

Welcome to the USF Genomics Training



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COLLEGE OF MARINE SCIENCE



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USF Genomics RNAseq Laboratory Workshop

Instructors Introduction

USF Genomics Core



Min Zhang
Core Manager



Matthew Mercurio
Core Staff

Illumina



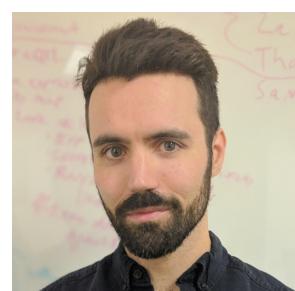
Ryan Gentry
Sequencing specialist

Evelyn Griffin,
egriffin@illumina.com
Account Manager FL

Genomics Omics Hub



Jenna Oberstaller
Head of Omics Hub



Justin Gibbons



Charley Wang

10th RNAseq Laboratory Workshop Virtual Training Agenda

Time	10th USF Genomics RNASeq Laboratory Workshop - Virtual Training Agenda (Monday, Feb. 1st, 2021)	Instructor
9:00 AM - 9:15 AM	Trainee-introductions (Brief summary of your research interests)	Instructor and Trainees
9:15 AM - 10:00 AM	Introduction to the USF Genomics Core and iLab system	Min Zhang /USF Genomics Core
10:00 AM - 10:30 AM	Introduction to the USF Genomics Omics Hub, functional genomics, data-processing overview	Jenna Oberstaller/USF Omics Hub
10:30 AM - 11:15 AM	1) Illumina Sequencing overview	Ryan Gentry / Illumina Sequencing specialist
11:15 AM - 12:00 PM	2) Library Preparation for Next-Gen Sequencing	Min Zhang /USF Genomics Core
12:00 PM - 1:00 PM	Lunch Break	
1:00 PM - 1:45 PM	3) Sequencing set up and Illumina Customer Resources Overview	Mathew Mercurio / USF Genomics Core
1:45 PM - 2:30 PM	4) Library QC, Trouble-shooting, Sequencing Data QC and Illumina BaseSpace Tour	Min Zhang /USF Genomics Core
2:30 PM - 3:00 PM	5) iLab User Training	Mathew Mercurio / USF Genomics Core

Welcome to the USF Genomics Training Course

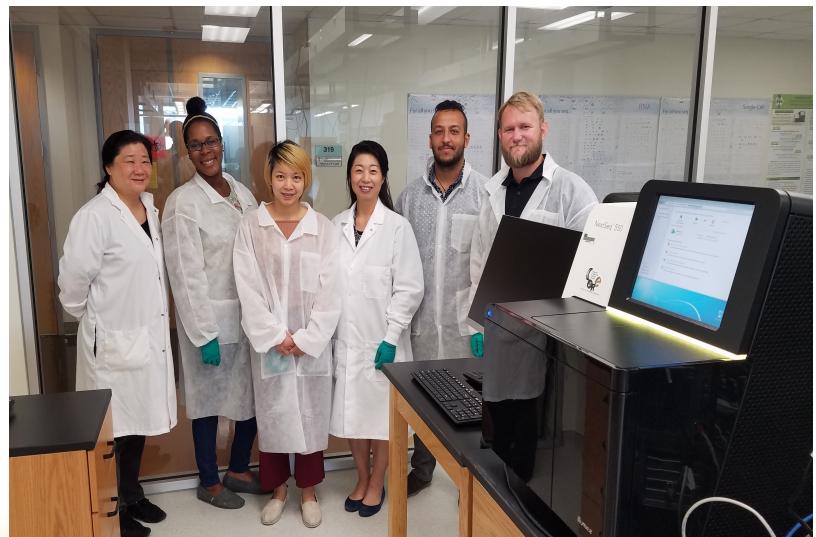
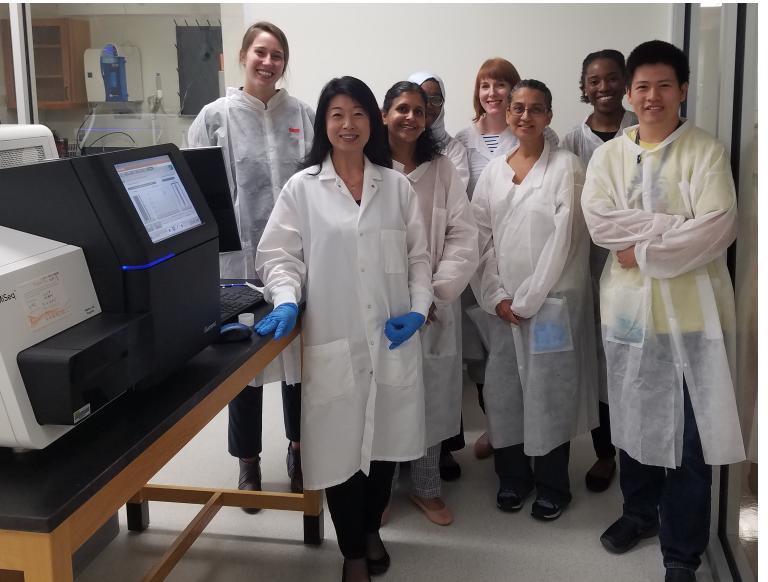
RNAseq Laboratory Workshop: Monday, Feb. 01, 2021, 9:00 AM - 4:00 PM

RNAseq Data Analysis Workshop: Tuesday - Friday, Feb. 02 - 04, 2021
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 December 2021.

