool to acquire information about sizing (identity), purity and specially the multimeric state of a protein of interest. To speed up analysis and keep sample and buffer consumption at minimum, prepacked gel filtration columns of 3 ml (5 mm diameter x 150 mm length) has been used. These columns make it possible to run rapid analysis (6-12min/run) with minimal sample (4-50µl) and buffer consumption. Results from a rapid screening of optimal conditions for HIC purification of antibody to minimize dimer and higher aggregate content, assessment of integrity of a recombinant protein and fast purity check of two different *Strep*-tag II proteins will be presented.

V55-S2 Top-down Protein Characterization by ETD/PTR

A. Schneider, M. Lubeck, R. Hartmer, C. Baessmann, A. Ingendoh

Bruker Daltonik GmbH, Bremen, Germany

Conventional collision induced dissociation (CID) has drawbacks in terms of a limited applicable molecular weight and the dependence of the fragmentation efficiency on the individual bond strength and amino acid sequence. Electron transfer dissociation (ETD) and its related proton transfer reaction (PTR) are alternative ways of fragmenting peptides and proteins, mostly applied in ion trap systems. ETD became the preferred method for the analysis of common post-translational modifications (PTM) in proteins. Reversible phosphorylation is known to be one of the most important functions in eukaryotic cells, but its detection and characterization is often difficult. In most cases the conventional CID results in the neutral loss of phosphoric acid, resulting in missing information about its binding site and sometimes in low further fragmentation of the peptide chain itself. ETD keeps the labile PTM bonds intact, in contrast, by generating a prompt dissociation at the amino acid backbone and allows therefore the facile localization of the PTM. For fragmentation via electron transfer dissociation, an excess of radical anions - generated in a negative chemical ionization source – is added to multiply charged peptide cations in the ion trap. PTR is a most useful addition for the fragmentation of larger peptides or even small proteins in the top-down or mid-down approach. Intact proteins can be identified and/or sequenced without any prior enzymatic digestion. While ETD is still used for the dissociation itself, the PTR anions reduce the charge states of the highly charged fragments into "ion trap readable" numbers. Presented here will be PTM elucidation of peptides and intact proteins in mixtures. N-terminal sequencing of intact proteins allows the direct identification by database search. An interesting model for the usefulness of these techniques is, e.g. histones whose biological function strongly depends on the attached modification.

V56-S2 Top-Down Localization of PEGylation Sites of Therapeutic Peptides by MALDI-ISD

R. Schäfer[1], D. Suckau[1], C. Yoo[2], V. Sauerland[1], M. Ronk[2], M. Ma[2]

[1]*Bruker Daltonik GmbH, Bremen, Germany;* [2]*Analytical R&D, Amgen Inc., Thousand Oaks, CA, USA*

In this study, a new approach to localize PEGylation sites is described that utilizes Matrix-Assisted Laser Desorption/Ionization In-Source Decay (MALDI-ISD) mass spectrometry. The sample was a 31mer peptide (3.5 kDa) with blocked N- and C-termini. It was conjugated to 20 kDa PEG for use as a therapeutic peptide (Amgen, disclosed under WO 2007/048026). Peptide and conjugate were prepared with sinapinic acid matrix in a seeded crystallization. The native as well as the conjugated peptide were analyzed using MALDI-ISD in reflector mode on an ultraflex III MALDI-TOF/TOF instrument (Bruker) as described in detail in. The free peptide provided sequence information for large parts of its sequence based on intense y, z+2 and c ions. Without precursor ion selection, the ISD spectrum allowed to confirm its intended structure with only Lys19 exposing a reactive epsilon-amino group as target structure for conjugation of the PEG-aldehyde. The peptide conjugate provided c- and y-ion series that were truncated excluding Lys19 from any sequence readout very clearly, indicating Lys19 as the proper substrate to the conjugation agent. This is remarkable, as an MS/MS analysis with its precursor ion selection was not possible by any method due to PEG oligomers of similar size. MALDI appeared advantageous in analyzing highly heterogeneous PEGylated peptide drugs with its ability to determine the site of PEGylation. No chemical or enzymatic steps were involved, providing for the high throughput that is beneficial for possible routine use.

Protein Sequencing

SR25-S2 Determining the N- and C-terminal Peptides of a Protein with Mass Spectrometry Via Isotopic Labeling

X. Yang[1] & T. Thannhauser[2]

[1]*Cornell University, Ithaca, NY, United States;* [2]*RWH Center for Agriculture and Health, USDA-ARS,Ithaca, NY, United States*

We have developed a method to determine the N- and C-terminal peptides of unknown proteins. This method is based on mass spectrometry, chemical modification and differential isotopic labeling. Once identified, the N and C terminal peptides are subjected to MS/MS analysis so that their amino acid sequences can be obtained through de novo sequencing protocols. This method will, when coupled with molecular biology techniques, allow us to discover the gene sequence of an unknown protein, even in the absence of a sequenced genome. Furthermore this approach will be particularly useful as a means to empirically annotate genomes.

V57-S2 MALDI Top-Down Sequencing: Calling N- and C-terminal Protein Sequences with High Confidence and Speed

D. Suckau

Bruker Daltonics, Bremen, Germany

This study describes the analysis of the 2 samples provided by ABRF-ESRG 2009 using Top-Down Sequencing on a MALDI-TOF/TOF (MALDI-TDS). Samples (50 pmol) were prepared using sDHB matrix and run on a MALDI-TOF/TOF (Ultraflex III, Bruker) by in-source decay. Reflector mode ISD spectra (reISD) were directly analyzed by database searching using Mascot. Only in dedicated cases, such as analyzing unique fusion sites, interactive or automatic de-novo sequencing was added to the simple standard procedure. Both proteins (~ 40 kDa) provided sequence calls from the N-terminus and the C-terminus from the same dataset permitting their identification. Up to 70 sequence calls from either terminus were obtained and, in addition, point mutations/ database errors were obtained. c-type ions or y-type ions were used to call protein N-termini and C-termini, respectively. ADH1_YEAST and G3P_RABIT were identified in the study. As reISD spectra allowed monitoring the termini themselves only indirectly by the position of sequence tags on the mass axis, T^3-Sequencing [2] was used to directly confirm the protein sequences of the N-termini. MALDI-TDS appears to be very useful for the N-terminal and C-terminal sequencing of proteins. Even a blocked N-terminus does not prevent obtaining an N-terminal sequence as the sequence ladder is not generated by a sequential chemical reaction requiring a free ?-amino group, but rather by prompt reactions at the site of electronic excitation in the MALDI ion source. It matches Edman sequencing in some regards and even adds significant analytical capabilities.

V58-S2 In-tact Protein N-terminal Sequencing by Mass Spectrometry; Alternatives to Edman Degradation

I. Mohtashemi, Z. Hao, A. Huhmer

Thermo Fisher Scientific, Walnut Creek, CA, United States

N-terminal sequencing remains a robust and reliable tool to identify proteins, determine expression dynamics in recombinant systems and implement quality control for signal peptide processing and protein degradation. Despite the fact that the traditional Edman technique is very robust and provides de novo capabilities the method suffers from several limitations namely, throughput, sensitivity, cost and specific data interpretation expertise. Mass spectrometry has been utilized as an alternative to Edman sequencing and results have thus far been variable. Most of these mass spec-based methods utilize the bottom-up approach in which the proteins are reduced/alkylated and digested into peptides prior to analysis using the mass spectrometer. However, these labor-intensive methods also have limitations in terms of digestion efficiency and efficient capture of the N-terminal peptides using various isolation methods such as immunoaffinity chromatography. In this work, we present data on N-terminal sequencing using top-down approaches in which the protein is directly infused into the mass spectrometer and fragmented via electron transfer dissociation (ETD). Using the appropriate ion/ion reaction conditions, charge state-reduced species are prominently evident, however a consecutive series of c-ions are obtained by ETD that provide unambiguous sequence determination of the protein N-terminus with minimal sample manipulation. Data from the ABRF ESRG samples will be presented.

V59-S2 Protein N- and C-terminal Sequencing Using Electron Transfer Dissociation Mass Spectrometry

Z. Hao, A. Huhmer, R. Biringer

Thermo Fisher Scientific, San Jose, CA, United States

Mass spectrometry has drawn more and more attention as an alternative technology to traditional protein N-, as well as C-terminal sequencing. Electron transfer dissociation (ETD) mass spectrometry is particularly advantageous for sequencing applications because ETD is relatively insensible to the size, the amino acid composition and post-translational modifications of proteins, therefore randomly cleaves protein or peptide backbone bonds. ETD of intact proteins performs with high efficiency, generating very informative, yet extremely complex spectra which contain highly charged product ions that are difficult, or even impossible to resolve at unit resolution. ETD was recently implemented in a hybrid linear ion trap - orbitrap mass spectrometer facilitating the analysis of intact proteins using ETD with its high resolution and accurate mass capabilities. For unit resolution instruments, proton transfer reaction (PTR) following ETD was developed to reduce spectral complexity. PTR removes protons from multiply charged product ions, generating a simplified spectrum that contains product ions of resolvable charge states at unit resolution. In this study, ETD was applied to protein N- and C-terminal sequencing both in hybrid linear ion trap and in unit-resolution linear trap. ETD with accurate mass and high resolution was employed to study optimized reaction conditions for protein N- and C-terminal sequencing. Our results using intact proteins ranging in size from 8 kDa to 46 kDa indicated that optimized ETD reaction time for N- and C-terminal sequence coverage was longer than that for maximum protein sequence coverage. Extended ETD reaction leads to near complete N- and C- terminal sequence covering 30 to 50 amino acids while the overall protein sequence coverage decreased. The optimized ratio of analyte to ETD reagent was also investigated. In a unit-resolution instrument, PTR following ETD of intact proteins up to 30 kDa increased N- and C- terminal sequence coverage when compared to ETD alone.

V60-S2 Straight Protein QC by Benchtop MALDI-TOF Top-Down Sequencing

A. Resemann

Bruker Daltonik GmbH, Bremen, Germany

We describe for the first time the straight identification/ sequence validation of intact proteins by MALDI top-down sequencing with a benchtop MALDI-TOF. A routine application for protein sequencers is the validation of proper protein structures, in particular from recombinant work. We show that a small benchtop MALDI can actually provide meaningful N- and C-terminal sequence information from undigested proteins. Protein samples (ABRF-ESRG 2009 study samples 1 and 2) were mixed in sDHB or SA matrix and approx. 20 pmol were applied to the MALDI steel target. A benchtop MALDI-TOF (microflex LRF, Bruker) with N2 laser was used in reflector mode to acquire in-source decay spectra. Protein identification by standard MS/MS search using

Mascot 2.2 (Matrix Science). The proteins in the study (~40 kDa) were identified as ADH1-YEAST and as G3P_RABIT. The latter protein was identified as fusion protein with an N-terminal His-tag beta-Gal Vector. Uniquely in this approach, a single reflector mode ISD spectrum of the undigested protein as provided by the organizers was used for the analysis of each sample. Typically the near terminal fragments were monitored by ISD (residues: ~10-50 from either terminus). However, a match of the retrieved protein sequence to the ISD dataset confirmed the proper sequence of the terminal residues although they were not observed on a residue by residue base. A straightforward, quick and cheap approach to QC isolated proteins — recombinant and natural alike – was described and demonstrated. The approach utilized a benchtop MALDI-TOF and standard sequence database searching. For QC type problems with existing sequence candidates, the sequencing requirement would be dwarfed to merely match the sequence and the experimental ISD fragments — a matter of seconds.

V61-S2 MALDI-ISD-FTICR MS for Rapid Top-Down Analysis of Protein Termini

S. Fu, J. Fuchser, M. Witt

Bruker Daltonik, Bremen, Germany

For the first time, MALDI-ISD-FTMS MS was used for Top-Down protein characterization. Fast and reliable assignment of N- as well as C-terminal protein sequences is an increasingly important task in protein biochemistry for, e.g., quality control of recombinant proteins, and even within the field of proteomics. Here, we introduce MALDI-Top-Down-Sequencing on an FTMS instrument, on which so far only the ESI-based ECD fragmentation has been introduced as Top-Down analysis method. Matrix-Assisted Laser Desorption/Ionization In-Source Decay (MALDI-ISD) has been shown to efficiently produce c- and z+2-type ions for rapid analysis of both termini of a protein. The workflow consisted of MALDI target preparation, MALDI-ISD mass measurement at slightly elevated laser fluence and subsequent data analysis. Time-consuming digests or LC separations were not involved. Database searches (Mascot) were triggered with low ppm mass tolerances providing ADH1 and G3P identities with respective sequence calls from the ABRF-ESRG 2009 study samples. Using Fourier Transform Ion Cylclotron Resonance Mass Spectrometry (FTMS) for protein identification and assignment of protein termini provided unambiguous assignment of C- and N-terminal sequences. Uniquely, due to the low ppm mass tolerances used in this approach, even Q. vs. K were safely assigned from undigested proteins. MALDI-ISD-FTMS is a new technique complementary to classical ESI-ECD, which rapidly identifies proteins with high confidence calling on their terminal sequences even from 40 kDa and larger proteins.

Proteomics / Protein Sample Preparation

SR26-S2 Resolution and Quantification of Protein Isoforms in Complex Genomes

D.B. Weatherly[1], J. Atwood III[1], R. Tarelton[2], X. Zhu[2], R. Orlando[2]

[1]BioInquire, Athens, GA, United States; [2]University of Georgia, Athens, GA, United States

One of the difficulties in analyzing the results of shotgun proteomics experiments is the differentiation of protein isoforms from the identified peptides. This problem arises when the sequence database of the organism of study contains many similar (but unique) proteins, either because the genome contains related families of genes or because the database was constructed from different sequenced isolates and thus contains many gene isoforms. In this work we demonstrate how to effectively resolve protein isoforms in complex proteomic analyses by combining molecular weight information, protein grouping, peptide filtering and set logic. To illustrate this approach, a proteomic analysis was performed on membrane proteins isolated from Trypanosoma cruzi (T. cruzi, blood stream form trypomastigotes. T. cruzi is the causative agent of human Chagas disease and with over 30% of its genome being comprised multi-copy gene familes, the proteome is highly redundant and difficult to discern by traditional proteomic methos. Using this approach, 2601 total proteins were identified, including validation of expression for greater than 700 large gene family members.

SR27-S2 N-Terminal Enrichment — Developing a Protocol to Detect Specific Proteolytic Fragments

A.A. Schepmoes, Q. Zhang, B.O. Petritis, W. Qian, R.D. Smith

Pacific Northwest National Laboratory, Richland, WA, United States

Proteolytic processing events are essential to physiological processes such as reproduction, development, and host responses, as well as regulating proteins in cancer; therefore, there is a significant need to develop robust approaches for characterizing such events. The current mass spectrometry (MS)-based proteomics techniques employs a "bottom-up" strategy, which does not allow for identification of different proteolytic proteins since the strategy measures all the small peptides from any given protein. The aim of this development is to enable the effective identification of specific proteolytic fragments. The protocol utilizes an acetylation reaction to block the N-termini of a protein, as well as any lysine residues. Following digestion, N-terminal peptides are enriched by removing peptides that contain free amines, using amine-reactive silica-bond succinic anhydride beads. The resulting enriched sample has one N-terminal peptide per protein, which reduces sample complexity and allows for increased analytical sensitivity compared to global proteomics. We initially compared the peptide identification and efficiency of blocking lysine using acetic anhydride (a 42 Da modification) or propionic anhydride (a 56 Da modification) in our protocol. Both chemical reactions resulted in comparable peptide identifications and ~95 percent efficiency for blocking lysine residues. However, the use of propionic anhydride allowed us to distinguish *in vivo* aceylated peptides from chemically-tagged peptides. In an initial experiment using mouse plasma, we were able to identify >300 unique N-termini peptides, as well as many known cleavage sites. This protocol holds potential for uncovering new information related to proteolytic pathways, which will assist our understanding about cancer biology and efforts to identify potential biomarkers for various diseases.

SR28-S2 IVICAT for the Masses: An Improved Technique for Permethylation of Peptides

J. Farmar, H. Nika, F. Che, L. Weiss, R. Angeletti

Albert Einstein College of Medicine, Bronx, NY, United States

To determine the levels of post-translational modifications of parasite proteins, in particular those of Toxoplasma gondii and Cryptosporidium parva, we needed a quantitative technique that would allow comparison of the amounts of acetylated versus mono-, di and tri-methylated lysines

in histones and other proteins. Recently, several laboratories determined relative quantities of peptides using iodomethane and deuterated iodomethane and showed derivitization of peptides to give a trimethylated amino terminus and/or trimethylated lysines.(1) These trimethylated peptides showed increased signal intensity in the mass spectrometer. The power of this approach, In Vacuo Isotope Coded Alkylation Technique (IVICAT), is offset by its technical difficulty: a hot torch must be used to seal the glass sample tube while it is under vacuum and cooled by liquid nitrogen. Also, the pH of the peptide solution has to be carefully adjusted prior to lyophilization. Using model peptides and proteins, we have simplified and improved the technique by employing a glass screw cap vial equipped with an inert cap and valve. A standard lyophilizer served as the vacuum source. We were able to use lower amounts of peptides and react multiple peptide samples within the same vial. We permethylated with high recovery lyophilized peptides, rotary concentrated peptides and peptides bound to reverse phase chromatography media packed into pipette tips.(2) Several aspects of the chemistry and mass spectrometry of the permethylated peptides will be presented. Our improved technique makes IVICAT accessible to most proteomics laboratories and may be used to provide more complete sequence coverage of proteins and proteomes in addition to quantitation. This work was supported by NIH/NIAID Contract HSN266200400054C.

SR29-S2 Searching for Efficient and High-Throughput Alternatives for Essential Sample Preparation Techniques in Mass-Spectrometry-Based Functional Proteomics

A.R. Ivanov, E.M. Freeman, Y. Margolin

Harvard School of Public Health, Boston, MA, United States

Good control, efficiency, and reproducibility of protein extraction from cells and tissues are essential for diverse biological and basic research applications. Effective and specific proteolytic digestion of proteins prior to mass spectrometry (MS) analysis is one of the fundamental techniques most commonly used in any proteomics laboratory. However, neither routine procedures for cell and tissue lysis nor for in-solution and in-gel protein digestion have been significantly altered in common practice for over a decade. Here, we have tested and optimized several alternative techniques for preparing lysates of mammalian cells, as well as in-solution and in-gel enzymatic digestion compatible with downstream qualitative and quantitative MS-based proteomics applications. Specifically, we used alternating hydrostatic pressure (Pressure Cycling Technology, or PCT) and specialized organic solvents for disruption of cells, micelles and membrane fragments and efficient protein recovery from cultured cells. Using high performance LC-MS analysis, we have tested the role of pressurization, organic solvents, chaotropic agents, reducing reagents, enzyme/substrate ratios, and incubation time on efficiency, selectivity, and throughput of proteolytic digestion. The tests were performed using a mixture of protein standards and the most effective conditions were applied to HepG2 cell lysates. The optimized conditions for pressure-assisted in-solution digestion were adapted to in-gel digestion of 1D-PAGE fractionated HepG2 lysates and protein identification results were compared to those acquired using optimized digestion conducted at atmospheric pressure. Application of PCT resulted in significant improvement of throughput and reproducibility of sample preparation for proteomic analyses. Superior extraction rate for cytosolic and membrane associated proteins, as well as for proteins related to many other gene ontology terms, biological pathways and interaction networks, were observed at pressure-assisted sample preparation.

