

Welcome to the USF Genomics Training

11th RNAseq Laboratory Workshop Virtual Training

USF GENOMICS TRAINING COURSES



UNIVERSITY OF SOUTH FLORIDA

USF Genomics Program – Genomics Training Courses

Virtual RNAseq Workshop - Laboratory Training Illumina RNAseq Library Prep and QC

Date: June 7th, 2021

Venue: Online (Blackboard Collaborate)

Time: 9:00 am – 5:00 pm

Registration Deadline: May 26th, 2021*

Introduction:

The virtual RNAseq Workshop-Laboratory Training will be split into an online-session and separate hands-on training. The online-session will consist of a full day of training and includes an introduction to the USF Genomics Equipment Core, RNA sequencing and NGS workflow overview. After the online-session is complete, the participant will become a certified user to submit work-requests through the USF Genomics Equipment Core iLab system. The hands-on training will be delivered as part of the user's first work-request with the core staff.

Online-Session

- Overview of Illumina NGS workflow
- Understanding Illumina SBS technology
- NGS library preparation and validation
- Sequencing set up
- Sequencing data QC and troubleshooting
- iLab user training

Hands-On Training

- Submit work Request
- Schedule sequencing work and training
- Library Prep under supervision
- Sequencing with the core staff

Enrollment Requirements:

- The hands-on training must be submitted within one year of the online-session completed.
- We strongly recommend that you enroll *only if you plan to submit a sequencing work request with the USF Genomics Equipment Core within a year* from the workshop date.
- Enrollment in the one-day virtual workshop is limited to 20 participants.

Online Registration Information:

<https://health.usf.edu/publichealth/ghidr/genomics/genomics-training>

USF Genomics Contact: genomics@usf.edu, (813) 974-7566

*Registration does not guarantee enrollment in this workshop. A separate email will be sent to confirm enrollment. Registrants not enrolled in the current session will be added to a waitlist.

If you have previously registered for the USF Genomics RNAseq Training, you will be sent an email about enrolling in a specific session.



UNIVERSITY OF SOUTH FLORIDA

USF Genomics Program – Genomics Training Courses

RNAseq Workshop - Computational Training RNAseq Data Analysis

Date: June 8th, 2021 – June 11th, 2021

Venue: Online (Blackboard Collaborate)

Time: 9:00 am – 5:00 pm

Registration Deadline: May 26th, 2021*

Overview: This 4-Day course will instruct participants on theoretical and practical concepts related to RNA sequencing data-analysis, enabling them to perform these analyses independently.

Day 1: An intro to data analysis using Linux ensuring that all participants can follow the practical sections of the training, followed by hands-on practice.

Day 2: An overview of the advantages and disadvantages of current sequencing technologies and their implications for data analysis, followed by hands-on analysis (pre-processing and QC of raw sequencing reads, read-mapping, visualization).

Day 3: Intro to the R statistical computing and visualization programming language, and *Bioconductor*, an R-based toolshed for analyzing genomic data, followed by hands-on quantification of gene-expression to identify differentially expressed genes.

Day 4: Putting it all together: a full day of hands-on RNAseq analysis using the TUXEDO pipeline.

Participants:

- Admittance is limited to 20 participants.
- Recommended prerequisites: basic knowledge of biochemistry/molecular biology; basic familiarity with computers; a need for RNA sequencing analysis for current research projects.
- *Prior sequence analysis or command-line experience are not required.*
- Participants will be required to complete a short online module prior to the workshop.
- Participants must have their own laptop to access the USF Research Computing infrastructure for hands-on sessions.

Online Registration Information: <https://health.usf.edu/publichealth/ghidr/genomics/genomics-training>

USF Genomics Contact: genomics@usf.edu, (813) 974-7566

Training Instructors: Jenna Oberstaller, PhD; Charley Wang, PhD; Justin Gibbons, PhD

*Registration does not guarantee enrollment in this workshop, a separate email will be sent to confirm enrollment. If not enrolled in this workshop, registrations will be added to a waitlist.

If you have previously registered for the USF Genomics RNAseq Training, you will be sent an email about enrolling in a specific session.

Workshop Structure:	Illumina Sequencing Refresher	Introduction to RNA sequencing	Introduction to R and Bioconductor	RNAseq: TUXEDO Pipeline Hands-on
	Linux for Bioinformatics	RNAseq Alignment and Visualization	Expression and Differential Expression	

Tuesday Wednesday Thursday Friday

USF Genomics RNAseq Laboratory Workshop

Instructors Introduction

USF Genomics Core



Min Zhang
Manager of
Genomics Core



Matthew Mercurio
Core Staff

Illumina

Matt Angel
Sequencing specialist

Meredith Millis, Field
Application Scientist

Evelyn Griffin,
egriffin@illumina.com
Account Manager FL

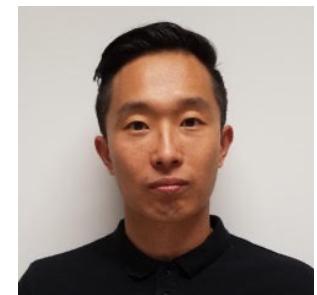
Genomics Omics Hub



Jenna Oberstaller
Director of
Omics Hub



Justin Gibbons



Charley Wang

11th RNAseq Laboratory Workshop Virtual Training Agenda

Time	11th USF Genomics RNASeq Laboratory Workshop - Virtual Training Agenda (Monday, June 7th, 2021)	Instructor
9:00 AM - 9:15 AM	Trainee-introductions (Brief summary of your research interests)	Instructor and Trainees
9:15 AM - 9:45 AM	Introduction to the USF Genomics Core, Illumina sequencing workflow overview	Min Zhang /USF Genomics Core
9:45 AM - 10:15 AM	Introduction to the USF Genomics Omics Hub, functional genomics, data-processing overview	Jenna Oberstaller/USF Omics Hub
10:15 AM - 11:00 AM	Understanding Illumina sequencing technology (SBS)	Meredith Millis, Field Application Scientist/ Illumina
11:15 AM - 12:00 PM	NGS library preparation overview	Min Zhang /USF Genomics Core
12:00 PM - 1:00 PM	Lunch Break	
1:00 PM - 1:45 PM	Library validation, QC and troubleshooting	Min Zhang /USF Genomics Core
1:45 PM - 2:30 PM	Sequencing set up	Mathew Mercurio/USFGenomics Core
2:30 PM - 2:45 PM	Illumina sequencing reagents & Customer Resources Overview	Evelyn Griffin, Account Manager/Illumina
2:45 PM - 3:30 PM	Illumina BaseSpace Tour and Sequencing data QC	Matt Angel, Sequencing Specialist/ Illumina
3:00 PM - 4:00 PM	iLab User Training	Mathew Mercurio/USF Genomics Core

