NIH NATIONAL CANCER INSTITUTE

Empowering your research with services from the CCR Genomics Core

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Staff Scientist (Office of Science Technology and Research, OSTR)
Building 41, Room D310

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Disclaimer

We did not receive any financial support from any suppliers or manufacturers mentioned and do not have any conflict of interest

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Background

- 3 NCI CCR Genomics/ sequencing cores
  - Sequencing Facility (Bao Tran, Frederick)
  - Genomics Technology Laboratory (Xiaolin Wu, Frederick)
  - Genomics Core (Liz Conner, Bethesda)

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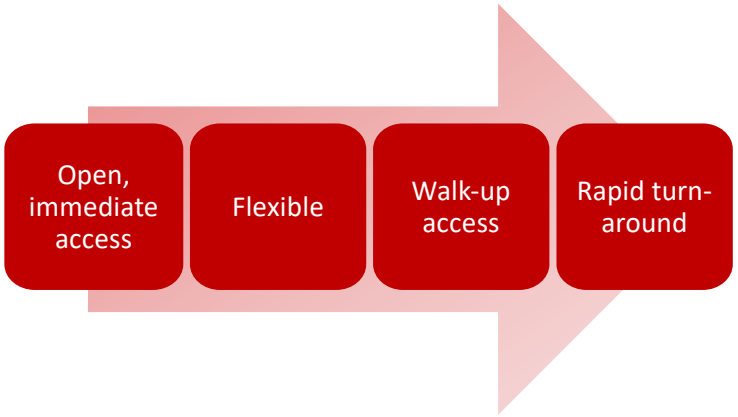
Background

- "Open access" facility with goal of providing efficiency and quality of a centralized facility with speed and convenience
- Help identify, evaluate and make available new technologies as they emerge
- Provide services to investigators in NCI CCR scientific community, the Clinical Center and other NIH institutes including NIAID, NHGRI, NHLBI, NINDS, NEI, NIA, NIMH, NIDDK, and NICHD



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Staff & Location

Bldg. 41, Room D310

Collaborative Protein Technology Resource (CPTR)

Single Cell Analysis Facility (SCAF)

Genomics Core (GC)

Core members:

- Liz Conner, Manager
- Steve Shema
- Qin Wei
- Madeline Wong
- Desiree Tillo, Bioinformatics Consultant

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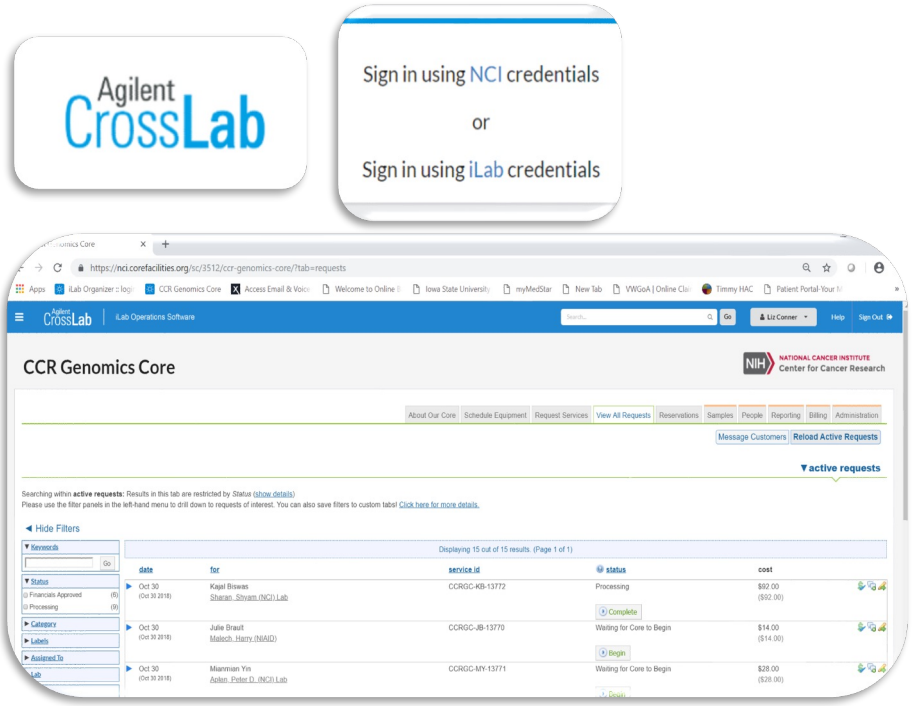
Facility Usage and Access

