

USF Genomics Equipment Core

Introduction to the USF Genomics Core & Sequencing Services



❖ 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)**

USF GENOMICS CORE FACILITIES AND RESOURCES

❖ Instrumentation for Library-prep and Quantifications



Agilent 4200



Agilent 2200



Roche LightCycler 96 System



M220 Focused-ultrasonicator



Qubit® 4 Fluorometer

What We Can Provide:

- ❖ Sequencing Services
- ❖ Genomics Training
- ❖ Quotation - Sequencing Price
- ❖ Consultation- Method/Cost estimate
- ❖ Letter of Support for Proposal Prep
- ❖ Collaboration- NGS Method Development

USF Genomics Equipment Core Service Guideline

Certified User Service – ‘Self-Service’

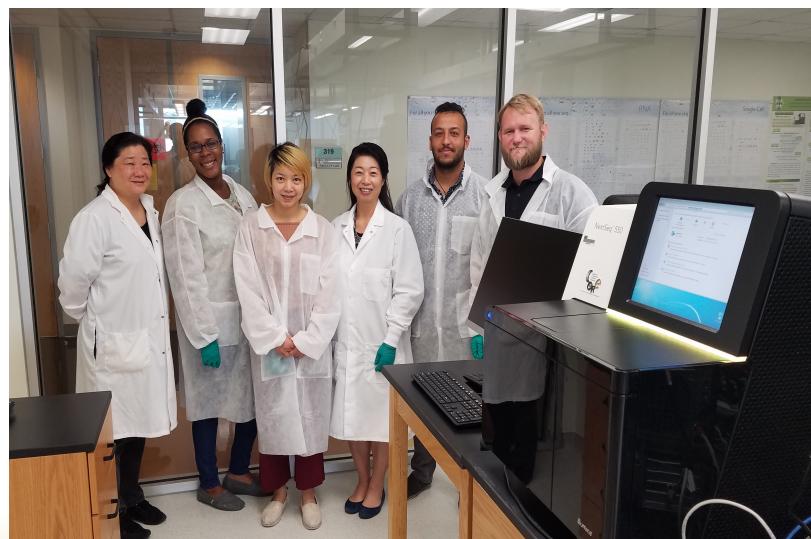
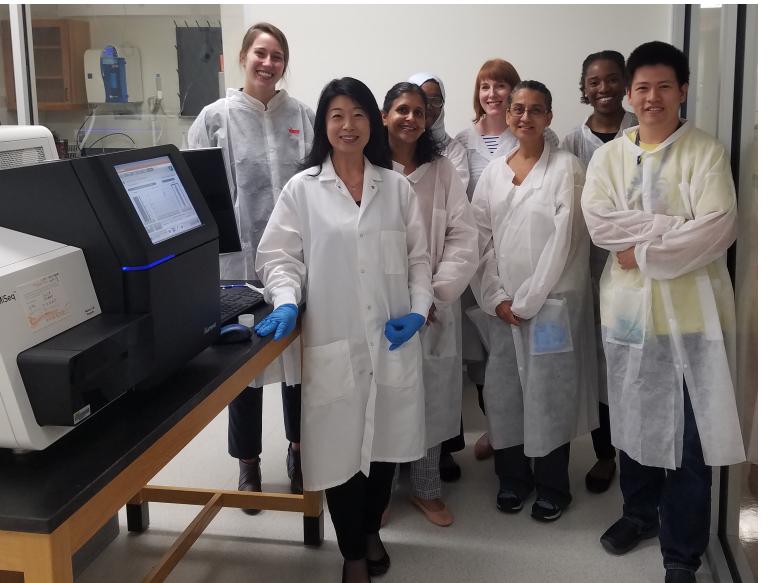
- The USF faculty, staff, and students who wish to use the USF Genomics Equipment Core ‘Certified User Service’ must complete the USF Genomics Training ‘RNAseq Illumina Sequencing Laboratory Workshop’.
- All sample preparation, library prep and sequencing works will be performed by the ‘Certified User’ under the core staff supervision. The ‘Self-Service’ price will be calculated based on the USF Genomics Equipment Core rate sheet, which includes necessary reagents, materials and two hours core staff labor for the library-QC and instrument use.

