

Next Generation Sequencing Platform, Bern

- Who are we and what do we offer?



Next Generation Sequencing
Platform

UNIVERSITY of BERN

www.ngs.unibe.ch

University of Bern

Pamela Nicholson

17th November 2020



Next Generation Sequencing Platform

-who are we?



Small History

- A core facility born out of the Institute of Genetics, Vetsuisse, University of Bern
- Initiated in 2011
- By December 2020, we will be a team of 8 people

Core Facilities

The University of Bern houses several state-of-the-art core facilities in order to enable researchers at the university to carry out scientific research at the highest level.

Clinical Cytomics Facility
Clinical Metabolomics Facility
Clinical Trials Unit (CTU) Bern
Comparative Pathology Platform (COMPATH)
Cytometry Laboratory / FACSlab
ESI, Experimental Surgery Unit
Genomics
Live Cell Imaging (LCI)
Liquid Biobank Bern (LBB)
Mass Spectrometry and Proteomics Laboratory
Medical Rapid Prototyping

A Core facility –what does this mean and why does it matter?

1. A cost-effective means for making state-of-the-art instrumentation and services available to researchers
2. Enable researchers to move their research programs in new directions by facilitating and supporting interdisciplinary strategies
3. Serve as a nexus that encourages collaborations between internal and external researchers, thereby expanding the impact of research programs
4. Maximise the institutional investment by sharing capacity with external researchers, when available. Commercial users pay higher fees, thereby contributing an important revenue source for facilities that help support their operations.



Next Generation Sequencing Platform

-who do we serve?

Next Generation Sequencing
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 INSELSPITAL
UNIVERSITÄTSSPITAL BERN
HÔPITAL UNIVERSITAIRE DE BERNE



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UNIVERSITÄT
BERN



UNI
FR
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UNIVERSITÉ DE FRIBOURG
UNIVERSITÄT FREIBURG

....And many external researchers/customers, both academic and commercial such as Agroscope, Eawag, Microsynth, CSL Behring etc.



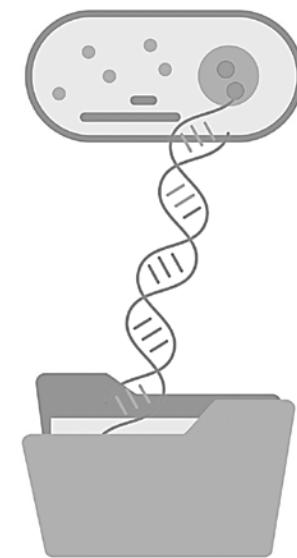
Next Generation Sequencing Platform

-what do we do?



We deliver high-throughput, next generation sequencing data from Illumina and Pacific Biosciences sequencing platforms.

We operate as a full-service facility that offers expertise in all aspects of the experimental process including evaluation of sample quality, construction of sequencing libraries, qualifying and normalising libraries and sequencing.



NGSP - A Technical Core Facility



illumina NovaSeq 6000
illumina MiSeq
illumina iSeq 100
Access to illumina NextSeq and additional MiSeq instruments
10X Genomics Chromium Controller (x 2)
PacBio Sequel

Beckman Coulter Biomek i7 Automated Workstation -*pipetting robot*

Advanced Analytics Fragment Analyzer CE12 -*QC for nuclei acids*
Advanced Analytics FEMTO Pulse CE -*QC for nucleic acids*

Covaris E220evolution Focused Ultrasonicator -*gDNA shearing 6-20kb*
Diagenode Megaruptor 2 -*DNA fragmentation 3-75kb*

Sage Science Pippin HT & Blue Pippin -*size selection*
Life Technologies Qubit 2.0, 4.0 and Flex Fluorimeter -*quantification of nucleic acids*
DeNovix DS-11 FX Spectrophotometer & Fluorometer

DeNovix CellDrop Automated Cell Counter

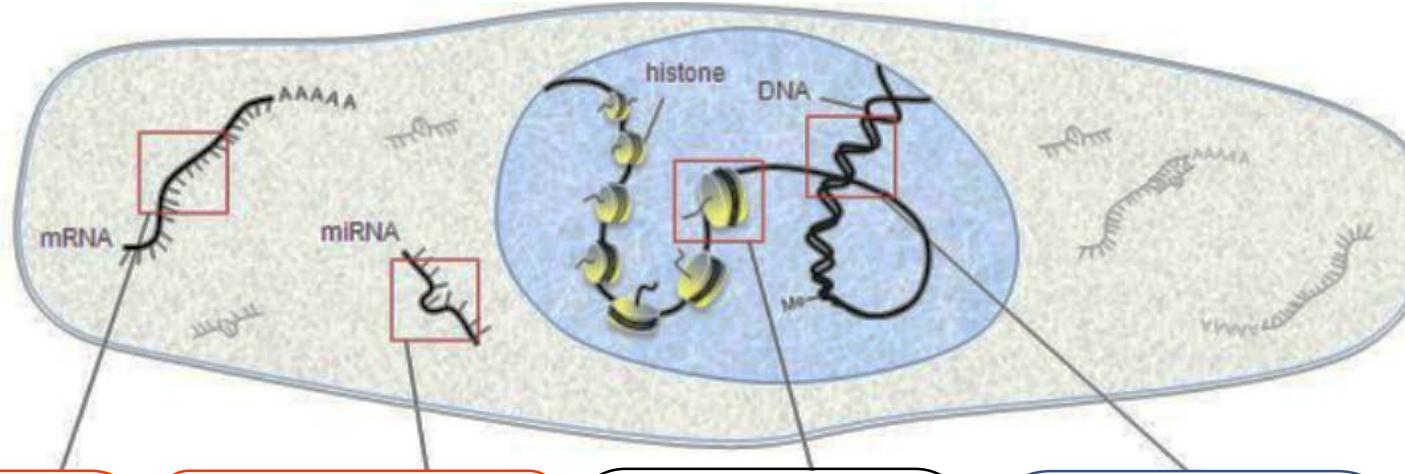
Access to Bio-Rad CFX Connect Real Time System -*qPCR*

**LIMS - designed and implemented to track
and store experimental
information and enable data distribution**



Next Generation Sequencing Platform

-Applications



Transcriptomics

RNA-Seq:

-De novo

-Reference based

Small RNA-omics

- Small RNA-Seq

Epigenomics

-WGBS

-RRBS

-ChIP-Seq

-ATAC-Seq

Genomics

WGS:

-De Novo

-Reference based

-Shotgun Sequencing

-Amplicon Sequencing

-CRISPR screening

Metagenomics

-WSMS

-indexing for 16S (-ITS)

amplicons

-V1-V2 16S (-ITS)

-V3-V4 16S (-ITS)

-Full-length 16S(-ITS)