V62-S2 Comparison of Cellular Phosphoprotein Responses with High Quantitative Confidence Using Pulsed High Pressure Proteolytic Digestion and Automated Phosphopeptide Enrichment

R. Biringer[1], M. Rosenblatt[2], D. Hughes[2], K. Rampalli[2], A. Hühmer[1], J. Rogers[2]

[1]Thermo Fisher Scientific, San Jose, CA, United States; [2]Thermo Fisher Scientific, Rockford, IL, United States

The analysis of the regulatory elements and dynamics of cellular signaling events present multiple challenges. Proteomic approaches to understanding signal transduction provide an unbiased and highly comprehensive analysis of global events. However, phosphoprotein expression profiling is often limited by the technical difficulty and reproducibility associated with sample preparation. We have treated A431 and HeLa cells with growth factors in the presence and absence of kinase inhibitors or siRNA. Protein from these cells was extracted and digested (with multiple proteases) using a novel pulsed high-pressure lysis apparatus. Peptides were then labeled with Tandem Mass Tags (TMT), desalted, and then fractionated using strong cation exchange (SCX) chromatography. Automated Phosphopeptide enrichment was performed using magnetic TiO2 beads. We have used these protocol enhancements to identify, quantify, and profile phosphorylation sites across technical replicates with good reproducibility. These results demonstrate the ability to quantitatively profile complex phosphoprotein mixtures in different cancer types with statistical confidence using a combination of protocol and reagent enhancements.

V63-S2 A Multi-Laboratory Study Assessing Reproducibility of a 2D-DIGE Differential Proteomic Experiment

A. Borthwick[1], M. Wells[1], J. Bech-Serra[2], N. Colomé[2], ProteoRed[3], J.P. Albar[4], F. Canals[2]

[1]Nonlinear Dynamics, Newcastle upon Tyne, UK; [2]Vall d'Hebron University Hospital Research Institute, Barcelona, Spain; [3]ProteoRed consortium. Spanish National Institute of Proteomics; [4]Centro Nacional de Biotecnologia-CSIC, Madrid, Spain

Although 2DE-electrophoresis has been long used to study differential proteomics, its reproducibility has been always a major concern. In recent years, different methodological improvements have contributed to more robust 2DE workflows: use of immobilized IEF strips, fluorescence based difference gel electrophoresis (DIGE), new software tools, etc. In order to assess the reproducibility of 2DE experiments across laboratories, we set up a multi-laboratory study, performed at 11 laboratories of the ProteoRed network (Spanish network of proteomics facilities). All participating labs received two protein extracts, prepared from cultured human adenocarcinoma MDA-MB-468 cells, treated or not with 50 ng/

ml EGF (Epidermal Growth Factor) for 24h. Differential analysis was performed by a 4-gel 2D-DIGE experiment, using 4 technical replicates of each sample, with Cy dye swapping. Strictly defined 2DE conditions were followed by all labs. Each lab selected the 30 spots presenting the highest fold variations (with $p<0.05$), and attempted MS protein identification.

The results demonstrate a very good within lab and across lab reproducibility. Within labs, 75-85% detected spots present %CV <10%, and 40-60 %CV <5%. Across all labs, around 60% and 15% of spots show %CV <10% and <5%, respectively. Selection of differentially expressed spots shows good reproducibility across labs, although there is a certain degree of subjectivity in the selection, as each lab applied its own filtering criteria. Overall, 24 spots were ranked among the top-30 by at least 3 labs, and 14 by at least 4. MS protein identification was, on average, 60% successful, with 22 spots identified by at least 3 different labs. In those cases, identical gel locations corresponded to the same protein Id. In conclusion, the results of the study show the robustness of the methodology used, and demonstrate the feasibility of across lab validation schemes, pointing towards development of inter-lab QC strategies for proteomic research.

V64-S2 Enrichment with High Specificity of Phosphopeptides at Low pH Prior to Mass Spectrometry Analysis

H. Hedlund, A. Nissfolk, J. Öhman, G. Risberg, M. Albenius, M. Sjödahl

GE Healthcare Bio-Sciences AB, Uppsala, Sweden

A new product will be presented designed for single use small-scale phosphopeptide enrichment. The chromatographic mechanism is based on IMAC (immobilized metal ion affinity chromatography) and the beads are precharged with Fe^{3+}. The binding of phosphopeptides to is pH-dependent and the advantage of performing the binding at an extremely low pH has been utilized for a high-purity and reproducible enrichment of phosphopeptides. Different types of protein samples were used in the study. First, a model peptide from trypsin digested bovine β-casein, spiked into a background of albumin tryptic fragments was tested. Secondly, different complex samples, such as cell extract from *S. cerevisiae*, HeLa cells and also *E. coli* extract spiked with model proteins were analyzed. Each cell extract was diluted in 50 mM glycin-HCl, 50% acetonitrile, pH 2.0. After tryptic cleavage the sample was loaded and captured on microspin columns packed with the new IMAC medium and eluted with 1% phosphoric acid, 50% acetonitrile, pH 1.7. All eluates were analyzed with mass spectrometry. To avoid the need for methylesterfication of acidic protein/peptides, several experiments were performed to optimize the binding of the peptides at low pH. According to the mass spectrometry analysis, a high signal/noise ratio was obtained for the phosphopeptides in a complex sample. The spiked model proteins were identified in the clarified *E.coli* extract and seventeen phosphopeptides were identified with high significance ($p < 0.001$). For the *S. cerevisiae* extract, forty-one phosphopeptides encoding thirty-eight proteins were detected with high significance ($p < 0.001$) in the eluted material. Data from the study shows that during low pH conditions in sample binding and elution the new $Fe3^{+}$ precharged IMAC medium has a high specificity for phosphopeptides and can be used for enrichment of phosphopeptides from complex samples with a high level of reproducibility.

V65-S2 Parallel Isoelectric Focusing of Tryptic Peptides by the Digital ProteomeChip

R. Garlick, J. Dasch, B. Skea, P. Kandola

Protein Forest, Inc., Lexington, MA, United States

MS based proteomics of complex samples has evolved from single spot analysis of 2D gels to high throughput methods such as MudPit, where thousands of proteins are identified from a single sample. For these techniques, fractionation methods are usually employed to reduce the complexity of the sample prior to LCMS thus providing an increase in protein coverage. The objective of this study is to investigate the use the parallel isoelectric focusing of tryptic peptides as a sample prep method for LCMS proteomics. Parallel isoelectric focusing using the digital ProteomeChip dPC ™was used to separate tryptic peptides from a complex mix in 30-60 minutes. The anodic and cathodic buffers contained octoglucopyranoside, added for maximal resolution and solubility during the separation. The peptide amount may range between 20-400 ugm. After pI separation, the peptides were passively are eluted from the gel plugs. We show that this sample matrix is directly compatible with ESI MS. Also, fluorescent labeled peptides were used to demonstrate reproducible pI separation between multiple dPC lots. In conclusion, we demonstrate that parallel isoelectric focusing of peptides provides a rapid, reproducible and easy to use pI based separation for proteomic applications.

Proteomics/Protein Quantitation

SR30-S2 Using IEF Fractionation of Tryptic Peptides to Reduce False Protein Identifications and to Minimize Estimates of False Discovery Rates in Proteomics Experiments

S. Hucko[1], T.W. Thannhauser[2]

[1]Cornell University, Ithaca, NY, United States; [2] R.W. Holley Center for Ag. & Health, USDA-ARS, Ithaca, NY, United States

Searching massive protein sequence databases with large sets of tandem mass spectrometry data invariably leads to false peptide/protein identifications. Estimates of the false discovery rate (FDR) associated with an experiment can be made by searching suitable decoy databases. However, such an approach cannot be used to flag any of the putatively identified peptides as likely false positives. A strategy that can be employed to deal more effectively with the problem of false discovery is to determine additional physical constants of the peptides found within the samples. These can then be used as supplementary filters for the data. Here we show that a comparison between the peptide isoelectric point (pI), determined experimentally by using "off-gel" IEF as a pre-fractionation step prior to LC MS/MS analysis, and the pI calculated for all putative peptide hits can be used to flag specific peptide identifications as "likely to be false". A similar comparison for putative hits to a decoy database is shown to reduce the estimated FDR by factor of 3 in samples isolated from tomato fruit. LC-MALDI-MS/MS is recommended to analyze the IEF fractions, particularly when the isoelectric focusing is run at standard ampholyte concentrations. LC-ESI-MS/MS can also be used, providing the fractionation is run at greatly reduced ampholyte concentrations. Using reduced ampholyte concentrations reduces the correlation between the measured pH of the fractions and the published pI range of the IPG strips. Nevertheless, it is shown that reasonable estimates of the

isoelectric points of the peptides found in the fractions can be made from the published pI range of the IPG strip. Since peptide IEF fractionation provides an experimentally determined estimate of the peptides' pI it is a superior 1st dimension separation strategy to SCX chromatography in shotgun proteomics applications.

SR31-S2 Proteome Changes between Genetically-Related Helicobacter Pylori That Differ in Carcinogenic Potential Revealed by DIGE/MS

D.B. Friedman, A.T. Franco, D.A. Israel, R.M. Peek Jr.

Vanderbilt University, Nashville, TN, United States

Helicobacter pylori represents the strongest known risk factor for gastric adenocarcinoma, yet only a fraction of infected persons develop cancer. We use DIGE/MS to identify differentially-expressed proteins between a non-carcinogenic *H. pylori* strain and its carcinogenic derivative. Independent quadruplicate samples were pre-fractionated and analyzed by pH 4-7 and pH 7-11 DIGE using 8-gel sets coordinated by a Cy2-labeled sample mixture to provide every resolved form with a unique internal standard for cross-gel normalization/quantification. 842 resolved protein features (including charged isoforms) were matched across the resulting 16 pH 4-7 proteome maps, for which Principal Component Analysis (PCA) clearly indicated that the major source of variation (PC1, 80.3%) distinguished the cytoplasmic and membrane samples, as expected. An additional 5.2% of variance (PC2) separated the carcinogenic strain 7.13 from the non-carcinogenic strain B128. Similar results were obtained for an additional 300 protein features resolved in the pH 7-11 range. Unsupervised Hierarchical Clustering similarly demonstrated high reproducibility between independent replicate samples and an extremely low level of background noise (technical variation), and highlighted a discrete subset of protein expression changes representing candidate proteins potentially involved in carcinogenesis. Subsequent MS-based protein identification indicated multiple classes of proteins that displayed strain-specific differences, including oxidative stress response, evasion of the immune system, and bacterial replication. One notable difference was found for the FlaA flagellar protein. Peptide mass mapping clearly demonstrated a unique peptide ion at *m/z* 732.38 that was specific to FlaA from strain B128. The resulting TOF/TOF fragmentation pattern was consistent with the sequence GC(296)LNLR, whereas an arginine is normally present at position 296. This residue maps to a region predicted helical region, and mobility and ultrastructural analysis indicated that the R-to-C mutation correlated with decreased motility and increased flagellar adherence, consistent with a motility-based model of carcinogenic potential.

SR32-S2 Proteomic Comparisons of Differentiating Embryonic Stem Cells, an In Vitro Model of Embryogenesis

R. Orlando[1], P. Angel[1], D.B Weatherly[2], J. Atwood III[2]

[1]CCRC, University of Georgia, Athens, GA, United States; [2]BioInquire, Athens, GA 30602, United States

Embryonic stem cells, early primitive ectoderm-like cells, and embryoid bodies may be used as an in vitro model of embryogenesis. These cells are representative of the in vivo 4.5 d.p.c. inner cell mass, the 5.5 d.p.c. primitive ectoderm, and the 6-8 d.p.c differentiated embryo, respectively. Here, we use progressively differentiated embryonic stem cells as an in vitro model of embryogenesis. We explore protein regulation of these differentiating cells using shotgun proteomics and spectral counts. Peptides identified from each population were clustered to proteins at a ?1% FDR. A total of 2911 proteins (1013 protein homology groups) were identified for the ES proteome, 1852 proteins (568 protein homology groups) for the EPL proteome, and 3328 proteins (1038 protein groups) were identified in the embryoid body proteome. Uniquely identified proteins were grouped according to comparative and single proteomes, forming a total of 1509 proteins shared across all three proteomes. Proteins shared between cell states represented over 50% of the protein population. These proteins formed four comparative proteome groups consisting of proteins shared between ES and EPL, ES and EB, EPL and EB, and between all three proteomes (ES, EPL, and EB). Average spectral counts were used as a semi-quantitative measure of protein abundancy for comparative proteomes. Proteins typically used as loading controls during western blotting were used as control proteins to evaluate protein normalization techniques. For the general population of identified proteins, variations in the %RSD ranged from 1% RSD to 151% RSD. Ingenuity software was used to investigate functionality between comparative proteomes. Top functions were those identified at a ?95% confidence level. Some of these functions were cell growth (ES/EPL), RNA post transcriptional modification (ES/EB), and cell movement (EPL/EB). Regulation of proteins in these identified functional groups could be correlated to embryogenetic events.

SR33-S2 Quantitative Proteomics in Biomedical Research

S. Chen, S. McClung, M. Chow, R. Zheng, A. Chung, C. Bryant, N. Zhu

Proteomics Core Facility, Interdisciplinary Center for Biotechnological Research, University of Florida, FL, United States

Proteomics and mass spectrometry have provided unprecedented tools for fast, accurate, high throughput biomolecular separation and characterization. Studying at the protein level allows researchers to investigate how proteins, their dynamics and modifications affect cellular processes and how cellular processes and the environment affect proteins. Here we present our capabilities in quantitative proteomics and MS analysis. The tools include gel-based 2D-DIGE (Two Dimentional Difference Gel Electrophoresis) and gel-free iTRAQ (Isobaric Tags for Relative and Absolute Quantitation) technologies. Along with our capacity of separating proteins and characterizing differential protein

expression, we have a suite of mass spectrometers available for biomedical research, including a 4700 TOF-TOF Proteomics Analyzer, a quadrupole/time-of-flight (QSTAR XL), and a quadrupole-linear ion-trap (4000 QTRAP). These instruments are mainly used for protein identification, posttranslational modification characterization and protein expression analysis. In addition to 2D-DIGE and iTRAQ, we have recently explored the multiple reaction monitoring (MRM)-based protein quantification. Our facility is fully set up to synthesize and purify peptides. With synthetic standard peptides, MRM technology, which is a gold standard for drug analysis, enables sensitive and absolute quantification of proteins.

V66-S2 ICPL 4-plex: Isotopic Protein Labeling for Quantitative Protein Analysis by nano-LC-MALDI-TOF/TOF

B. Owens, S. Hahner, S. Steckbeck, W. Evers, A. Resemann, D. Suckau

Bruker Daltonics, Bremen, Germany

Mass spectrometry based quantitative proteomics has become an important component of biological/clinical research. Quantitative protein analysis requires methods that are able to provide accurate/reproducible differential expression values for proteins in two or more biological samples. Stable isotopic labeling with LC-MALDI-MS/MS-analysis has emerged as a powerful tool to measure the relative quantitative differences with high accuracy. The new 4-plex isotope coded protein labeling (4-plex ICPL) approach is based on the labeling of intact proteins by amine-specific ICPL reagents (Bruker). The labeling step is typically applied prior to protein–pre-fractionation/separation steps and prior to protein digestion. It, therefore, uniquely supports Top-Down proteomics studies involving, e.g., SDS-PAGE protein separations. Various protein mixtures with different complexity were subjected to the new 4-plex ICPL reagent. Four different low complex protein samples (10 proteins) were successfully analyzed by nano-LC-MALDI-TOF/TOF analysis. After the MS analysis of the complete LC-run peptide peaks with appropriate labeling were determined by the processing software and quantification ratios were calculated using median statistics. For identification of the regulated peptides, the most abundant peptide was automatically MS/MS analyzed. The WARP-LC 1.2 software (Bruker) was used for the quantification of the 4-plex ICPL-labeled proteins, which is used for the quantification of iTRAQ and SILAC labeled peptides and proteins as well. Due to the common request for the determination of quantitative differences among > 2 biological samples, the 4-plex ICPL approach was also subjected to the quantification of several target proteins present in the more complex *E.coli* proteome.

V67-S2 Ruggedness of Nanobore LCMS for Qualitative and Quantitative Biomarker Analysis using an Automated Emitter Position and Rinsing System

A. Berg, C. Marshall-Waggett, G. Valaskovic

New Objective, Inc., Woburn, MA, United States

Nanobore LC-MS is a technique used for qualitative analysis of complex proteomes extracted from biological matrices including plasma, cell lysates and tissue extracts. As the need for qualitative nanobore LC-MS shifts to quantitative analyses, system robustness is critical. Complex mixture analysis presents significant challenges, particularly when operating under nanoflow conditions. The lifetime of nanobore components (traps, column, emitters) can be significantly compromised when analyzing crude samples extracted from biological matrices. A systematic investigation of contamination resulting from the analysis of plasma was conducted. Contamination from the thermal degradation of biochemicals (proteins, peptides, lipids) is a factor because the nanospray emitter is typically in close proximity to the heated atmospheric pressure inlet of the mass spectrometer. The contamination manifests as a coating on the exterior surface of the emitter. Such contamination can change the surface wetting properties of the mobile phase, negatively impacting spray stability. An automated digital control positioning system enables the exterior of the emitter to be washed with a suitable solvent at regular intervals, minimizing the buildup of contamination on the exterior of the emitter. The performance improvements of washing the emitter were tested by analyzing samples derived from canine plasma samples spiked with two quantitative controls, one a small molecule and the other, a peptide. The recovery of these two standards was monitored throughout sequential injections and plotted. Improved robustness as a result of regular emitter rinsing is under long-term investigation.

V68-S2 Intelligent Use of Retention Time for Higher Order Multiple Reaction Monitoring Multiplexing – *Scheduled* MRM™ Algorithm

S. Mollah, C.L. Hunter

Applied Biosystems, Foster City, CA, United States

Targeted peptide quantitation is a rapidly growing application within proteomics mass spectrometry due to its widespread utility in biomarker verification, protein/peptide confirmation and characterization, as well as pathway mapping. The utility of Multiple Reaction Monitoring (MRM) on triple quadrupole based MS systems for these studies is currently an active area of investigation, driven by the well known sensitivity and selectivity attributes of this type of MS approach. As more extensive protein panels need to be monitored in a targeted way across multiple samples, higher MRM multiplexing is becoming essential for throughput. The need for rapid assay development, higher multiplexing and more robust assays are some of the key challenges. In this work, the unique combination of triple quadrupole and ion trapping capabilities of the hybrid triple quadrupole - linear ion trap mass spectrometer (QTRAP® System) has been utilized to create 100s of high quality, specific MRM transitions for multiple peptides to many plasma proteins. Human plasma was separated by reversed-phase HPLC on an Eksigent Ultra nanoLC System. MRM analysis was performed using the NanoSpray® source on an Applied Biosystems/MDS Analytical Technologies QTRAP® 5500system. By using the MRMPilot™ Software coupled with the MIDAS™ workflow, all MRM transitions to plasma proteins were designed. Using MRMPilot, iterative MRM analysis was performed which provided rapid refinement of MRM parameters without requiring synthetic peptides. Intelligent use of retention time using a new acquisition software (Scheduled MRM) enabled many more MRM transitions to be included in a single acquisition method, while maintaining good peak area reproducibility. Here, using an MRM method created with Scheduled MRM™, 784 MRM transitions to 98 proteins in human plasma was monitored in a single analysis. Ten replicate analysis was performed to test for reproducibility. The plasma protein peptides were labeled with mTRAQ™ reagent, so 4 MRM transitions to the light version and 4 to the heavy version of each peptide were monitored. In spite of a large number of MRMs monitored in a single run, over 90% of peptide peak areas and ratios (light over heavy) had reproducibility better than 5% CV. This was made possible by using Scheduled MRM™ algorithm which separated the MRMs by retention time and thus sampled only a portion of the MRMs over a specific time window versus sampling all 784 MRMs every cycle over the whole gradient. The usage of MRMPilot and Scheduled MRM has automated and simplified the complete workflow of taking discovery

data, to refinement of MRMs and to creation of a method containing the final list of a large numbers of MRMs along with its elution time. These processes would be very laborious and time comsuming if done otherwise or maually. In addition, it would not be feasible to sample a large number of MRMs in one analysis without sacrificing the MRM peak quality which would affect the quantitation accuracy. Presented here are the details of the workflow of using MRMPilot and Scheduled MRM™ to generate a large list of MRMs and its results are discussed.