Welcome to the USF Genomics Training Course

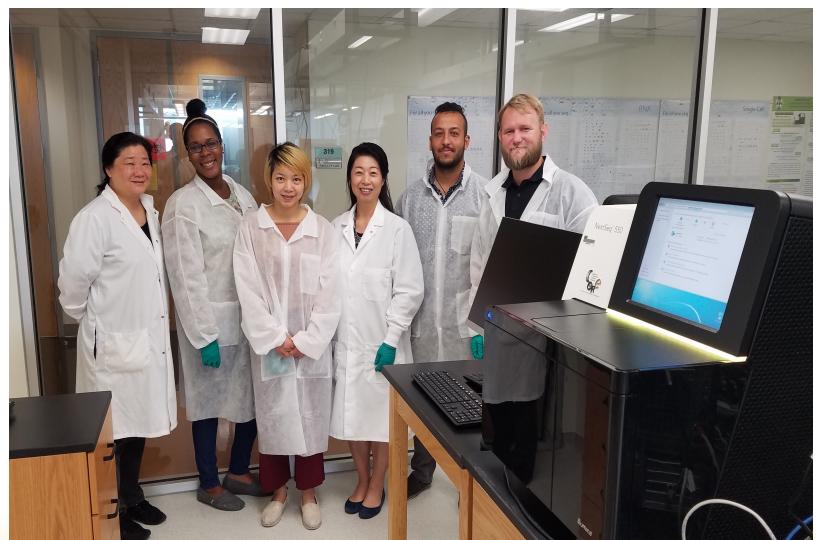
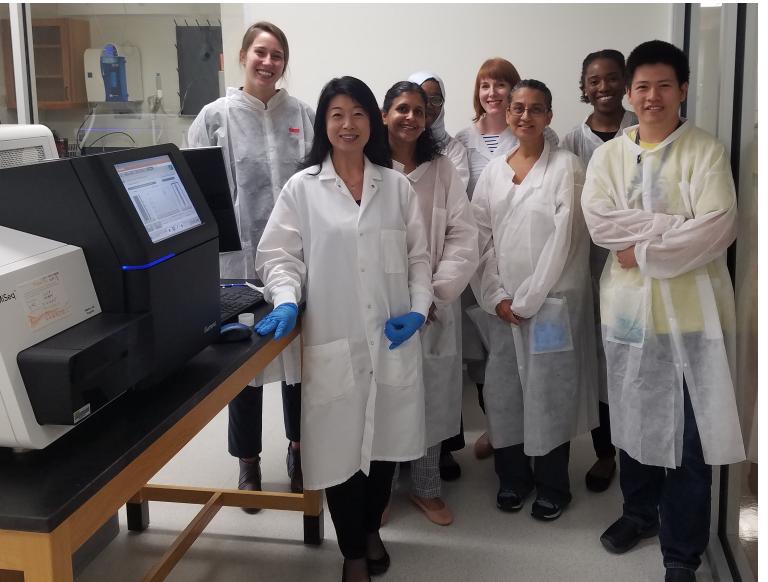
RNAseq Laboratory Workshop: Monday, 9:00 AM - 4:00 PM

RNAseq Data Analysis Workshop: Tuesday - Friday, 9:00 AM - 4:00 PM each day

- The virtual RNAseq Laboratory Training will be split into an online session and separate hands-on training. The online session will consist of a full day of training includes: introductions of USF Genomics Core and Omics Hub; overview of Illumina sequencing; NGS library preparation workflow overview and sequencing technology.
- After the online session is complete, you will become a certified user to submit work request through the USF Genomics Equipment Core iLab system.
- The hands-on training will be received as part of your first work request with the core staff. This work request and hands-on training must be completed within 1 year. We strongly recommend that you complete hands-on training with the USF Genomics Equipment Core before June 2022.

USF GENOMICS CORE AND RESOURCES

RNA Sequencing Laboratory Workshop



USF Genomics RNAseq Laboratory Workshop

Self Introductions

(Brief summary of your research interests)

11th RNAseq Laboratory Workshop Virtual Training Participants

First Name	Last Name	Lab Role	PI Name	Affiliation
Pranjal	Tyagi	Graduate Student	Chengqi Charley Wang	College of Public Health
Ahmad	Shakri	Post-doc	Liwang Cui	Morsani College of Medicine
Emily	Peterson	Graduate Student	Angele Parent	Morsani College of Medicine
Kathleen	Scott	Faculty	Kathleen Scott	College of Arts and Sciences
Jana	Wieschollek	Graduate Student	Dr. Kathleen Scott	College of Arts and Sciences

USF Genomics RNAseq Laboratory Workshop

Introduction to the USF Genomics Core and iLab System

USF Genomics Program

USF Genomics Equipment Core (Genomics Core)

Located on the 3rd floor of IDRB. The ~3000 sq. ft. BSL2 laboratory space



USF Genomics Program

USF Genomics Program has membership from colleges across USF

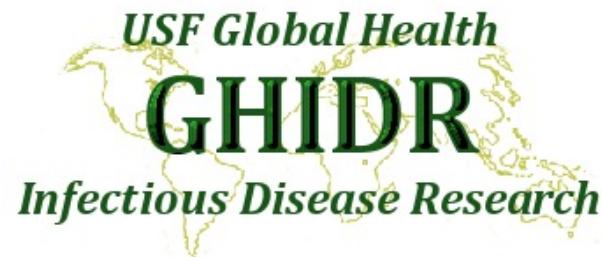
College of Public Health

College of Medicine

College of Nursing

College of Arts and Sciences

College of Marine Science



USF Genomics Researchers – 2016

Naturally Interdisciplinary

John Adams – COPH

Amy Alman – COPH

Mya Breitbart – CMS

Charles Chalfant – CAS

Liwang Cui – MCOM

Larry Dishaw – MCOM

Maureen Groer – CON

Thao Ho – MCOM

Rays Jiang – COPH

Kami Kim – MCOM

Stephen Liggett – MCOM

Xiaoming Liu – COPH

Lynn Martin – CAS

Thomas McDonald – MCOM

Jason Rohr – CAS

Kathleen Scott – CAS

Lindsey Shaw – CAS

Xingmin Sun – MCOM

Monica Uddin – COPH

Thomas Unnasch – COPH

Michael White – MCOM

Derek Wildman – COPH

Program Goals for USF Genomics

- Sustain & grow USF research programs and current research projects
- Establish a forum for genomics research activities
 - Engage students at all levels
 - Be a community resource
- Support for on campus hands-on training and protocol development
- Support for computational analysis of genomics for new projects and renewals
 - Attract new computationally-gifted students/post docs by presence of genomics talent pool and broad interdisciplinary character of the program
 - Improve on-campus infrastructure for specialized applications with Research Computing

What We Can Provide:

- ❖ **Genomics Training Courses**
- ❖ **Sequencing Services and Consultation – Genomics Core**
- ❖ **Computational Services and Consultation – Omics Hub**
- ❖ **Grant-writing Support**
- ❖ **Collaboration**

USF Genomics Core Services

Submit Work Request through the iLab

Sequencing Services :

- Whole-Genome Sequencing
- RNA Sequencing
- 16S rRNA Sequencing
- Metagenomic Sequencing
- Targeted Gene Sequencing
- Gene Expression Profiling
- miRNA & Small RNA Analysis
- Single-Cell Sequencing
- DNA, RNA Quantification

Submit Forms via genomics@usf.edu

Other Supporting Services:

- Genomics Training
- Consulting Request
- Quotation Request
- Letter of Support Request
- Specific Training Request
- Internship Request
- Collaboration Request

USF Genomics

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- [Undergraduate Opportunities](#) >
- [Annual Symposium](#)
- [College of Arts and Sciences News](#)
- [Genomics Training](#)
- [Request Services](#)

Request Services

The USF Genomics Program has the capability to assist with both sample sequencing and computational analysis for research projects at USF.