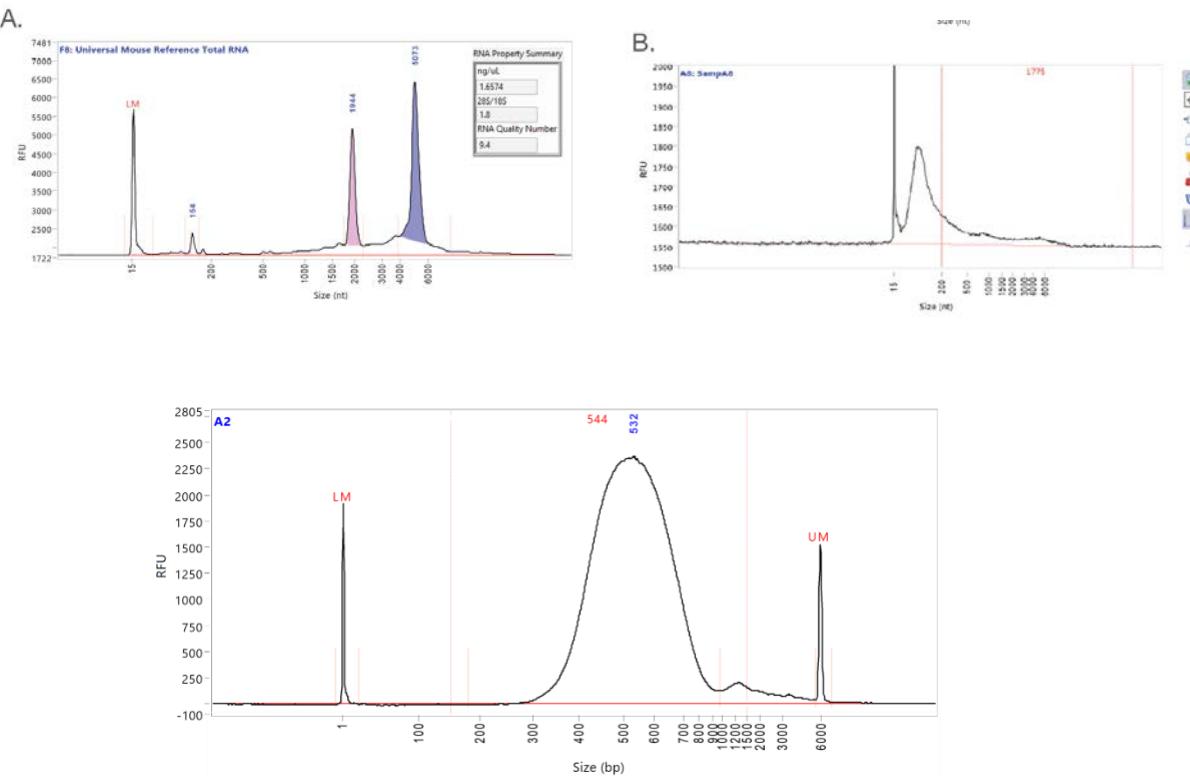
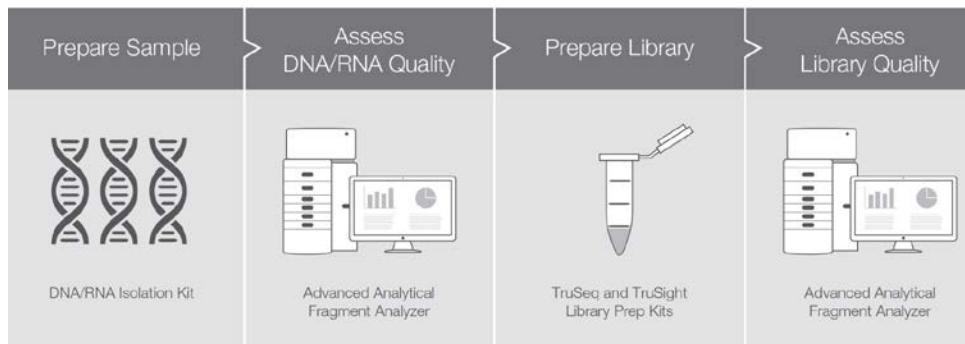
(illumina)

-Full-length 16S

(PacBio)

Accessory Services

- DNA shearing
- DNA Size Selection
- Bead based & column DNA/RNA clean & concentrate
- RNase or DNase treatments
- Nucleic acid assessments:
 - Spectrophotometry –*purity*
 - Fluorometry-*quantity*
- Capillary Electrophoresis –*Integrity and size*
- Pulsed-field capillary electrophoresis –*long-read NGS QC, gDNA, small RNA, or cfDNA analysis from low concentration samples.*



Nucleic Acids Detected By Fragment Analyzer

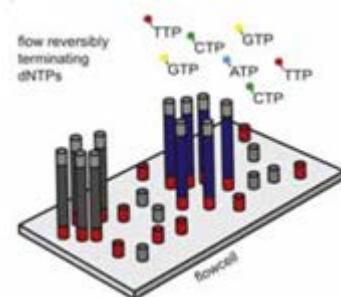
- | | | |
|-----------------------------|------------------------------|--|
| ● PCR Amplicons | ● Total RNA | ● Plasmids (Supercoiled & Linear) |
| ● CRISPR/Cas9 | ● microRNA | ● TILLING |
| ● Genomic DNA | ● Small RNA | ● SSR/Microsatellite Fragments |
| ● cfDNA | ● Messenger RNA (mRNA) | ● NGS Library Preparations for All Platforms |
| ● High Molecular Weight DNA | ● Restriction Enzyme Digests | |

Next Generation Sequencing Platform

-Complementary Sequencing Options

Illumina

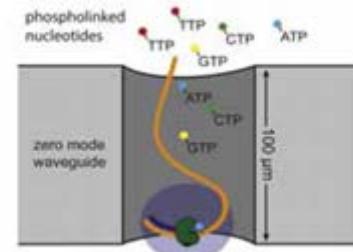
- DNA templates -> attached to a flow cell and amplified
- Sequencing = light-signal based
- successive rounds of base incorporation, imaging
- Base composition based on four-color images



<http://illumina.com>

PacBio

- DNA template -> pushed through a single polymerase
- Sequencing = light-signal based
- Base incorporation emits a fluorescent signal captured in a video



<http://pacbio.com>

First generation

- Sequence by synthesis

Sanger sequencing

Detects DNA fragments by capillary electrophoresis resulting from the selective incorporation of fluorescently tagged "chain-terminating" ddNTPs

Second generation

- Clonal amplification
- Massively parallelized sequencing
- Short sequence read lengths
- Direct detection without electrophoresis

Pyrosequencing

454/Roche

Detects luminescence proportional to the release of pyrophosphates from DNA polymerization

Reversible termination

Illumina

Detects fluorescently labeled, reversible-terminator dNTPs incorporated during DNA polymerization