V69-S2 Quantitative Differentiation of Secreted Proteins from Mycobacterial Strains

M.M. Champion[1], P.A. Champion[2], K. Chan[2], J.S. Cox[2]

[1]Applied Biosystems, Foster City, CA, United States; [2]University of California San Francisco, CA, United States

In microbial pathogenesis, virulence originates from multiple sources; genetic content, changes in phylogeny, and differences in protein localization. Mycobacterium tuberculosis is an acid-fast bacillus responsible for 1.75 million deaths annually. A major aspect of mycobacterial virulence includes the secretion of virulence proteins. The precise role of these exported factors in virulence is unclear. Because M. tuberculosis is a slow-growing mycobacteria, other, more rapidly growing pathogenic mycobacteria, including M. marinum are often used as models for studying M. tuberculosis. Here, we employed a unique combination of iTRAQ and mTRAQ chemistries to label the secreted proteomes from M. tuberculosis and M. marinum. The goal of this study was to identify quantitatively specific differences in the secreted proteomes. These differences determined by isobaric labeling were then further characterized and defined by stable isotopic labeling of substrates determined to be different between different strains of WT tuberculosis and related model-organisms. We were able to determine that many known virulence factors exhibited distinct quantitative changes between different strains. The output of this work is to create a method that would enable the quantitative differentiation from clinical isolates of tuberculosis. The results of this screen and the correlation of substrate quantitation with virulence will be discussed.

V70-S2 Novel Protein Expression Assays Using qPCR for the Detection and Relative Quantification of Protein Markers in Human Embryonic Stem Cells

T. Settineri, D. Ruff, M. Shannon, S.Chen

Applied Biosystems, part of Life Technologies, Foster City, CA, United States

Quantitative PCR (qPCR) has revolutionized the characterization of nucleic acids in cells, and several classes of cellular nucleic acids are routinely analyzed by qPCR assays, including genomic DNA, mRNA and microRNAs. Proximity ligation assay (PLA) technology extends qPCR applications now to the detection of cellular proteins through the amplification of a surrogate DNA template. PLA is a three-step process that involves, 1) binding of paired antibody-oligonucleotide probes to a protein target in biological samples, 2) templated ligation of the oligonucleotides in proximity, and 3) qPCR detection. We have optimized this technique for crude cell lysates utilizing a simple, one-step sample lysis approach to release all classes of proteins, and combined it with gold standard TaqMan® chemistry to create a highly sensitive and specific process for measuring protein expression in small samples. One application of this assay is the detection and relative quantification of markers in pluripotent and differentiated stem cells. Stem cell characterization typically relies on determining the presence and amount of stage specific protein markers such as OCT4, NANOG, SOX2, and LIN28. Protein expression results confirm cell-stage specific changes in protein expression of these key stem cell markers, and the data can be directly compared with published mRNA expression profiles for the same cell lines. We have engaged a number of researchers as test sites for these assays and are gathering input and feedback. Our findings illustrate how this new assay system expands the scope of qPCR to protein detection and quantification, an important area of cell biology.

SR49-S2 Dynamics of the MAPK Insulin Signaling Pathway Using a Label-Free Platform Based on MS/MS Average TIC

J.M Asara[1]; A. Friedman[2]; P. Hong[3]; J. Locasale[2]; N. Perrimon[2]

[1]Beth Israel Deaconess Medical Center, Boston, MA, United States; [2]Harvard Medical School, Boston, MA; 3Brandeis University, Waltham, MA, United States

Large-scale proteomics experiments similar to those first introduced by Gavin et. al. are needed in order to interrogate protein-protein interactions in cellular signaling pathways. We approached this question by performing TAP-MS experiments on 15 key nodal bait proteins in the MAPK pathway in drosophila SR+ cells with and without insulin and EGF (Spitz) stimulation. Data dependent LC/MS/MS experiments were run using a Proxeon EasynLC coupled to a Thermo LTQ-Orbitrap XL for at least two replicates of each bait condition for a total of 94 LC/MS/MS experiments including TAP vector controls. The data was searched against the reversed Flybase protein database due to its completeness using Sequest within Thermo's Proteomics Browser Software suite. In order to quantify changes in protein levels between bait conditions, we developed a software suite called Naked Quant v1.0 based on the "Spectral TIC" approach that averages the TIC values from each identified MS/MS spectrum (spectral count) per protein identified. The method was previously compared to SILAC and spectral counting in a proteomics screen (Asara et. al., Proteomics, 2008). The software utilizes several features including protein grouping across experiments, normalization as well as fold change and ratio calculations based on spectral counts, TIC sum and TIC average. From the output, different networks were assembled based on the dynamic average TIC signal change using several different criteria including simple fold changes between basal and stimulated conditions using common identified proteins and pair wise changes in signal between basal and stimulated conditions where at least two or more proteins need to change together. The network revealed many canonical interactions and several novel interactions in the MAPK signaling pathway. Several of these novel interactions have been verified biochemically and correlate with a previously published RNAi screen using pERK as the readout under basal and insulin treatment (Friedman and Perrimon, Nature, 2007). These data show that novel interactions in signaling pathways through protein-protein interaction studies can be effective using label-free mass spectrometry approaches.

SR34-S2 Identification of a Novel Pathway in Stimulated Human T-Cells That Drives Macrophage Activation and Cytokine Production

K.R. Jonscher[1], L. Li[2], C.K. Edwards[2]

[1]University of Colorado Denver, Department of Anesthesiology, Denver, CO, United States; [2]University of COlorado Denver, Department of Dermatology, Denver, CO, United States

The NAD-dependent deacetylase SirT1 regulates lipid and glucose metabolism in liver and increased SirT1 activity in caloric-restricted models has been linked to extended life span in several species. Deacetylation of transcription factors and co-modulators, such as p53, NF-κB, C/EPBβ and PGC-1α, allows SirT1 to sense and regulate energy levels. The SirT family can also deacetylate mitochondrial proteins, suggesting that posttranslational modification by sirtuins may have global effects on energy metabolism especially gluconeogenesis and lipogenesis. Livers from mice fed either control or a high fat (HF) diet (45 kcal % as fat, 12 wks) were harvested and homogenized. SirT1 levels were measured in whole cell lysates by Western blot. Proteins modified by lysine acetylation were immunoprecipitated with anti-acetyllysine antibody and subsequently separated by one dimensional gel electrophoresis. Bands showing differential staining between the control and HF fed mice were excised and proteins were digested with trypsin. Tandem mass spectrometry using an Agilent Ultra quadrupole ion trap generated product ion spectra that were searched with SpectrumMill against the SwissProt database. The levels and acetylation of identified proteins were validated by immunoprecipitation and Western blotting. Mice on the HF diet were obese, with fatty livers and reduced SirT1 activity as assessed by the NAD+/NADH ratio. SirT1 protein expression levels did not significantly change, however. Acetylation of a subset of the proteins identified, such as carbamoyl-phosphate synthase, uricase, pyruvate carboxylase and ATP synthase, has been previously reported. Interestingly, peroxiredoxin, catalase, and Hsp70, proteins involved in redox and the stress response, were hyperacetylated in the livers of obese mice. We postulate that *modification of those proteins could influence the ability of obese mice to modulate oxidative stress, gluconeogenesis and lipogenesis.* Globally surveying lysine acetylated proteins using immunoprecipitation and gel electrophoresis/tandem mass spectrometry provides insights into how obesity impacts liver metabolism.

SR35-S2 Proteomic Analyses of Pancreatic Cyst Fluids

A. Yeung, E.Ke, B. Patel, T. Liu, X. Li, O. Haluszka, J. Hoffman, H. Ehya, N.A. Young, J.C. Watson, D. Weinberg, M. Nguyen, S.J. Cohen, N.J. Meropol, S. Litwin, J.L. Tokar

Fox Chase Cancer Center, Philadelphia, PA., United States

There are currently no diagnostic indicators that are consistently reliable, obtainable, and conclusive for diagnosing and risk-stratifying pancreatic cysts. Proteomic analyses were performed to explore pancreatic cyst fluids to yield effective diagnostic biomarkers. We have prospectively recruited 20 research participants and prepared their pancreatic cyst fluids specifically for proteomic analyses. Proteomic approaches applied were: 1) MALDI-TOF (matrix-assisted laser-desorption-ionization time-of-flight) mass spectrometry peptidomics with LC/MS/MS (HPLC-tandem mass spectrometry) protein identification. 2) 2D gel electrophoresis. 3) GeLC/MS/MS (tryptic digestion of proteins fractionated by SDS-PAGE and identified by LC/MS/MS). Sequencing of over 350 free peptides showed that exopeptidase activities rendered peptidomics of cyst fluids unreliable; Protein nicking by proteases in the cyst fluids produced hundreds of protein spots from the major proteins, making 2D gel proteomics unmanageable; GeLC/MS/MS revealed a panel of potential biomarker proteins that correlated with CEA (carcinoembryonic antigen). Two homologs of amylase, solubilized molecules of four mucins, four solubilized CEACAMs (CEA-related cell adhesion molecules), and four S100 homologs, may be candidate biomarkers to facilitate future pancreatic cyst diagnosis and risk-stratification. This approach required less than 40 microliters of cyst fluid per sample, offering the possibility to analyze cysts smaller than 1 cm diameter.

SR36-S2 High Mobility Group Box 1 (HMGB1) In Eosinophil Activation

C. Straub, K. Pazdrak, A. Kurosky

The University of Texas Medical Branch at Galveston, Galveston, TX, United States

Eosinophils have been implicated in allergic inflammation and certain parasitic and viral infections. Our proteomic studies of non-activated and activated eosinophils identified the expression and release of HMGB1. HMGB1 plays a prominent role in immunoregulatory cell activation including granulocyte activation. Our experiments were designed to determine whether eosinophil-derived HMGB1 contributes to the autocrine activation of human peripheral blood eosinophils. HMGB1-presence in eosinophils was determined using western blotting and 2-dimensional gel electrophoresis. Pro-Q Diamond phosphoprotein stain and Sypro Ruby protein stain were used for detection of phosphoproteins or proteins, respectively. Cell survival rates and expression of CD69 were determined after stimulation of eosinophils with GM-CSF, rHMGB1, and various HMGB1 inhibitors. Eosinophils stimulated with either GM-CSF, rHMGB1, and eosinophil-derived HMGB1 showed significantly higher viability and expression of CD69. Pre-treating eosinophils with glycyrrhizin, a specific inhibitor of HMGB1 activity, partially inhibited prolongation of survival as well as upregulation of CD69. This upregulation was significantly inhibited by treatment with glycyrrhizin or anti-RAGE2 antibody. The phosphoproteomic profile of HMGB1 stimulated eosinophils also changed. The phosphoproteomic pattern and the proteomic pattern of eosinophils treated with HMGB1 and GM-CSF vary as compared to control cells and compared to each other. Our studies demonstrate the autocrine activation of eosinophils through HMGB1. These findings indicate a significant immunoregulatory role for eosinophils and provide a novel mechanism for the characterization of eosinophil-associated pathologies. This study was supported by the NIH/NHLBI Proteomics Initiative NO1-HV-28184 (AK).

SR37-S2 The Effects of *Cryptococcus neoformans* on Protein Expression in a Human Brain Endothelial Cell Line

R.A. Eigenheer[1], K. Vu[2], A. Gelli[2], B. Phinney[1]

[1]Proteomics Core Facility, Genome Center, University of California, Davis, CA, United States; [2]Department of Pharmacology and Toxicology, Medical School, University of California, Davis, CA, United States

Cryptococcus neoformans is an invasive opportunistic fungal pathogen that causes life-threatening cryptococcal meningitis in immune-suppressed patients. Untreated cryptococcal meningitis is fatal in normal hosts, and this organism causes high mortality in patients with reduced T-cell-dependent immune function. Unfortunately, treatments are limited because this brain infection remains significantly understudied, and the current antifungal drugs have toxicity and efficacy issues. A number of cellular factors in the brain endothelium are believed to respond and ultimately facilitate the invasion of cryptococcal cells into the CNS; few of these have been identified to date. We have begun to explore the differential protein expression in human brain endothelial cells with or without exposure to *C. neoformans*. Cells were grown to confluence on standard glass microscope slides, then the cells removed and peptides extracted. Peptides were directly loaded on a Michrom PolySulfoethyl Aspartamide SCX-enrichment microtrap, then sequentially eluted onto a Agilent ZORBAX 300SB C_{18}, reversed phase trap cartridge using (increasingly concentrated) salt injections of ammonium formate. After each salt injection the Agilent C_{18} trap was switched in-line with a Michrom Magic C_{18} AQ 100 um x 150 mm C_{18} column connected to a Thermo-Finnigan LTQ iontrap mass spectrometer. All MS/MS data were analyzed using X! Tandem, then Scaffold to validate peptide and protein identifications. Protein identifications were accepted if they could be established at greater than 95.0% probability and contained at least 2 identified peptides. Our initial analysis identified approximately 500 brain endothelial cell proteins, with approximately 10% of these proteins either up-regulated or down-regulated in response to cryptococcal cells. The proteins identified belong to several different functional groups including cytoskeletal/mobility, immune system, cell signaling, mitochondria-related and oxidative defense. These findings suggest that the interaction between the brain endothelium and cryptotoccal cells is a dynamic process.

SR38-S2 Comparison of 2-Dimensioal Protein Gels from Wild Type and Mutant Yeast Strains as Analyzed by 2 Different Instrument Systems

M. Zianni[1], E. Jones[1], J. Jackman[2]

[1]Plant-Microbe Genomics Facility, The Ohio State University, Columbus, OH, United States; [2]Department of Biochemistry, The Ohio State University, Columbus, OH, United States

Proteomic analysis of biological samples is increasing in its importance for various research applications. Part and parcel of this increase in interest is an influx of commercial formats available for utilizing this technology. Because cost and application are generally limiting factors when choosing among the various instrumentation available, one challenge for a core facility is then to choose among these available formats one which can fulfill the needs of both client and facility. To this end a robust comparative analysis of competing platforms would be beneficial. Our experimental models were a tRNA methyltransferase (Trm10) deletion strain of *Saccharomyces cerevisiae* and its otherwise isogenic wild-type parent strain. The *trm10Δ* strain is hypersensitive to the presence of the anti-tumor agent 5-fluorouracil (5-FU), but the biological basis for this hypersensitivity is not known, and identification of proteomic changes that result from growth in the presence of 5-FU could provide important clues to the mechanism of 5-FU action in cells. Following overnight treatment with a sub-lethal dose of 5-FU, total soluble protein from both strains was isolated and a portion was labeled using the GE Healthcare DIGE labeling system and subjected to separation by 2D PAGE. The resulting gels were (1) subsequently visualized on the Bio Rad Versa Doc 4000MP Imaging platform and analyzed with PDQuest and (2) imaged with the GE Healthcare Typhoon system and analyzed with DeCyder for comparison of the platforms. The same protein isolates were also separated by 2D PAGE and stained with SYPRO Ruby protein stain and subsequently visualized on both imaging platforms. Images were analyzed using the GE Healthcare DeCyder and Bio Rad PD Quest 8.0 analysis software packages as a further comparison group.

SR39-S2 Dual Mass Spectrometry Approach for the Detection and Quantitation of Active Ricin in Foods

S.C. McGrath[1], D.M. Schieltz[1], L.G. McWilliams[2], S.R. Kalb[1], J.R. Barr[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States; [2]Battelle, Columbus, OH, United States

The toxin ricin has gained notoriety due to wide availability and potential use as a bioterror agent. Ricin toxin comprises 1-5% of castor beans by weight and is relatively easy to extract. Because of this, there is concern over deliberate contamination of the food supply. Ricin poisoning by ingestion is estimated at 20ug/kg in humans. Ricin is a 64kDa ribosome-inactivating protein that causes cell death by blocking protein translation. Several methods exist for detecting ricin, including cell death assay, cell-free translation, PCR, ELISA, and mouse bioassay. Though some of these methods are fast and have low detection limits, they may give false positives, detect only genetic material, or require live cell cultures or animal facilities. None combine quantitation of ricin with a test for activity at the sensitivity and selectivity required. The method described here achieves the goals of speed, sensitivity, and selectivity for ricin analysis in food. Ricin was added to food matrices such as water, half-and-half, 2% milk, and apple juice, and extracted from samples using polyclonal antibodies bound to magnetic beads. To test for activity, the beads were mixed with buffer containing a 12-mer ssDNA substrate and allowed to incubate. Depurination of the substrate was confirmed by MALDI-TOFMS in positive linear mode. Bound ricin was then digested using trypsin and absolute quantitation performed by isotope dilution mass spectrometry. Seven isotopically-labeled tryptic peptides from ricin A and B chains were synthesized and mixed with digests of captured ricin. Two of the peptides allow ricin to be distinguished from its agglutinin. Samples were detected using an LTQ Orbitrap XL operating in product-ion-monitoring mode. Using this method, ricin was successfully extracted from each food matrix tested. Activity of recovered ricin was assessed and quantitation achieved with an LOD of 50fmol/mL in less than 8 hours.

SR41-S2 ITRAQ Proteomics Revealed Functional Differentiation of Guard Cells and Mesophyll Cells

S. Chen[1,2], M. Zhu[1], S. Dai[1], S. McClung[2]

[1]Department of Botany and Zoology, University of Florida, FL, United States; [2]Proteomics Core Facility, Interdisciplinary Center for Biotechnological Research, University of Florida, FL, United States

Guard cells are highly specialized cells, forming tiny pores called stomata on leaf surface. The opening and closing of stomata control leaf gas exchange and water transpiration. Mesophyll cells are specialized for photosynthesis. Despite of the phenotypic and functional differences between the two types of cells, the full protein components and their functions have not been explored, but are addressed here through a comparative proteomic approach using iTRAQ tagging and 2D LC-MS/MS, we have identified 1458 non-redundant proteins in the guard cells and mesophyll cells of canola leaves. Numerous proteins were found to be differentially expressed between guard cells and mesophyll cells. Proteins involved in energy (respiration), transport, transcription (nucleosome), cell structure, and signaling are preferentially expressed in guard cells. By contrast, proteins involved in photosynthesis, starch synthesis, disease/defense/ stress, and other metabolism are preferentially represented in mesophyll cells. This work represents the most extensive proteomic description of canola guard cells and has improved our knowledge of the functional specification of guard cells and mesophyll cells.

V71-S2 Biomarkers for Acute Contusive Spinal Cord Injury in Rats

S. Sun[1], A. Chen[2], J.E. Springer[2]

[1]Applied Biosystems Framingham MA United States; [2]University of Kentucky, Lexington, KY, United States

Traumatic injury to the spinal cord initiates a host of pathophysiological events that are secondary to the initial insult leading to neuronal dysfunction and death; yet, the molecular mechanisms underlying its dysfunction are poorly understood. The lack of biomarkers for monitoring spinal cord injury (SCI) makes accurate diagnosis and evaluation of SCI progression difficult. In this study, a proteomic approach was used to look for biomarkers from rat spinal cord tissue using iTRAQ labeling and 4800 TOFTOF instrument. Several biomarkers involving in energy metabolism, signaling pathways, protein degradation, inflammatory response, stress response, DNA damage, neuronal functions and blood-spinal cord barrier disruption were identified.