Investigators can register and submit samples through our website at: https://nci.corefacilities.org/account/login



- Users complete iLAB request for Sanger samples
- Other applications the Core Staff will enter the iLAB request
- Billing every quarter of each fiscal year (DEC, APR & AUG) via CAN transfer
- NO POTS order and any other type of approval

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Services

- Analytical & Preparative Electrophoresis
- Droplet Digital PCR (ddPCR)
- Digital Gene Expression (Nanostring nCounter)
- Sanger Sequencing
- Next-Generation Sequencing (NGS) – illumina platforms
- Digital Spatial Profiling (NanoString GeoMx DSP)
- Next-Generation Sequencing (NGS) – Oxford Nanopore

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Service: Analytical & Preparative Electrophoresis

sage science

PippinHT

High Throughput Sizing

- Up to 24 samples in one run

- 3% agarose, 100-250 bp
- 2% agarose, 100-600 bp
- 1.5% agarose, 300-1.5 kb
- 0.75% agarose, High-pass size selection

- **Cost recovery service ($10.00/sample) with same day sample delivery**

Agilent Technologies

4150 & 4200 Tape Station

Automated Electrophoresis for DNA & RNA samples to analyze size, quantity & integrity (RNA RIN number)

- Genomic DNA Tape
- D1000 DNA standard and high sensitivity
- D5000 DNA standard and high sensitivity
- RNA standard and high sensitivity

- **Cost recovery service ($5 - 6.00/sample) with same day data delivery**

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Service : Droplet Digital PCR (ddPCR)

BIO-RAD X200 Droplet Digital PCR System

- Provides absolute quantification of target molecules without the use of standard curves
- Chemistries
  - EvaGreen
  - Probe
- Common Applications
  - Expression
  - Mutation
  - Copy Number Variation

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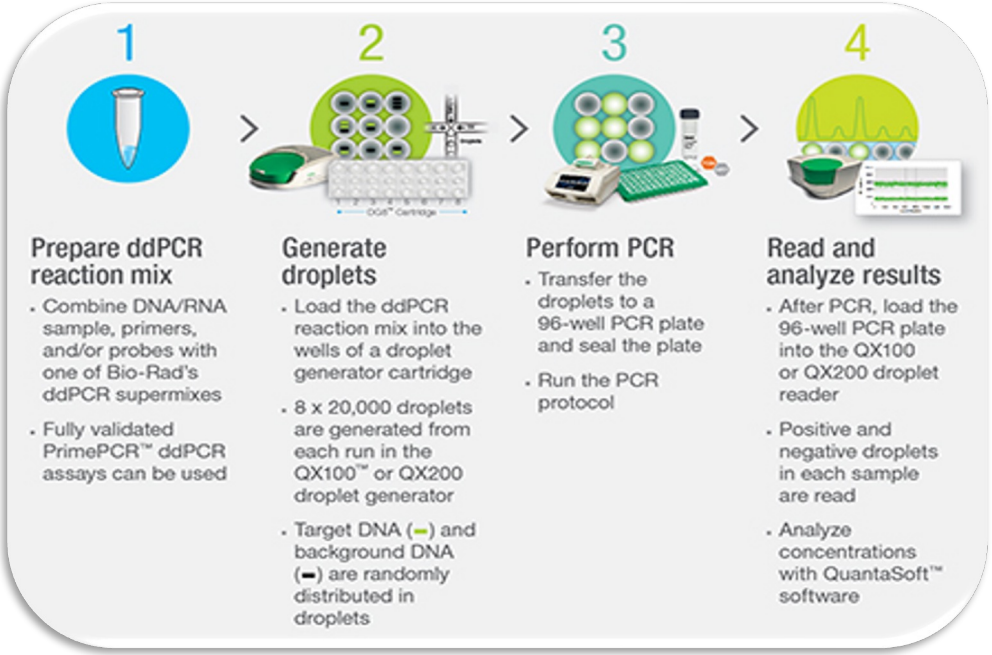
Service : Droplet Digital PCR (ddPCR)