USF Genomics Core



USF Genomics Hub

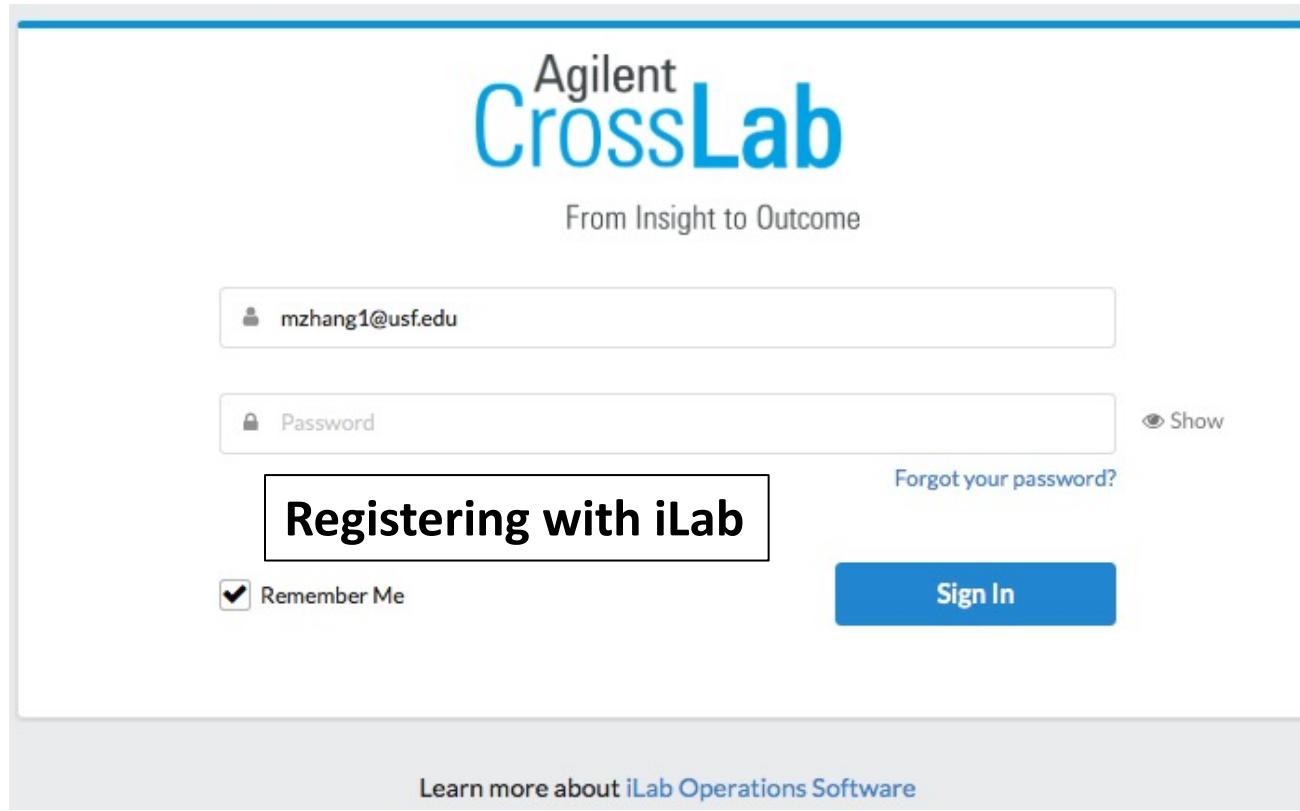


Grant Resources

iLab User Training

Matthew Mercurio
USF Genomics Core Staff

iLab Operation Software



- 1) Accessing the URL by the USF Genomics Core**
- 2) Click 'Register', bring you to the first step of the Registration**
- 3) Verify the CAPTCHA requirement, agree to iLab's privacy and policies.**

USF Genomics Equipment Core Service Guideline

Certified User Service – ‘Self-Service’

- All USF faculty, staff, and students who wish to use the USF Genomics Equipment Core Certified User Service – ‘Self-Service’ must complete the USF Genomics Training ‘RNAseq Illumina Sequencing Laboratory Workshop’ and be authorized as certified users.
- All sample preparation and QC, library prep, and sequencing work will be performed by the certified user under core staff supervision. A core staff member will be available during appointments with the USF Genomics Equipment Core to assist as necessary.
- The ‘Self-Service’ price will be calculated based on the USF Genomics Equipment Core rate sheet and two hours of core staff labor for the library-QC and instrument use/sequence QC and instrument maintenance.

Non-Certified User Service – ‘Full-Service’

- All USF faculty wishing to use the USF Genomics Equipment Core but have no ‘Certified User’ available in their lab may submit a sequencing request for Non-Certified User Service – ‘Full-Service’.
- All sample-QC, library prep and sequencing work will be performed by the core staff.
- The ‘Full-Service’ price will be calculated based on the USF Genomics Equipment Core rate sheet and the core staff labor cost will be invoiced for the time to complete the work request.

Genomics Equipment Core Sequencing Services

NGS Services:

- ❖ Whole-Genome Sequencing
- ❖ RNA Sequencing
- ❖ Gene Expression Profiling
- ❖ 16S rRNA Sequencing
- ❖ Metagenomics Sequencing
- ❖ Targeted Gene Sequencing
- ❖ Gene Expression Profiling
- ❖ miRNA & Small RNA Analysis
- ❖ Single-Cell Sequencing
- ❖ DNA, RNA Quantification

❖ Next-Gen Sequencers and Single-Cell System



Illumina - MiSeq System

12 – 15 million reads (v2)

22 – 25 million reads (v3)

Read Length:

2 x 75; 150; 250; 300

\$950 - \$1,600



NextSeq 550 System

~ 130 million reads (Mid)

~ 400 million reads (High)

Read Length:

2 x 75; 150; 300; or 1 x 75

\$1,500 - \$5,000



10x Chromium SC

Cell: 500 – 10,000

**~3,000 genes per cell
(medium)**

❖ Instrumentation for Library-prep and Quantifications



Agilent 2200



Agilent 4200



Roche LightCycler 96 System



M220 Focused-ultrasonicator



Qubit® 4 Fluorometer

❖ Instrumentation for Cell Culture

- ✓ Class II Biosafety Cabinets x 4
- ✓ O₂/CO₂ 3 Gas Incubators x 4
- ✓ Microscopes x 3
- ✓ Centrifuge x 3
- ✓ Refrigerators 4°C, -20°C and -80°C freezers
- ✓ Liquid nitrogen tank cell storage -120°C