Non-Certified User Service – ‘Full-Service’

- The USF faculty, staff, and students who has no ‘Certified User’ available in the lab may submit ‘Full-Service’ for sequencing .
- All sample-QC, library prep and sequencing works will be performed by the core staff. The ‘Full-Service’ price will be calculated based on the USF Genomics Equipment Core rate sheet, which includes necessary reagents, materials and the core staff labor cost depends on the work request.

USF GENOMICS CORE AND RESOURCES

RNA Sequencing Laboratory Workshop



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

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

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 – Through the iLab

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.

USF Genomics Core Services

Submit Work Request through the iLab

Sequencing Services :

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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

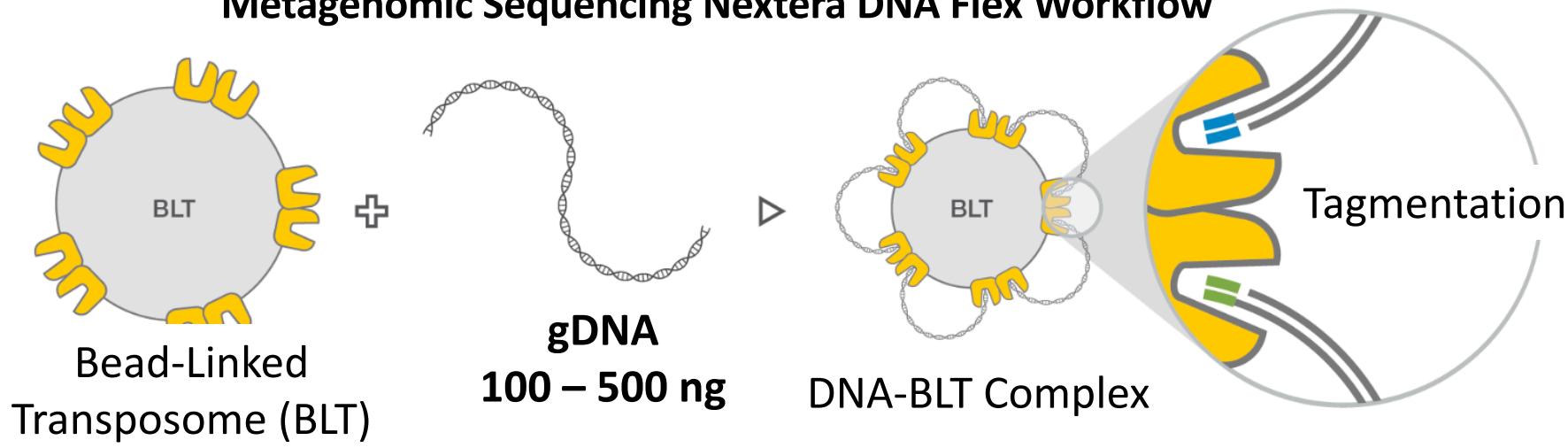
Services Description: Full Service-Sequencing	Rate/Unit	Qty	Amount
Sequencing use NextSeq V2.5 (High-Output - 300 Cycle)	\$6,461.00	1	\$6,461.00
Instrument Use & Sequence QC (core staff labor)	\$47.00	2 hours	\$94.00
Sub-Total: Sequencing Services (8 samples)			\$6,461.00
Price Per Sample (40 Million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