BIO-RAD X200 Droplet Digital PCR System

- Users provide DNA template with primers and Probe (FAM & HEX Probe Assay: 2 genes) or DNA template with primers (EvaGreen Assay)
- Core Staff adds the Supermix, Generate droplets, Perform PCR and Read
- Biorad Analysis software provided free to Users
- Users will analyze their results with Biorad support if needed



- **Cost recovery service ($7.00/sample in batch of 8 samples) with same day or next day data delivery**

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Service : Droplet Digital PCR (ddPCR)

BIO-RAD QX200 Droplet Digital PCR System

- Probe Assay – 2 colors systems (FAM & HEX)

COMING SOON

BIO-RAD QX600 :
6 plex (colors) system

Increase power of experiment – multiplex to detect > 2 genes of interest in a single well

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Services

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Service : NanoString Digital Gene Expression

nanoString nCounter Analysis System

- Novel digital color-coded barcode technology (direct multiplexed measurement of gene expression)
- High levels of precision and sensitivity
- Users contact NanoString directly to purchase or design a CodeSet tailored to their experiment (Pre-made panels are available for Gene Expression, Gene Fusions, miRNA, CNV, SNV and Protein)
- Samples are processed after hybridization in the Core

1 Hybridize

- Flexible sample requirements
- Only 4 pipetting steps
- No Amplification
- 800 hybridizations in a single tube

2 Purify

3 Count

- Sensitive
- Precise
- Quantitative
- Simple

- **Cost recovery service ($24.00 / sample in batch of 12 samples) with results within 2 business days from the start of hybridization**

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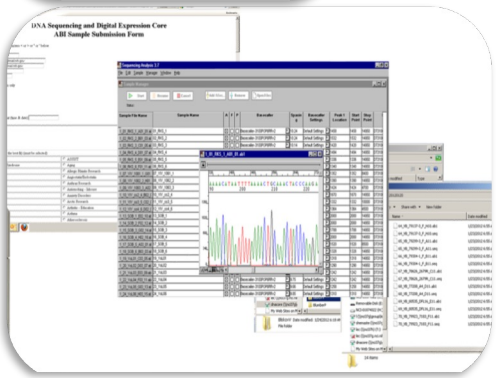
Service : Sanger Sequencing



- Applied Biosystems DNA Analyzer
  - 24-capillary 3500XL (2 units)

ThermoFisher SCIENTIFIC

- Standard plasmid and PCR products as well as bacterial genomic DNA, BACs and cosmids. Fragment analysis – analyze amplified PCR products
- Investigators provide us with DNA template + primer and we perform the reaction and electrophoresis or customers do their own reactions for electrophoresis only
- Data is analyzed and placed in a secure folder on the NIH network and investigators are notified via email when their data becomes available

- **Cost recovery service ($2 - 6.00/sample) & typically 1 business day data delivery for samples received by 1 pm**

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Service : Next-Generation Sequencing (NGS)

illumina platforms

iSeq

- 1 kit
- 5 M max clusters
- Up to 2 x150 bp

MiSeq

- 8 different kits available
- 25 M max cluster
- Up to 2 x 300 bp

NextSeq550

- 5 different kits available
- 400 M max clusters
- Up to 2 x 150 bp

NextSeq2000

- 11 different kits available
- 1.1 B max cluster
- Up to 2 x300 bp

- **Cost recovery service ($400 – 5700/kit) & data delivery < 1 week**

- Library provided by Users (RNASeq, ChIPSeq, NanoString DSP, 10X Genomics – 3', 5', Immune and Visium, Amplicon or Custom Library prep)
- Library prepared by us (RNASeq, ChIPSeq, Bacterial genome, NanoString DSP)
- Different Users' samples are not