USF GENOMICS CORE AND RESOURCES

RNA Sequencing Laboratory Workshop



USF Genomics RNAseq Laboratory Workshop

Self Introductions

(Brief summary of your research interests)

10th RNAseq Laboratory Workshop Virtual Training Participants

First Name	Last Name	Lab Role	PI Name	Affiliation
Mariana	Argenziano	Faculty	Thomas McDonald	Morsani College of Medicine
Neha	Arora	Post-doc	George Philippidis	Patel College of Global Sustainability
Syed	Hussain	Graduate Student	Xingmin Sun	Morsani College of Medicine
Jessica	Martin	Graduate Student	George Philippidis	College of Arts and Sciences
Peter	Radulovic	Undergraduate Student	Shyam Mohapatra	College of AS, College of Pharmacy
Hyacinth	Burrowes	Undergraduate Student	Becky Willis	College of Arts and Sciences
Teresa	Darcey	Graduate Student	Hana Totary-Jain	Morsani College of Medicine
Preston	McDonald	Graduate Student	Mark Margres	College of Arts and Sciences
Sofia	Bhatia	Post-doc	Manas Biswal	Taneja College of Pharmacy
Matthew	Gamache	Graduate Student	John Parkinson	College of Arts and Sciences
Chukwuemeka	Ogbu	Graduate Student	Amy Alman	College of Public Health
Surendra Kumar	Kolli	Post-doc	Dr. John H. Adams	College of Public Health
Pradeep	Annamalai Subramani	Post-doc	Prof John Adams	College of Public Health
Hiran Malinda	Lamabadu W. Patabendige	Staff	Xingmin Sun	Morsani College of Medicine

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



Core Staff:

Matthew Mercurio, Sr. Biological Scientist
Min Zhang, Core Manager/Scientific Researcher

Administrator:

Paige P. Hunt, Academic Program Specialist
Judy Sommers, Financial Administrator

USF Genomics Program

USF Genomics Program has membership from colleges across USF

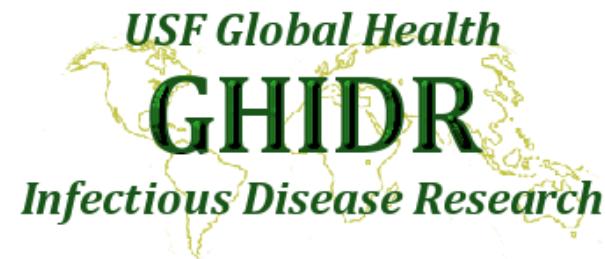
College of Public Health

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 Trainings and Services

Genomics Core - Laboratory

Services:

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

Omics Hub - Computational

Services:

- Training & Consulting
- Genome assembly and annotation
- Phylogenomics
- Transcriptomics
- Epigenomics
- Custom scripts or analysis pipelines
- Data visualization

RNA Sequencing Workshops

Laboratory Workshop – RNA-seq Library Preparation

Computational Workshop – RNA-seq Data Analysis

Three Sessions:
February
May
September

USF Genomics Program- Genomics Training Courses

Laboratory Workshop — Illumina RNA-seq Library Preparation
Computational Workshop — RNA-seq Data Analysis

Laboratory

Goal: 4-day extensive hands-on training to enable you to confidently prepare high-quality sample libraries for sequencing

Day 1: Illumina sequencing technology overview, RNA sample Preparation

Day 2: Library Prep (1): mRNA purification; 1st, 2nd strand cDNA synthesis

Day 3: Library Prep (2): A-Tailing, ligation, PCR amplification, Beads Purification

Day 4: qPCR quantification, Normalize & pool, Set-up Miseq run, Base-Space

Introduction: This training course includes presentations on technology theory and Practical experience with entire laboratory sample prep workflow. Experienced trainer will lead you using TapeStation, Miseq equipment. By the end of this training, you will be able to: complete Illumina library preparation process, set up a sequencing run using Illumina Miseq Control Software.

Participants

- To maximize effectiveness, this training is limited to 6 participants of each time.
- A basic knowledge of biochemistry and molecular biology, familiarity with laboratory equipment usage are requisites.
- Participants will need to bring your own micropipettes (10µl, 200µl and 1000µl)
- If participants wish to include their own experimental samples, each participant can prep two samples.

Way to Apply

Online registration: <https://goo.gl/forms/DINXXKV4Xvr15nem1>

Scan for Registration: 

Phone Number: 813-974-6672

Email: genomics@health.usf.edu

Computational

Goal: 4-day in-depth course will instruct participants on the theoretical and practical concepts related to RNA sequencing analysis, enabling to perform analyses independently.