V72-S2 Rapid Pathogen Identification by MALI-TOF Mass SPE Spectrometry/ Saramis Database in Clinical Microbiological Routine Diagnostics

F. Xiang[1], J. Gielen[2], M. Erhard[2], W. Kallow[3], M. Krönke[2], O. Krut[2]

[1]Shimadzu Biotech, Pleasanton, CA, United States; [2]Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Goldenfelsstr, Cologne, Germany; [3]AnagnosTec GmbH, Am Mühlenberg, Germany

MALDI-TOF MS / SARAMIS is a straightforward, rapid, robust, and, inexpensive method for the routine identification of bacteria and fungi in clinical microbiology laboratories. Automated identification systems are widely-used in medium-to-high-throughput clinical microbiology laboratories. However, such systems are relatively slow because they depend on bacterial growth and metabolic activity. Bacterial identification by MALDI-TOF mass spectrometry provides a promising way to accelerate pathogen identification, since it can be performed in a few minutes from small samples. In this study we compared with 1,400 clinical routine samples the performance of MALDI-TOF MS coupled to SARAMIS (Spectral ARchiving And Microbial Identification System, AnagnosTec, Germany) with established methods (VITEK2/API, BioMérieux) in the clinical microbiology routine diagnostics.

V73-S2 MALDI imaging: Interpretation of gastric cancer MALDI images by hierarchical clustering

C. Klein[1], S. Deininger[1], M. Ebert[2], M. Becker[1], A. Füttterer[1], C. Röcken[3]

[1]Bruker Daltonik GmbH, Bremen, Germany; [2]Department of Medicine II, Klinikum rechts der Isar, Technische Universität München; [3]Institute of Pathology, Charité University Medicine, Berlin

As MALDI imaging draws more attention in the clinical research community, mass spectrometrists are increasingly requested to perform these analyses. The resulting datasets can only be interpreted in the histological context by a pathologist. In a collaboration, the mass spectrometrist is asked to provide the results in an accessible format that allows a quick and intuitive analysis by the pathologist.

A quick way for analyzing MALDI imaging data based on similarity of spectra rather than on individual mass signals is highly desirable. Here, we present hierarchical clustering as a tool to interpret MALDI imaging results and compare them to the histology in an interactive way. Tissue sections (human gastric cancer and mouse organs) were cut on a cryostat and thaw mounted on conductive, ITO-coated glass slides. The MALDI matrix was applied with an imagePrep station. The mass spectra were subjected to hierarchical clustering. Images were reconstructed by selecting nodes in the dendrogram and assigning a color to the respective mass spectra (pixels). Hierarchical clustering is a technique that sorts the mass spectra in a dendrogram according to overall similarity. We found it useful to select individual tree nodes in the full dendrogram and to highlight the respective spectra on the image. This allowed a semi-supervised interactive annotation of the datasets. The results were generally in good agreement with the histology and allowed in some cases even the assignment of histological structures invisible in individual ion

images. Compared to the analysis of MALDI imaging results in a tedious and time-consuming peak-by-peak fashion, the hierarchical clustering allowed a complete annotation of the images in a few minutes, which suits perfectly with the limited time a clinical researcher can spend on this type of work.

Bioinformatics — Protein Analysis Related

SR42-S2 Automating Commercial Mass Spectrometry Search Engines

D.L. Helseth Jr., H. Xu, A.B. Schilling

CBC/RRC Proteomics & Informatics Services Facility, Chicago, IL, United States

Commercial database search engines for tandem mass spectrometric data offer "black box" capabilities but often ship with additional features that can simplify result preparation for high-throughput core facilities. We demonstrate round-trip sample submission to a Sorcerer PE V3.4 appliance (Sage-N Research, Inc.) that submits and retrieves search results from a Mascot server (version 2.2, Matrix Science), automatically merges results into a consolidated Scaffold result file ready for the user. We installed XCalibur and ReAdW.exe on Sorcerer's preinstalled CrossOver virtual machine per the Sorcerer installation instructions. This allows direct submission of Thermo .RAW files without prior mzXML conversion. A dedicated user account was created on our Mascot server, and the same databases loaded on each server. Using Sorcerer's installed TPP software (version 3.4) we generated MGF files from resulting mzXML files using MzXML2Search and submit these files to our Mascot search engine using X11 tunneling to launch an internet browser from the Sorcerer server. Resulting .DAT files were downloaded from Mascot to the "original" folder generated on Sorcerer for the initial search, and ScaffoldBatch2 was invoked to generate a Scaffold result file incorporating both Sorcerer/SEQUEST and Mascot results. The workflow described above has also been automated using an in-house developed Python program. The Python program monitors all the searches submitted to the Sorcerer, converts mzXML to mgf and submits those searches to Mascot server using the Mascot command-line interface, retrieves the results from the Mascot server to the Sorcerer server using SFTP, and generates the merged Scaffold files. This process is fully automated and can be performed without human supervision. The Python scripts and other documentation will be shared upon request and involve no modification to proprietary vendor code. Supported in part by a grant from The Searle Funds at the Chicago Community Trust to the Chicago Biomedical Consortium

SR43-S2 Database Analysis of Electron Transfer Dissociation Mass Spectrometry Data

R.J. Chalkley, P.R. Baker, A.J. Lynn, A.L. Burlingame

Department of Pharmaceutical Chemistry, University of California, San Francisco, CA, United States

Electron Transfer Dissociation (ETD) is a fragmentation mechanism that has recently become widely available in commercial mass spectrometers. The radical-based fragmentation mechanism produces different fragment ion types to the more ubiquitous collision-induced dissociation (CID) fragmentation. Initial reports talked of production of 'picket-fence' c and z ion series, but the reality is more complicated, with b and y ions sometimes observed (especially if supplemental activation is used), formation of z+1 and c-1 ions and even amino acid side-chain cleavages. In this poster we will present our experiences analyzing ETD acquired in an LTQ and an Orbitrap by database searching using Protein Prospector, discussing ion types observed and problems with mass accuracy of peaklists. This work is funded by NIH NCRR grant RR001614 and the Vincent J. Coates Foundation.

SR44-S2 The PRIDE Database: A Hub for Proteomics Data and Associated Tools

L. Martens

EMBL-EBI Hinxton, Cambridge, United Kingdom

One of the early problems encountered by proteomics when it evolved into a high-throughput discovery platform, was the management and dissemination of the accumulated data. Over the past few years, several different data repositories have sprung up to alleviate this need. We here present one of these, the Proteomics Identifications Database (PRIDE), which has been one of the key repositories in the community since its inception in 2005. Since then, PRIDE has evolved with the field in terms of standards compliance, data contents, and available tools. PRIDE is one of the few proteomics repositories today that readily supports secure peer reviewing of submitted data prior to publication, and submission to PRIDE of supporting data for manuscripts is therefore increasingly recommended by high-ranking journals in the field. PRIDE also puts heavy emphasis on sufficient metadata annotation, to ensure the correct interpretation of submitted data. To this end, the widely used Ontology Lookup Service (OLS) was developed within the team. At the same time, PRIDE has greatly expanded the ways in which users can browse and query data, for instance using the powerful PRIDE BioMart and the immensely popular Protein Identifier Cross Referencing (PICR) service. The latter is unique in that it can translate protein identifiers across namespaces as well as across time, seamlessly updating older accession numbers to their newer equivalents. Submitting data to PRIDE also has never been easier, with the freely available PRIDE Converter wizard which efficiently creates fully annotated PRIDE XML from a variety of input formats. Finally, PRIDE also plays an important role in ProteomExchange, the large-scale collaboration to exchange proteomics data between different repositories. The PRIDE database has thus become a real hub for proteomics data and associated tools, and is set to evolve further alongside the field of proteomics over the next years.

SR45-S2 IDPicker 2.0: Improved Protein Assembly with High Discrimination Peptide Identification Filtering

Z. Ma[1], M.C. Chambers[2], M.D. Litton[2], S.M. Sobecki[2], L.J. Zimmerman[2], D.L. Tabb[1, 2]

[1]Department of Biomedical Informatics, Vanderbilt University Medical Center, Nashville, TN, United States; [2]Mass Spectrometry Research Center, Vanderbilt University Medical Center, Nashville, TN, United States

Tandem mass spectrometry-based shotgun proteomics has become a widespread technology for analyzing complex protein mixtures. A number of database searching algorithms have been developed to assign peptide sequences to tandem mass spectra. Assembling the peptide identifications to proteins, however, is a challenging issue because many peptides are shared by multiple proteins. IDPicker is an open-source protein assembly tool that derives a minimum protein list from peptide identifications filtered to a specified False Discovery Rate. Here we update IDPicker to increase confident peptide identifications by combining multiple scores produced by database search tools. By segregating peptide identifications on both charge state and the number of tryptic termini in validation, IDPicker yields more confident identifications for protein assembly. The new version achieves higher accuracy by requiring additional novel peptides in the parsimony process, especially when searching against multiple-species databases. IDPicker has been tuned for incorporation in many identification workflows by the addition of a graphical user interface and the ability to read identifications from the pepXML format. These advances position IDPicker for high peptide discrimination, reliable protein assembly, and highly-scalable proteomics studies.

SR46-S2 TagRecon: Identification of Amino Acid Mutations in Complex Samples Using Peptide MS/MS Sequence Tags

S. Dasari, M.C. Chambers, D.L. Tabb

Department of Biomedical Informatics, Vanderbilt University, Nashville, TN, United States

Shotgun proteomics is the preferred tool for large-scale characterization of protein modifications present in complex samples. Numerous search algorithms were developed to characterize known and unknown protein modifications using tandem MS/MS. Peptide sequence tag based search algorithms (like InsPect) can efficiently identify peptides with unanticipated modifications. However, the efficacy of sequence tagging for identifying unanticipated amino acid mutations has not been demonstrated. We developed TagRecon, a novel sequence tag based search engine, to identify amino acid mutations present in complex LC/MS/MS mixtures. TagRecon rapidly scans a database for candidate peptides using short sequence tags derived from a MS/MS spectrum. Candidates are matched to spectra by comparing prefix and suffix masses on either side of a matching tag while allowing for one mass mismatch to occur. Mass mismatches are interpreted as either amino acid mutations or modifications. Finally, probabilistic scores for candidate matches are computed using matched fragment ion intensities and their m/z fidelities. Proteins are inferred from peptide identifications using the IDPicker protein assembly tool. A simulated mutation dataset was generated by analyzing the defined Sigma UPS protein mixture using an LTQ, and introducing random mutations in their corresponding FASTA sequence database. TagRecon was used to match the experimental peptides to the database peptides containing simulated mutations. A total of 93 peptides containing simulated amino acid mutations were identified. 85% of identified simulated mutations were localized to correct residues (accuracy), 4% were incorrectly localized, 4% were chemical modifications mis-interpreted as simulated mutations (at the same mass), and the rest (7%) were false positives. TagRecon reads spectra and outputs peptide identifications using standard formats (mzML and pepXML). It also takes advantage of multi-core CPUs and multi-node clusters. These advances, along with its accuracy, make TagRecon an ideal tool for large-scale detection of amino acid mutations present in complex samples.

SR47-S2 A Free Web-Based Database Search Engine for Tandem Mass Spectrometric Data of Proteins and Peptides

H. Xu[1], D.L. Helseth Jr.[1], A.B. Schilling[1], M.A. Freitas[2]

[1]CBC/RRC Proteomics & Informatics Services Facility, University of Illinois at Chicago, Chicago, IL, United States; [2]Department of Molecular Virology, Immunology and Medical Genetics, the Ohio State University, Columbus, OH, United States

In the report, we describe a free web-based database search engine for tandem mass spectrometric data of proteins and peptides. The web-based search engine consists of two main components 1) MassMatrix database search program (http://www.massmatrix.net), and 2) a web interface for MassMatrix. MassMatrix is a database search software package for tandem mass spectrometric data. It has additional capabilities that set it apart from other database search engines. It is capable of direct searching of tandem mass spectrometric data of proteins and peptides with intact disulfide bonds or chemical cross-links. The algorithm is also capable of searching through hierarchical MSn (n>=3) spectra (useful in phosphopeptide analysis) where higher confidence in peptide ID can be achieved over MS2 alone. A robust linear regression based algorithm has also been included in MassMatrix to perform automated evaluation of peptide identifications by use of LC retention times to improve the sensitivities and specificities. New features of the automated data analysis for the quantitation of proteins and peptides by use of isobaric MS/MS tag (iTRAQ and Tandem Mass Tag) and N15 labeling have been added to MassMatrix. The web interface used to access MassMatrix is also a generic web interface for other database search engines. It can be adapted for any database search engine with minor modifications and be run on a PC or a cluster under the Windows or Linux operating systems. The web interface allows users to configure the database search engine, perform searches, organize and view their results. All settings, configurations, and search results on the web server are user specific and only accessible to privileged users. Both MassMatrix search program and the web interface are free and accessible at http://searcher.rrc.uic.edu running on a six-node cluster.

SR48-S2 Integration of Chromatography, LC-MS/MS Data Acquisition, and Peptide Identification Performance Metrics into a Proteomics Software Pipeline

P. Rudnick[1], L. Kilpatrick, D. Tchekhovskoi, K. Clauser, C. Kinsinger, D. Liebler, S. Stein

[1]NIST, Gaithersburg, MD, United States; [2]The Broad Institute of MIT and Harvard, Cambridge, MA, United States; [3]NCI, Bethesda, MD, United States; [4]Vanderbilt University Medical School, Nashville, TN, United States

Analytical variability is an often overlooked reality for most proteomics workflows. Failure to identify and measure it can undermine biomarker discovery efforts. In this report, we describe a panel of performance metrics targeting 6 specific areas of LC-MS/MS proteomics platforms. These categories include liquid chromatography, ion source, dynamic sampling, MS, MS/MS and peptide identification. Variability in each these categories is dissected with specific and quantitative metrics which can be used to evaluate technical repeatability (within a series) and reproducibility (between series). These metrics have been implemented in a freely available software pipeline. The pipeline directly processes Thermo Fisher .raw files (from LTQ and LTQ-hybrid mass spectrometers) and includes: (1) a converter/feature-finding algorithm for extracting peak lists, and approximating peak widths and peak heights, (2) a peptide identification engine (SpectraST or OMSSA), (3) a program that calculates all of the metrics using the output files, and (4) a program generating summary statistics reported in a text file. The programs are implemented in C or C++ and the pipeline script is written in Perl. An example application using increasing loading amounts of a yeast reference material will be presented. This software represents a new tool for assessing technical variability and optimizing performance in shotgun proteomics.

V74-S2 Web-Based Solutions for Scientific Associations, Core Labs and Shared Research Resource Laboratories

D. Hexter

World Design Group, Inc., Ithaca, NY, United States

We have created specific Web-based technology for scientific associations and institutions, core labs and shared research resource laboratories. The Web-based technology provides these institutions the ability to manage multiple informational Web sites through one centralized content management (CMS) system. This centralized CMS solution saves administration time, and provides efficiencies in managing different Web sites for one organization. The system administrator can assign any user the ability to edit any set of pages across different Web sites. The granular permissions structure is group based to simplify the routine changing of staff access over time. Page viewing permissions can also be set which restricts the content to a defined set of users.

An online grant application and reviewing module provides the ability to assign grant applications to reviewers, who can then securely review these applications online. The administrator (s) can then electronically approve or reject the grants, and notify the submitters of the decision. The information collected by the grant application can be easily modified as needed. The system supports budgets in a spread sheet format, and all data can be exported for offline analysis. Research and working group functionality allow a collaborative method for posting content and documents to any site. This module is powered by the same users' permission module as the rest of the technology. Web-based technology developed specifically for the needs of scientific associations, core labs and shared research resource laboratories, can streamline the management of their online presence — saving time and money.

V75-S2 Identification of Mosquito Salivary Gland Proteins and Determination of Parasite Infection

M. Macht[1], A. Sharma[2], R. Hebeler[1]

[1]Bruker Daltonik GmbH, Bremen, Germany; [2]National Institute for Malaria Research, New Delhi, India

Mosquito salivary glands are organs specialized in the production of a mixture of molecules that facilitate blood feeding by the lubrication of mouthparts and the inhibition of homeostasis. This is relevant for Malaria research since the Plasmodium sporozoites invade salivary glands and are injected with the saliva into vertebrate hosts during blood feeding. To get insights into the proteomics of mosquito salivary glands, the organ was isolated from mosquitos and the proteins separated by 1D-PAGE after cell lysis. The gels were silver stained, bands were cut from the gel and subsequently digested using trypsin. The bands were analyzed by nanoLC-ESI-QTOF-MS/MS on a Bruker micrOTOF-Q II system and all the data transferred into the ProteinScape database system for further data analysis. As expected, in the course of this project, a number of mosquito proteins specific for salivary glands could be identified yet surprisingly not only mosquito-originating proteins were identified but also three proteins originating from the nematode parasite Brugia malayi, which was not expected in the sample. This parasite causes the disease Elephantiasis and the combination of 1D-PAGE and nLC-MSMS analysis of the salivary glands allowed the identification of the parasite infection in the mosquitos. Since no P. falciparum related proteins were identified, it can be regarded as a confident identification of the parasite infection, accidential identification of three different proteins from a known mosquito parasite can be regarded as very unlikely. ProteinScape performs handling and processing of the data from multiple LC-MS/MS datasets in a fast, convenient and automated manner and allows for automated decoy validation of the results. This removes a major bottleneck in complex proteomics experimental setups with multiple separations, multiple types of MS analyses and the use of multiple search engines.

V76-S2 A Novel Approach to Extract and Share Biological Knowledge from Proteomics Data

C.R. Ingrell, A. Podtelejnikov, M. Damsbo

Proxeon, Odense, Denmark

Proteomics today generates vast amounts of peptide and protein identification data with high accuracy. A growing issue in proteomics is data interpretation, particularly analyzes of protein and peptide identified made by automated database search engines, the subsequent extraction of biologically meaningful information from mass spectrometry experiments

and sharing this results with co-workers and collaborators. This poster shows the application of a new bioinformatics tool, ProteinCenter, that manages these protein and peptide lists and puts them in a biological context and enables the sharing of the results. The tool was developed specifically to help researchers rapidly obtain a biologically-relevant overview in large-scale proteomics studies by using biological annotations from multiple resources. Within minutes, output generated by protein database search engines can be translated into biological information and shared with collaborators. Here we presented the express bioinformatic analysis and comparison of large scale proteomics datasets derived from PRIDE database including an organ-specific proteome map for Arabidopsis thaliana and HUPO projects. Protein identifications were clustered using ProteinCenter algorithms based either on indistinguishable proteins or sequence homology. The results of ProteinCenter data processing will be presented including statistical analysis of over- and under-represented features like gene ontology categories, PFAM annotated proteins, Signal peptide proteins, TM annotated proteins, Enzymes, involvment in KEGG pathways and others.