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Service : Next-Generation Sequencing (NGS)

illumina platforms

- For >1.1B reads, Users are advised to discuss with the Sequencing Facility in Frederick for production scale NovaSeq run

NovaSeq6000

- 20B max cluster
- Up to 2 x250 bp

NovaSeq X

- 52B max cluster
- Up to 2 x150 bp

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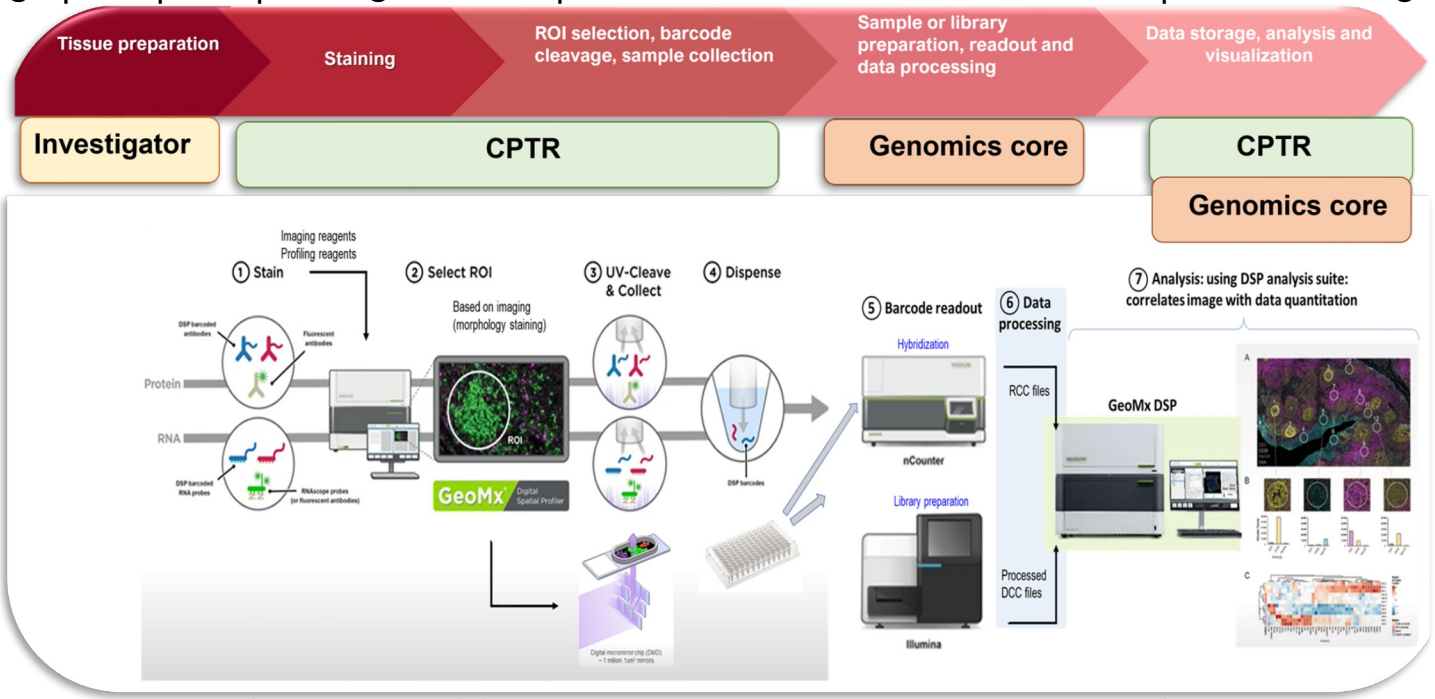
Service : GeoMx Digital Spatial Profiler (DSP)

nanoString

- Shared between Collaborative Protein Technology Resource (CPTR) and Genomics Core based on expertise
- High-plex spatial profiling to assess protein and/or RNA within tissue samples from a single FFPE or