USF Genomics Equipment Core
3720 Spectrum Blvd, suite 316
Tampa, FL 33612

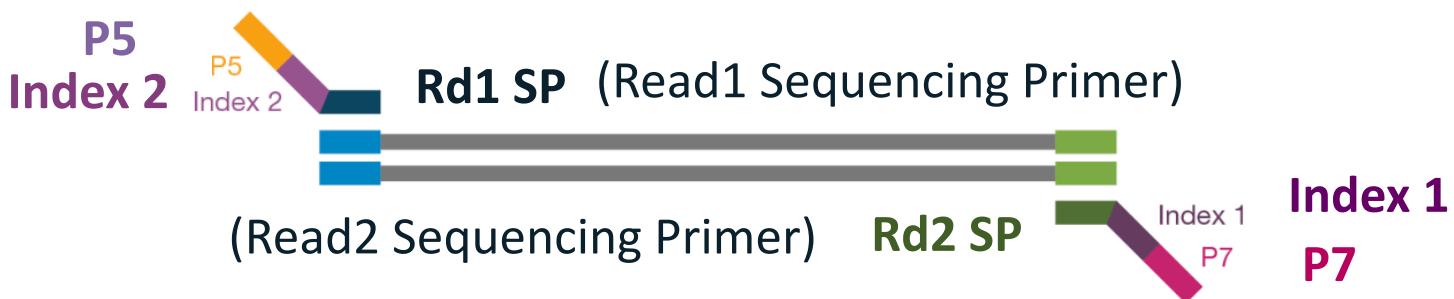
University of South Florida
Email: genomics@usf.edu

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

Metagenomic Sequencing Nextera DNA Flex Workflow



↓ PCR amplification



↓

P5 Index 2 Rd1 SP **DNA** **Rd2 SP Index 1 P7**

p5 Index2 Rd1 SP DNA Rd2 SP Index1 p7

Sequencing-ready fragment (~600 bp)

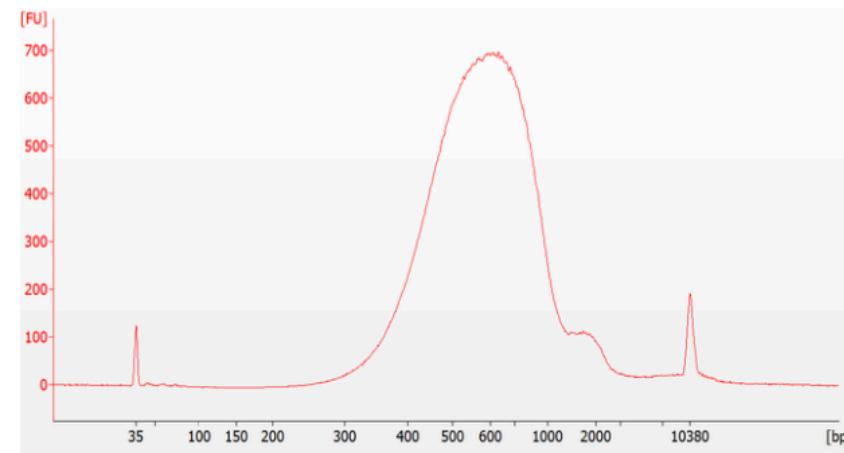
NextSeq 500/550 High Output Kit v2.5 (300 cycles)

❖Whole Genome Metagenomic Sequencing

NexTera DNA Flex Library Prep

Library average size ~600 bp

Figure 3 Example Bioanalyzer Trace



Sequencing System	Starting Concentration (nM)	Final Loading Concentration (pM)
HiSeq 2500 and HiSeq 2000 (high output modes)	2	12
HiSeq 2500 (rapid run mode)	2	8.5
HiSeq X, HiSeq 4000, and HiSeq 3000	2–3	200-300
iSeq 100	2	200
MiniSeq	2	1.2–1.3
MiSeq (v3 reagents)	4	12
NextSeq 550 and NextSeq 500	2	1.2–1.3
NovaSeq 6000	2	See document # 1000000019358 (NovaSeq 6000 System Guide)

Sequencing Kits We Recommend to Use:

Illumina Library-Prep Kits and Indexes Would be Recommended

Sequencing Data QC,
Index Distribution QC,
FASTQ Data Generation



Illumina BaseSpace
Sequence HUB

❖ RNA Sequencing: TruSeq® Stranded mRNA Library Prep
(48 Samples) 20020594



❖ 16S rRNA Sequencing:

Amplicon PCR: 16S Amplicon PCR Primers (Forward, Reverse)