V77-S2 A New Integrated Bioinformatics Platform for Quantitative Proteomics Studies

W. Jabs[1], M. Lubeck[1], M. Behrens[1], U. Schweiger-Hufnagel[1], P. Hufnagel[1], D.C. Chamrad[2], K. Marquart[2], M. Blueggel[2], B. Sitek[3], B. Korte[3], S. Link[3], C. Stephan[3], K. Stühler3, H.E. Meyer[3], C. Baessmann[1]

[1]Bruker Daltonik GmbH, Bremen, Germany; [2]Protagen AG, Dortmund, Germany; [3]Medizinisches Proteom-Center, Bochum, Germany

Comprehensive quantification of changes in protein abundances is one of the most important but also among the most challenging tasks in proteomics. Different kinds of separation techniques, of mass spectrometer types, of identification strategies, and of quantification strategies (e.g. dyes, chemical labeling, label free) do all have their particular strength and weaknesses in terms of protein identification rate as well as quantification accuracy and precision. Therefore, a combination of these techniques is necessary to approach a comprehensive and validated description of biological systems. Due to the high complexity of these workflows and vast amounts of data they produce, a bioinformatics platform is required which allows for efficient data acquisition, analysis, interpretation, validation as well as data reduction and linked information storage. We are developing such a platform based on a central database which allows for sophisticated data warehousing and data mining strategies. The benefits of this system is presented in a study using a cell culture model: Lung carcinoma cells treated and not treated with TGF-beta are analyzed with two distinct technologies: First, label free MS quantification using a high resolution LC-ESI-Q TOF system and a list of regulated targets for protein identification is applied. Second, we investigate the biological system after ICPL labeling for MS quantification, SDS-PAGE separation, and protein identification with an high capacity ion trap mass spectrometer operating in AutoMS(n) mode. Database queries are used to compare the results of both workflows in regard of protein identification and quantification. Combining quantification data from several proteomics workflows based on an integrated hard- and software platform is applied for an in depth study of changes in carcinoma cells.

V78-S2 Generation, Validation and Publication of Proteomics Results Following Publications Guidelines

U. Schweiger-Hufnagel, D. Suckau[1], J. Glandorf[1], H. Thiele[1], S. Weise[2], P. Hufnagel[1]

[1]Bruker Daltonik GmbH, Bremen, Germany; [2]Protagen AG, Dortmund, Germany

Several initiatives have emerged during the last 2 years that try to establish standards to the Proteomics research community. Results are e.g. the HUPO PSI data formats (such as mzML) or minimal information requirements (such as MIAPE) and publication guidelines of Proteomics journals such as MCP, Proteomics and others. In addition, initiatives provided first platforms on the web to accept data along with publication of proteomic data, such as PRIDE. We analyzed the effect of TGF beta on a human lung carcinoma cells in a SILE quantitation experiment. The lysates of treated and untreated cells were labeled using ICPL and separated by means of SDS PAGE. Bands were excised and proteins were digested. The extracted peptides were submitted to RP LC-MS/MS runs. Following separate protein database searches the identification results of all gel bands have been integrated into an overall protein list by the ProteinExtractor algorithm. Following a Decoy validation and quantitative analysis, our bioinformatics platform collected the relevant results and exported them into dedicated report files. These contained the relevant information in the required formats to submit it to journals along with the publication draft.

V79-S2 From: Workflow for Maximizing Protein Identifications on the LTQ Orbitrap XL ETD

B.P. Shan[2], M. Zeller[1], B. Delanghe[1], T. Ueckert[1], C. Wigmore[2]

[1]Thermo Fisher Scientific, Bremen, Germany; [2]Bioinformatics Solutions Inc., Waterloo, ON, Canada

Identifying a maximum number of proteins with the largest sequence coverage in a complex sample with a minimum of effort has always been a challenge. Hereby we describe a method that combines an automated intelligent acquisition method on an LTQ Orbitrap XL using different dissociation techniques with a data processing workflow that uses multiple search engines to increase the number of identified proteins to a maximum. The acquisition method consists of a Decision Tree Data Dependent method, where activation type, CID or ETD, is determined for each peptide based on m/z and charge state to maximize the chance of being identified. The sample is injected multiple times. After each injection all precursors that have been selected for MS2 are added to the exclusion list of the next run automatically. The consecutive runs are searched in Proteome Discoverer with 4 different algorithms, Sequest, ZCore, Mascot and Peaks DeNovo and summarized.

CF2-M Cornell University Life Sciences Core Laboratories Center

G. Grills, J. VanEe, P. Schweitzer, Wang W., Y. Li, S. Zhang, C. Kinsland, R. Williams, K.Y. Deng, J. Pillardy, Q. Sun, T. Stelick, E. Paronett, J. Spisak, L. Cote, R. Cameron, J. Zhao, B. Hover, M. Figueroa, R. Shaknovich, Y. Xin, S. Monni, R. Sherwood, S. Baumgart, J. Mattison, C. Mottler, C. Bayles, G. Xia, L. Ponnala, S. Stefanov, R. Bukowski, C. Myers, D. Ripoll, J. Flaherty, A. Manocchia, J. Dick, E. Dodge, K. Smith, A. Clark, D. Lin, C. Aquadro, W. Zipfel, T. Begley, J. Rose, P. Soloway, A. Melnick

Life Sciences Core Laboratories Center, Cornell University, Ithaca, NY, United States

The Cornell University Life Sciences Core Laboratories Center (CLC) provides an array of genomics, proteomics, imaging and informatics shared research resources and services to the university community and to outside investigators. With a concentration of advanced instrumentation and expertise in their applications, the CLC is a key resource for life sciences basic research and medical research investigators at Cornell University and at other academic institutions and commercial enterprises.

CF3-M Information technology Services Facility of the Cornell University Life Sciences Core Laboratories Center

J. VanEe, J. Flaherty, A. Mannochia, J. Dick, E. Dodge, K. Smith

Cornell University, Ithaca, NY, United States

The Information Technology Services Facility of the Cornell University Life Sciences Core Laboratories Center (CLC) provides desktop and network support, software license management, and LIMS maintenance and development to our core facilities as well as a diverse array of Cornell University life sciences investigators and academic units.

CF4-M New in the CGRB Core Laboratories

C. Rosato, M. Dasenko, A. Girard, A. Krupkin, S. Drake, S. Givan, C. Sullivan, J. Carrington

Oregon State University, Corvallis, OR, United States

The Center for Genome Research and Biocomputing Core Lab at Oregon State University provides services for fee in genomic technologies (DNA sequencing, DNA fragment analysis (genotyping)) and in functional genomic technologies (microarray). Sequencing is provided both for traditional Sanger sequencing on an AB 3730 and Ultra highthroughput sequencing on the Illumina Genome Analyzer. This year we added the Paired End module and upgraded to the GAII. DNA fragment analysis is performed on the AB 3100. Our microarray services include Affymetrix and NimbleGen platforms. Sample labeling, hybridization and scanning are offered. NimbleGen capabilities were added this year. The Agilent BioAnalyzer is a service often tied to the microarray services. We also have a BioRobotics Microgrid for spotting custom microarrays. Our biocomputing infrastructure has grown to include over 110 machines and over 450 processors. We maintain over 100TB of shared file storage, gigabit-ethernet network switches with a 10gig backbone, an 800/1600GB tape backup system and standardized Linux cluster node software and operating systems. Policies have been developed to manage the massive data output of the Illumina Genome Analyzer to allow researchers adequate time to analyze their data and to allow Center staff to rotate new data into the infrastructure and older data off. The CGRB also maintains multi-user instruments available to researchers. These include a Zeiss LSM510Meta microscope, an AB7500 qPCR, a Storm 820 Phosphoimager, a Nanodrop, a Genetix Q-Pix colony picker, an Axon Genepix 4200A microarray scanner, and a fluorescent plate reader.

CF5-M Idaho State University Molecular Research Core Facility

E. O'Leary-Jepsen, C. Ryan

Idaho State University, Pocatello, Idaho, United States

The Idaho State University Molecular Research Core Facility (ISU MRCF) provides shared research instrumentation and services to ISU faculty, staff, students, and external researchers. The MRCF was established in 1994 through a National Institutes of Health/EPSCoR Institutional Development Award. Since 1994 the MRCF has undergone two expansions and currently comprises nearly 1200 square feet in the Gale Life Sciences Building. Facility staff maintain and provide training for 30 scientific instruments. Our current space is in contrast to 173 square feet and six instruments in 1995. The MRCF operates under both the Office of Research and the ISU Biomedical Research Institute and receives support from an Advisory Committee comprised of the MRCF Director, Facility Director, and faculty members representing the Departments of Biological Sciences and Biomedical and Pharmaceutical Sciences. The facility provides DNA sequencing and fragment analysis services using an Applied Biosystems 3130XL Genetic Analyzer. Researchers submit requests and receive data in a streamlined fashion through our Geospiza Finch server, which allows for after hours order requests and around the clock remote access to data files. Shared instrumentation includes the following: PCR, quantitative-PCR, gel imager, microarray, bioanalyzer, DNA/RNA/Protein spectrophotometer, centrifuges, phosphorimager, liquid scintillation analyzer, microplate reader, liquid handling robot, and fluorescence and deconvolution microscopes. Software designed for downstream data analysis is available on several computer kiosks. Authorized researchers have 24 hour access through a card tracking security system. In addition to the critical research component, the MRCF participates in educating the next generation of scientists by providing tours and demonstrations. For example, in November 2008 over 400 students toured the MRCF and were given demonstrations on a variety of instruments. The MRCF also sponsors workshops and seminars such as the annual ISU Bioinformatics Workshop.

CF6-M The SOLiD at Penn State — Implementation of a New Technology in a Core Facility

D. Grove, C. Price, C. Praul

The Pennsylvania State University, University Park, PA, United States

The SOLiD next generation sequencer performs short read sequencing by ligation using DNA amplified on beads and then deposited on a slide. The investment in the SOLiD instrument, accessories, reagents, and computer systems can reach $600,000 and higher. As we implement the SOLiD technology we have been working on reducing costs to the researcher, establishing an efficient work-flow, and optimizing protocols. In particular we have found that sample quality is important in obtaining sufficient yield during library preparation. Accurate titration of the library immediately prior ePCR was the biggest impediment to getting good and best beads. Therefore, we investigated various quantification methods which included the Agilent Bioanalyzer, Nanodrop spectrophotometer, qPCR by SYBR Green and the Qubit fluorimeter. In order to reduce costs we have also been assessing protocols for incorporating bar-coding of libraries which will allow multiplexing up to 16 samples per slide section. Another area of cost reduction is to use less Taq Polymerase in the emulsion PCR and we have included PCR mini procedures as routine sample preparation. To aid in data distribution to researchers as well as facilitate further data analysis, a "pipeline" to the galaxy site set up by Anton Nekrutenko here at PSU has been established. Costs and charges will also be discussed.

CF7-M Searchable Core Facility Database: Building Resource Bridges

B. Fleming, T. Hunter, J. Vincent

Vermont Genetics Network/University of Vermont, Burlington, VT, United States

The Vermont Genetics Network was established in 2001 and is funded by the National Center for Research Resources (NCRR), and is part of a National Institutes of Health (NIH) initiative called IDeA Networks of Biomedical Research Excellence (INBRE) to build biomedical research infrastructure. We identify the need for and develop facilities at the University of Vermont (UVM), our lead institution and promote and encourage access for the scientific community throughout the state. Not all infrastructure needs are required by a broad base of local researchers and in these cases would be beneficial to explore accessing these technologies regionally. To explore what services or technologies that are accessible regionally is a desirable goal of the Vermont Genetics Network. This would avoid duplicating a resource that would not be cost effective at one site or state, but could still be accessed regionally. To explore regional networks, an online core facility searchable database has been developed. The database will only be useful if it is populated. A representative of the Vermont Genetics Network will have computers accessible to all ABRF attendees to view and populate. Researchers will be able to search online by service offerings and location to find a facility regionally that will best meet their needs. The data is also available in an excel readable XML file.

CF8-M The Genomic Technologies Facility at Iowa State University

L. Gao, P.S. Schnable

Center for Plant Genomics, Iowa State University, Ames, IA, United States

Established in 1999, the Genomic Technologies Facility of the Center for Plant Genomics of the Plant Sciences Institute provides training and fee-based services for biological research at the genomic level. Services include but not limited to the production and analysis of custom spotted microarrays for global mRNA profiling experiments; high-throughput genotyping and quantitative gene expression analysis using Sequenom MassARRAY, Stratagene Mx4000 and Roche LightCycler 480 instruments. The Genomic Technologies Facility not only provides expertise and equipment to investigators and collaborators within Iowa State University, but also offers our services outside. The Genomic Technologies Facility and the Schnable research group have combined microarray technology with laser capture microdissection to generate a series of SAM (shoot apical meristems)-enriched maize B73 cDNA gene chips. About 2,800 genetically mapped cDNAs have been included on the maize chips, and the MicroArray Data Interface (MADI) is available online to facilitate researchers in querying and viewing information associated with spots printed on these chips. In addition, the Schnable research group has validated more than 1,000 SNPs between the two inbred lines B73 and Mo17, which are available for use in a wide variety of maize genetic experiments in the Genomic Technology Facility.

CF9-M CIAN — Cell Imaging and Analysis Network

E. Kuster-Schock, G. Lesage, J. Lacoste, S. Bunnell

Department of Biology, McGill University, Montreal, Quebec, Canada

The Cell Imaging and Analysis Network (CIAN) provides services and tools to researchers in the field of cell biology from within or outside the McGill University community. CIAN is composed of six scientific platforms: Cell Imaging (confocal and fluorescence microscopy), Proteomics (2-D, DiGE and fluorescent protein analyses), Automation-High throughput screening (Pinning robot and liquid handler), Protein expression and antibody production, Genomics (microarray and real-time PCR), and Data storage/analysis (cluster, server and workstations). Users submit project proposals and can obtain training in any aspect of the facility. Since its opening, CIAN served 150 users from 60 labs with 25 affiliations. CIAN is designed to facilitate training, enhance interactions, and share resources and expertise.

CF10-M The Center for Functional Genomics Core facilities at the University at Albany-SUNY

S.V. Chittur, J.A.Tine, B.Parr and Q.Lin

Center for Functional Genomics, University at Albany-SUNY, Rensselaer, NY, United States

The Center for Functional Genomics (www.cfgbiotech.org) is a fee-for-service basic research laboratory providing services to hundreds of researchers at companies, universities, and government agencies around the world, helping them obtain robust, reliable results quickly and affordably. This state-of the-art facility is comprised of multiple technology based core laboratories that offer services in molecular genetics, proteomics, mass spectrometry, microarrays, transgenics, cell culture, laser capture microdissection and flow cytometry. The microarray services include expression analysis using Affymetrix, Agilent and Nimblegen arrays, Eppendorf DualChips or Custom spotted cDNA arrays; ChIP-Chip, RIP-Chip, genotyping using Affymetrix SNP and Targeted Genotyping techonolgies, and qPCR validation. The molecular genetics core provides services in DNA sequencing, genotyping, gene cloning/vector construction, qPCR and gene/promoter isolation. The transgenics core provides transgenic mice and gene targeting technologies for in vivo gene function analysis and generation of animal models. This is done by either pronuclear injection, or mouse ES cell electroporation and subsequent blastocyst injection. The proteomics and mass spectrometry core offers expertise in 2D gel electrophoresis; mass-spec based quantitative protein profiling, post translational protein modification and metabolite ID and quantitation. The flow cytometry core supports instrumentation for multicolor phenotyping, cell cycle analysis and cell sorting. The co-localization of these core facilities offers a seamless and comprehensive approach to drug discovery and health research.

CF11-M Molecular Resource Facility, UMDNJ-New Jersey Medical School

R.J. Donnelly, S. Kuppasani, K. Dhawan

UMDNJ-New Jersey Medical School, Newark, NJ, United States

The Molecular Resource Facility of the New Jersey Medical School was established in April 1995 to enhance the resources available to the research community within the medical school. It serves to provide services to the research community and as a source of information on molecular techniques and research strategies involving molecular biology. Our facility now provides services in many areas including DNA sequencing, protein sequencing, peptide synthesis, qPCR and others. Our services are available to any laboratory requiring these services.

CF12-M Proteomics, Biological Mass Spectrometry, and High Throughput Inhibitor Screening at the University of Cincinnati

R. Rathore, W.D. Haffey, K. Rask, H. Shu, M. Wyder, K.D. Greis

Proteomics Laboratory, University of Cincinnati, Department of Cancer and Cell Biology, Genome Research Institute, Cincinnati, OH, United States

The University of Cincinnati Proteomics laboratory offers a full array of proteomics and biological mass spectrometry capabilities both on a fee-for-service basis and as grant-supported collaborative research projects. During the initial consultation with Proteomics Laboratory personnel, the needs of the investigator are addressed and then the appropriate strategy to solve the problem at hand is presented. Through these initial discussions, the overwhelming majority of problems that often plague core laboratories are circumvented thus setting appropriate expectation for the investigator and maximizing the use of the core resources. The capabilities offered range from simple QA analysis of isolated proteins and peptides, to more complex comparative profiling of proteins from 2 or more biological conditions, and finally to the development technologies in global phosphorylation changes and drug discovery applications of mass spectrometry for high throughput inhibitor screening. For comparative profiling of cells or tissues, both 2-D gel electrophoresis approaches and quantitative mass spectrometry methods are offered. 2-D gel electrophoresis can be done using the DIGE method for pair wise comparisons or via silver-stained methods and comprehensive image analysis for multivariate experiments. Protein identification from gel spots with sensitivity in the low femtomole to attomole range is routinely done in the laboratory using both nanoLC-MS/MS and MALDI-TOF/TOF methods. Using the same mass spectrometry techniques, identification and characterization of isolated protein complexes are also available. For MS-based quantitative proteomics, currently the preferred method is via isotope labeling using iTRAQ reagents from Applied Biosystems, but analysis for SILAC-labeled culture samples can also be provided. Furthermore, this presentation highlights our expertise in global mapping of phosphorylation sites from complex mixtures by affinity enrichment (TiO2 or IMAC) followed by ion exchange separation and nanoLC-MS/MS. Finally, an emerging application for mass spectrometry-based enzyme assays and inhibitor screening is presented with an eye toward attracting new customers and collaborators outside of the University of Cincinnati who are interested in screening a target enzyme for new inhibitors.

CF13-M Trudeau Institute Molecular Biology Core Facility

P.S. Adams, A.E. Moquin

Trudeau Institute, Saranac Lake, NY, United States

The core facility paradigm is constantly changing. The Molecular Biology Core Facility (MBCF) at the Trudeau Institute thrives by providing a variety of services to the investigators at the institute while recovering the majority of its expenses. The MBCF offers an assortment of services that are specifically tailored to the Institute mission and personalizes each service to the individual investigator. A five-year core grant, as part of a program project, helps to cover the cost of developing new techniques. Services provided include DNA sequencing, spectratyping of the T-cell repertoire (fragment analysis), production, purification and labeling of Major Histocompatibility (MHC) Class I and Class II multimers for Fluorescent Activated Cell Sorter (FACS) analysis, real-time PCR measurement of gene expression and viral loads, knock-out mouse and Mycoplasma screening, RNA quality analysis by using "Lab on a Chip" technology, DNA haptenation and recombinant protein expression / purification for antibody detection and in vivo vaccine development strategies. A variety of simple services such as primer design, primer ordering, stock primers, peptide ordering and Taq production save the investigators time, effort and money. Education and training are provided for all techniques and for using MBCF instrumentation, such as spectrophotometers, real-time PCR equipment and image capture/ analysis equipment. Not only does the MBCF provide the service, the personnel assist in the planning and analysis to maximize proper usage of the technique. The MBCF at Trudeau Institute prospers through versatility and customization.

CF14-M From Bench to Bedside: Patient-Oriented Core Programs at Nemours Biomedical Research

J. Holbrook, K. Sol-Church

COBRE-Center for Pediatric Research, Wilmington, DE, United States

Nemours is one of the nation's largest medical group practice devoted to pediatric care, education, and research. It is the mission of Nemours to provide leadership, institutions, and services to improve the health of children. As a part of that mission, Nemours Biomedical Research has a long-standing commitment to scholarly and scientific endeavors directed towards improving the diagnosis and treatment of pediatric medical conditions. With locations in Wilmington DE, as well as Jacksonville, Orlando, and Pensacola FL, more than 40 different research programs and laboratories support the medical and surgical staff in restoring and improving the health of acutely and chronically ill children. Nemours has invested in patient-oriented and science based research that bridge the gap between bench and bedside, as well as in programs and services that utilize this knowledge, to impact medical care and disease prevention.
This poster will describe the different CLIA and CAP certified diagnostic cores available for clinicians to help with diagnostic and care of pediatric patients. We will also present an overview of the cores and shared facilities available at the Center for Pediatric Research, a COBRE-funded research center which was created at the Alfred I duPont Hospital for Children to foster the development of translational research programs covering a broad range of pediatric disorders.