- Interested Users would contact CPTR or Genomics Core to obtain more information

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Service : GeoMx Digital Spatial Profiler (DSP)

nanoString

Assay types:

PROTEIN

- **Protein with nCounter readout**
  - **Human** Immuno oncology
  - **Human** Neuroscience
  - **Mouse** Immuno oncology
  - **Mouse** Neuroscience
- **Protein with NGS readout (illumina platforms)**
  - **Human** core + immuno, oncology or neuro panels

RNA

- **RNA with nCounter readout: 94 transcripts** (73 core panel genes + 5 housekeeping + 6 neg control + 10 custom)
  - **Human** Immune pathways
- **RNA with NGS readout (illumina platforms)**
  - **Human** Cancer Transcriptome Atlas (hu CTA): ~ **1800** transcripts (~5 probes per gene)
  - **Human** Whole Transcriptome Atlas (hu WTA): ~ **18,000** transcripts (one probe per gene)
  - **Mouse** Whole Transcriptome Atlas (m WTA): ~**18,000** transcripts (one probe per gene)

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Service : Next Generation Sequencing (NGS)

Long-read sequencing

PacBio

Sequencing Facility in Frederick

SEQUEL IIe SYSTEM

Oxford NANOPORE Technologies

Sequencing Facility in Frederick

Genomics Core

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Service : Next Generation Sequencing (NGS)

Oxford NANOPORE Technologies

Long-read sequencing

	Flongle	MinION	GridION	P2	PromethION
WGS - small genomes	Yes: Low to medium plex	Yes: Low to medium plex	Yes: Low to medium plex	Yes: Highly multiplexed	Yes: Highly multiplexed
WGS - large genomes	Low pass	Low pass	Yes	Yes	Yes
Targeted sequencing	Yes: Low to medium plex, and adaptive sampling			Yes: Highly multiplexed and adaptive sampling	
Metagenomics	Yes: Quantitative species ID			Yes: Quantitative species ID	
RNA sequencing	Yes: Isoform & expression from same experiment			Yes: Isoform & expression from same experiment, including low abundance transcripts and single cell	
Epigenetics	Yes - when native DNA used			Yes - when native DNA used	

NIH NATIONAL CANCER INSTITUTE

Service : Next Generation Sequencing (NGS)

Long-read sequencing

Oxford NANOPORE Technologies

Kits

FLO-MIN106D flow cells are suitable for most sequencing kits:

- Ligation Sequencing Kit (SQK-LSK110)
- Ligation Sequencing Kit (SQK-LSK109)
- Cas9 Sequencing Kit (SQK-CS9109)
- PCR-cDNA Sequencing Kit (SQK-PCS111)
- PCR-cDNA Sequencing Kit (SQK-PCS109)
- PCR-cDNA Barcoding Kit (SQK-PCB109)
- Direct cDNA Sequencing Kit (SQK-DCS109)
- Direct RNA Sequencing Kit (SQK-RNA002)
- Rapid Sequencing Kit (SQK-RAD004)
- Rapid Barcoding Kit (SQK-RBK004)
- Rapid PCR Barcoding Kit (SQK-RPB004)
- 16S Barcoding Kit (SQK-RAB204)
- PCR Sequencing Kit (SQK-PSK004)
- PCR Barcoding Kit (SQK-PBK004)
- Field Sequencing Kit (SQK-LRK001)
- Ligation Sequencing Kit (SQK-LSK112) - *legacy*
- Ligation Sequencing Kit XL (SQK-LSK112-XL) - *legacy*
- Native Barcoding Kit 24 (SQK-NBD112.24) - *legacy*
- Native Barcoding Kit 96 (SQK-NBD112.96) - *legacy*

FLO-MIN114 flow cells can only be used with the V14 Sequencing Kit:

- Ligation Sequencing Kit V14 (SQK-LSK114)

N ^{6}-methyladenosine (m^6A) mRNA modification

A specific gene expression

Whole plasmid sequencing

Viral genome sequencing

Software

Basecalling:

- MinKNOW
- Guppy

as .fast5 and FASTQ files.