Day 1: Illumina Sequencing Refresher, Linux for Bioinformatics

Day 2: Introduction to RNA sequencing, RNA-seq Alignment and Visualization

Day 3: RNA-seq Alignment and Visualization, Introduction to R and Bioconductor

Day 4: Introduction to R and Bioconductor Expression and Differential Expression

RNA-seq: TUXEDO Pipeline Hands-on

Topics:

- ❖ Module 1: A hands-on introduction to date analysis using Linux, designed for all computational skill levels;
- ❖ Module 2: Discussion of advantages and disadvantages of current sequencing technologies and their implications on data analysis.
- ❖ Module 3: Introduction to preprocessing raw reads, read mapping, visualization of mapped reads and one proceeds with first hands-on analyses (QC, mapping, visualization).
- ❖ Module 4: Introduction to 'R' a programming language to perform statistical computing and visualization, and 'Bioconductor' an R based toolshed for analyzing genomic data.
- ❖ Module 5: Quantification of gene expression and predict differentially expressed genes, followed by a whole day hands-on RNA-Seq analysis pipeline.

Participants

- Admittance to the course is limited to 15 participants.
- A basic knowledge of biochemistry and molecular biology, a basic familiarity with computer usage and a need for RNA sequence analysis for current ongoing research are recommended prerequisites.
- Participants will need to have their own laptop to access the USF research Computing infrastructure for hands-on sessions.

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USF Genomics

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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

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

❖ 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.

Pre-reading materials for the RNAseq Laboratory Workshop

Part 1: Library construction for NGS sequencing

Pre-class reading:

1.Understanding the NGS workflow: Main Steps in Next-Generation Sequencing.

View an Example Workflow:

<https://www.illumina.com/science/technology/next-generation-sequencing/beginners/ngs-workflow.html>

Part 2: Understanding Illumina sequencing technology

Pre-class reading:

2.How Does Illumina Sequencing Work: Understanding Sequencing by Synthesis (SBS) Technology.

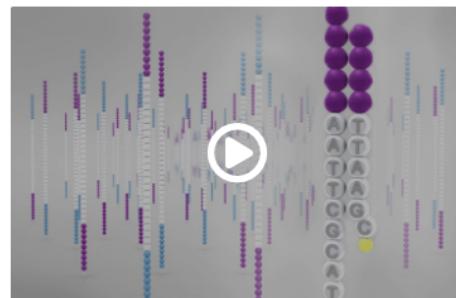
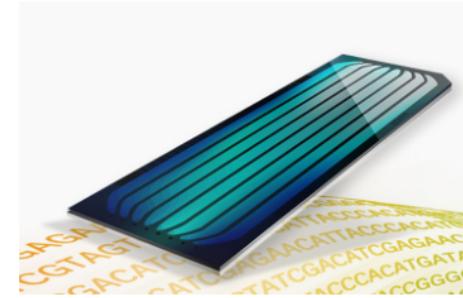
View Introduction to SBS Technology:

<https://www.illumina.com/science/technology/next-generation-sequencing/sequencing-technology.html>

Science and Education / Technology / Next-Generation Sequencing:
Sequencing by Synthesis (SBS) Technology

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).

Pre-class reading:

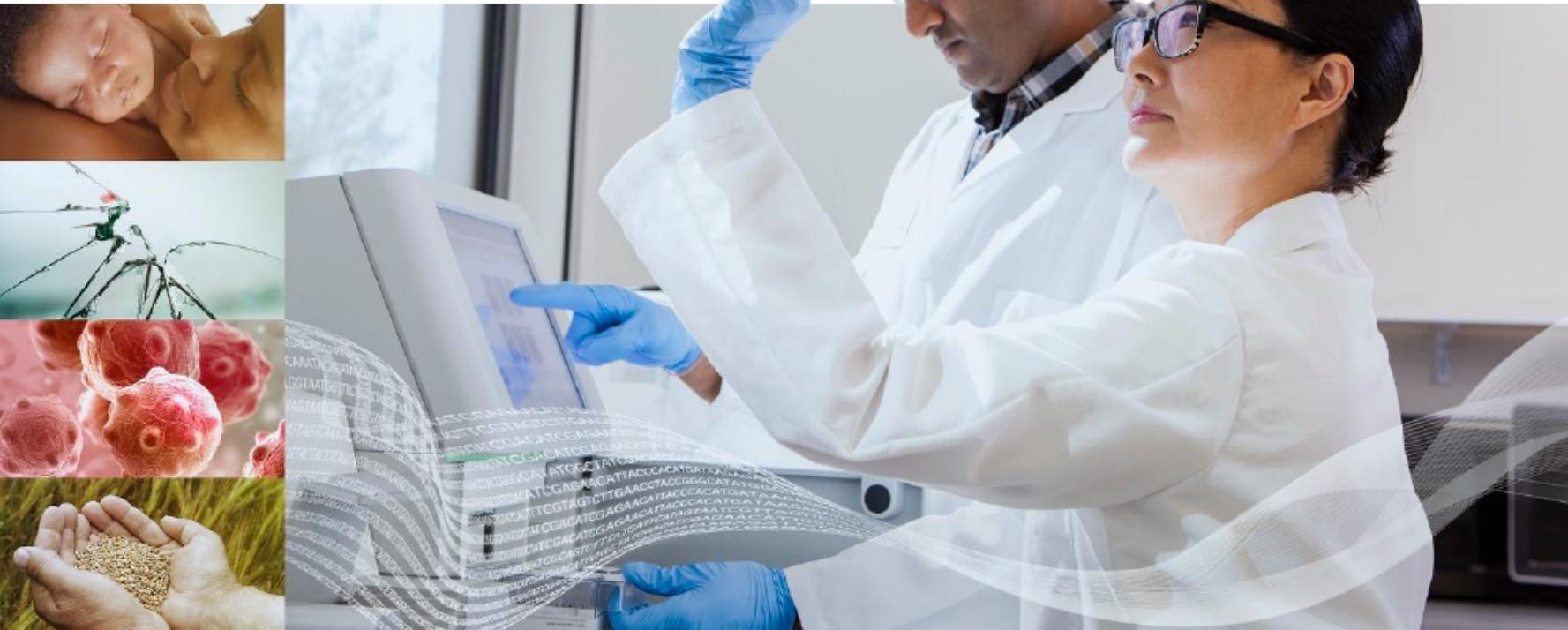
2. How Does Illumina Sequencing Work: Understanding Sequencing by Synthesis (SBS) Technology.

