ABRF 2009:
Optimization and Application of Existing and Emerging Biotechnologies
February 7-10, 2009 – Memphis, Tennessee

SATELLITE EDUCATIONAL WORKSHOP PROGRAM
(sw1) Next Generation DNA Sequencing
(current as of 12/12/08)

Saturday, February 7, 2009
8:00 am – 4:00 pm
Memphis Cook Convention Center

Massively Parallel Sequencers in the Core Facility: Applications and Computation

Workshop sponsor: **Illumina**
Session sponsors: **Roche Applied Science and Applied Biosystems**
Speaker sponsors: **Fluidigm, Geospiza, GenomeQuest, and DNAStar**

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 - 8:00 am	**CONTINENTAL BREAKFAST** - *Ballroom Foyer*
	MORNING SESSION: PLATFORMS AND APPLICATIONS Peter Schweitzer, Cornell University (organizer)
8:00 - 8:20 am	**From Reads to Data Sets: Why Next Gen is not like Sanger Sequencing** Todd Smith, Geospiza
8:20 - 9:10 am	**Sequencing with the Roche 454 FLX** Alvaro Hernandez, University of Illinois at Urbana-Champaign
9:10 - 10:00 am	**Sequencing with the Applied Biosystems SOLiD**
10:00 - 10:30 am	**Refreshment Break** - *Ballroom Foyer*
10:30 - 11:20 am	**Sequencing with the Illumina Genetic Analyzer** James Hadfield, Cambridge Research Institute, United Kingdom
11:20 - 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 - 1:00 pm	**LUNCH** - *Ballroom Foyer*

AFTERNOON SESSION: COMPUTATION AND ANALYSIS

Charles Nicolet, University of California at Davis (organizer)

1:00 – 1:20 pm	**Subnet Architecture for MPS: a Core Manager's Computational Awakening** Charles Nicolet, University of California at Davis
1:20 - 1:50 pm	**Building a Core Infrastructure for High-Throughput DNA Sequencing** Scott Givan, Oregon State University
1:50 - 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 - 2:45 pm	**REFRESHMENT BREAK** - *Ballroom Foyer*
2:45 - 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 - 3:35 pm	**Sponsor Presentation** Roche Applied Science Applied Biosystems
3:35 - 4:00 pm	**Ask the Experts** Informal discussion between speakers, attendees and sponsors

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SATELLITE EDUCATIONAL WORKSHOP PROGRAM
(sw2) Proteomics Instrumentation
(current as of 11/28/08)

Saturday, February 7, 2009
8:00 am – 4:00 pm
Memphis Cook Convention Center.

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

***Session sponsor*: Applied Biosystems**

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

7:00 am - 12:00 pm	**REGISTRATION OPEN** - *Lobby*
7:00 - 8:00 am	**CONTINENTAL BREAKFAST** - *Ballroom Foyer*
8:00 - 8:15 am	**INTRODUCTION TO PROTEOMICS**
8:15 - 9:00 am	**MAJOR TECHNOLOGY PLATFORMS OVERVIEW:** **LC/MS/MS, 2D-GEL/MS, PROFILING/IMAGING**
9:00 – 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 - 10:30 am	**REFRESHMENT BREAK** - *Ballroom Foyer*
10:30 – 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 – 12:00 am	**QUALITATIVE & QUANTITATIVE LC/MS/MS APPLICATIONS (PART I):** **STABLE ISOTOPE LABELING**
12:00 - 1:00 pm	**LUNCH** - *Ballroom Foyer*
1:00 – 1:45 pm	**QUALITATIVE & QUANTITATIVE LC/MS/MS APPLICATIONS (PART II):** **LABEL-FREE, MRM/SRM**
1:45 – 2:00 pm	**QUALITATIVE AND QUANTITATIVE GEL-BASED APPLICATIONS (PART I):** **2DE AND DIGE**
2:00 - 2:30 pm	**REFRESHMENT BREAK** - *Ballroom Foyer*
2:30 – 3:30 pm	**QUALITATIVE AND QUANTITATIVE GEL-BASED APPLICATIONS (PARTI I):** **DIGE/MS, MULTIVARIATE STATISTICS, VARIATION, POWER ANALYSIS**
3:30 - 4:00 pm	**ASK THE EXPERTS SESSION** Informal discussions between presenters, organizers, and attendees

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SATELLITE EDUCATIONAL WORKSHOP PROGRAM
(sw3) Recombinant Protein Laboratory
(current as of 12/1/08)

February 6-7, 2009
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 - 8:00 am **CONTINENTAL BREAKFAST** – *St Jude, DTRC Room T4*

8:00 - 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 - 1:00 pm **LUNCH** – *St. Jude, E1004*

1:00 – 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 – 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 - 8:00 am **CONTINENTAL BREAKFAST** – *St. Jude, E1004*

8:00 - 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 - 1:00 pm **LUNCH** – *St. Jude, E1004*

1:00 - 1:30 pm **ASK THE EXPERTS SESSION –** *St. Jude, E1004*

Round-table discussion on protein expression/purification as a shared resource

1:30 - 4:00 pm **LAB WORK –** *St. Jude, Lab E8066*

A. SDS-PAGE Analysis of Purified Protein

B. Biochemical Assay of Activity

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SATELLITE EDUCATIONAL WORKSHOP PROGRAM
(sw4) HPLC Theory and Practice
(current as of 11/28/08)

Saturday, February 7, 2009
8:00 am – 4:00 pm
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 - 8:00 am **CONTINENTAL BREAKFAST** - *Ballroom Foyer*

8:00 - 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 - 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 - 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 - 1:00 pm **LUNCH** - *Ballroom Foyer*

1:00 - 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 - 2:30 pm **REFRESHMENT BREAK** - *Ballroom Foyer*

2:30 - 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 - 4:00 pm **ASK THE EXPERTS SESSION**
Informal discussions between presenters, organizers, and attendees

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SATELLITE EDUCATIONAL WORKSHOP PROGRAM
(sw5) Proteome Informatics
(current as of 11/28/08)

Saturday, February 7, 2009
8:00 am – 4:00 pm
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 - 8:00 am	**CONTINENTAL BREAKFAST** - *Ballroom Foyer*
8:00 - 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 - 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 - 10:30 am	**REFRESHMENT BREAK** - *Ballroom Foyer*
10:30 - 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 - 1:00 pm **LUNCH** - *Ballroom Foyer*

1:00 - 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 - 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 post-translational modifications, or if they contain mutations.

2:20 - 2:50 pm **REFRESHMENT BREAK** - *Ballroom Foyer*

2:50 - 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 - 4:00 pm **ASK THE EXPERTS SESSION**
Informal discussions between presenters, organizers, and attendees