Downstream analysis:

tools and pipelines

ustomer-developed tools and pipelines

User analyzing the data

The gene was not detected

User analyzing the data

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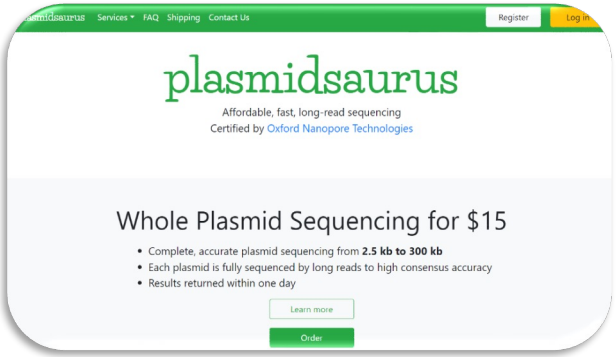
Service : Next Generation Sequencing (NGS)

Oxford NANOPORE Technologies

Long-read sequencing

- Rapid PCR Barcoding Kit (SQK-RPB004) ← **Whole plasmid sequencing**



- Advantage to the CCR community if we offer the service – no POTS order or any other approval – will be similar to requesting Sanger sequencing
- Maybe useful for difficult to assemble plasmids

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Service : Next Generation Sequencing (NGS)

Oxford NANOPORE Technologies

- Rapid PCR Barcoding Kit (SQK-RPB004) ← **Whole plasmid sequencing**

Sample: p304_ABE_E59A_SpG

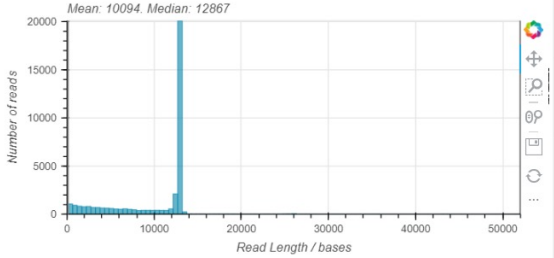
Completed successfully

raw downsampled

36253
No. reads

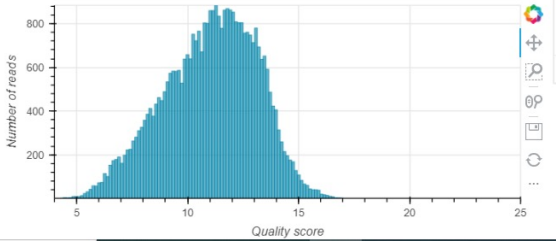
Read length distribution.