View Introduction to SBS Technology:

<https://www.illumina.com/science/technology/next-generation-sequencing/sequencing-technology.html>

1) Illumina Sequencing Overview: Library Prep to Data Analysis

Ryan Gentry M.S.
Sequencing Specialist



QB7845

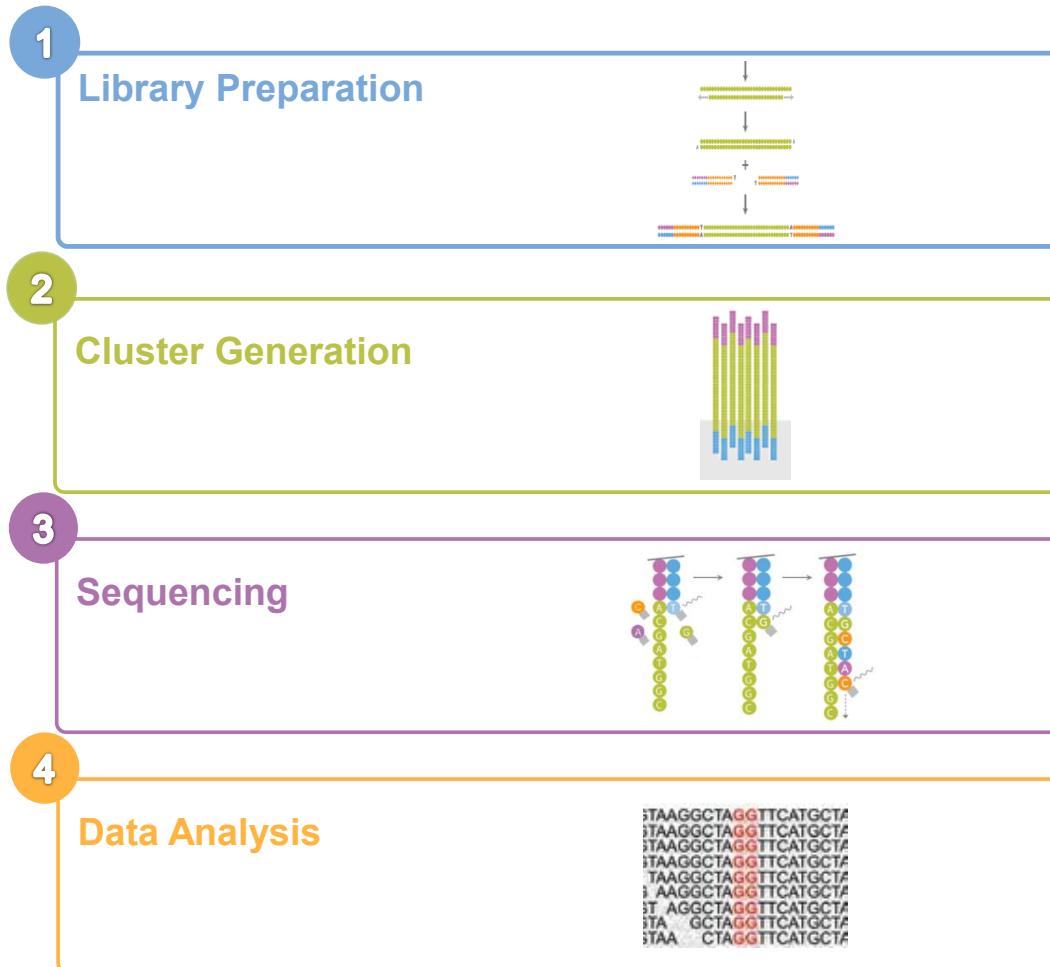
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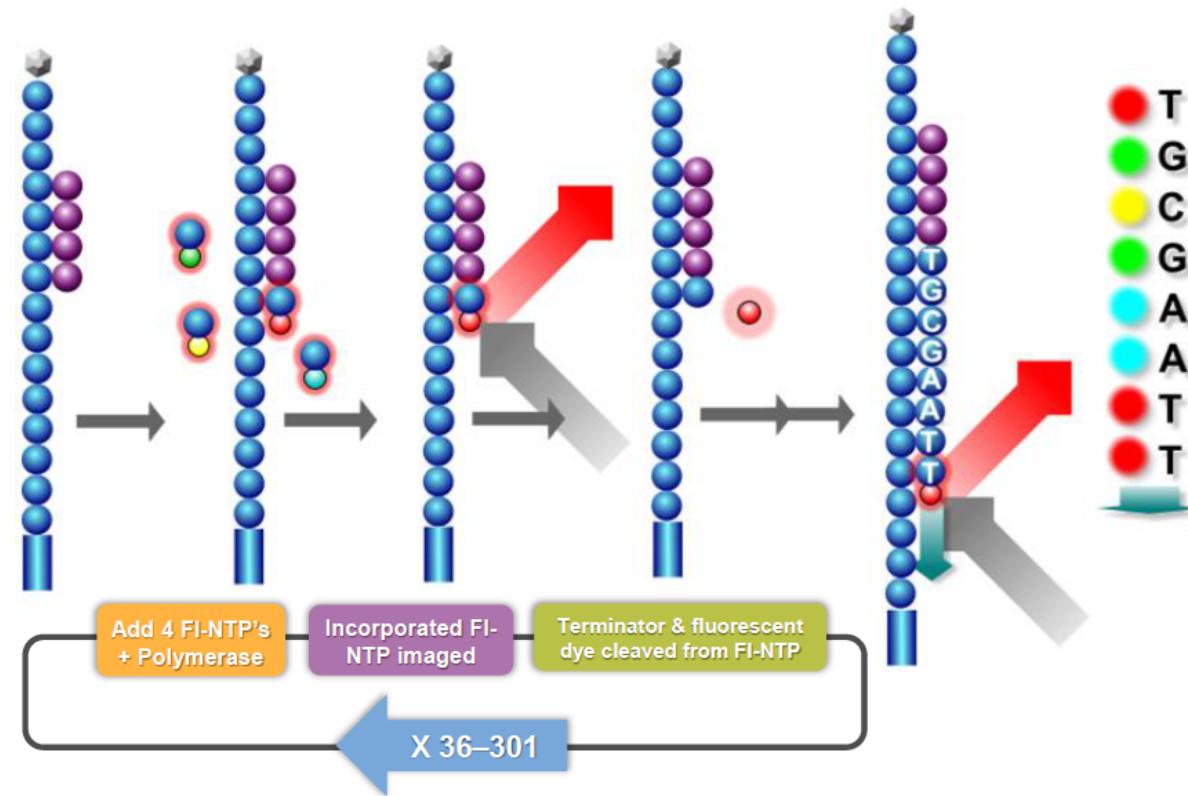
Illumina, 24Sure, BaseSpace, BeadArray, BlueFish, BlueFuse, BlueGnome, cBot, CSPro, CytoChip, DesignStudio, Epicentre, ForenSeq, Genetic Energy, GenomeStudio, GoldenGate, HIScan, HiSeq, HiSeq X, Infinium, iScan, iSelect, MiSeq, MiSeq DR, MiSeq FGx, NeoPrep, NextBio, Nextera, NextSeq, Powered by Illumina, SureMDA, TruGenome, TruSeq, TruLight, Understand Your Genome, UYG, VeraCode, verifi, VeriSeq, the pumpkin orange color, and the streaming bases design are trademarks of Illumina, Inc. and/or its affiliate(s) in the US and/or other countries. All other names, logos, and other trademarks are the property of their respective owners.