Index PCR: Nextera XT Index kit (2 Primers N7xx, S5xx) from the Index Kit

2x KAPA HiFi HotStart ReadyMix

❖ Whole Genome Metagenomic Sequencing

Nextera DNA Flex Library Prep Kit, Nextera DNA CD Indexes

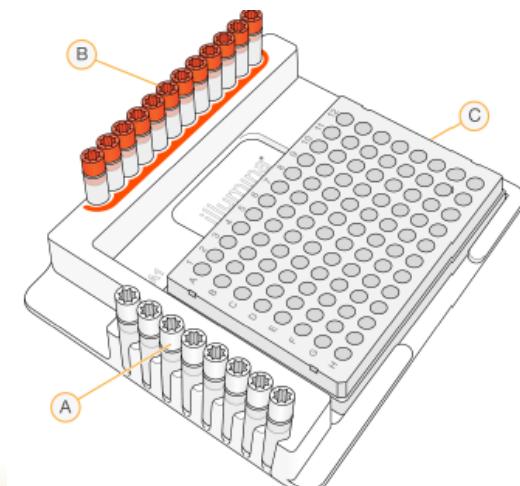
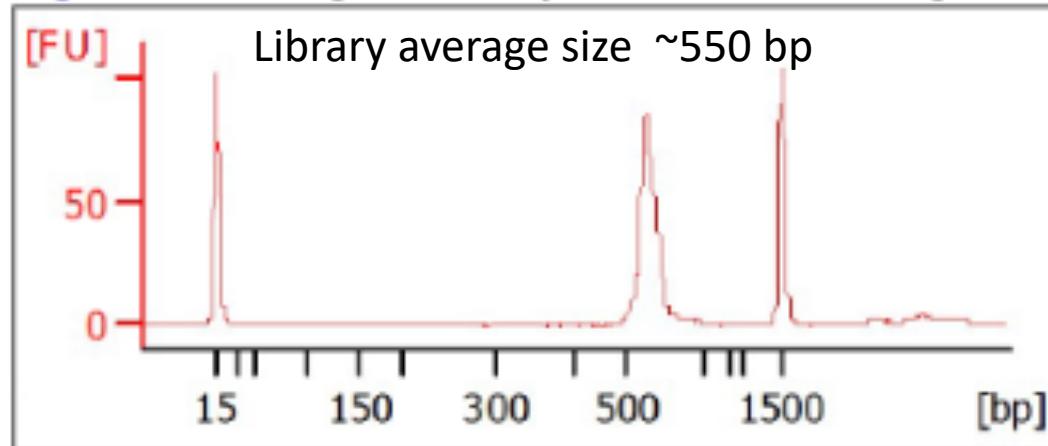


Figure 3 Example Bioanalyzer Trace after Amplicon PCR Step



Dilute Denatured DNA

- 1 Dilute the denatured DNA to the desired concentration using the following example:



NOTE

Illumina recommends targeting 800–1000 K/mm² raw cluster densities using MiSeq v3 reagents. It is suggested to start your first run using **a 4 pM loading concentration and adjust subsequent runs appropriately.**

Final Concentration	2 pM	4 pM	6 pM	8 pM	10 pM
20 pM denatured library	60 µl	120 µl	180 µl	240 µl	300 µl
Pre-chilled HT1	540 µl	480 µl	420 µl	360 µl	300 µl

❖16S rRNA Sequencing:

16S Metagenomic Sequencing Library Preparation

Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System

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- 3 Sequence on MiSeq—Using paired 300-bp reads, and MiSeq v3 reagents, the ends of each read are overlapped to generate high-quality, full-length reads of the V3 and V4 region in a single 65-hour run. The MiSeq run output is approximately > 20 million reads and, assuming 96 indexed samples, can generate > 100,000 reads per sample, commonly recognized as sufficient for metagenomic surveys.**

Specifications for the MiSeq System

MiSeq offers short sequencing run times and long read lengths while maintaining high data quality