New Instrument Purchase

eppendorf
epMotion® 5075tc NGS



arriving in late July

Cellometer K2 Image Cytometer
Optimized Analysis of Primary Cells



Pipette 20µl

Cellometer® K2

❖ Grant-writing Support and Collaboration

We Can Help You:

- ✓ Providing support letter and core facilities/resources
- ✓ Consultations
- ✓ Experimental design
- ✓ Estimates the cost
- ✓ Preliminary data generation
- ✓ Find the collaborators

USF GENOMICS CORE FACILITIES AND RESOURCES

Laboratory: The USF Genomics Core laboratory facilities are on the 3rd floor of the Interdisciplinary Research Building (IDRB) in the USF Research Park. The ~3000 sq. ft. of laboratory space is equipped for cell biological and molecular biological experiments. The USF Genomics Core facility offers/encourages one-on-one consultation with a team of experienced bench and computational scientists on experimental design, sample preparation and data analysis for USF researchers.

The USF Genomics Core laboratory facilities house the following instrumentation:

Next-Gen Sequencers:

1. Illumina - NextSeq-550
2. Illumina - MiSeq
3. Single Cell RNA-seq instrument - 10x Chromium

Instrumentation for library-prep and quality-assurance:

1. Covaris M220 - DNA fragmentation
2. Agilent TapeStation 2200 - DNA and RNA quality assessment
3. Roche LightCycler 96 qPCR System – DNA library quantification
4. Invitrogen Qubit 2.0 fluorometer – DNA and RNA sample quantitation
5. Bio-Rad thermal cycler - PCR
6. Bench top centrifuges, heat blocks, vortexers

Instrumentation for cell culture:

1. Class II Biosafety Cabinets x 3
2. O₂/CO₂ Incubators x 4
3. Microscopes x 3
4. Centrifuges x 3
5. Refrigerators 4°C, -20°C and -80°C freezers
6. Liquid nitrogen tank cell storage -120°C

Computational: The USF Genomics Core computational facilities are on the 4th floor of the IDRB and comprise a 1400 sq. ft. Genomics Core computational consultants run many Mac, Linux and Dell workstations and have access to USF's High-Performance Computing (HPC) cluster and a variety of both proprietary and open-source, command-line based analysis software. Data generated from the Core sequencers, as well as any data to be analyzed by the Core, are securely stored on HIPAA-compliant architecture of USF's HPC cluster. HPC infrastructure and hardware supporting USF Genomics and the USF Genomics Core Facility is managed by the USF Department of Research Computing.

USF Genomics Core - Quotation Request Form

CONTACT INFORMATION - *All Fields Required*

Date:

College/Department:

Principal Investigator's Name (*Last, First*):

PI's Email:

PROJECT INFORMATION - *All Fields Required*

Title of the Proposal:

Project Timeline:

Brief Description of the Proposal:

SEQUENCING SAMPLES INFORMATION - *All Fields Required*

Type of Sequence (e.g. DNA, RNA or 16s rRNA, Metagenomic, Single-cell):

Number of Samples:

Sample Description (Common Name):

Sequence Length Request:

Number of Reads per Sample Request:

Species Name of the Reference Genome:

Size of the Genome in Mbp:

Has anyone completed RNAseq-Laboratory-Workshop in your lab? (Yes/No)

(if yes, please fill in name of certified user who will do the library prep in the core):

COMMENTS (Please specify your request)

Do you need data analysis support from USF Genomics Omics Hub? (Yes/No)



USF Genomics Equipment Core, USF Genomics Program

(813) 974-2005, genomics@usf.edu

Thank you for using the USF Genomics Equipment Core. Here is the Quotation according to your work request. Please let us know if we can assist you in any other way. Thanks.

Quotation Request: Dr. XXXX

Date: XXXX

Sequence Type: Metagenomic sequencing

Number of Samples: 600

Reads per Sample: 20 million

Sequence Length: Pair End 2X 150 bp

Total Price Per Sample: \$693.25 (Library Prep: \$238.92 per sample; Sequencing \$454.33 per sample)

Total Cost for 100 Subjects and 600 samples: \$415,950.00

Services Description: Full Service-Library Prep	Rate/Unit	Qty	Amount
DNA Quantification by Qubit	\$3.50	6	\$9.50
DNA QC by TapeStation	\$7.00	6	\$42.00
Nextera DNA Library Prep (Nextera DNA Flex Kit)	\$144	6	\$864.00
High-Sensitivity DNA Library QC	\$8.00	6	\$48.00
Library QC and Instrument Use (core staff labor)	\$47.00	2	\$94.00
Full-Service (core staff labor)	\$47.00	8	\$376.00
Sub-Total: Sequencing Services (Library Prep)			\$1,433.50
Price Per Sample (Library Prep)			\$238.92

Services Description: Full Service-Sequencing	Rate/Unit	Qty	Amount
Sequencing use NextSeq V2.5 (Mid-Output - 300 Cycle)	\$2,632.00	1	\$2,632.00
Instrument Use & Sequence QC (core staff labor)	\$47.00	2 hours	\$94.00
Sub-Total: Sequencing Services			\$2,726.00
Price Per Sample (20 Million reads per sample)			\$454.33

USF Genomics Equipment Core

3720 Spectrum Blvd, suite 316

Tampa, FL 33612

University of South Florid

Email: genomics@usf.edu

Website: <https://health.usf.edu/publichealth/ghdr/genomics/request-services>



USF Genomics Equipment Core, USF Genomics Program
(813) 974-2005, genomics@usf.edu

Thank you for using the USF Genomics Equipment Core. Here is the Quotation according to your work request. Please let us know if we can assist you in any other way. Thanks.

Quotation Request: XXXX

Date: XXXX

Sequence Type: Metagenomic sequencing

Number of Samples: 600

Reads per Sample: 40 million

Sequence Length: Pair End 2X 150 bp

Total Price Per Sample: \$1,046.55 (Library Prep: \$238.92 per sample; Sequencing \$807.63 per sample)

Total Cost for 100 Subjects and 600 samples: \$627,930.00

Services Description: Full Service-Library Prep	Rate/Unit	Qty	Amount
DNA Quantification by Qubit	\$3.50	6	\$9.50
DNA QC by TapeStation	\$7.00	6	\$42.00
Nextera DNA Library Prep (Nextera DNA Flex Kit)	\$144	6	\$864.00
High-Sensitivity DNA Library QC	\$8.00	6	\$48.00
Library QC and Instrument Use (core staff labor)	\$47.00	2	\$94.00
Full-Service (core staff labor)	\$47.00	8	\$376.00
Sub-Total: Sequencing Services (Library Prep)			\$1,433.50
Price Per Sample (Library Prep)			\$238.92