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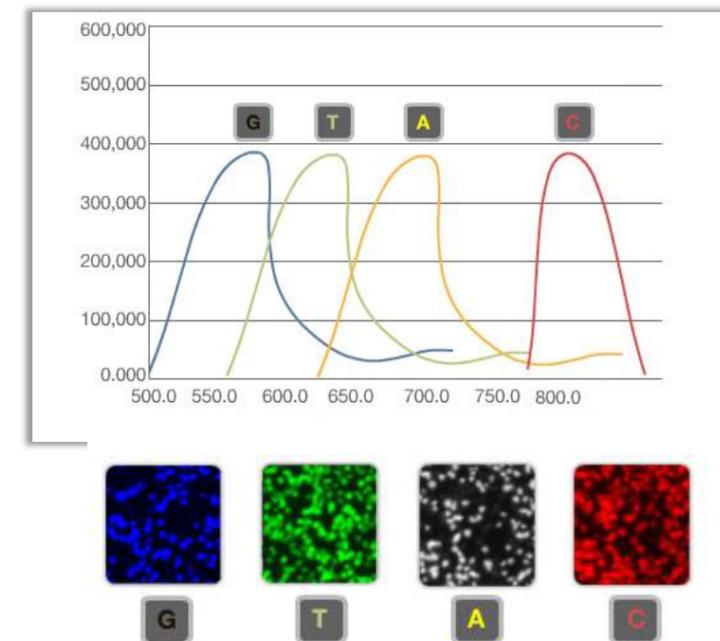
Illumina Sequencing Workflow