	MiSeq Reagent Kit v2				MiSeq Reagent Kit v3	
Read Length	1 × 36 bp	2 × 25 bp	2 × 150 bp	2 × 250 bp	2 × 75 bp	2 × 300 bp
Total Time*	~4 hrs	~5.5 hrs	~24 hrs	~39 hrs	~21 hrs	~56 hrs
Output	540–610 Mb	750–850 Mb	4.5–5.1 Gb	7.5–8.5 Gb	3.3–3.8 Gb	13.2–15 Gb

	MiSeq Reagent Kit v2 Micro		MiSeq Reagent Kit v2 Nano	
Read Length	2 × 150 bp		2 × 250 bp	2 × 150 bp
Total Time*	~19 hrs		~28 hrs	~17 hrs
Output	1.2 Gb		500 Mb	300 Mb

* Total time includes cluster generation, sequencing, and base calling on a MiSeq System enabled with dual-surface scanning.

Reads Passing Filter**

	MiSeq Reagent Kit v2	MiSeq Reagent Kit v3	MiSeq Reagent Kit v2 Micro	MiSeq Reagent Kit v2 Nano
Single Reads	12–15 million	22–25 million	4 million	1 million
Paired-End Reads	24–30 million	44–50 million	8 million	2 million

** Install specifications based on Illumina PhiX control library at supported cluster densities (865–965 k/mm² clusters passing filter for v2 chemistry and 1200–1400 k/mm² clusters passing filter for v3 chemistry). Actual performance parameters may vary based on sample type, sample quality, and clusters passing filter.

USF GENOMICS CORE FACILITIES AND RESOURCES



Illumina - MiSeq System

12 – 15 million reads (v2)

22 – 25 million reads (v3)

Read Length: PE 2X

75; 150; 250; 300

\$1,000 - \$2,000



NextSeq 550 System

~ 130 million reads (Mid Output)

~ 400 million reads (High Output)

Read Length: PE 2X

75; 150

\$2,000 - \$6,800

USF Genomics Equipment Core, USF Genomics Program
 (813) 974-7566, genomics@usf.edu

Thanks for using the USF Genomics Equipment Core. Here is the RNA sequencing report of the Nextseq run 20210325-GP-039. Please let us know if we can assist you in any other way.

1. Overview of Work Request

Work Request by	
PI	
Species Name	
Sample Type	Total RNA
Sample Number	14
Library Type	Truseq stranded mRNA, Illumina
Reads per sample:	20 Million
Sequencing Kit:	Nextseq High-output kit, 150 cycles
Read Length (bp)	76/76
Type of Read	Paired-End

2. Sequencing Report

Name of the run	20210325-GP-039
Completion date	26-Mar-21
QC status	QC Passed
Overall density	291 K/mm ²
>= Q30 (%)	88.94
Passed QC filter (PF reads %)	77.79
PF Reads Generation	580 Million
FASTQ Generation	90.98 Gbp

3. Index QC of the Samples

Sample ID	Index ID	Index 1	Index 2	% Reads (PF)
Ctrl-R1	A002	CGATGT	n/a	7.62
Ctrl-R2	A004	TGACCA	n/a	7.36
A6-R1	A005	ACAGTG	n/a	8.66
A6-R2	A006	GCCAAT	n/a	8.99
A10-R1	A007	CAGATC	n/a	7.56
A10-R2	A012	CTTGTA	n/a	8.95
M6-R1	A013	AGTCAA	n/a	7.78
M6-R2	A014	AGTTCC	n/a	5.07
M10-R1	A015	ATGTCA	n/a	9.03
M10-R2	A016	CCGTCC	n/a	6.46
H6-R1	A018	GTCCGC	n/a	3.33
H6-R2	A019	GTGAAA	n/a	3.25
H10-R1	A001	ATCACG	n/a	6.58
H10-R2	A003	TTAGGC	n/a	4.13

Below are shared links for checking the RUN, and download the FASTQ data files thru the Illumina Basespace.

RUN: 20210325-GP-039

<https://basespace.illumina.com/s/lQK1tlRx045b>

Project: 20210325-GP-039 FASTQ DATA

<https://basespace.illumina.com/s/sBCCQOu6fi5R>

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>



Thanks!