Services Description: Full Service-Sequencing	Rate/Unit	Qty	Amount
Sequencing use NextSeq V2.5 (High-Output - 300 Cycle)	\$6,461.00	1	\$6,461.00
Instrument Use & Sequence QC (core staff labor)	\$47.00	2 hours	\$94.00
Sub-Total: Sequencing Services (8 samples)			\$6,461.00
Price Per Sample (40 Million reads per sample)			\$807.63

USF Genomics Equipment Core

3720 Spectrum Blvd, suite 316

Tampa, FL 33612

University of South Florid

Email: genomics@usf.edu

Website: <https://health.usf.edu/publichealth/ghidr/genomics/request-services>

USF Genomics Core Letter of Support Request Form

CONTACT INFORMATION - *All Fields Required*

Date:

College/Department:

Principal Investigator's Name (*Last, First*):

PI's Email:

PROJECT INFORMATION - *All Fields Required*

Title of the Proposal:

Project Timeline:

Brief Description of the Proposal:

SEQUENCING SAMPLES INFORMATION - *All Fields Required*

Type of Sequence (e.g. DNA, RNA or 16s rRNA, Metagenomic, Single-cell):

Number of Samples:

Sample Description (Common Name):

Sequence Length Request:

Number of Reads per Sample Request:

Species Name of the Reference Genome:

Size of the Genome in Mbp:

Has anyone completed RNAseq-Laboratory-Workshop in your lab? (if yes, please fill in name of certified user):

Do you need data analysis support from USF Genomics Omics Hub?

COMMENTS (Please specify your request)

Letter of Support for Proposal Prep



Oct. 26, 2020

XXXX, PhD
Professor, Genomics Program
College of Public Health
University of South Florida
3720 Spectrum Blvd.
Tampa, FL 33612

Dear Professor XXX,

Re: Letter of Support for NIH R01 proposal "XXXXXXXXXXXXXX".

I am delighted to write in support of the above exciting proposal. As described in your proposal, this proposed research plans including single-cell sequencing. The University of South Florida Genomics Program Equipment Core is well equipped to conduct the studies you have described. Our core facility is equipped with the sequence systems including Illumina NextSeq-550 and 10x Chromium with Single-Cell Controller. We offer training, consulting and service for all Illumina based sequencing experiments. USF Genomics Equipment Core is dedicated to supporting research at USF.

We will be happy to assist you and your students with any kind of genomics training and consulting for both experimental design and sequencing. We assure you that the core will provide advice and service as needed. We wish you success with your research proposal, and look forward to working with you on this fascinating project.

Sincerely,

USF Genomics Equipment Core Manager
3720 Spectrum Blvd, suite 316
Tampa, FL 33612
University of South Florid
Email: genomics@usf.edu
Website: <https://health.usf.edu/publichealth/ghidr/genomics>

USF Genomics Program Genomics Equipment Core Internship Application Form

PERSONAL INFORMATION - All Fields Required *

Name:

Email:

College/Department:

Principal Investigator's Name (*Last, First*):

PI's Email:

RESEARCH BACKGROUND - All Fields Required *

Major:

Areas of Interest:

Research Experience:

INTERNSHIP INTEREST - All Fields Required *

Why are you interested in this program?

How will you benefit from this internship?

ADDITIONAL INFORMATION - If You Want to Let Us Know

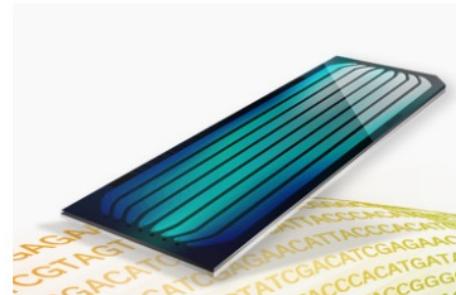
Understanding Illumina sequencing technology (SBS)

The screenshot shows the Illumina website's navigation bar with links for Overview, NGS for Beginners, NGS vs. Sanger, NGS vs. qPCR, RNA-Seq vs. Microarrays, Experiments & Protocols, and More. Below the navigation is a breadcrumb trail: Science and Education / Technology / Next-Generation Sequencing: Sequencing by Synthesis (SBS) Technology.

Meredith Millis,
Field Application Scientist,
Illumina

Introduction to SBS Technology

Illumina sequencing technology, sequencing by synthesis (SBS), is a widely adopted next-generation sequencing (NGS) technology worldwide, responsible for generating more than 90% of the world's sequencing data.¹ Illumina sequencing instruments and reagents support massively parallel sequencing using a proprietary method that detects single bases as they are incorporated into growing DNA strands.