Sequencing By Synthesis 2nd Read



Four Channel SBS Chemistry: GA, HiSeq, MiSeq



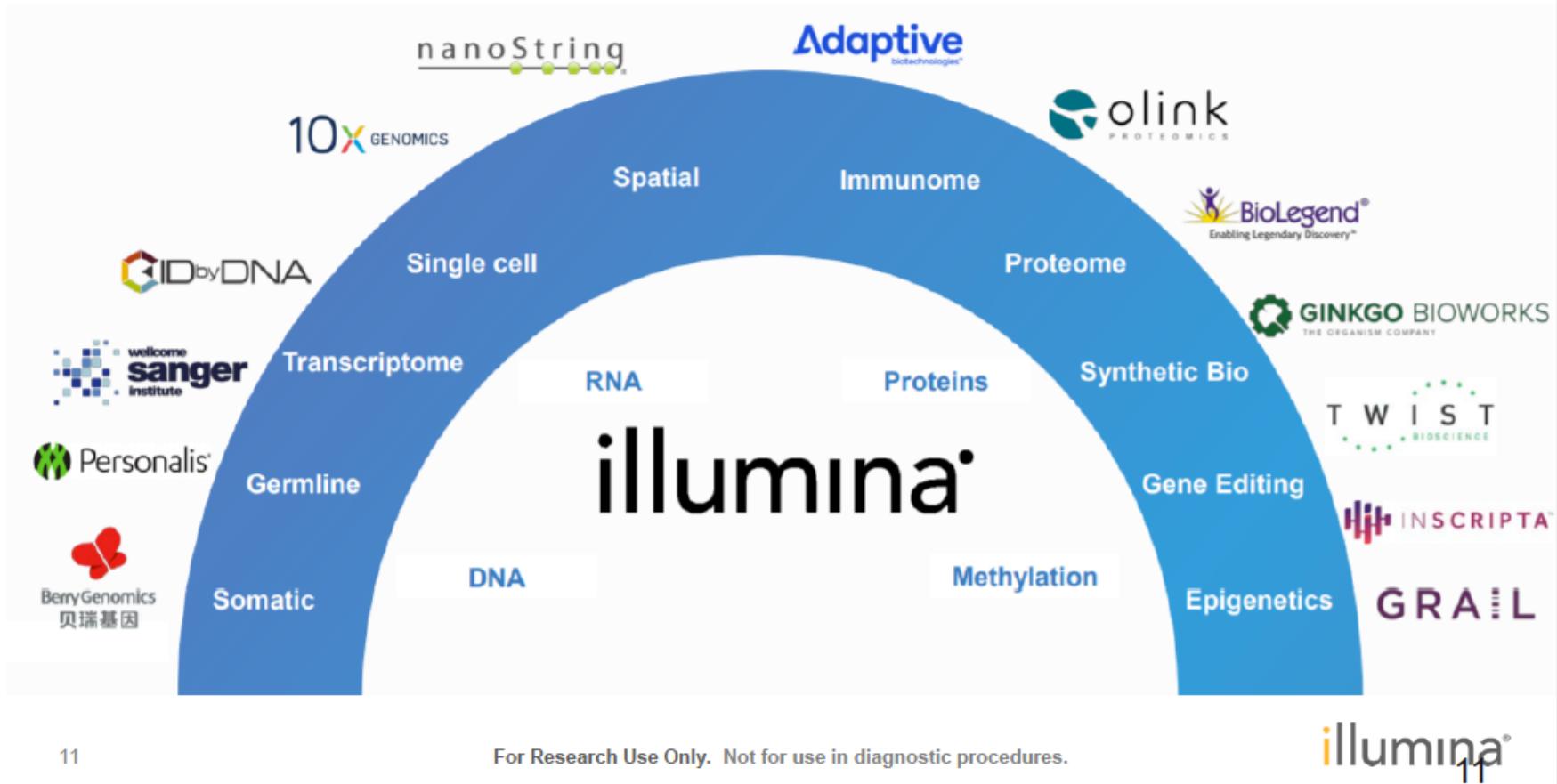
Each of the 4 DNA bases emit an intensity of a unique wavelength

Collects 4 images

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

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One Technology, Many Applications