Mean: 10094. Median: 12867

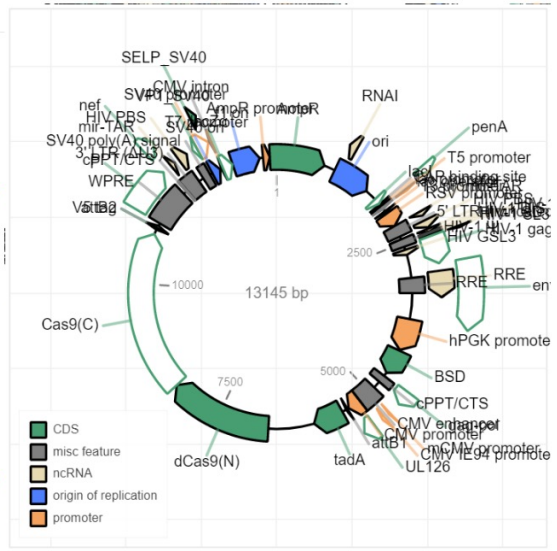


Read quality score

Mean: 11. Median: 11



- obtained single assembly



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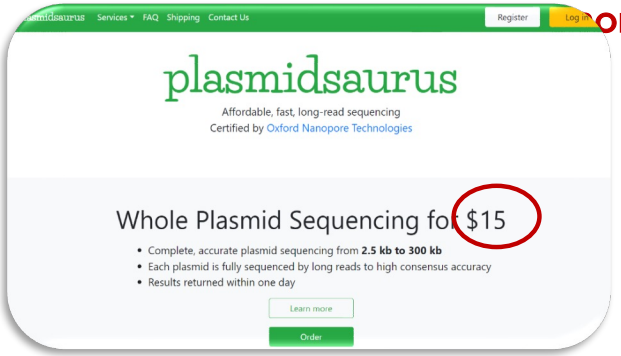
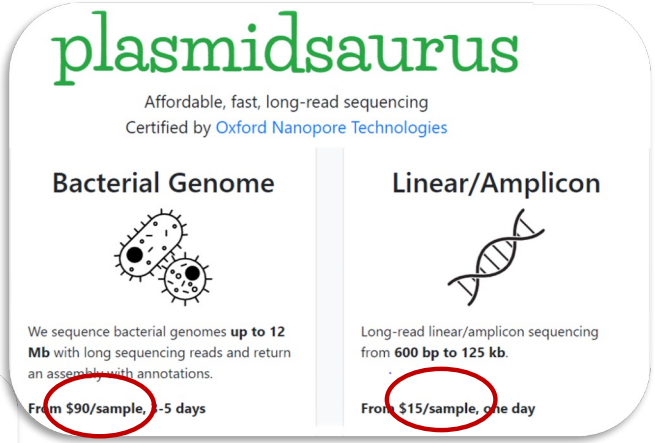
Service : Next Generation Sequencing (NGS)

Oxford NANOPORE Technologies

Long-read sequencing

- Rapid PCR Barcoding Kit (SQK-RPB004) ← Whole plasmid sequencing, amplicon or bacterial ome





- The cost – we are unable to match the price unless we have enough samples to

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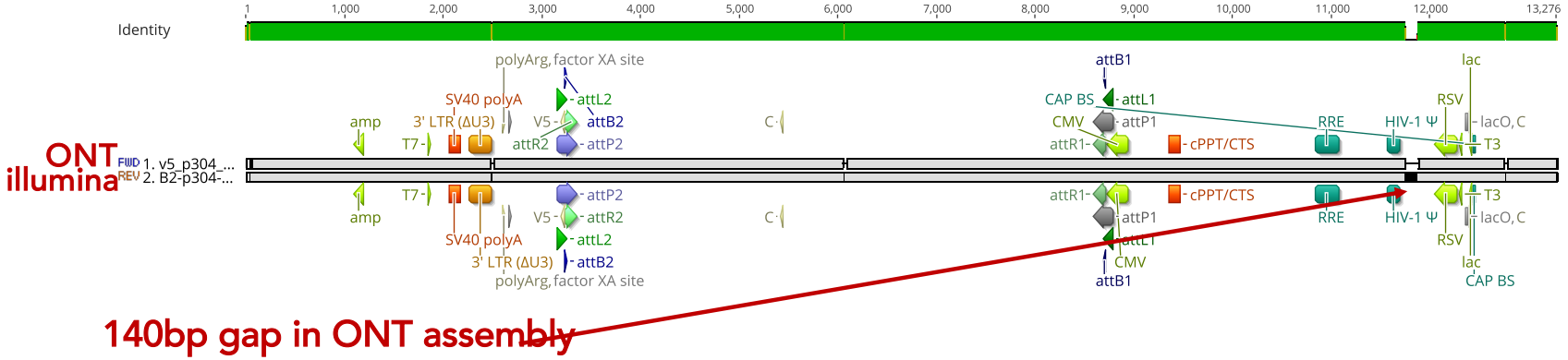
Service : Next Generation Sequencing (NGS)

Oxford NANOPORE Technologies

Long-read sequencing

- Rapid PCR Barcoding Kit (SQK-RPB004) ← **Whole plasmid sequencing**
  - Currently comparing the Sanger, short-read illumina and long-read Oxford Nanopore data

    -higher error rate, especially around homopolymers (illumina generally comparable to Sanger)
  - Need more testing



140bp gap in ONT assembly

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Service : Next Generation Sequencing (NGS)