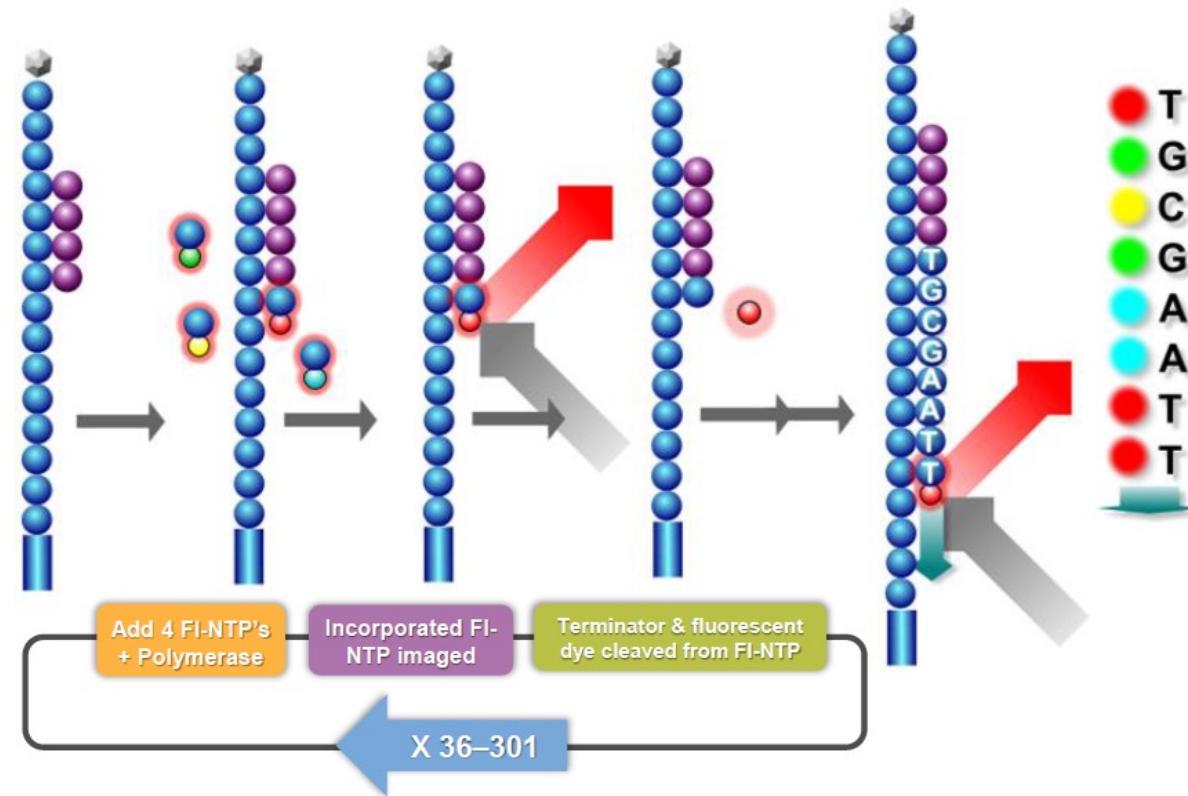


SBS Chemistry

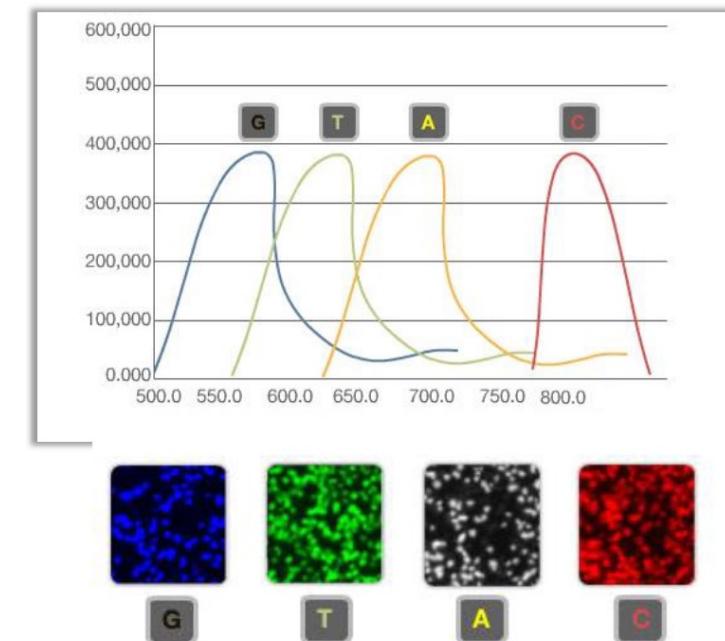
A fluorescently labeled reversible terminator is imaged as each dNTP is added, and then cleaved to allow incorporation of the next base. Since all 4 reversible terminator-bound dNTPs are present during each sequencing cycle, natural competition minimizes incorporation bias.²

The end result is true base-by-base sequencing that enables accurate data for a broad range of applications. The method virtually eliminates errors and missed calls associated with strings of repeated nucleotides (homopolymers).

Sequencing By Synthesis 2nd Read



Four Channel SBS Chemistry: GA, HiSeq, MiSeq



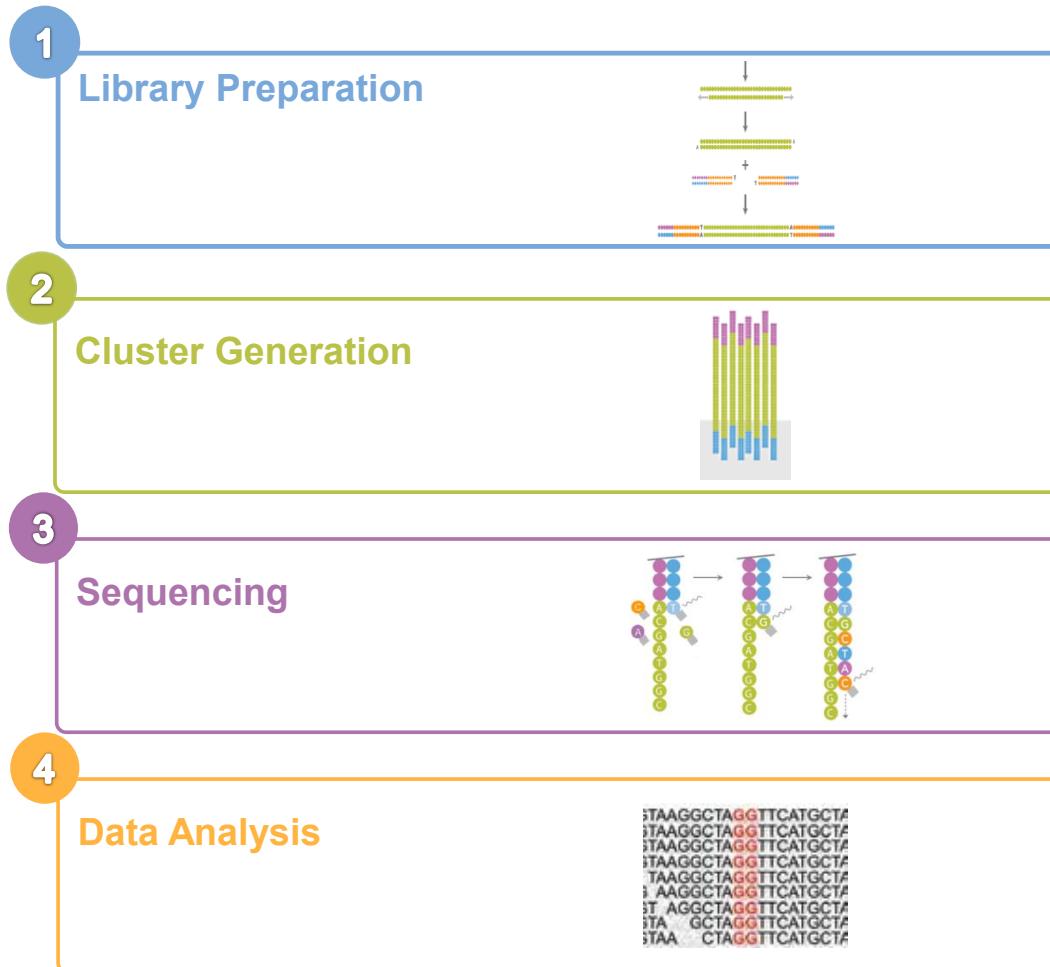
Each of the 4 DNA bases emit an intensity of a unique wavelength