Sequencing by ligation

ABI/SOLiD

Detects sequentially ligated, fluorescently labeled 8-mer primers

Semiconductor sequencing

Ion Torrent

Detects hydrogen ions released during DNA polymerization

Third generation

- Single-molecule sequencing
- Ultralong sequence reads

SMRT sequencing

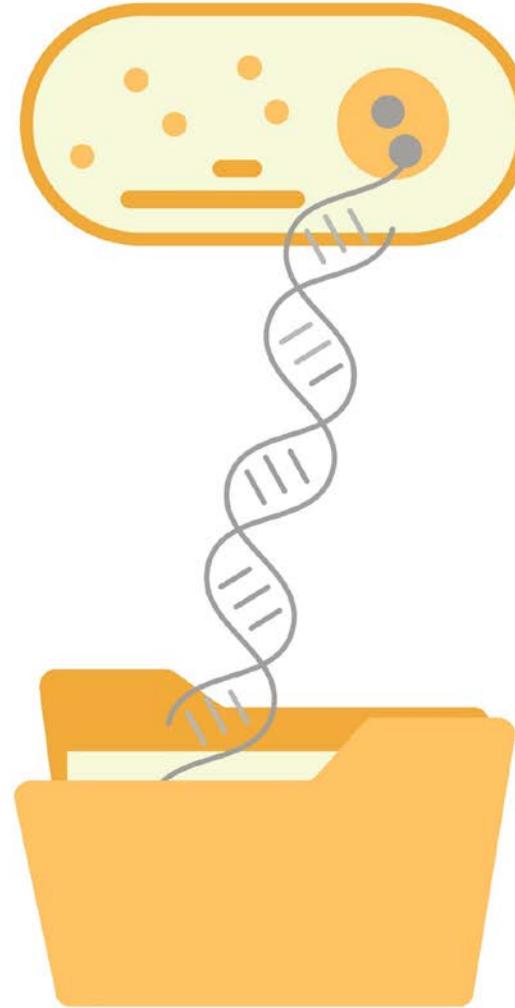
PacBio

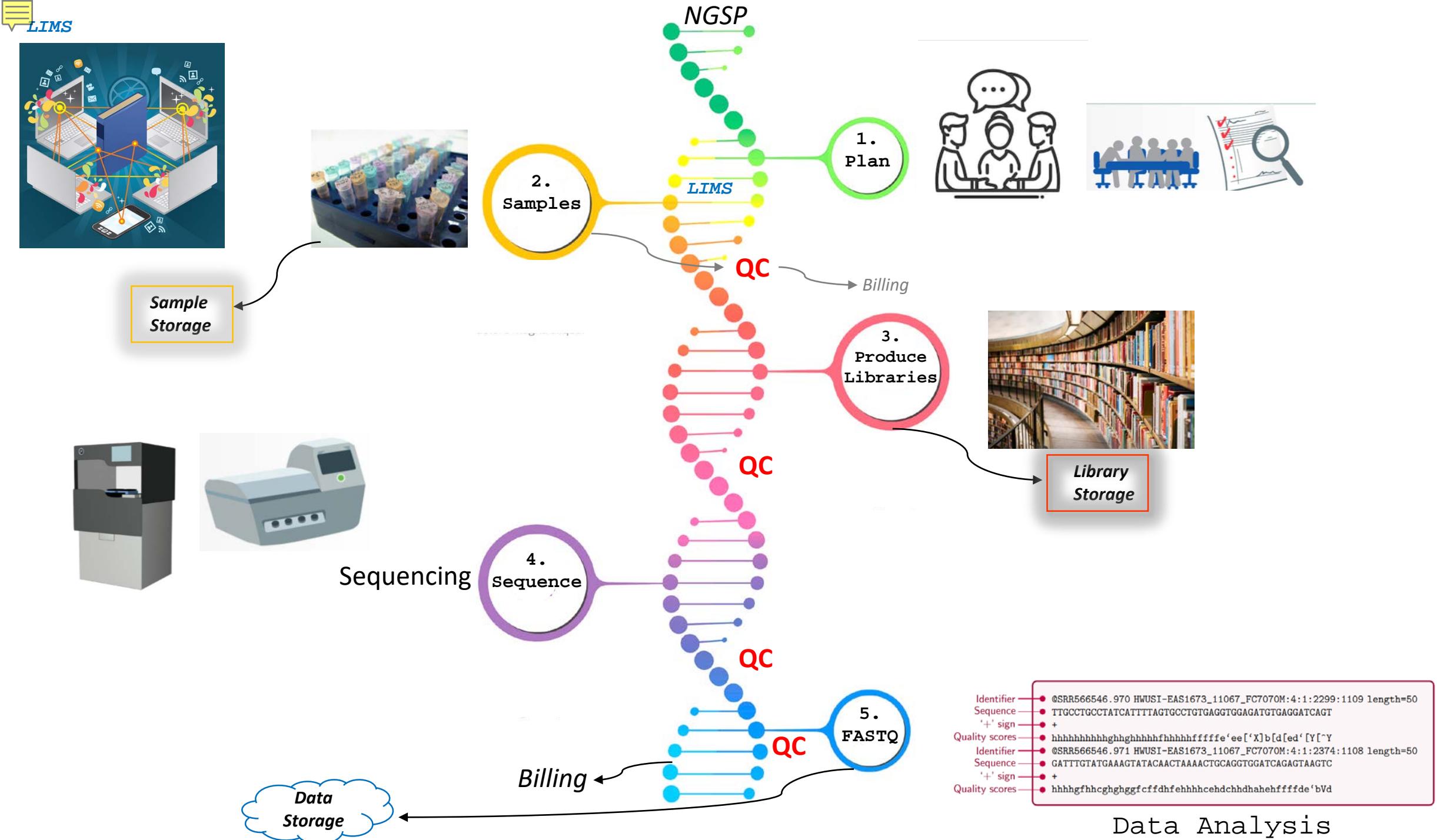
Detects fluorescently labeled nucleotides being incorporated from an affixed DNA polymerase on a zero-mode waveguide

Nanopore sequencing

Oxford

Detects changes in ionic current from differing nucleotides occupying a nanopore



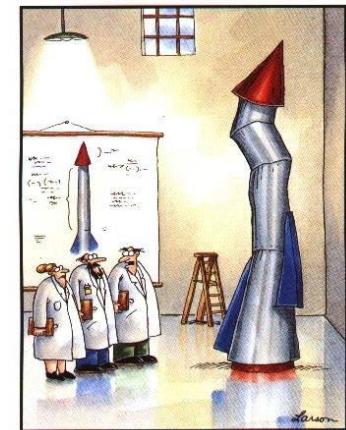


1. Project planning



Meetings to discuss your NGS project

- For complicated projects or projects using very new applications—invite everybody involved
- We are very open to new or non-standard applications—collaborative meetings
- Please give as much information as possible in initial emails –so we can prepare
- For quote requests -please provide all the necessary information for your project
- Price list is on the website—*updated as of October 2020*



An Nicholson, Pamela (VETSUSS) x

Cc

Sequencing

Dear Pamela,

I study antibiotic resistance and would like to sequence the *I. Scottii* genome using illumina. Please can you send me a price by tomorrow.

Thanks!

Alexander Fleming

Next Generation Sequencing Platform

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UNIVERSITÄT
BERN

Internal Price List for UniBe, UniFr & Inselspital

All prices in CHF

-Library Preparation Services

Sequencing libraries compatible with illumina platforms

DNA Sequencing Libraries	Input	Price	Cat. No.
DNA PCR-free library	1 µg gDNA	215	LIBD01 ¹
DNA PCR-free library - Low input	100 ng gDNA 10 ng cfDNA	240	LIBD02



Our LIMS



vetsrv03.campus.unibe.ch/table/runs

UHTS LIMS

Home Facility data User data Tracking Query projects Query runs Admin Account

Tables

Laboratories Projects Samples User Requests Libraries Bioanalysts Runs Base Cellines / Demultiplexins Alignments / QC Genomes File Transfers

Runs

All runs Active runs Find runs New runs

ID	Run folder	Cycle Nb	Run type	Run date	Release date	Status
111	191021_A00574_0120_BHF3WDRXX	50	paired-end reads	2019-10-21		Active
510	191021_A00574_0129_AHGKC3DRXX	50	paired-end reads	2019-10-21		Active
509	191018_A00574_0127_BHQG7WDRXX	50	paired-end reads	2019-10-18	2019-10-22	Released
508	191018_A00574_0128_AHF5CDRXX	150	paired-end reads	2019-10-18	2019-10-22	Released
507	191015_M02973_0201_0000000000-CNFBK	150	single read	2019-10-15		Active
506	191010_M02973_0202_0000000000-CNKBB	300	paired-end reads	2019-10-10		Active
505	191007_M02973_0199_0000000000-CLNN9	150	paired-end reads	2019-10-07	2019-10-09	Released
504	191002_M02973_0199_0000000000-CN9P9	230	paired-end reads	2019-10-02	2019-10-04	Released
503	190930_M02973_0197_0000000000-CN97D	250	paired-end reads	2019-09-30		Failed
502	190928_A00574_0124_BHF7WDRXX	50	paired-end reads	2019-09-26	2019-09-27	Released
501	190926_A00574_0125_AHCVC5CDRXX	100	single read	2019-09-26	2019-09-27	Released
500	190924_A00574_0122_BHFWMDRXX	100	single read	2019-09-24	2019-09-26	Released
499	190924_A00574_0123_AHFWNTDRXX	150	paired-end reads	2019-09-24	2019-09-26	Released
498	190920_A00574_0120_Arunnumbertakenbycgl	150	paired-end reads	2019-09-20		Failed
497	190923_M02973_0195_0000000000-CNLL6	75	paired-end reads	2019-09-23	2019-09-24	Released
496	190920_A00574_0121_BHFMHWDSXX	150	paired-end reads	2019-09-20	2019-09-23	Released
495	190913_A00574_0119_BHMTNDMXX	100	paired-end reads	2019-09-13	2019-09-16	Released
494	190913_A00574_0118_AHF7CWDXX	100	paired-end reads	2019-09-13	2019-09-16	Released
493	190909_A00574_0117_AHCVC5CDRXX	150	paired-end reads	2019-09-09	2019-09-11	Released
492	190905_A00574_0116_BHMMWWDMXX	50	paired-end reads	2019-09-05	2019-09-09	Released
491	190904_A00574_0115_AHCYC5WDRXX	100	single read	2019-09-04	2019-09-05	Released
490	190830_A00574_0113_BHCK32DRXX	100	single read	2019-08-30	2019-09-03	Released
489	190830_A00574_0114_AHCYLCDRXX	150	paired-end reads	2019-08-30	2019-09-03	Released
488	190828_M02973_0194_0000000000-CH2SD	150	paired-end reads	2019-08-28	2019-08-29	Released
487	190826_M02973_0193_0000000000-CNK86	150	single read	2019-08-26		Failed
486	190823_M02973_0192_0000000000-CLMVR	250	paired-end reads	2019-08-23	2019-08-26	Released
485	190820_A00574_0111_BHK3VTD5XX	150	paired-end reads	2019-08-20	2019-08-26	Released
484	190820_A00574_0110_AHN3YND5XX	150	paired-end reads	2019-08-20	2019-08-26	Released
483	190820_M02973_0190_0000000000-CLNN9	250	paired-end reads	2019-08-20	2019-08-23	Released
482	190815_A00574_0109_BHCGCJDRXX	150	paired-end reads	2019-08-15	2019-08-19	Released
481	190813_A00574_0108_AHGCG3DRXX	150	paired-end reads	2019-08-13	2019-08-16	Released
480	190812_A00574_0107_AHCVNHDXX	100	single read	2019-08-12	2019-08-21	Released
479	190812_A00574_0106_BHK677DMXX	100	single read	2019-08-12	2019-08-13	Released
478	190802_A00574_0104_BHCCYYDRXX	100	paired-end reads	2019-08-02	2019-08-12	Released
477	190802_A00574_0103_AHF3W5DRXX	150	paired-end reads	2019-08-02	2019-08-12	Released
476	190731_A00574_0102_AHF5KCDRXX	50	paired-end reads	2019-07-31	2019-08-05	Released
475	190729_M02973_0189_0000000000-CDN65	250	paired-end reads	2019-07-29	2019-07-31	Released
474	190726_A00574_0101_BHL2LHDXX	50	paired-end reads	2019-07-26	2019-07-30	Released
473	190726_M02973_0188_0000000000-CHM3	150	paired-end reads	2019-07-26	2019-07-29	Released
472	190726_A00574_0100_AHKL3DSXX	150	paired-end reads	2019-07-26	2019-08-06	Released