CF15-M Quality Assurance and Quality Control in the Core Genotyping Facility

B.D. Hicks, L. Burdett, T. Creavalle, C.L. Dagnall, K.A. Haque, A. O'Neil

Core Genotyping Facility, Division of Cancer Epidemiology and Genetics, Advanced Technology Program, SAIC Frederick, Inc, NCI-Frederick, Frederick, MD, United States

The Core Genotyping Facility's Quality Assurance and Quality Control Group provides the necessary resources in support of a controlled laboratory environment, resulting in efficient DNA sample handling, robust, reliable and reproducible genotyping, and streamlined data analysis. Members of this group work closely with the staff of the Production Genotyping Team, Dedicated Scientific Operations, and Bioinformatics groups to create Standard Operating Procedures for laboratory and analysis workflows. In addition to SOP development and training, members are also responsible for laboratory automation, equipment maintenance, manufacturing of sample handling and genotyping controls and standards, and new assay validation. Select members of this group also review pre-genotyping sample eligibility and perform post-genotyping quality control analyses, creating a highly informative dataset for genetic epidemiological analyses. Together, this group ensures that the product delivered meets the customer's requirements for high quality data, and that data is generated following the most efficient and cost-effective laboratory and analytical procedures.

CF16-M DNA Sequencing and Genotyping Facility

P. Schweitzer, T. Stelick, E. Paronett, J. Spisak, L. Cote, R. Cameron, C. Aquadro

Life Sciences Core Laboratories Center, Cornell University, Ithaca, NY, United States

The DNA Sequencing and Genotyping Facility of the Cornell University Life Sciences Core Laboratories Center (CLC) provides an array of shared research resources and services to the university community and to outside investigators. The facility offers a concentration of advanced instrumentation and expertise in their applications. Services include the ABI3730xl platform for Sanger sequencing of plasmid and PCR products. Resources for massively-parallel, "next generation" sequencing include the Roche 454 GS FLX and the Illumina Genome Analyzer II. The Sequenom MassArray is available for SNP genotyping, methylation and gene expression studies. The ABI 7900HT is available for RT-PCR studies. The facility also provides support for SNP genotyping using automated sample processing pipelines for ABI SNPlex, Illumina GoldenGate and Infinium, and Affymetrix SNP and Targeted Genotyping projects. The goal of the facility is to meet the increasing need of investigators for rapid and accurate DNA sequencing and genotyping.

CF17-M Applying Next Generation Sequencing Technologies as Shared Research Resources

G. Grills, P. Schweitzer, Q. Sun Q, W. Wang, T. Stelick, R. Bukowski, L. Ponnala, J. VanEe

Life Sciences Core Laboratories Center, Cornell University, Ithaca, NY, United States

New DNA sequencing technologies presents an exceptional opportunity for novel and creative applications with the potential for breakthrough discoveries. To support such research efforts, we have implemented the Illumina Solexa Genome Analyzer II and the Roche 454 Genome Sequencer FLX platforms as academic core facility shared research resources. We have established sample handling methods and informatics analysis pipelines in support of these new technologies. Our DNA sequencing and genotyping core laboratory provides sample preparation and data generation services and in collaboration with the gene expression and informatics core facilities, provides both project consultation and analysis support for a wide range of possible applications, including de novo or reference based genome assembly, detection of genetic variation, transcriptome sequencing, small RNA profiling, and genome-wide measurements of epigenomic protein-nucleic interactions. Implementation of next generation sequencing platforms as shared resources with multi-disciplinary core facility support enables cost effective access and broad based use of these emerging technologies.

CF18-M Services offered by the DNA Sequencing and Genomics Core Facility at the University of Utah

M. Powers, M. Klein, H. Escobar

DNA Sequencing and Genomics Core Facility, University of Utah, Salt Lake City Utah, United States

The University of Utah DNA Sequencing and Genomics Core Facility offers a variety of genetic analysis services to both on-campus and off-campus researchers. The details of these services and the workflow associated with them will be discussed. Our sequencing facility provides DNA sequencing using an Applied Biosystems 3730xl instrument. A standardized sample submission process increases workflow efficiency and Geospiza's web based system allows users to securely view their sequencing data online. In addition to standard DNA sequencing, we offer mitochondrial genome sequencing, a mutation detection service to identify single nucleotide polymorphisms (SNPs), insertions, and deletions. Researchers can select genes or regions of interest to which our facility designs and optimizes the PCR primers, performs the initial PCR, runs the sequencing reactions, and analyzes the data using SoftGenetics Mutation Surveyor software. The genomics facility provides full service genotyping from PCR setup through analysis. Our facility has commercial and custom sets of fluorescently labeled microsatellite markers that can be used for whole genome linkage studies and fine mapping projects. Particular sets of markers can be used for loss of heterozygosity and microsatellite instability studies. An Applied Biosystems 3130xl instrument is used to run genotyping samples and Applied Biosystems GeneMapper software is used for data analysis. The 3130xl instrument is also available for researchers with fluorescently labeled PCR products that are ready to run. Taqman genotyping assays and higher throughput custom SNP genotyping can know be achieved using the Illumina Bead Express system. To meet the real-time PCR gene expression demands of our customers, two Applied Biosystems 7900HT instruments are available. Additionally, we have two automated fluid handling systems, a Biomek FX and a Velocity 11 VPrep for researchers needing automated fluid handling for their experiments.

CF19-M SOLiD Sequencing Enables Studying the Whole Methylome

C. Bormann Chung[1], M. Barker[1], V. Boyd[1], Y. Sun[1], L. Jones[1], M. Rhodes[1], C. Monighetti[1], S. Stanley[1], V. Bashkirov[1], H. Peckham[2], F. De La Vega[1], K. McKernan[2]

[1]Life Technologies, Foster City, CA, USA; [2]Life Technologies, Beverly, MA, USA

Through the addition of methyl groups to Cytosine (C) at CpG sites (Cytosine and Guanine separated by a phosphate), transcription of the DNA is inhibited. Thus methylation plays an important role in the regulation of gene expression in both the normal and dysfunctional cell. It can be found in numerous cell processes, such as differentiation, chromosome stability, the transcription prevention of repetitive or alien sequences, and cancer development. During bisulphite conversion and subsequent PCR amplification, unmethylated Cs are converted into Thymine (T), while methylated Cs will not be converted. Sequencing of this bisulphite treated DNA, enables the characterization of the methylation status for each CpG site. However only the introduction of next generation sequencing technologies have overcome the limited analysis of CpGs in specific regions, thus providing the opportunity for hypothesis-free study of the entire methylome. Here we present a technique that utilizes the high throughput of the SOLiD™ System with the single base level resolution of bisulphite sequencing. A novel adaptation to the fragment library protocol enabled the DNA library to be treated with bisulphite after the addition of sequencing adapters. The resulting bisulphite converted library was then subjected to emulsion PCR and sequencing using standard sequencing protocols. The conversion of unmethylated C to T during the bisulphite reaction essentially reduces the DNA to a three base genome, typically making mapping of short reads very challenging. However using the SOLiD 2 base encoding system, the three base bisulphite converted genome retains all four colors in color space, thus providing the complexity required for accurate mapping.

CF20-M RWJMS DNA Core Facility Provides Fast and Accurate DNA Sequencing and Genetic Analysis Services

L. Schein

RWJMS DNA Core Facility, UMDNJ, Piscataway, NJ, United States

The RWJMS DNA Core Facility provides automated DNA sequencing and genotyping services, oligonucleotide synthesis, DNA fragment analysis, SNP analysis, Human cell marker identification services, Taqman low density arrays, quantitative Real-Time PCR services and an ABI Freezer Program. The Facility runs an ABI PRISMÂ® 3100 Genetic Analyzer and a 3130XL Genetic Analyzer, state of the art machines for automated DNA capillary electrophoresis sequencing and genotyping. The Core utilizes an ABI 7900HT Real-Time PCR System that can accommodate any real-time PCR application including Taqman ® Assays. This high-throughput system contains robotics enabling multiple 384 or 96 well plates to be loaded automatically. The Core also has an agreement with Integrated DNA Technologies, an innovative leader in DNA synthesis, to provide fast, quality DNA and RNA oligonucleotides at a greatly reduced cost with no shipping charges. In addition, the DNA Core Facility purchases select Applied Biosystems reagents in bulk. We are able to pass this cost-savings onto our users with our ABI Freezer Program. The DNA Core Facility is a shared resource of the Cancer Institute of New Jersey.

CF21-M NCI Core Genotyping Facility: Technology Enables Genome-Wide Association Studies

A.A. Hutchinson, M. Beerman, J. Boland, L. Burdett, B. Hicks, M. Rivera-Silva, M. Yeager

Core Genotyping Facility, National Cancer Institute, Division of Cancer Epidemiology and Genetics, Advanced Technology Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD, United States

With remarkable advances in genomic technologies, the National Cancer Institute established the Core Genotyping Facility (CGF) to investigate the contribution of germline genetic variation to cancer susceptibility and outcomes. Working in concert with epidemiologists, biostatisticians and basic research scientists in the intramural research program, the CGF has developed the capacity to conduct genome-wide association studies (GWAS) and candidate gene approaches to identify the heritable determinants of various forms of cancer. Utilizing technologies by Illumina®, Affymetrix®, Applied BioSystems®, Fluidigm®, and Roche/454®, the CGF has created a laboratory facility capable of high-throughput genotyping and detailed scientific follow-ups studies. In the current environment, the CGF is capable of taking collected samples from the epidemiologist and following a circular path of genetic discovery, analysis, reproduction, and reporting using the laboratory and analytic tools contained within the organization. This allows rapid-response to large scale discoveries. As the climate of genetic research changes, the CGF has added the Roche/454® next-generation sequencing platform to allow deep re-sequencing and follow-up of regions of interest found with the various SNP genotyping techniques. Funded by NCI Contract HHSN261200800001E.

CF22-M Epigenomics Facility

Y. Li, W. Wang, P. Schweitzer, M. Figueroa, R. Shaknovich, Y. Xin, S. Monni, T. Stelick, E. Paronett, J. Zhao, B. Hover, G. Grills, C. Aquadro, D. Lin, A. Melnick

Life Sciences Core Laboratories Center, Weill Cornell Medical College, New York, NY and Cornell University, Ithaca, NY, United States

The Epigenomics Facility of the Weill Cornell Medical College and the Cornell University Life Sciences Core Laboratories Center (CLC) provides an array of epigenomics research resources and services to the university community and to outside investigators. The facility offers a concentration of advanced instrumentation and expertise in their applications. This is an inter-campus facility, with resources and services located both on the Cornell University campus in Ithaca, NY, and the Weill Cornell Medical College in NYC, NY. DNA methylation profiling and protein-nucleic acid association analysis (ChIP-Chip and ChIP-Seq) resources include the NimbleGen Illumina, Affymetrix, and Agilent microarray platforms, the Sequenom MassArray, the ABI 3730xl, and the Roche 454 GS FLX and Illumina Genome Analyzer II. The goal of the facility is to meet the increasing need of investigators for rapid and accurate epigenomics project design, sample preparation, data generation, and data analysis.

CF23-M Core Biology Facility at North Dakota State University

J.S. Haring

Center for Protease Research, North Dakota State University, Fargo, ND, United States

The Core Biology Facility (CBF) was established at NDSU in 2003 with NCRR-NIH-COBRE grant funding to provide access to equipment and specialized training in the areas of molecular biology and tissue culture in order to enhance the biomedical research environment at NDSU. The CBF is also instrumental in testing compounds synthesized by NDSU's Chemistry faculty for potential inhibition of matrix metalloproteinases and histone deacetylase enzymes. To assist clients in performing their experiments, the CBF is equipped with a Gemini EM microplate fluorimeter, a Thermo Multiskan spectrophotometer, an Agilent 2100 Bioanalyzer, two ABI 7500 Fast Real Time PCR machines, two ABI 2720 thermocyclers, an Axon 4000B Genepix microarray scanner, an Accuri C6 Flow Cytometer, a computer workstation with FlowJo 8.7.1 and access to the IPA Ingenuity Database, and a Leica DMIL inverted microscope with digital color camera, in addition to basic molecular biology and tissue culture instrumentation. The CBF also provides investigator or student training and consultations on experimental design and data interpretation.

CF24-M Quantitative PCR Services in SeqWright

L. Fu, D. Buck, F. Lu

SeqWright, Inc., Houston, TX, United States

SeqWright since its inception 14 year ago has grown to become a premier genomics service provider offering its customer access to the latest cutting edge technologies in the genomics service arena. Quantitative PCR(QPCR) services were offered as a natural addition to the SeqWright portfolio of services which included DNA sequencing, complex gene cloning, cell line ID, and genotyping. SeqWright's QPCR service is run in a state of the art facility by seasoned industrial scientists with many years of experience. With the recent acquisition of the ABI 7900HT to increase capacity, we are now able to handle any scale of project. With a reputation for excellence, SeqWright has recently become a preferred ABI QPCR service provider.

We have established SOP and validation of all the major applications for QPCR and can offer services at both research and regulatory levels. We offer the following QPCR services: copy number determination of DNA or RNA targets, absolute or relative quantitation with a choice of normalizing methods, gene expression assays on over 800,000 genes in 9 species using the pre-designed TaqMan® assays, microRNA assays and microRNA profiling for a broad range of species, microarray study validation as a companion service to its world leading microarray service, post-PCR SNP genotyping and plus/minus assays.

Benefits of SeqWright's QPCR service include: dedicated Ph.D. project manager providing careful consideration of assay design, template preparation, and analytical methods for each individual QPCR project. Capacity and process automation required to handle any scale of project. Support for both research and regulatory levels of compliance.

CF26-M Danforth Center: Proteomics & Mass Spectrometry Core Facility

L.M. Hicks, S. Alvarez, B. Zhang

Donald Danforth Plant Science Center, St. Louis, MO, United States

The Proteomics and Mass Spectrometry Facility at the Donald Danforth Plant Science Center (http://www.danforthcenter.org/pmsf/) is equipped with state-of-the-art technologies for the detailed study of a wide range of biomolecules. The facility provides both full- and self-service capabilities to both internal and external clients at competitive rates. The facility offers fast, high quality specialized analytical services including: protein extractions, liquid chromatographic separations; high resolution 1D/2D gel electrophoresis; gel image analysis and protein expression analysis; high-throughput protein spot excision; in-solution and in-gel protein digestion; high-throughput protein identification; accurate protein molecular weight analysis; protein covalent/non-covalent complex analysis; biomolecule interactions (surface plasmon resonance); small molecule separation/structure determination; protein post-translational modification analysis, and peptide de novo sequencing. Major instrumentation includes: QSTAR XL Q-TOF MS/MS system (Applied Biosystems), 4000QTRAP LC-MS/MS system (Applied Biosystems), Voyager-DE STR MALDI-TOF MS (Applied Biosystems), GCQ GC-MS (ThermoFinnigan), nanoflow HPLCs (LC Packings/Eksigent), System Gold HPLC (Beckman Coulter), Shimadzu HPLC system (Shimadzu), Ultra Performance LC (UPLC) (Waters Inc.), Biacore2000 (Biacore Inc.), 1D and high resolution 2D gel electrophoresis systems (BioRad and Amersham Biosciences), Typhoon 9410 variable mode imager (Amersham Biosciences), GelPix high throughput protein spot excision system (Genetix Inc.), and a MultiProbe II automatic protein digestion and handling system (Perkin-Elmer Inc.). Protein intact mass, identification and characterization are a few of the many applications that the facility performs. For proteomics applications, the Q-TOF and MALDI-TOF instruments are well-suited for analyzing both small peptides and large proteins. The QTOF instrument can perform exact mass measurements for molecular formula determination and can be coupled with on-line separations using nano-LC for de-novo sequencing and modification analysis. The 4000QTRAP system serves as a powerful instrument for metabolomic profiling, label free peptide quantitation and advanced postranslational modification analysis. We currently have 4000 QTRAP methods for plant hormone quantitation, folate analysis, and ionic compound profiling. Additionally, we have a Waters UPLC system set-up to perform both free and hydrolyzed amino acid analyses.

CF27-M University of Massachusetts Worcester Foundation Campus Laboratory for Proteomic Mass Spectrometry, Shrewsbury, MA

J. Leszyk

University of Massachusetts Worcester Foundation Campus Laboratory for Proteomic Mass Spectrometry, Shrewsbury, MA, United States

The laboratory was originally established in 1989 with a grant from the W.M. Keck Foundation given to the former Worcester Foundation for Biomedical Research. The laboratory's technology focus has evolved over the last decade from a largely micro chemical approach to characterizing proteins (Automated Edman Degradation) to a mass spectrometry based approach. Our services are offered to a large clientele from both academic and corporate entities in the US and abroad. The laboratory offers both ESI and MALDI based ionization mass spectrometry services however we have focused on the development of very high sensitivity gel based analyses using advanced state-of-the-art MALDI TOF mass spectrometry. The two MALDI TOF instruments from Shimadzu Biotech (Axima QIT, and Axima TOF2) provide both accurate MS and true tandem (MS/MS) capabilities at sub femptomole levels for peptides. This poster will give some examples of the kinds of problems addressed utilizing the various instrumentation systems. Such examples will include protein identification, de novo sequencing, and the localization of both chemical and post-translational modifications.

CF28-M FC2: Analytical Instrumentation — Biological Mass Spectrometry

M.D. Person

University of Texas at Austin, Austin, TX

The Analytical Instrumentation Facility Core is a biological mass spectrometry facility that performs service work and collaborative research for the biology, pharmacy and biomedical researchers at the University of Texas at Austin and members of the Center for Research in Environmental Disease at M.D. Anderson Cancer Center and the UT School of Public Health in Houston. The core has MALDI-TOF, MALDI-TOF/TOF, ESI-ion trap, ESI-triple quadrupole-linear ion trap, and GC-quadrupole mass spectrometers. In addition, there are HPLC systems from analytical to nanoflow range and an HPLC system coupled to an electrochemical (EC) detector. Our focus is on biological molecules ranging from metabolites, drugs, and nutrients to protein analysis. We conduct quantitative analysis using GC-MS, HPLC-EC and multiple reaction monitoring on small biomolecules such as 8-oxo-deoxyguanosine, homocysteine, and fluconazole. We also provide protein and peptide molecular weight analysis and sensitive protein identification service. We collaborate on proteomics projects to understand the mechanisms of carcinogenesis. Collaborative projects have successfully characterized protein modifications including chemical adducts, phosphorylation, acetylation, methylation and inhibitor binding sites. The core works to develop robust compound extraction and storage protocols for samples from tissues and biofluids. We test, implement and modify new services in response to researcher interests and innovations in mass spectrometry and sample preparation. The core serves as a resource for UT-Austin and CRED by providing high-end instrumentation and technical expertise as tools for advancing biological and biomedical research.