Collects 4 images

- During each cycle, each cluster appears in only 1 of 4 images

illumina®

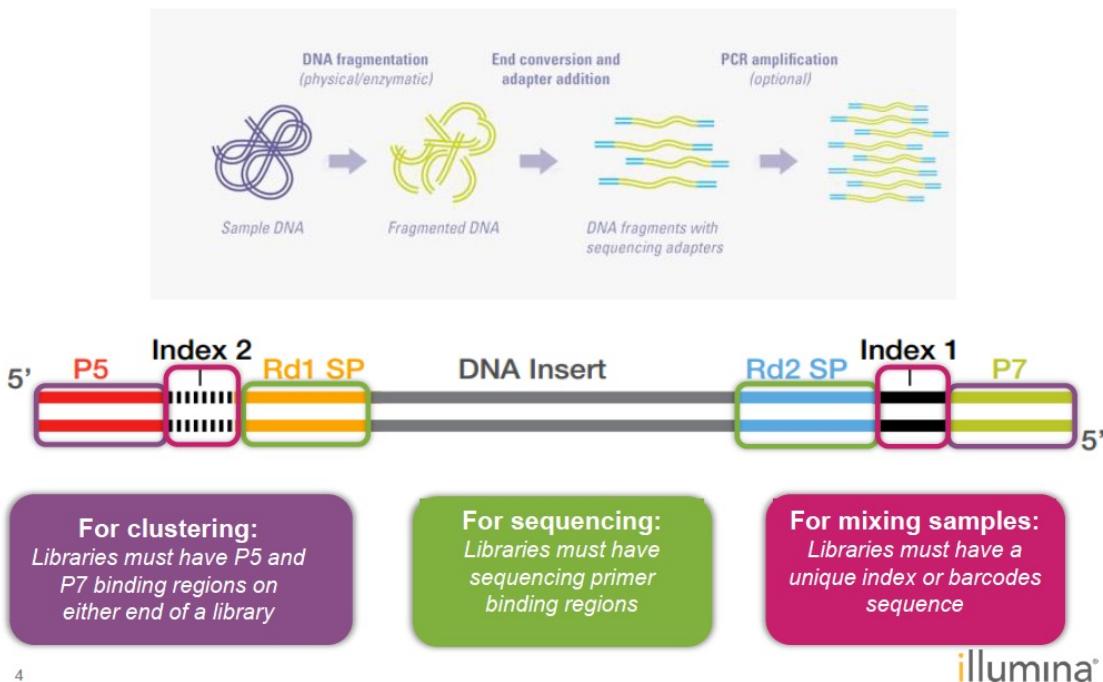
Illumina Sequencing Workflow



Next-Gen Sequencing Library Preparation Overview

Min Zhang
USF Genomics Core
Manager

Library Prep is Critical for Successful Sequencing

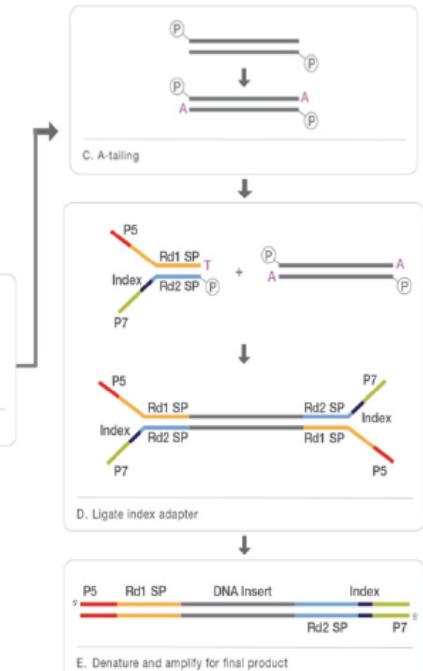
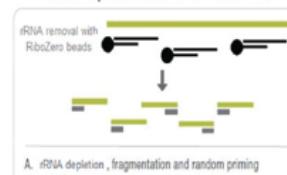