Laboratory Information Management System

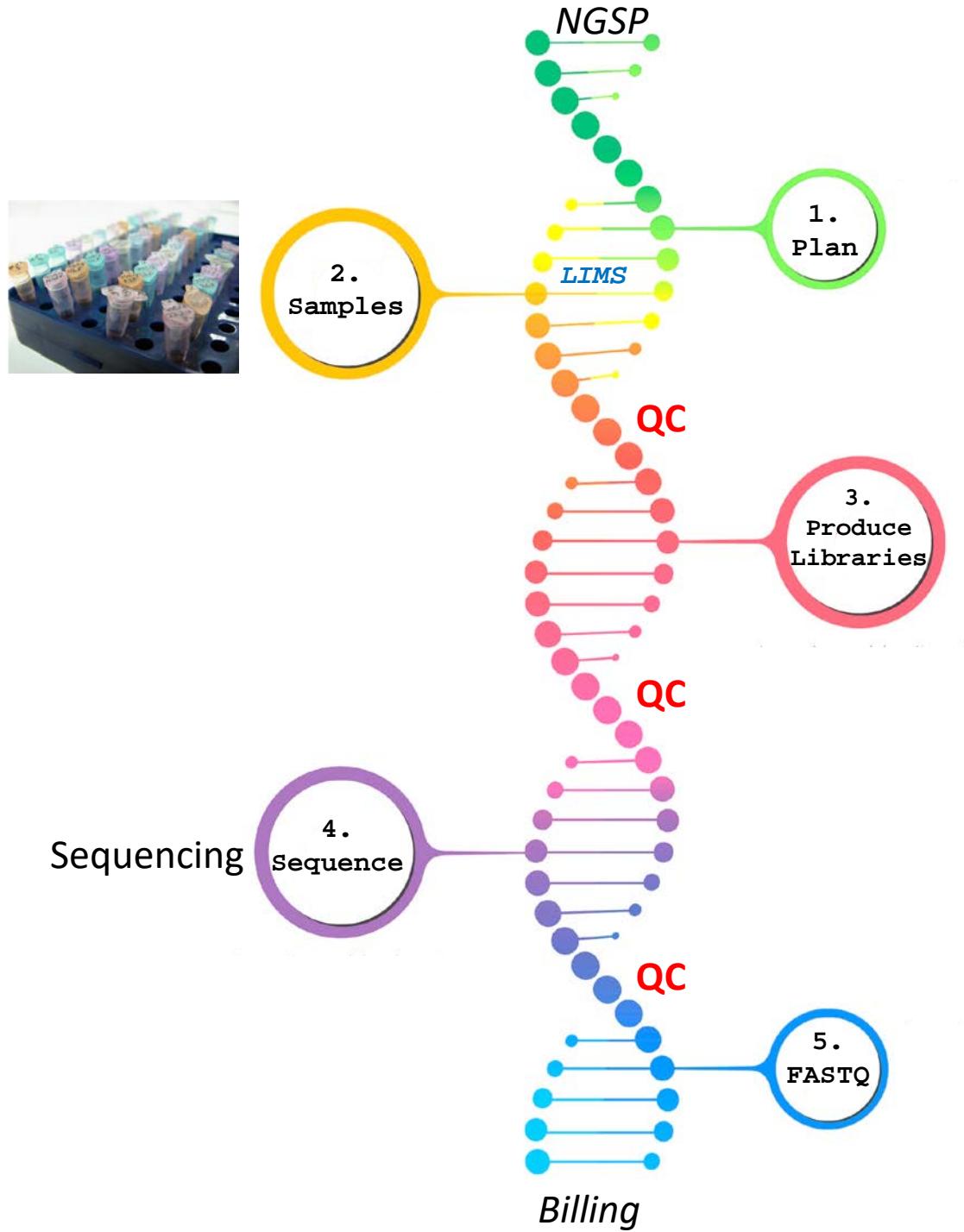
-Enables workflow automation which can in turn reduce human error

-Centralise access and storage of data

-It is the thread that runs through the whole process of a project from sample to libraries to sequencing to downloading fastq files.

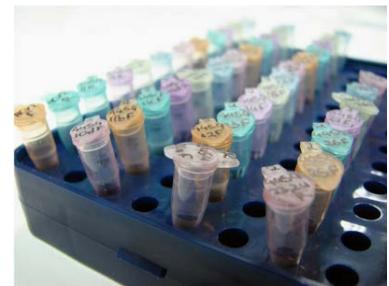
Start-to-end in the same place with access from anywhere.

<http://vetsrv03.campus.unibe.ch/>





2. Samples



- The quality of a result depends on the starting material - this is particularly true with NGS
 - A key challenge in sample prep for NGS is quality and quantity - quality is the more important factor
 - All downstream applications have clear sample requirements (concentration, minimum volumes, DNA quality, RNA quality, RNase/DNase treated and so on...)

Quantity - Qubit Fluorometer

Quality - Advanced Analytics Fragment Analyzer CE12, Advanced Analytics FEMTO Pulse CE & DeNovix FX-11 Spectrophotometer (260/230nm & 260/280nm ratios)

- Not just about what is in the tube but also what they are named and how they are labelled....



Sample Requirements - *illumina*

Library Type	Notes	Recom. Quantity	Concentration	Volume	Lowest Quant.	Preferred Buffer	260/280 ratio	260/230 ratio
DNA Library Preps	all samples have to be RNA-free							
Genomic DNA library		>= 1 ug	>= 20ng/ul	>= 50 ul	>= 100 ng	EB	1.8-2	>2

DNA samples need to be *RNA-free* and dissolved in **EB** buffer or **TLE** buffer. Molecular biology grade water is also OK for Illumina samples.