CF29-M The Vanderbilt Mass Spectrometry Shared Facilities

D.L. Hachey, D.B. Friedman, W.H. McDonald, M.L. Reyzer, E.H. Seeley, M.W. Calcutt, R.M. Caprioli

Vanderbilt University, Nashville, TN

The Vanderbilt Mass Spectrometry Research Center (MSRC) provides an integrated bioanalytical service facility to Vanderbilt researchers in addition to a comprehensive MS research component. The synergies achieved by merging research and service components provide investigators with state-of-the-art proteomics, tissue profiling/imaging and bioanalytical MS technologies that are more comprehensive than usually available in most academic laboratories. These cores are managed by a professional staff of six faculty members and nine research assistants, bioinformatics specialists and an instrument engineer. The Proteomics Laboratory supports multiple technology platforms, including HPLC peptide separations and 2D gel separations of intact proteins, followed by ESI-linear ion trap/orbitrap and MALDI-TOF/TOF MS and supporting bioinformatics for protein identification and targeted characterization. We routinely utilize single- and multi-dimensional LC/MS/MS for protein cataloguing and differential-expression studies (using spectral counting), and Difference Gel Electrophoresis (DIGE) for large-scale expression studies on simple and complex proteomes. The tissue imaging core provides tissue sectioning, staining, and MS directly from tissue sections via either high resolution imaging across an entire tissue section, or higher-throughput histology-directed profiling using specific tissue areas, followed by biostatistical analysis of the MS data. Both of these cores work closely with users at all stages of experiments including detailed post hoc informatics consultations, but generally operate as limited-access facilities where users prepare samples and core technical staff performs the analyses. The bioanalytical MS core provides instrumentation to perform a wide variety of analyses (e.g., identification and structural analysis of biological molecules, qualitative and quantitative assays of drugs and metabolites). The MS core operates in an open access environment where users are encouraged to run their own samples with the advice and assistance of Core personnel. The MSRC also offers a variety of educational instrument operation and training classes throughout the year to facilitate optimal core usage.

CF30-M Automated Electron Transfer Dissociation of Large Peptides and Medium Size Proteins in a QTOF Instrument on an LC Timescale

A. Schneider, C. Stoermer, R. Hartmer, D. Kaplan, M. Park

Bruker Daltonik GmbH, Bremen, Germany

The combination of liquid chromatography (LC) and tandem mass spectrometric (MS) analysis of peptides produced by enzymatic digestion is an established technique for protein identification approaches. For the analysis of such biopolymers tandem mass spectrometers with electrospray ionization (ESI) are commonly used. Electron transfer dissociation (ETD) has become an important technique maintaining fragile modifications of biomolecules during the fragmentation process and is now well established on ion trap instruments. The high resolution and accuracy of orthogonal time-of-flight (oTOF) instruments will extend the possible applications to larger peptides and medium-size proteins. ETD in a hybrid quadrupole time-of-flight (QTOF) instrument has already been demonstrated. Major steps to enable LC applications of ETD in QTOF instruments will be presented: (i) the adaption of ETD reaction times to several kHz repetition rate of the TOF analyzer; (ii) automated precursor ion selection taking into account the charge state of the analyte ions, (iii) optimized ETD sequence timing. With this ETD QTOF prototype instrument the amino acid sequence of several peptides and small proteins were characterized.

CF32-M Fannnie E. Rippel Biochemistry and Biotechnology Facility

A. Yeung, Y. Chen, X. Li, C.G. Miller

Fox Chase Cancer Center, Philadelphia, PA, United States

The Fannie E. Rippel Biochemistry and Biotechnology Facility (BBF) is a full-service, one-stop shopping, facility, from sample preparation to mass spectrometry, from biochemistry to data interpretation. A previous successful component, Real-time PCR facility, has been moved to a genomics core. Technologies supported now include sample preparation, chromatography purification, metabolite and drug analytical chemistry, mass spectrometry, proteomics, and DNA synthesis.

CF33-M We will highlight services available at the CBC/RRC Proteomics and Informatics

D.L. Helseth Jr., H. Xu, B. Hendrickson, A.B. Schilling

CBC/RRC Proteomics & Informatics Services Facility, University of Illinois at Chicago, Chicago, IL, United States

Services Facility (PISF), the core mass spectrometry facility for the Chicago area located at the University of Illinois at Chicago. Through funding from a grant from The Searle Funds at the Chicago Community Trust to the Chicago Biomedical Consortium we provide support to users from Northwestern University and the University of Chicago in addition to the University of Illinois at Chicago. Our instrumentation and services will be summarized with emphasis on new services and new equipment. We offer training to CBC users including a one-day overview and an annual four-day hands-on proteomics and informatics training course. We also host a CBC-sponsored monthly Proteomics Club for proteomics users in the Chicago area. Our facility offers self-serve instrument access for trained users in addition to routine samples and special project submission. OFFGel isoelectric focusing is provided as a service, along with MuDPIT, iTRAQ and other custom services. Please visit our website at http://proteomics.rrc.uic.edu/ for additional details about our services, to submit samples, for recommended protocols for sample submission or for facility information formatted for grant submission. We're available for consultation by phone, e-mail, Access Grid or in person.

CF34-M iTraq Labeled Protein Quantification Using the TSQ Vantage LC-MS/MS

B. Smith, R. Alvarado, J. Presley, P. Kent, K. Nugent, B. Phinney

UC Davis Molecuar Structure Facility, Davis, California, United States

Accurate quantification of proteins and peptides prior to analysis on sensitive mass spectrometers (MS) can be very useful in many proteomics applications – such as spectral counting and differential mass spectrometry (dMS). Accurate quantification of protein digests prior to MS analysis allows for proper loading of the sample and can prevent MS system damage or column overloading. Current methods of quantifying protein digests prior to MS analysis, such as near and far UV, fluorescence labeling, and dye assays, are very limited and are effective for proteins but not protein digests. Amino acid analysis (AAA) is a very common and reliable method of quantifying peptides/proteins. Traditional ion exchange amino acid analysis, however, requires a relatively large amount of sample (~2 nmol) and can take some time to yield results. More sensitive pre-column derivatization AAA can be performed but still offers only a small increase in sensitivity and is subject to matrix effects during derivatization. Because most proteomics experiments are sample limited, it is important to be able to quantify proteins and peptides using minute amounts of material. We will compare AAA sensitivity and accuracy using a triple quadrapole mass spectrometer (combined with Applied Biosystems iTraq reagents and Michrom Bioresources ADVANCE Plug-and-Play nano-spray source) to traditional post-column ninhydrin AAA. Our facility possesses Hitachi high-performance amino acid analyzers and over 20 years of AAA experience – allowing us to quickly and confidently verify the accuracy of the MS protein/peptide quantification.

CF35-M RI-INBRE Centralized Research Core Facility

A. Ahmed, N. Nous

Center for Molecular Toxicology, College of Pharmacy, University of Rhode Island, Kingston, RI, United States

The RI-INBRE Centralized Research Core Facility inaugurated in July 2003, is being supported by the Rhode Island IDeA Network of Biomedical Research Excellence (RI-INBRE) grant and by the participating institutions that include: University of Rhode Island, Brown University, Rhode Island College, Providence College, Salve Regina University, and Roger Williams University. This facility is located in the College of Pharmacy at the University of Rhode Island's Kingston campus. The Core Facility is providing access to research instrumentation and training support to RI-INBRE participants as well as other scientists affiliated with academic institutions and the private sector throughout the state of Rhode Island. Instrument reservation and scheduling are available, through the core facility's website (wwww.uri.edu/inbre/corelab), on a first come, first served basis. All new users are supported with operator assisted access to the equipment. Independent access to the equipment is also available to all trained users. In addition, full service access via sample submission is provided particularly in ICP-MS, LC/MS/MS and N-terminal protein sequencing. After-hours access is granted through the University of Rhode Island I.D. card or by use of a key code password. This poster will present a detailed listing of Core Facility equipment and services. Supported by NIH-NCRR Grant # 1P20RR16457.

CF36-M Instrumentation for Core Facilities: the NIH SIG and HEI Programs

M.A. Tingle

National Institutes of Health, Bethesda, MD, United States

For many years, the NIH has provided expensive state of the art equipment to the biomedical community through the NCRR Shared Instrumentation Grant (SIG) and more recently, the High-End Instrumentation (HEI) programs. In many cases, these instruments are located in institutional core facilities which provide access and the high level technical expertise in cutting edge technologies and complex analytical procedures required for both basic and translational studies. Although the SIG and HEI programs fund instrumentation that span the technology spectrum, some instruments are placed in DNA sequencing, microarray and mass spectrometry cores managed by facility directors who are members of the ABRF. This talk will summarize the funding levels and trends in equipment for both the SIG and HEI programs.

CF37-M The Hartwell Center

G. Neale, A. High, J. Li, S. Malone, J. Morris, D. Naeve, A. Nourse, J. Obenauer, C. Obert, V. Pagala, K. Rakestraw, P. Rodrigues, C. Naeve

St. Jude Children's Research Hospital, Memphis, TN, United States

The Hartwell Center is a unique integration of high-throughput biotechnology resources, bioinformatics resources, and computing resources; designed to provide state-of-the-art tools for biomedical discovery. The Biotechnology laboratories provide cutting edge services in four areas: Macromolecular Synthesis, DNA Sequencing, Functional Genomics and Proteomics. They are supported by a staff of 30, including 9 Ph.D.-level scientists with expertise in all disciplines encompassed by the Center. The Macromolecular Synthesis group provides a variety of research reagents via automated synthesis of oligonucleotides and peptides using standard and custom chemistries. The DNA sequencing laboratories provide investigators access to genome-wide sequence analysis using Roche FLX and Illumina technologies, as well as Sanger DNA sequence analysis, fragment size analysis, and SNP detection technologies. The Functional Genomics group provides microarray analysis services using commercial and custom-built arrays. The Proteomics group offers a range of protein chemistry capabilities including protein identification, intact mass measurement, peptide modification analysis, and molecular interaction analyses by surface and solution-based technologies. All of these resources are highly integrated and readily accessible using our Web-based on-line ordering, tracking, invoicing, and data retrieval system. Research Informatics within Information Sciences provides crucial bioinformatics and computing resources that support the high throughput data generated by the Hartwell Center and other technologies. This group includes 10 Ph.D.-level bioinformatics scientists and 5 staff members that maintain and operate a high performance computing facility. Computational resources include an 810 CPU Linux cluster and more than 100 physical and virtual servers with 368 TB of disk storage. These computer systems provide access to a wide range of bioinformatics applications for genomic, proteomic and structural biology analyses. The wealth of resources offered by the Hartwell Center provide a broad range of chemistry, biomolecular, and bioinformatics services in support of research programs at St. Jude Children's Research Hospital.

CF38-M The University of Oklahoma Health Sciences Center Laboratory for Genomics and Bioinformatics

A.F. Gillaspy

University of Oklahoma Health Sciences Center, Department of Microbiology and Immunology, Oklahoma City, OK, United States

The University of Oklahoma Health Sciences Center's Laboratory for Genomics and Bioinformatics (LGB) is a full service facility offering a wide range of services for DNA sequencing (custom and genome-scale projects), genotyping, fragment analysis, gene expression (Real Time PCR and microarrays), protein analysis and bioinformatics. The LGB welcomes sample submission from any academic or industrial entity and we offer easy online submission and data retrieval via our facility webpage (http://microgen.ouhsc.edu). The laboratory is well equipped with the necessary robotics to serve customers requiring high-throughput services for PCR setup, plasmid preparation, and sample normalization in addition to the specialized services listed above. Available equipment includes two ABI 3730xl sequencers, an ABI SOLiD system, BiaCore T100, ABI 7500 Fast qPCR machine, Beckman NX liquid handler, an Agilent BioAnalyzer, and a Beckman PF2D Proteome lab and PA800 for protein analysis needs. In addition to the wet bench techniques we also offer Bioinformatics support including database development and maintenance, custom script writing, data processing and analysis, and the development of web based tools. To date, we have completed or are participating in the whole genome sequencing of 18 microorganisms (most are pathogens of human or agricultural importance), draft sequencing of 4 additional microbes, and EST sequencing. We also have provided microarray hybridization and data analyses for several gene expression studies in a variety of organisms, including human, mouse and several microorganisms.

CF39-M Core Facility and Outreach Integration through the Vermont Genetics Network

J.M. Murray

Vermont Genetics Network, University of Vermont, Burlington VT, United States

The Vermont Genetics Network (VGN) is funded by the National Center for Research Resources (NCRR), and is part of a National Institutes of Health (NIH) initiative called IDeA Networks of Biomedical Research Excellence (INBRE) to build biomedical research infrastructure. The VGN Program developed three core facilities at the University of Vermont (UVM); microarray, bioinformatics and proteomics. UVM acts as the lead institute in a partnership with 5 baccalaureate colleges throughout Vermont. VGN outreach develops educational modules based on the technologies available in the VGN sponsored cores. We use our outreach program to inspire Vermont undergraduates within and outside our partner colleges by providing visits from VGN staff and faculty, who work closely with students and college faculty to implement cutting edge experiments in their course settings. This program has lead to numerous collaborations between faculty at Vermont baccalaureate colleges and the DNA Analysis and Microarray Cores at UVM. One of the goals of Microarray Outreach is the integration of this technology into the science curriculum at colleges throughout the state. We are seeing this goal being achieved with subsequent deliveries at several colleges. The bioinformatics outreach tutorial was developed in 2006 in association with Dr. William Barnes on sabbatical from Clarion University of Pennsylvania. The module has been delivered by the outreach program since the Fall of 2007. This module is also being quickly integrated into the baccalaureate colleges' curriculum. The proteomics module is schedules for beta testing in the spring of 2009 at UVM All of the VGN outreach activities invest in the level of biomedical research throughout the State of Vermont. The outreach modules and the networking that is achieved through these modules helps to bring about sustainable changes in how we, in Vermont, carry out research and educate our next generation of scientists.

CF40-M Proteomics and Glycomics at the Australian Proteome Analysis Facility (APAF)

B. McInerney, L. Woods, M. Baker

APAF, Macquarie University, Sydney, Australia

Whilst a PhD student at APAF in 1995, Marc Wilkins first coined the fusion word "proteomics". Since then APAF has been a leading institution in developing technology and providing proteomics services to the local Australian and international community. Traditionally strong in 2-D gel electrophoresis and classical protein chemistry (such as edman sequencing and amino acid analysis) APAF has evolved with the growth of proteomics and now provides a comprehensive range of proteomic MS-based techniques with particular focus on biomarker discovery through quantitative proteomics, MRMs and more recently glycomics services.

APAF has considerable experience in quantifying differences in protein expression by iTRAQ labelling and differential fluorescence gel electrophoresis (DIGE). For quantitation of targeted proteins in complex mixtures multiple reaction monitoring (MRM) analysis has been developed. Expertise is also available in label-free quantitation and peptide IPG-IEF "shotgun" methodologies. As well, we offer routine MS analyses to identify and sequence proteins from any biological source. Antibody-based removal of abundant proteins from samples, such as plasma, is available as a sample preparation technology. The importance of post-translational modifications (PTMs) is well recognised in enabling proteins to achieve full biological function. The most common, and largely ignored, PTM is glycosylation. Due to a recent investment in glycomics APAF now offers N- and O-linked glycosylation analysis by MS. This is proving particularly useful for characterising recombinant proteins and antibodies and supporting the regulatory approval process for biotech products. APAF has state-of –the-art, user-friendly facilities in mass spectrometry, gel electrophoresis, chromatography and bioinformatics. These provide competitive, high quality services to the scientific community. We have broad experience in a wide range of samples including the more difficult to analyse biofluids, such as plasma and urine, and plants proteomes like the wheat glutenins. The facility is available to researchers under access arrangements. Further details at www.proteome.org.au.

CF41-M Core Synthesis Facility at NDSU: Research and Capabilities

R.S. Murthy, G.R. Cook

Center for Protease Research, Department of Chemistry and Molecular Biology, North Dakota State University, Fargo, ND, United States

The Core Synthesis Facility (CSF) was established as a part of the Center for Protease Research at NDSU in April 2008 with funding from an NCRR-NIH-COBRE grant. The primary objective of CSF is to act as a strong in-house support system to facilitate biomedical researchers in accomplishing their research goals. The CSF is equipped with modern synthetic technologies and state of the art instrumentation. The research in the CSF is focused on organic synthesis of small molecules for biomedical applications and analytical characterization of substrates of interest. The current research projects involve synthesis of enzyme inhibitors, fluorescent labeling of peptides and amino acids, development of a cytotoxic drug delivery model and qualitative analysis of natural products by LC-MS/HRMS. The CSF also offers scientific consultation and provides student training. On a whole, the research in the CSF is dedicated towards the treatment of a variety of diseases and our future goal is to establish a nationwide clientele.

CF43-M Microscopy and Imaging Facility

C. Bayles, R. Williams, G. Xia, R. Doran, W. Zipfel

Life Sciences Core Laboratories Center, Cornell University, Ithaca, NY, United States

The Microscopy and Imaging Facility of the Cornell University Life Sciences Core Laboratories Center (CLC) provides an array of shared research resources and services relating to optical microscopy, including confocal microscopy, fluorescence imaging, spectrofluorimetry, ultrasound, image processing and analysis. The mission of the facility is to provide cutting edge technologies and high quality services that will significantly contribute to life sciences research, training and education programs.

CF44-M Laser Capture Microdissection

A. Liu

Fox Chase Cancer Center, Philadelphia, PA, United States

The state-of-the-art technology laser capture microdissection (LCM) provides the researcher with a means to isolate a pure population of cells from heterogeneous tissue specimens. The purified DNA, RNA or protein from captured cells can be used for a wide range of downstream applications such as gene expression analysis, loss of heterozygosity studies, or proteomic assays, etc. Many different types of molecular analyses have been successfully performed on cells procured by LCM and a number of new LCM technologies have been developed in our LCM facility, which will contribute to meeting the growing interdisciplinary research program at Fox Chase Cancer Center and potentially other ABRF members.

CF45-M The UC Davis Genome Center Proteomics Facility

B.S. Phinney, R.A. Eigenheer, R. Alvarado, B. Smith, J.M. Presley

University of California, Davis, CA, United States

The UC Davis Genome Center Proteomics Facility offers protein analysis via amino acid analysis, Edman sequencing and proteomic services including protein identification, post-translational modification discovery, label free quantitation and targeted protein analysis. The facility utilizes state of the art instrumentation, including a Thermo LTQ-FT Ultra, and TSQ Vantage mass spectrometers. Our group utilizes open source software and uses techniques and protocols that are made publicly available. Using open source software allows users to analyze their own data without significant monetary investment. We also offer data analysis and sample preparation classes to UC Davis and the community at large, including a week long hands on summer short course. Our group operates as an open core facility within the Genome Center on the UC Davis campus

CF47-M IDPicker 2.0: Improved Protein Assembly with High Discrimination Peptide Identification Filtering

Z. Ma[1], M.C. Chambers[2], M.D. Litton[2], S.M. Sobecki[2], L.J. Zimmerman[2], D.L. Tabb[1, 2]

[1]Department of Biomedical Informatics, [2]Mass Spectrometry Research Center, Vanderbilt University Medical Center, Nashville, TN, United States

Tandem mass spectrometry-based shotgun proteomics has become a widespread technology for analyzing complex protein mixtures. A number of database searching algorithms have been developed to assign peptide sequences to tandem mass spectra. Assembling the peptide identifications to proteins, however, is a challenging issue because many peptides are shared by multiple proteins. IDPicker is an open-source protein assembly tool that derives a minimum protein list from peptide identifications filtered to a specified False Discovery Rate. Here we update IDPicker to increase confident peptide identifications by combining multiple scores produced by database search tools. By segregating peptide identifications on both charge state and the number of tryptic termini in validation, IDPicker yields more confident identifications for protein assembly. The new version achieves higher accuracy by requiring additional novel peptides in the parsimony process, especially when searching against multiple-species databases. IDPicker has been tuned for incorporation in many identification workflows by the addition of a graphical user interface and the ability to read identifications from the pepXML format. These advances position IDPicker for high peptide discrimination, reliable protein assembly, and highly-scalable proteomics studies.