TruSeq Stranded RNA Workflows

TruSeq Stranded mRNA



TruSeq Stranded Total RNA



FOR RESEARCH USE ONLY

Library Validation, QC and Troubleshooting

Min Zhang
USF Genomics Core
Manager

Library Yield Problem

Problem: Low or No Yield

Poor Quality
Starting
Material

Poor AMPure
Bead
Technique

Improper
Reagent
Handling

Lid Not
Heated on
Thermocycler

Best Practices

Low or No Yield Causes:
Input Issues

Garbage in



Bad Sample

Garbage out



Bad Library

Bad Library → Bad Sequencing Data

Sequencing set up and Illumina Customer Resources Overview

Matthew Mercurio
USF Genomics Core
Staff

Benchtop Sequencers



iSeq 100



MiniSeq



MiSeq Series +



NextSeq 550 Series +



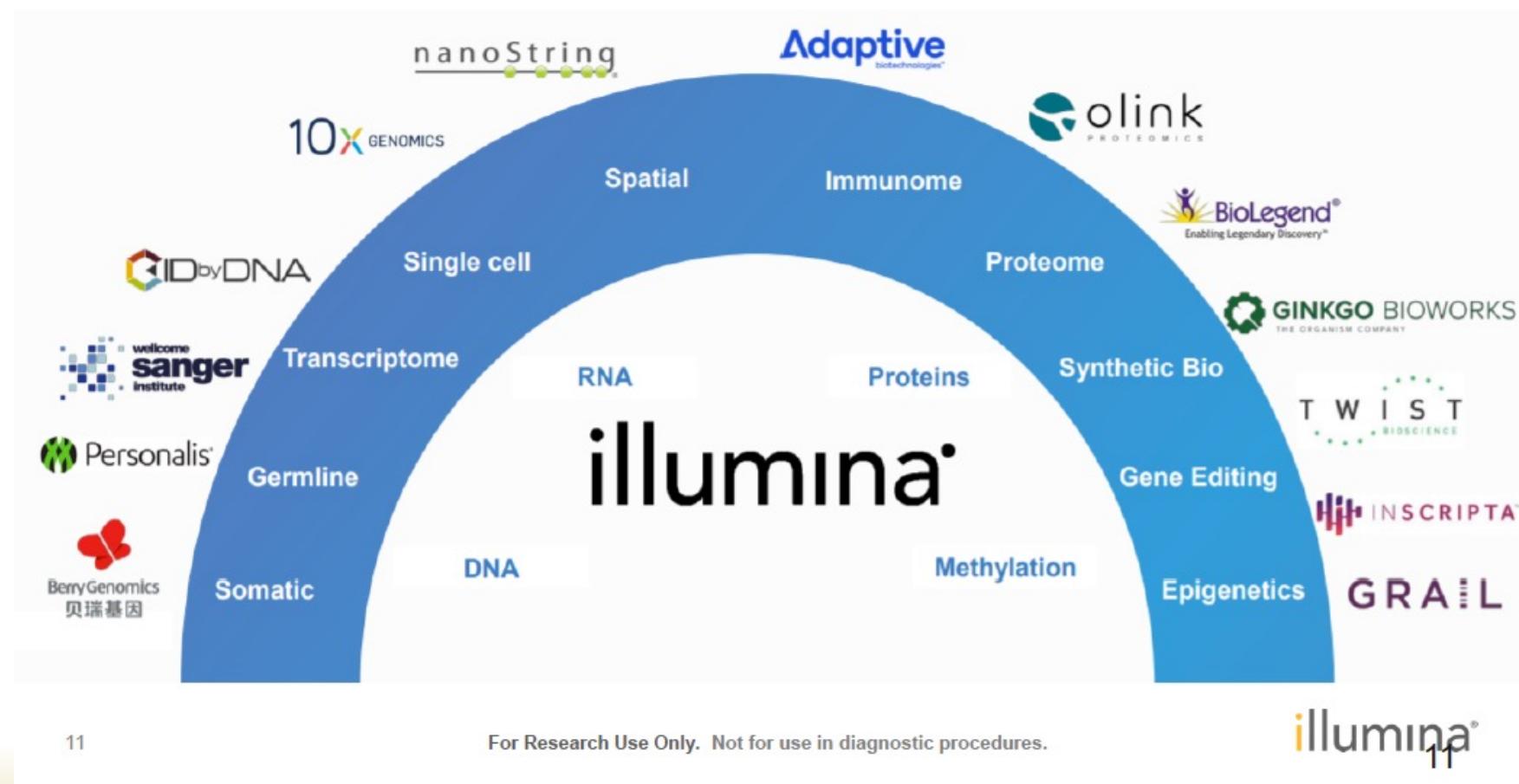
NextSeq 2000



Illumina Customer Resources Overview

One Technology, Many Applications

Evelyn Griffin,
Account Manager,
Illumina



Illumina BaseSpace Tour and Sequencing Data QC

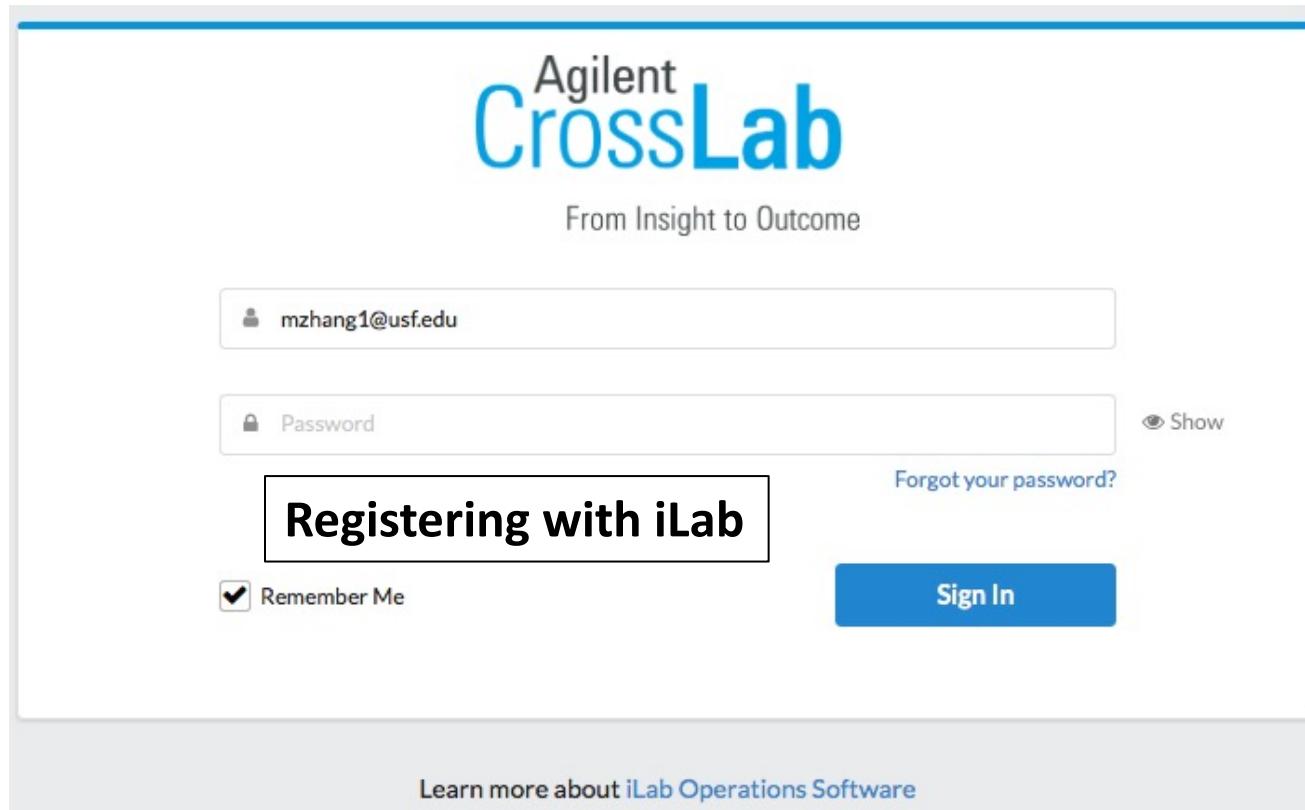
The screenshot shows the Illumina BaseSpace Sequence Hub interface. The top navigation bar includes links for HOME, RUNS, PROJECTS, ANALYSES, BIOSAMPLES, APPS, and DEMO DATA. A user profile for "John Adams" is visible on the right. Below the navigation, the title "Run: 20201221-BRT-033-P1: Charts" is displayed, along with tabs for SUMMARY, SAMPLES, CHARTS (selected), METRICS, INDEXING QC, SAMPLE SHEET, and FILES. The main content area displays several QC charts: "Flow Cell Chart" (heatmap of raw data), "Data By Cycle" (line graph of intensity over cycles), "QScore Distribution" (bar chart of total reads vs Q score), "Data By Lane" (line graph of density per lane), and "QScore Heatmap" (heatmap of Q scores across lanes and cycles). At the top left, text indicates "Flow Cell: HKLJ2AFX2 Extracted: 158 Called: 158 Scored: 158".

Matt Angel,
Sequencing Specialist,
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iLab User Training

Matthew Mercurio
USF Genomics Core
Staff

iLab Operation Software



- 1) Accessing the URL by the USF Genomics Core
- 2) Click 'Register', bring you to the first step of the Registration
- 3) Verify the CAPTCHA requirement, agree to iLab's privacy and policies.

USF Genomics Equipment Core Workflow

1. Complete 'Initial Request'

User – Complete the RNAseq Laboratory Training and register in the iLab as certified user.
User - Upload the '**PI signed the Policies and Procedures**' to the iLab.
Paige - DocuSign for the core, upload fully signed Policies and Procedures to the iLab.

2. Submit 'Service Request'

User - Upload the '**Billing Form**' and '**Sample Sheet**', submit the work request.
Judy – '**Financial Approval**', confirm with the core that Billing INFO is correct/ready to start.
Core staff – Generate the '**quote**', then submit the quote to User/PI for approval.
User - Upload the '**PI signed quote**', then clicks '**Agree**' to accept the work

3. Submit 'Reservations'

User - Submit the work '**Reservation**' through the iLab.
Core staff – Save and **approve 'Reservations'**, Click '**Begin**' when work starts.

4. Post work 'Completed'

Core staff – '**Reporting**', submit the report summary to PI and note the work completed.
Core staff – Create the '**Invoice**', and confirm with Judy '**Billing initiated**'.
Judy – Submit the '**Billing**' request to PI and financial officer, confirm with the core when payment completed.
Core staff – Click '**Finish Work**' once the work and payment are completed.



Thanks!

Questions?