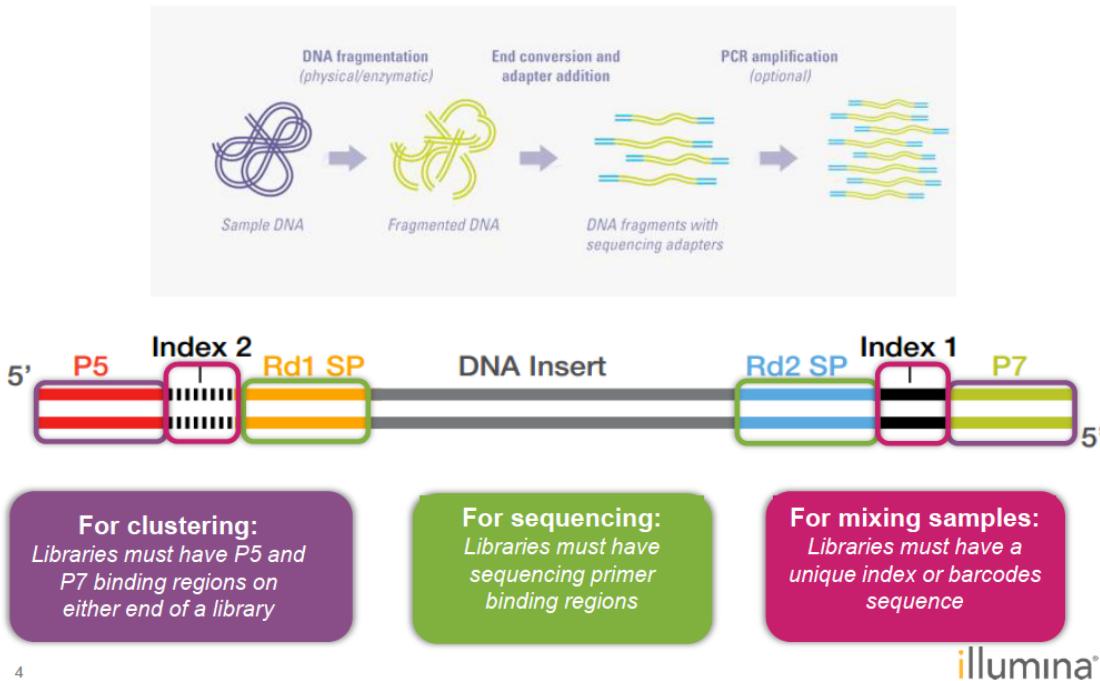


2)

Library Preparation for Next-Gen Sequencing

Min Zhang
USF Genomics Core
Manager

Library Prep is Critical for Successful Sequencing

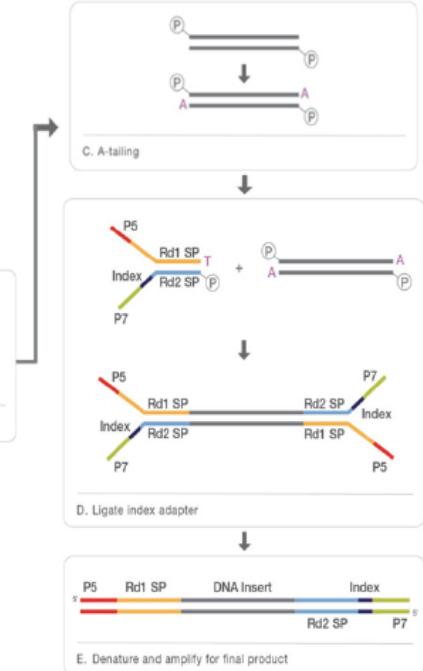
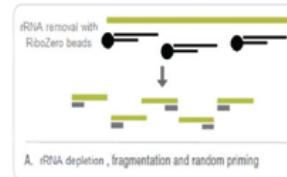


TruSeq Stranded RNA Workflows

TruSeq Stranded mRNA



TruSeq Stranded Total RNA



FOR RESEARCH USE ONLY

3)