	Notes	Total RNA Quantity	Concentration	Volume	Lowest Quant.	Preferred Buffer	260/280 ratio	260/230 ratio
RNA-seq Libraries Preps	all samples have to be DNA-free							
Strand-specific RNA-seq library (poly-A)	RIN score >8.0 (>7.0 plants)	>500 ng	>= 25 ng/ul	>= 20 ul	>100ng	H2O Mol. Biol. grade	1.8-2.1	>1.5

RNA samples need to be *DNA-free* and should be dissolved in **molecular biology grade water** (RNase-free; not DEPC treated).

Common buffers for DNA and RNA

EB-Buffer: 10mM TRIS (pH= 8.0-8.4) – e.g.
Qiagen EB Buffer
EBT-Buffer: 10mM TRIS, 0,1%Tween20
(pH=8.0-8.4)
TE-Buffer: 10mM TRIS, 1 mM EDTA
(pH=8.0-8.4)
TLE-Buffer: 10mM TRIS, 0.1 mM EDTA
(pH=8.0-8.4)

I. Sample requirements for PacBio sequencing

The Pacific Biosciences® library preparation process does not utilize amplification techniques and resulting library molecules are directly used as templates for the sequencing process. As such, the quality of the DNA starting material will be directly reflected in the sequencing results. Any irreversible DNA damage present in the input material (e.g., interstrand crosslinks, etc.) will result in impaired performance in the system. High-quality, high-molecular-weight genomic DNA is imperative for obtaining long read lengths and optimal sequencing performance.

Sample Requirements - *PacBio*

PacBio library prep requires microgram DNA sample amounts. The recommended amount of input DNA further correlates with the desired read length.

Since the PacBio technology interrogates single molecules, any defect (e.g. a nick, an abasic site, a DNA adduct) can interfere with the sequencing process. Thus, the integrity and purity of the DNA sample is of utmost importance. The DNA quality and the DNA amount will determine which library insert sizes are feasible and how many SMRT-cells can be sequenced. The DNA samples should fulfill these criteria:

- Minimal DNA purity: OD_{260/280} should be 1.8-2.0; OD_{260/230} should be >2.0
- Has undergone a minimum of freeze-thaw cycles.
- Has not been exposed to high temps (> 65°C for more than one hour can cause a detectable decrease in sequence quality).
- Has not been exposed to pH extremes (< 6 or > 9).
- Does not contain insoluble material.
- Is RNA-free.
- Has not been exposed to intercalating fluorescent dyes or ultraviolet radiation.
- Does not contain, divalent metal cations (e.g., Mg²⁺), denaturants (e.g., guanidinium salts, phenol), or detergents (e.g., heme, humic acid, polyphenols). Amplicon samples should be submitted in EB buffer (EDTA-free).
- Must be double-stranded. Single-stranded DNA cannot be converted into SMRTcell templates but can interfere with polymerase binding.

The sample requirements **will vary strongly depending on genome size**.

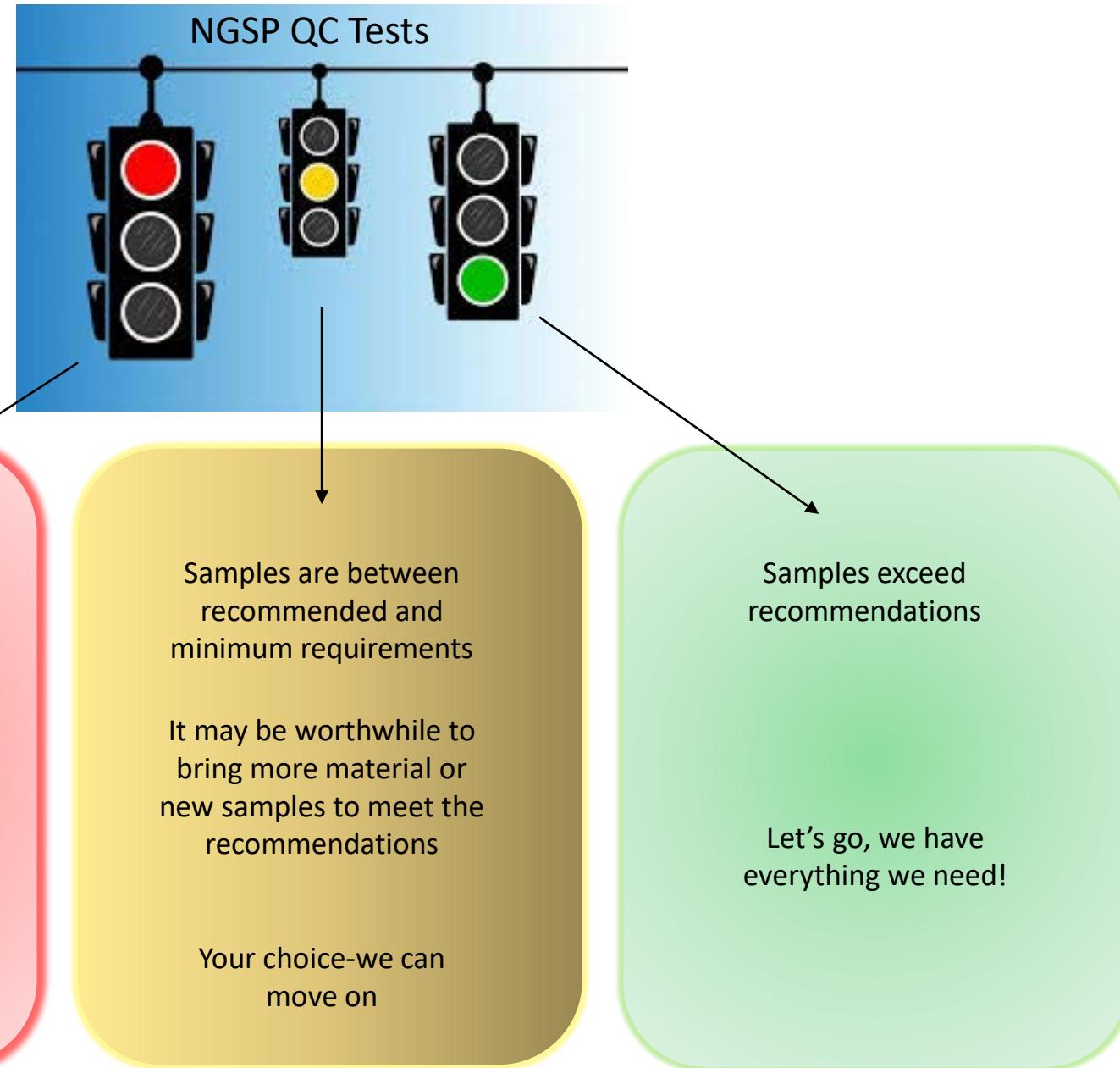
The table shows minimum DNA sample requirements:

PacBio Libraries	DNA Quant.	Concentration	Preferred Buffer	260/280	260/230
				ratio	ratio
inserts up to 3kb	2 ug	> 100 ng/ul	200 ul	TE	1.8-20 >2
inserts 3-10 kb	5 ug	> 100 ng/ul	200 ul	TE	1.8-20 >2
inserts 10-20 kb	20 ug	> 100 ng/ul	200 ul	TE	1.8-20 >2
inserts >20 kb	40 ug	> 100 ng/ul	200 ul	TE	1.8-20 >2
Amplicons	1 ug	> 20 ng/ul	50 ul	EB	1.8-20 >2

PacBio Iso-Seq:

We recommend submitting 1 ug (or more) per total RNA sample (DNA-free) at a concentration of 50 ng/ul or higher in molecular biology grade water. The nanodrop 260/230nm ratio should be >1.5 and the 260/280nm ratio 1.8-2.1. RIN-score 8 or higher. For plants 7 or higher.

Sample QC



Sample Requirements - *10x Genomics*

10X Chromium Single Cell suspension sample requirements:

-Minimum concentration of 100 cells/ μ L (400 to 700 cells/ μ L optimal range) in a volume of at least 30 μ L.

-If at all possible, please provide 70 μ L of single cell suspension (two attempts at chip loading in case of clog plus additional for cell QC). We will require 10 μ L sample for the cell counter.

-Maximum concentration of 1200 cells/ μ L.

Single cell suspension should be at least 70% live (Trypan Blue stained) and free of visible debris and doublets.