CF48-M Extending MALDI-QqQ-MS Enzyme Screening Assays to Targets with Small Molecule Substrates

R. Rathore[1], J.J. Corr[2], K.D. Greis[1]

[1]University of Cincinnati, Genome Research Institute, Cincinnati, OH, United States; [2]MDS Analytical Technologies, Concord, ON, Canada

Mass spectrometry-based high throughput screening has tremendous future potential as an alternative to current screening methods due to its speed, sensitivity, reproducibility and label-free readout. In addition, this method offers a direct readout of the substrate and product, thus minimizing the potential for false positive or false negative hits. We recently reported that a new generation matrixassisted laser desorption ionization-triple quadrupole mass spectrometer (MALDI-QqQ-MS) is ideally suited for a variety of enzyme assays and screening protocols. This instrument provides comparable speeds (at greater than a sample per second) with superior signal-to-background, better reproducibility and a reagent cost savings of greater than 90% as compared to typical fluorescence-coupled assays. Thus far the MALDI-based readout has been validated for a variety of enzyme classes (kinases, phosphatases, proteases, hydroxylases), however all these targets have peptide substrates that are readily monitored without interference from the MALDI matrix. To further extend the application of the MALDI-QqQ readout to enzymes with small molecule, non-peptide substrates, we evaluated this method for measuring enzyme activity and inhibition of acetylcholinesterase. Due to matrix interference in measuring these small molecules during the MALDI process, multiple reaction monitoring (MRM), available on the QqQ instruments, was used to generate a selective MS/MS transition and accurately measure both the substrate (acetylcholine) and the product (choline) of acetylcholinesterase. Importantly, accurate dose-dependant inhibition measurements were also demonstrated thus validating the MRM readout for enzymes with small molecule substrates and products. Collectively, these data demonstrate that a MALDI-QqQ-MS based readout platform is amenable for small molecule substrates and products and offers significant advantages over current HTS methods in terms of speed, sensitivity, reproducibility and reagent costs.

CF49-M High Mobility Group Box 1 (HMGB1) In Eosinophil Activation

C. Straub, K. Pazdrak, A. Kurosky

The University of Texas Medical Branch at Galveston, Galveston, TX, United States

Eosinophils have been implicated in allergic inflammation and certain parasitic and viral infections. Our proteomic studies of non-activated and activated eosinophils identified the expression and release of HMGB1. HMGB1 plays a prominent role in immunoregulatory cell activation including granulocyte activation. Our experiments were designed to determine whether eosinophil-derived HMGB1 contributes to the autocrine activation of human peripheral blood eosinophils. HMGB1-presence in eosinophils was determined using western blotting and 2-dimensional gel electrophoresis. Pro-Q Diamond phosphoprotein stain and Sypro Ruby protein stain were used for detection of phosphoproteins or proteins, respectively. Cell survival rates and expression of CD69 were determined after stimulation of eosinophils with GM-CSF, rHMGB1, and various HMGB1 inhibitors. Eosinophils stimulated with either GM-CSF, rHMGB1, and eosinophil-derived HMGB1 showed significantly higher viability and expression of CD69. Pre-treating eosinophils with glycyrrhizin, a specific inhibitor of HMGB1 activity, partially inhibited prolongation of survival as well as upregulation of CD69. This upregulation was significantly inhibited by treatment with glycyrrhizin or anti-RAGE2 antibody. The phosphoproteomic profile of HMGB1 stimulated eosinophils also changed. The phosphoproteomic pattern and the proteomic pattern of eosinophils treated with HMGB1 and GM-CSF vary as compared to control cells and compared to each other. Our studies demonstrate the autocrine activation of eosinophils through HMGB1. These findings indicate a significant immunoregulatory role for eosinophils and provide a novel mechanism for the characterization of eosinophil-associated pathologies. This study was supported by the NIH/NHLBI Proteomics Initiative NO1-HV-28184 (AK).

CF50-M Using targeted proteomics to assess the impact of sirtuins on liver metabolism in obesity

A. Kendrick[1], M. Choudhury[2], M. Rahman[2], J. Friedman[2], K.R. Jonscher[1]

[1]University of Colorado Denver, Deparment of Anesthesiology; [2]University of Colorado Denver, Deparment of Pediatrics

The NAD-dependent deacetylase SirT1 regulates lipid and glucose metabolism in liver and increased SirT1 activity in caloric-restricted models has been linked to extended life span in several species. Deacetylation of transcription factors and co-modulators, such as p53, NF-κB, C/EPBβ and PGC-1, allows SirT1 to sense and regulate energy levels. The SirT family can also deacetylate mitochondrial proteins, suggesting that posttranslational modification by sirtuins may have global effects on energy metabolism especially gluconeogenesis and lipogenesis. Livers from mice fed either control or a high fat (HF) diet (45 kcal % as fat, 12 wks) were harvested and homogenized. SirT1 levels were measured in whole cell lysates by Western blot. Proteins modified by lysine acetylation were immunoprecipitated with anti-acetyllysine antibody and subsequently separated by one dimensional gel electrophoresis. Bands showing differential staining between the control and HF fed mice were excised and proteins were digested with trypsin. Tandem mass spectrometry using an Agilent Ultra quadrupole ion trap generated product ion spectra that were searched with SpectrumMill against the SwissProt database. The levels and acetylation of identified proteins were validated by immunoprecipitation and Western blotting. Mice on the HF diet were obese, with fatty livers and reduced SirT1 activity as assessed by the NAD+/NADH ratio. SirT1 protein expression levels did not significantly change, however. Acetylation of a subset of the proteins identified, such as carbamoyl-phosphate synthase, uricase, pyruvate carboxylase and ATP synthase, has been previously reported. Interestingly, peroxiredoxin, catalase, and Hsp70, proteins involved in redox and the stress response, were hyperacetylated in the livers of obese mice. We postulate that *modification of those proteins could influence the ability of obese mice to modulate oxidative stress, gluconeogenesis and lipogenesis.* Globally surveying lysine acetylated proteins using immunoprecipitation and gel electrophoresis/tandem mass spectrometry provides insights into how obesity impacts liver metabolism.

NOTES

RG1-S1 The DNA Sequencing Research Group general survey, 2009: Second Generation Sequencing Instruments and Services in Core Facilities.

P. Schweitzer[1], D. Bintzler[2], K. Dewar[3], J. Kieleczawa[4], A. Perera[5], S. Singh[6], R. Steen[7], M. Zianni[8], M.M. Detwiler[9]

[1]Cornell University, Ithaca, NY, United States; [2]DNA Analysis, Inc., Cincinnati, OH, United States; [3]McGill University, Montreal, Quebec, Canada; [4]Wyeth Research, Cambridge, MA, United States; [5]Stowers Institute for Medical Research, Kansas City, MO, United States; [6]University of Minnesota, St. Paul, MN, United States; 7Harvard Medical School, Boston, MA, United States; [8]Ohio State University, Columbus, Ohio, United States; 9Roswell Park Cancer Institute, Buffalo, NY, United States

The ABRF DNA Sequencing Research Group (DSRG) has conducted a general survey to collect data on the current state of second generation sequencing instrumentation (often termed "massively-parallel" or "next generation" sequencers) and services offered by core facilities. The DSRG has monitored trends in sequencing platforms in core facilities by conducting surveys periodically. However, this survey was the first to focus on second generation sequencers since their introduction. The information gathered this year provided data about the widespread availability of this equipment and these services in core facilities. The future acquisition and expectations for such instruments were also assessed. For comparison, the survey gathered information on Sanger (first generation) sequencing operations to determine how these technologies are affected by the second generation technologies. The importance of this survey lies in the fact that it serves as an initial "snapshot" of the status of second generation sequencing services in core facilities while they are in their infancy, and therefore is a baseline for surveys in years to come.

RG2-S1 Nucleic Acid Research Group 2008-2009 Study: A Comparison of Different Priming Strategies for cDNA Synthesis by Reverse Transcriptase, as Evaluated by Real-Time RT-qPCR

S.V. Chittur[1], D.S. Grove[2], D.J. Hollingshead[3], T.C. Hunter[4], K.L. Knudtson[5], G.L. Shipley[6], K. Sol-Church[7], W.L. Taylor[8] and A.T. Yeung[9]

[1]University at Albany, Albany, NY, United States; [2]Penn State University, University Park, PA, United States; [3]University of Pittsburgh, Pittsburgh, PA, United States; [4]University of Vermont, Burlington, VT, United States; [5]University of Iowa, Iowa City, IA, United States; [6]UTHSC-Houston, Houston, TX, United States; [7]A.I. duPont Hospital for Children, Wilmington, DE, United States; [8]UTHSC-Memphis, Memphis, TN, United States; [9]Fox Chase Cancer Center, Philadelphia, PA, United States

Real-time reverse transcriptase quantitative PCR (RT-qPCR) is widely used for measuring transcription levels. Assay-specific primers, although ideal for cDNA synthesis, are not always practical. Priming strategy and reverse transcriptase enzyme affect the sensitivity and variability of RT-qPCR and microarray results. The Nucleic Acid Research Group (NARG) designed a study to determine the optimal priming strategy for RT-qPCR. The NARG 2008-09 study was an extension of the 2007-08 study in which we evaluated the effect of reverse transcription priming strategies on RT-qPCR results. The previous study suggested a relationship between the assay sensitivity using cDNA generated with oligo-dT primers and qPCR assay placement relative to the 3-prime end of the transcript. This year's study was designed specifically to compare oligo-dT and random priming strategies as the assay target site varied. Because the previous study identified random hexamers or nonamers as most efficient of those tested, this years study was designed specifically to compare oligo-dT, random 6-mers and 9-mers or gene specific primers and combinations. Four reverse transcriptases; Superscript II, Superscript III, Transcriptor and MultiScribe, were employed to determine the effect of enzyme. In addition, the qPCR assays looked at three genes of varying abundance, β-actin (high copy), β-glucuronidase (medium copy) and TATA binding protein (low copy) as well as varying distance from the 3-prime end for each transcript.

RG3-S1 Edman Sequencing Research Group (ESRG) Study 2009: Comparison of Edman and Mass Spectrometry Techniques for N-terminal Sequencing

R.S. Thoma[1], B. Hampton[2], J.S. Smith[3], W. Sandoval[4], P. Hunziker[5], N.D. Denslow[6]

[1]Monsanto Co., St. Louis, MO, United States; [2]University of Maryland School of Medicine, Baltimore, MD, United States; [3]University of Texas Medical Branch, Galveston, TX, United States; [4]Genentech Inc., South San Francisco, CA, United States; [5]University of Zurich, Zurich, Switzerland; [6]University of Florida, Gainesville, FL, United States

For decades, automated Edman sequencing has been the method of choice for determining the N-terminal amino acid sequence of proteins. However, the major but by no means only limitation of this technique is its inability to obtain amino acid sequence from N-terminally blocked proteins. Mass spectrometric techniques for protein sequence analysis do not suffer from this limitation though unequivocal determination of protein N-termini on a routine basis has been elusive. The advantages of mass spectrometric techniques have in recent years driven investigators to look beyond Edman chemistry to find alternative technologies to obtain N-terminal sequence. Several mass spectrometric methodologies have been published, primarily for proteomics analyses, which may be quicker, less costly and more sensitive than Edman sequencing. Because such techniques involve a range of biochemical and instrumental methodologies having different advantages and limitations the ESRG has created a study to ascertain how reliably they can produce N-terminal amino acid sequence information and to compare those results to those obtained by automated Edman sequencing.

The ESRG 2009 study was designed to allow the participants freedom to use their analytical technique of choice to obtain as much N-terminal amino acid sequence information as possible from two test proteins. Approximately one nanomole of each sample was provided so laboratories may attempt a variety of techniques with the goal of obtaining each protein's N-terminal sequence. Results of these analyses as well as a comparison of methodology, instrumentation and specific protocols used will be presented so that core facilities can gauge their competence and expectations for determining N-terminal amino acid sequences.

RG4-S1 PRG 2009 Study: Relative Protein Quantification in a Clinical Matrix

M.J. MacCoss[1], A.S. Chien[2], B. Friedman[3], D.Hawke[4], J.Krijgsveld[5], K.S. Lilley[6], R.E. Settlage[7], N.E. Sherman[8], C.W. Turck[9]

[1]University of Washington; [2]Stanford University; [3]Vanderbilt University; [4]UT MD Anderson Cancer Center; [5]EMBL Heidelberg; [6]University of Cambridge; [7]Virginia Bioinformatics Institute; [8]University of Virginia; [9]Max Planck Institute of Psychiatry

The Proteomics Research Group (PRG) of the ABRF developed the 2009 study to assess approaches that individual laboratories would use to determine the relative abundance of target proteins in a complex mixture. An increasingly common request for proteomics laboratories is the detection of a specific target protein of interest in a complex mixture. Likewise, most of these requests are also interested in knowing the abundance of the target protein relative to that in a control sample. While this type of analysis has traditionally been addressed using Western blots or other immunoaffinity assay, recent advances in targeted mass spectrometry-based analyses are beginning to be reported in the literature as an alternative.

For this year's study, four different proteins were spiked into a plasma background matrix at three different levels. Two of these proteins are commonly measured plasma protein biomarkers, and the remaining two had identical primary structure and differed by only a single phosphorylation site. The participants were shipped six samples in total (three samples in blinded duplicate) and asked to report the relative abundances of the four target proteins in the six samples. Results from analysis of the samples and survey responses will be used to assess the different approaches that are used by the proteomics community to determine the relative abundance of a target protein of interest. For this year's study, four different proteins were spiked into a plasma background matrix at three different levels. Two of these proteins are commonly measured plasma protein biomarkers, and the remaining two had identical primary structure and differed by only a single phosphorylation site. The participants were shipped six samples in total (three samples in blinded duplicate) and asked to report the relative abundances of the four target proteins in the six samples. Results from analysis of the samples and survey responses will be used to assess the different approaches that are used by the proteomics community to determine the relative abundance of a target protein of interest.

RG5-S1 sPRG2009 Study: Development of a Quantitative Proteomics Standards

J.G. Farmar[1], D. Arnott[2], A.R. Ivanov[3], J.A. Kowalak[4], W.S. Lane[5], K. Mechtler[6], B.S. Phinney[7], M.R. Raida[8], and S.T. Weintraub[9]

[1]A. Einstein College of Medicine, Bronx, NY, United States; [2]Genentech, Inc., South San Francisco, CA, United States; [3]Harvard School of Public Health, Boston, MA, United States; [4]National Institute of Mental Health, Bethesda, MD, United States; [5]Harvard University FAS Center for Systems Biology, Cambridge, MA, United States; [6]Research Institute of Molecular Pathology, Vienna, Austria; [7]Proteomics Core UC Davis Genome Center, Davis, CA, United States; [8]Experimental Therapeutics Centre, Singapore; [9]Univ. of Texas Health Science Center at San Antonio, San Antonio, TX, United States

The Proteomics Standards Research Group (sPRG) initiated a study in 2007 that focused on development of a mixture of standard proteins that contained appropriate stable isotope labeled (SIL) peptides and could be used as a model for quantitative plasma proteomics. A set of 350 human plasma proteins was evaluated extensively, with the goal of selecting 50 proteins that would be distributed over five orders of magnitude in concentration. After lengthy consideration of the project, it was decided that there would be too many challenges associated with analysis of this type of sample. Efforts have now focused on development of a simplified standard that is based on human plasma proteins and would be suitable for use in assessing a laboratory's capabilities for absolute quantitative analysis. Drawing from the information gained previously from evaluation of the 350 human plasma proteins, 10 candidate proteins were selected for further consideration. As a first step, the sPRG members digested and analyzed each of the individual proteins. From those results, a list of prospective peptides was generated and corresponding unlabeled and SIL peptides were synthesized. For the next stage, the sPRG analyzed individual samples containing the synthetic peptides (labeled and unlabeled) for each protein. Subsequently, a digest of a mixture of the proteins was analyzed in the presence of the SIL peptides, and the relative intensities of each unlabeled/labeled peptide pair were assessed. It was concluded from these experiments that better standardization was needed for the proteins and SIL peptides before a study sample could be prepared. This led to a timeline of presenting analysis of a newly-formulated study sample by sPRG members at the ABRF conference and having results from participating laboratories available in time for presentation at ASMS 2009.

RG6-S1 iPRG2009 Study: Testing for Qualitative Differences Between Samples in MS/MS Proteomics Datasets

B.C. Searle[1], D.L. Tabb[2], J. Falkner[3], J.A. Kowalak[4], K. Mayer-Arendt[5], L. Martens[6], M. Askenazi[7], P.A. Rudnick[8], S.L. Seymour[9], W.S. Lane[10]

[1]Proteome Software Inc., Portland, OR, United States; [2]Vanderbilt University Medical Center, Nashville, TN, United States; [3]University of Michigan, Ann Arbor, MI, United States; [4]NIMH, Rockville, MD, United States; [5]University of Colorado, Boulder, CO, United States; [6]EMBL-EBI, Cambridge, United Kingdom; [7]Dana-Farber Cancer Institute, Brookline, MA, United States; [8]NIST, Frederick, MD, United States; [9]Applied Biosystems, Foster City, CA, United States; [10]Harvard University, Cambridge, MA, United States

Determining significant differences between mass spectrometry datasets from biological samples is one of the major challenges for proteome informatics. Accurate and reproducible protein quantitation in complex samples in the face of biological and technical variability has long been a desired goal for proteomics. The ability to apply qualitative difference testing is a first step towards that goal, and is routinely used in tasks such as biomarker discovery. In this work the Proteome Informatics Research Group (iPRG) of the ABRF presents the results of a collaborative study focusing on the determination of significantly different proteins between two complex samples. In this study, datasets representing five technical replicates of each sample were provided to volunteer participants and their ability to evaluate reproducible differences was tested. A survey was used to determine the relative merits of spectrum counting versus MS intensity-based differentiation, whether sophisticated statistical methods are necessary, and if computer software must be augmented by scientific expertise and intuition. Results and survey responses were used to assess the present status of the field and to provide a benchmark for qualitative difference testing on a realistically complex dataset.

NOTES

AUTHOR INDEX

Please note that only primary authors are listed in this index.

P

R

S

T

V

W

X

Y

Z

MEMBERSHIP APPLICATION

Association of Biomolecular Resource Facilities
9650 Rockville Pike, Bethesda, MD 20814-3998 (USA)
Telephone: (301) 634-7306 Fax: (301) 634-7420 Email: abrf@abrf.org

Contact Information

Your Name

Company/Institution

Address

City State Zip Country

Phone Fax

Email

Important! Your membership confirmation will be sent via e-mail, along with other ABRF communications.

Membership Options

Please check one.

- ❑ Member, $85.00
- ❑ Student Member*, $35.00

**To qualify for Student membership you must be a full-time undergraduate, graduate or medical student. Your department chair or student advisor must complete the following information:*

I certify that the applicant is a full-time student in

Signature of Department Chair or Major Advisor

Additional Information

Are you part of a resource laboratory? ❑ Yes ❑ No

If yes, please list your director/manager's name:

Facility Name:

Payment Method

Please return this form with your full payment to assure proper credit. ABRF Tax ID: 56-1659510.

- ❑ Check enclosed for full amount made payable to ABRF. *(Check must be in U.S. dollars and drawn on U.S. Bank.)*

- ❑ Credit card *(full amount will be charged)*: ❑ VISA ❑ MasterCard ❑ American Express

Credit Card Number Expiration Date / /

Signature of Cardholder

Send this form with payment to: ABRF, 9650 Rockville Pike, Bethesda, MD 20814-3998 (USA)
Fax: (301) 634-7306 *(Faxed membership forms must include credit card information.)*