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

4) Library QC, Trouble-shooting, Best Practices

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

Low or No Yield Causes:
Input Issues

Garbage in



Bad Sample

Garbage out

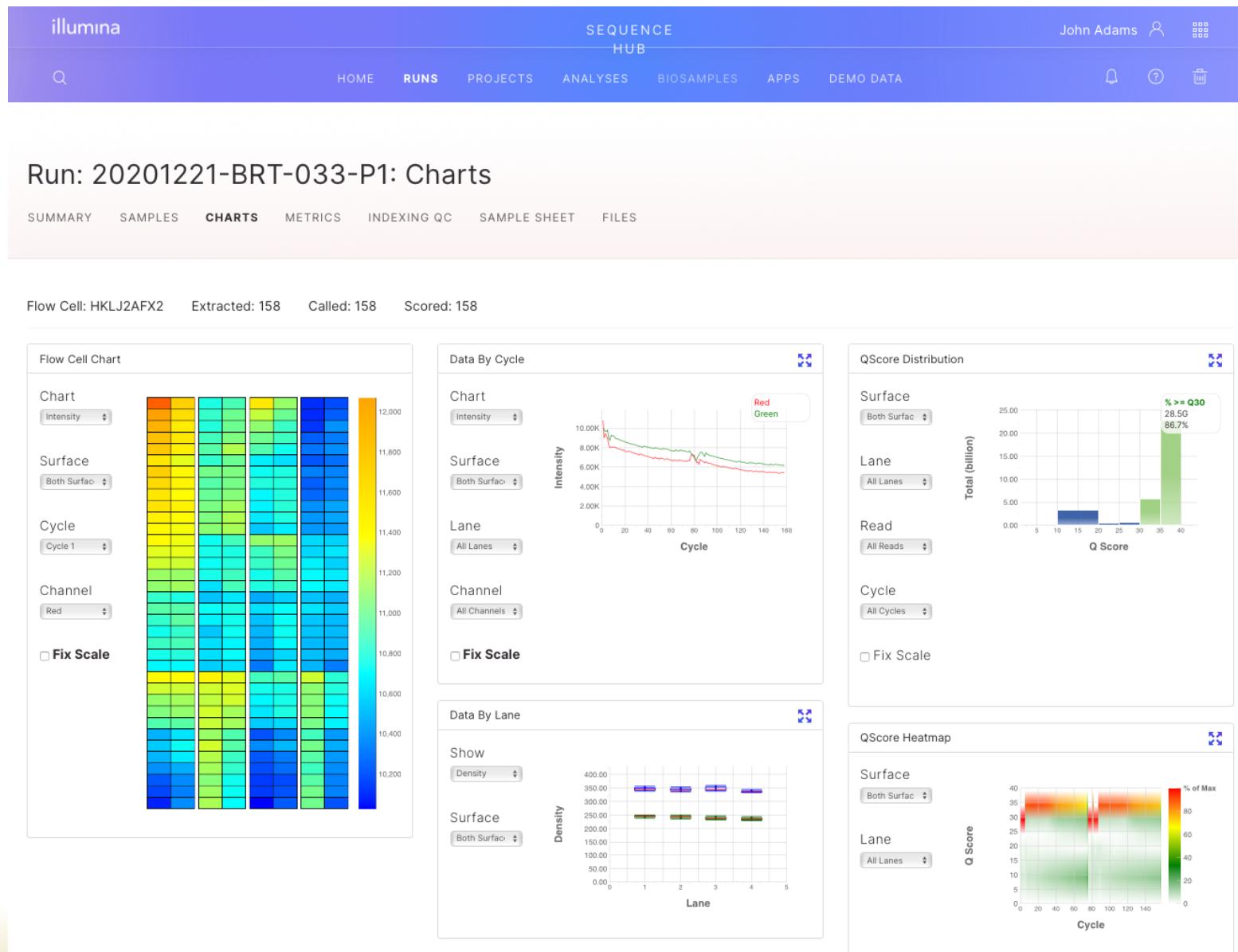


Bad Library → Bad Sequencing Data

4)

Sequencing Data QC, Illumina BaseSpace Tour

Min Zhang
USF Genomics Core
Manager

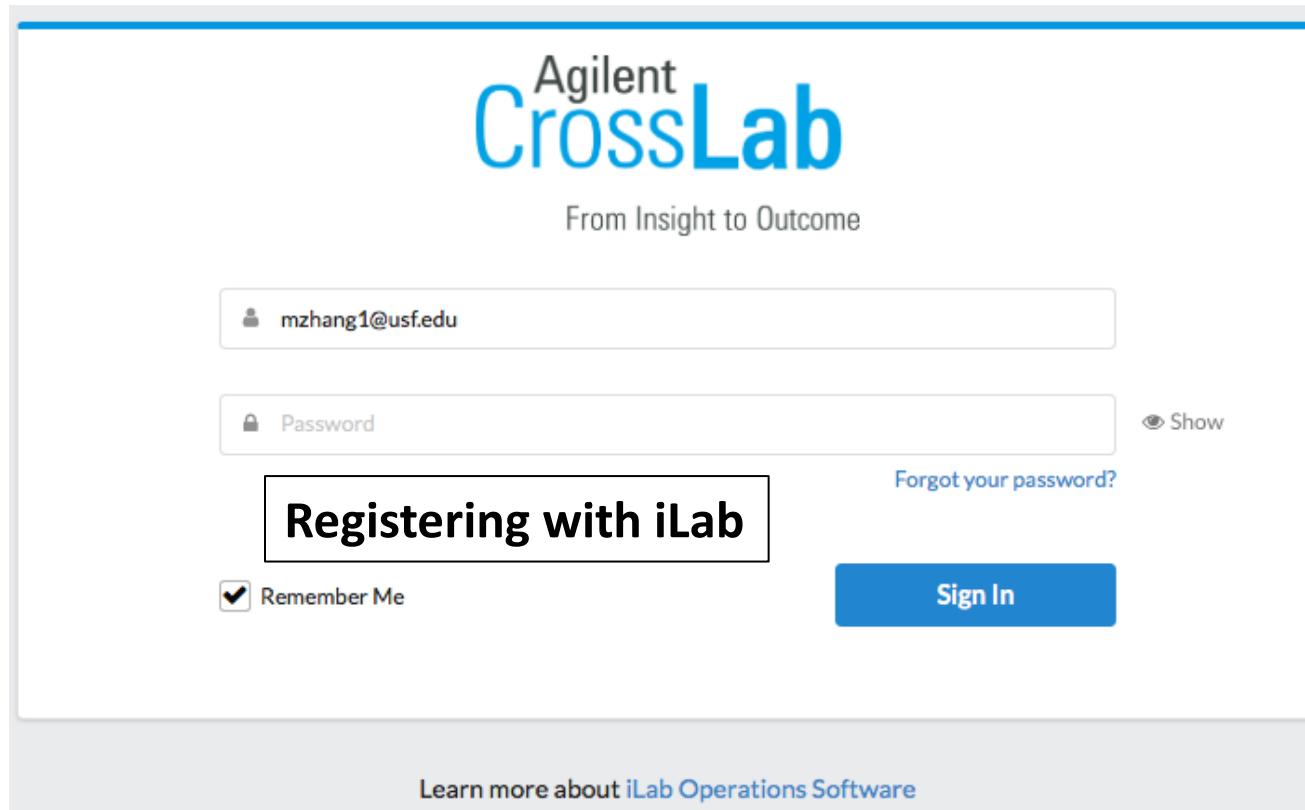


5)

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?