-For fresh samples submitting 10-40K cells total is best.

-For frozen samples, 100,000 cells are the minimum, but 1 million are better.

-To minimise physical damage to cells from shearing forces, it is critical to pipette gently and slowly during cell resuspensions. Try to use wide bore pipette tips.

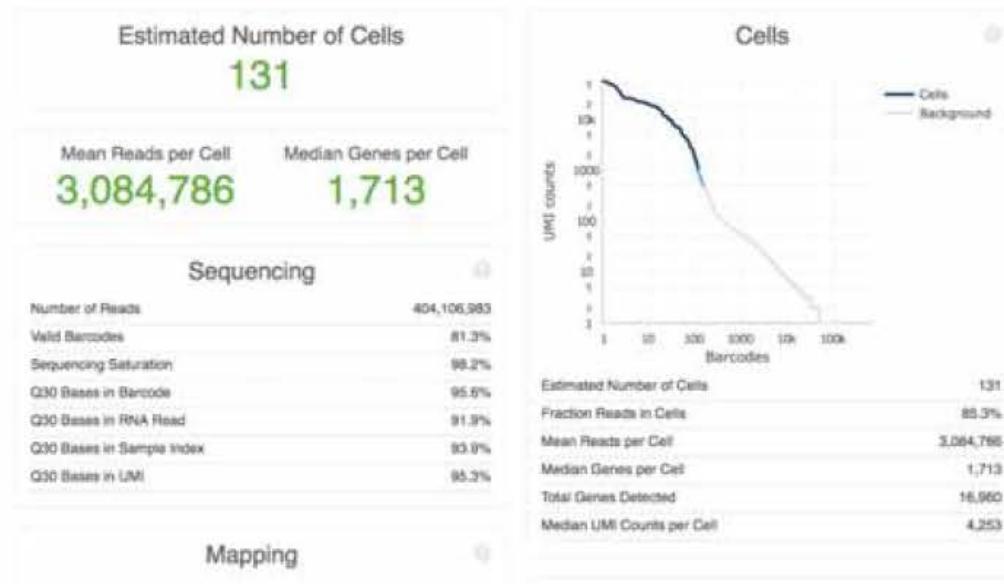
-Recommend cell suspension buffer is PBS/0.5% BSA. Cell suspension buffers should be free of EDTA and Mg++ as well as free of DNase to be compatible with single-cell assay. Up to 2% BSA content is OK.



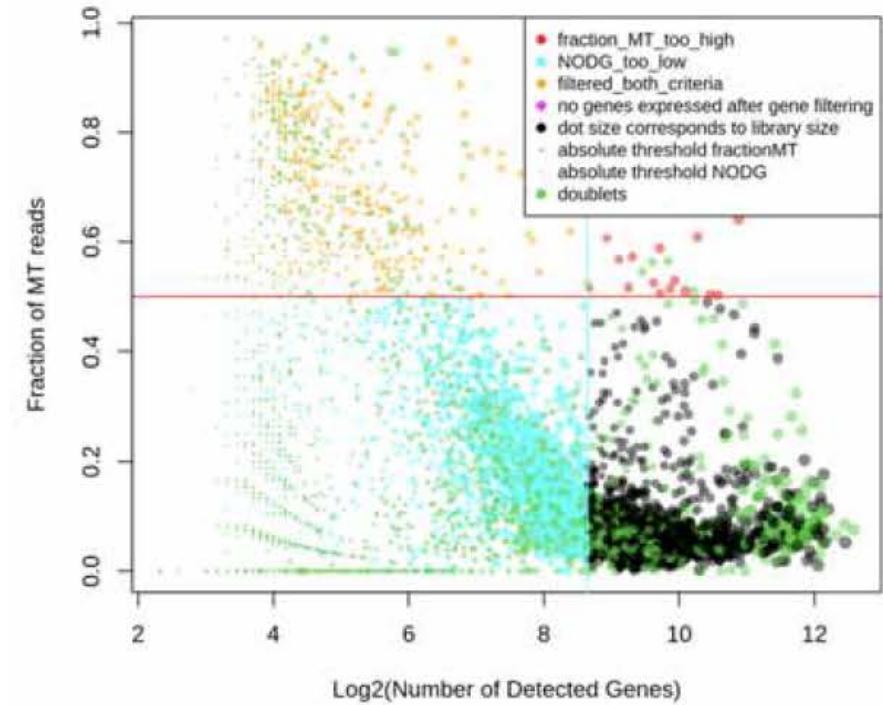
Metric	Control	Wide Bore (Rough)	Narrow Bore (Rough)	Vortex 5s
Number of Cells	1,118	846	1,012	983
Reads per Cell	50,000	50,000	50,000	50,000
Valid Barcodes	95.40%	95.50%	95.30%	95.50%
Fraction Reads in Cells	79.40%	72.80%	54.00%	63.10%
Reads Mapped Confidently to Transcriptome	70.50%	71.40%	71.80%	71.00%
Valid UMIs	99.40%	99.40%	99.40%	99.40%
Median Genes per Cell	3,137	3,180	2,833	2,934