Oxford NANOPORE Technologies

Long-read sequencing

- Hosted a seminar on Feb 15, 2023: Single cell sequencing with Oxford Nanopore and 10X Genomics
  - collaboration with SCAF
- Other applications

upgrade

WGS - small genomes	Yes: Low to medium plex	Yes: Low to medium plex	Yes: Low to medium plex	Yes: Highly multiplexed	Yes: Highly multiplexed
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Epigenetics	Yes - when native DNA used			Yes - when native DNA used	

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Service : Next Generation Sequencing (NGS)

Bioinformatics Support by Desiree Tillo, Bioinformatics Consultant

- Data delivery from NGS instruments

230217_VH01090_53_AACKM5HM5 Data Available

M mcintoshc@helix.nih.gov
To **NCI LEC DNA Core**

Reply | Reply All | Forward | ...
Mon 2/20/2023 11:30 AM

You forwarded this message on 2/20/2023 12:47 PM.
We removed extra line breaks from this message.

Content-Type: text/plain; charset="us-ascii"
MIME-Version: 1.0
Content-Transfer-Encoding: 7bit

Dear Core User,
Your NextSeq run on 2023-02-17 in data directory "230217_VH01090_53_AACKM5HM5" with experiment name "David Takeda Cell_Plasma ChIPSeq CS_RX 021723" completed successfully.

The data is accessible from the following URL:
http://cleversafetest.nci.nih.gov/SEQ37V/NEXTSEQ2000/230217_VH01090_53_AACKM5HM5.tar?Signature=kF2pIqkRepHF8Xw%2F10S%2BT7v6%2BXg%3D&Expires=1678119203&AWSAccessKeyId=l6JcnKaAlMCbhfDQpECs

The QC file is accessible from the following URL:
http://cleversafetest.nci.nih.gov/SEQ37V/NEXTSEQ2000/230217_VH01090_53_AACKM5HM5_qc.zip?Signature=1XlzSyA7dCJixg0jXOVH1UjT%2Ffc%3D&Expires=1678119203&AWSAccessKeyId=l6JcnKaAlMCbhfDQpECs

############################## Notes on Obtaining your data ############################## There are two ways to obtain your data files from an NIH Network attached computer:
1) Use the url link above.
2) Cut and paste the following command in a terminal window:
wget "http://cleversafetest.nci.nih.gov/SEQ37V/NEXTSEQ2000/230217_VH01090_53_AACKM5HM5.tar?Signature=kF2pIqkRepHF8Xw%2F10S%2BT7v6%2BXg%3D&Expires=1678119203&AWSAccessKeyId=l6JcnKaAlMCbhfDQpECs" -O 230217_VH01090_53_AACKM5HM5_Data.tar

################################ Obtaining your QC data ################################ There are two ways to

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Service : Next Generation Sequencing (NGS)

Bioinformatics Support by Desiree Tillo, Bioinformatics Consultant

- Data delivery from NGS instruments
- Experimental design consultations
- Interpretation of initial sequencing QC
- Provides advice on analysis resources for a variety of genomics applications (ChIP-seq, RNA-seq, ATAC-seq, variant analysis, GeoMX DSP transcriptome atlas)
- Long-term research projects on a collaborative basis in certain cases
    - development of custom workflows/analyses
    - project and schedule dependent

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Office of Science & Technology Resources (OSTR) Subsidy

- CCR investigators are eligible for NGS subsidy through OSTR from services from 3 NCI CCR Cores
  - Genomics Core (Bldg.41)
  - Single Cell Analysis Facility (SCAF) (Bldg.41)
  - Sequencing Facility (Fredericks)

  Subsidies provided to PIs who exceed $15,000 in cumulative sequencing costs during the fiscal year. After the initial $15,000 threshold, cumulative sequencing costs will be subsidized at 50% up to a total of $15,000 subsidy/year/PI
- CCR investigators are also eligible for Supplemental Technology Award Review System (STARS)
  - Pilot projects to get support for consumables

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Thank You

We look forward to
'Empowering your research with services from the CCR Genomics Core'

ncilecdnacore@mail.nih.gov