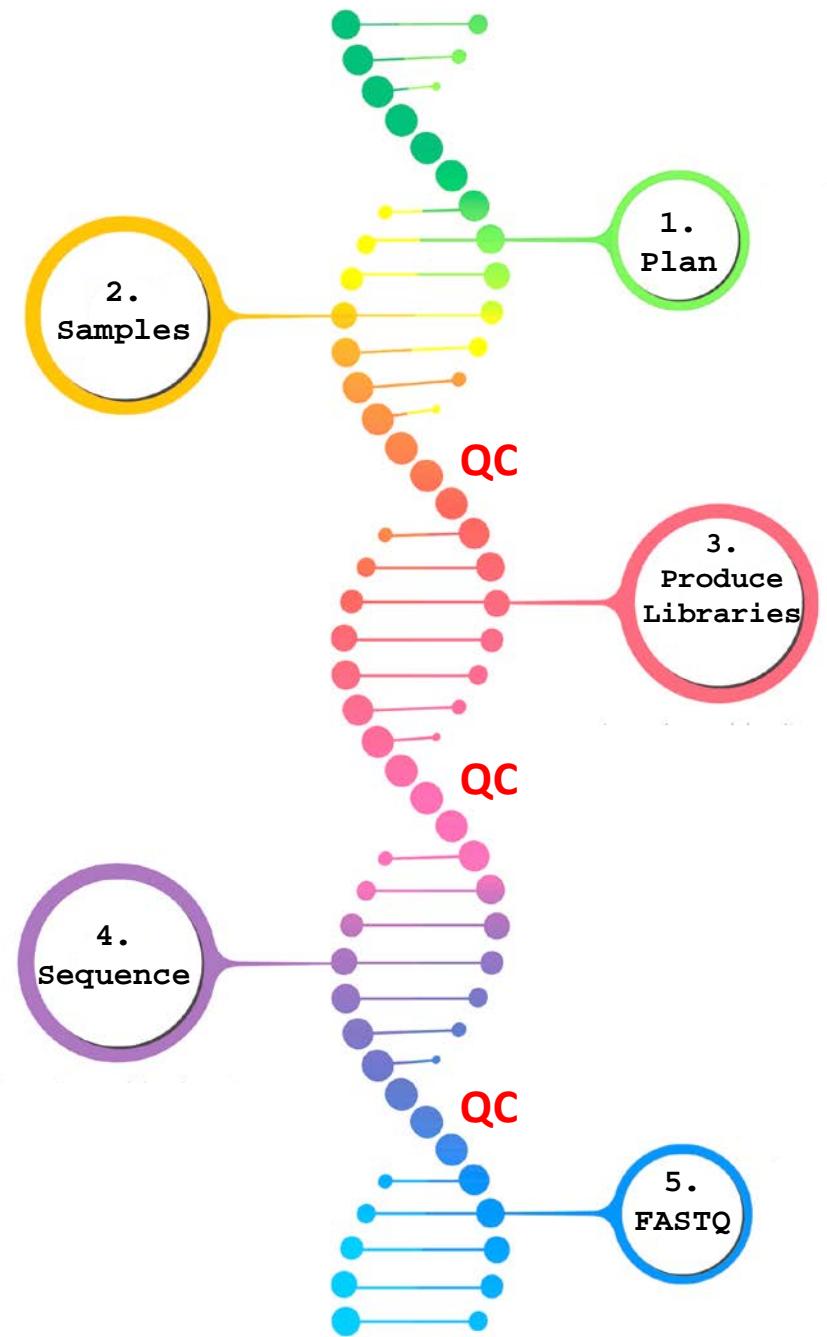
Sample Requirements - *10x Genomics*

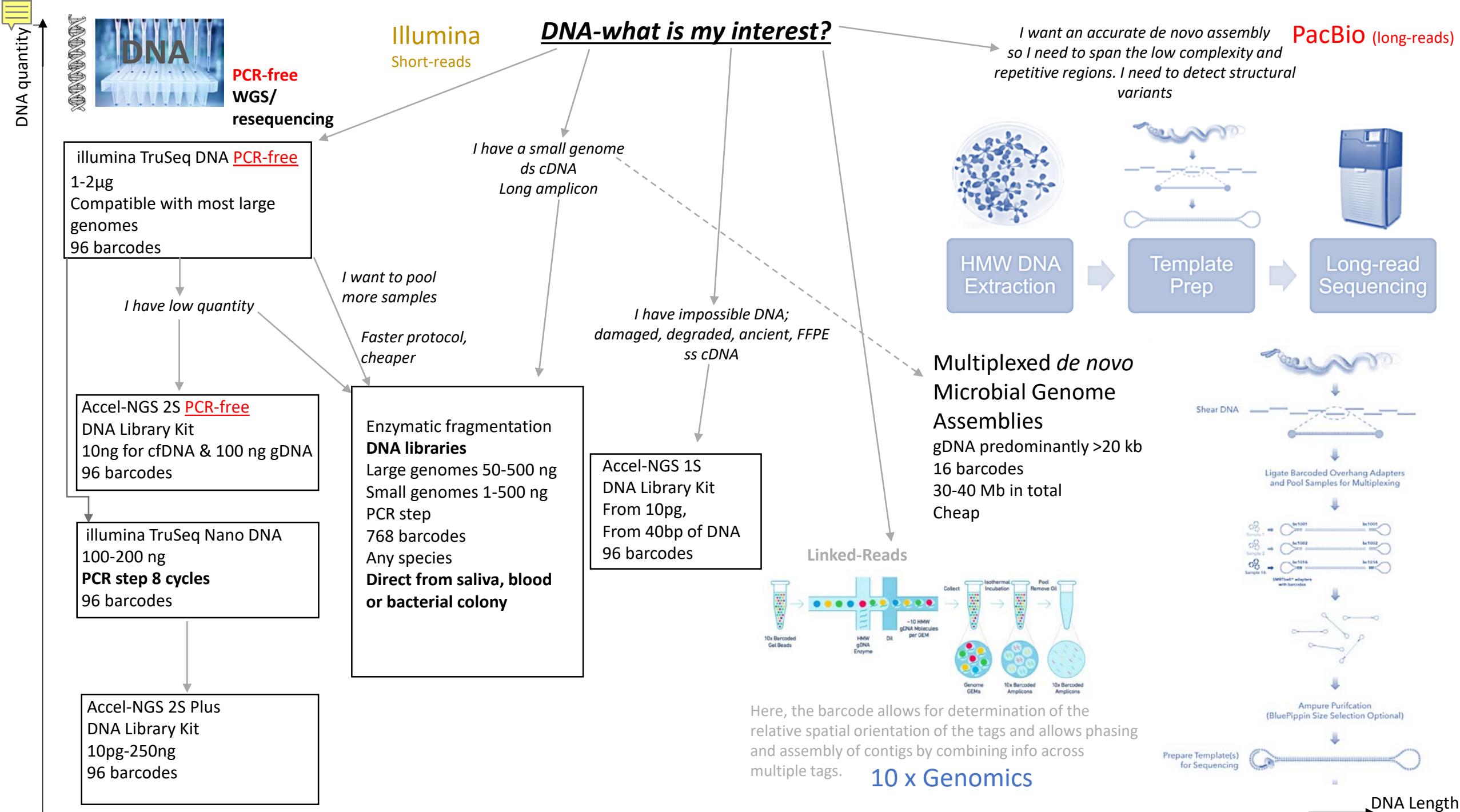
Too few cells..... + overamplified

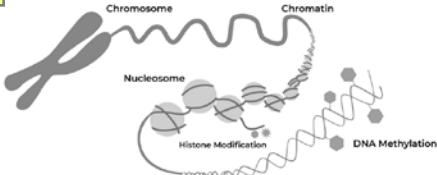


Too many cells..... + broken









Epigenomics

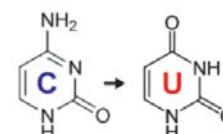
DNA Methylation

Direct detection of DNA methylation during **PacBio SMRT sequencing**-
(Bacterial samples)

-WGBS

Swift Biosciences Accel-NGS **Methyl-Seq** DNA

-we perform *bisulfite converted ssDNA* step

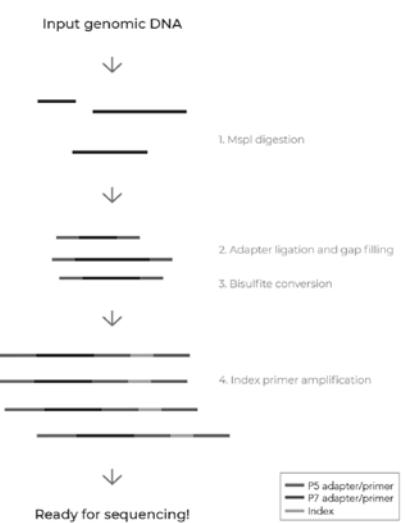


Sodium Bisulfite conversion of C to T
mC & hmC are not converted and are
read as C by whole genome NGS

-RRBS-Seq

Reduced Representation Bisulfite Sequencing
Zymo-Seq RRBS Library Kit

Generate libraries that cover a significant
percent ($\geq 70\%$ if using human genomic DNA)
of all CpG islands, promoters, and gene bodies
while largely reduces the sequencing cost,
making it ideal for high-throughput screening
and comparison experiments.



Chromatin Accessibility

Genome-wide chromatin profiling methods

-ChIP-Seq

Accel-NGS® 2S Plus DNA Library Kit

Reveals binding sites of TF, modified histones and their associated proteins

-ATAC-Seq

Report regions of open chromatin

Bulk

1. Get nuclei
2. Transpose using illumina's Tagment DNA TDE1 Enzyme and Buffer Kit followed by purification
3. Library generation based on illumina Nextera methods

Buenrostro *et al.*, *Nat Methods*.
2013;10(12):1213–1218.

New !

10 x Genomics

-single cell RNA & ATAC-Seq

1. Nuclei are transposed
2. Partitioning on a microfluidic chip into GEMs.
3. The transposed DNA of each individual nuclei is indexed with a unique 10x barcode.
4. Library generation

10 x Genomics

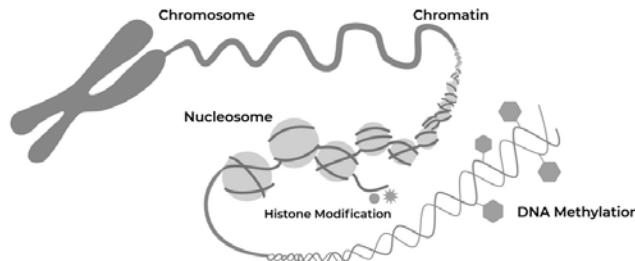
Single-cell

ChIP-Seq

Chromatin immunoprecipitation is frequently used to map the location of specific protein factors or epigenetic chromatin modifications.

- single-factor ChIPSeq is straightforward to process, investigators are increasingly use ChIPSeq in quantitative or comparative assays, which require more customized approaches
- three biological replicates per sample (antibody or experimental condition).
- For vertebrate genomes, 20 million reads should be a minimum target sequencing depth.
- Either paired-end or single-end sequencing strategies may be used; paired-end gives you the exact size of each chromatin fragment, while fragment size must be empirically estimated for single-end.
- When at all possible, ChIP should be checked by quantitative PCR at known loci prior to submission.

Macs2 ChIPseeker



WGBS

Detection and identification of methylated residues requires sequencing of bisulfite-treated DNA (or RNA), which converts unmethylated cytosine residues to uracil (and interpreted as a thymine in sequencing results), while methylated cytosine residues are unaffected.

- Sequence reads are therefore aligned to essentially two genomes in silico, converted and unconverted versions.
- Methylation occurs primarily at CpG contexts, but also less frequently at non-CpG contexts (mCHG or mCHH).
- Results are typically reported as percent methylation (number of unconverted C over total C) at any given position, but this should be thresholded to a minimum number of reads (usually 8 to 10 reads) before a call can be determined.
- Whole-genome bisulfite sequencing therefore requires at least 10X coverage (preferably much higher—we aim for 30-40X) at a significant cost.
- Bisulfite treatment is very damaging to DNA, so a lower rate of alignment is normally expected compared to traditional DNA sequencing. Furthermore, a higher percentage of Illumina PhiX control DNA is usually included to maintain reasonably expected levels of base composition, resulting in lower target genome yields.

Novocraft nonalign
Bismark
Biseq



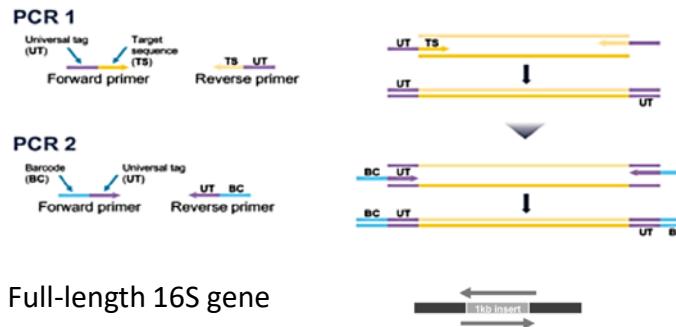
Metagenomics

16S Amplicon sequencing



PacBio Long-read

Barcoded Universal Primer



10 kb SMRTbell Libraries for Metagenomic Shotgun Sequencing

Requires 1.5 µg of input high-quality gDNA (majority >15kb) which is then sheared to 10 kb.

No PCR step but not high throughput

Sequel II

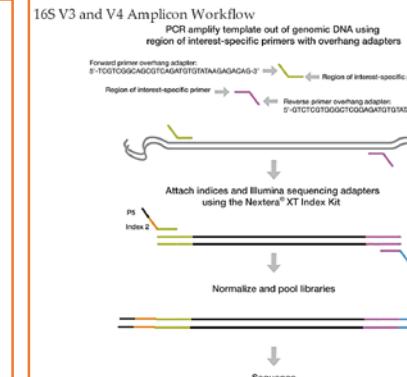
From input DNA to FASTQ files Service

Classical 16S-Seg



- | | |
|-----------|---|
| 16S V1-V2 | <ul style="list-style-type: none"> Better species-level resolution for many human-associated microbes when compared to the V3-V4 region. Excellent coverage for common genera such as <i>Methanobrevibacter</i>, <i>Bifidobacterium</i>, <i>Propionibacterium</i>, <i>Rickettsia</i>, <i>Chlamydia</i>, and <i>Treponema</i>. |
| 16S V3-V4 | <ul style="list-style-type: none"> Broader phylogenetic coverage than the V1-V2 region. Broad coverage for Archaea. Improved coverage for <i>Chloroflexi</i> and phyla of <i>Candidate Phylum Radiation</i> (CPR). |

Illumina Short-reads



Bring us 1st step PCR amplicons (16S &/or ITS) and we will perform clean-up, QC, indexing step, QC, pooling and MiSeq sequencing

From input DNA to FASTQ files Service

Full-length 16S



Swift Biosciences Panel -Multiplexed assay covers all variable regions of the 16S rRNA (or ITS1) genes in a single primer pool

Whole Shotgun Metagenomic Sequencing

illumina Nextera DNA Flex for scalable metagenomics studies

Input 1–500 ng –so works for low input material

PCR step

384 barcodes

Any species

Direct from saliva, blood or bacterial colony

Sequence using with run configurations of 2×150 or 2×250 bp for more coverage.

Full-length 16S sequencing short-read & long read

Full-length 16S PacBio

> Nucleic Acids Res. 2019 Oct 10;47(18):e103. doi: 10.1093/nar/gkz569.

High-throughput amplicon sequencing of the full-length 16S rRNA gene with single-nucleotide resolution

Benjamin J Callahan ^{1 2}, Joan Wong ³, Cheryl Heiner ³, Steve Oh ³, Casey M Theriot ¹, Ajay S Gulati ^{4 5 6}, Sarah K McGill ⁷, Michael K Dougherty ⁷

Affiliations + expand

PMID: 31269198 PMCID: PMC6765137 DOI: 10.1093/nar/gkz569

Free PMC article



High-throughput amplicon sequencing methodology based on PacBio circular consensus sequencing and the DADA2 sample inference method that measures the full-length 16S rRNA gene with single-nucleotide resolution and a near-zero error rate.

<https://pubmed.ncbi.nlm.nih.gov/31269198/>

16S rRNA - Microbial community analysis using **QIIME2**

Metagenome - Functional analysis using **HUMAnN2**

Metatranscriptome - Functional analysis of paired metagenome and metatranscriptome data using HUMAnN2

Full-length 16S illumina

Swift Resources for Data Analysis of 16S Panels (Accel and SNAP)

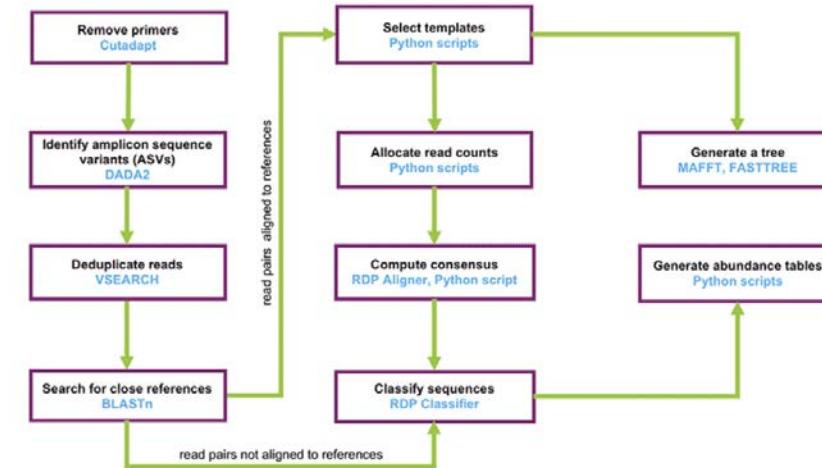
Swift 16S SNAP APP [Open Source Pipeline](#) is Swift's latest solution for studying microbial communities using Swift SNAP 16S v2 panel (see [tech note](#)). This pipeline starts with demultiplexed FASTQ files by a simple command and delivers the community composition table and reference phylogenetic tree. Not only does this pipeline encapsulate the standard but complex tasks, it is equipped for properly handling multi-V region amplicons to capture their collective advantages over the single amplicon 16S approach. Swift 16S SNAPP generates consensus sequences from multi-V region amplicons, and provides higher resolving power in quantifying microbial compositions

Swift 16S SNAP APP uses DADA2 for identifying amplicon sequence variants (ASCs) and RDP Classifier to taxonomic assignments.

For ITS1 data analysis support, please contact us at techsupport@swiftbio.com

<https://swiftbiosci.com/swift-normalase-amplicon-16s-its1-panels-snap/>

SNAP-APP for 16S multi-amplicon analysis



Swift 16S Swift 16S SNAP APP can be cloned from <https://github.com/swiftbiosciences/16S-SNAPP>

RNA – what is my interest?

RNA quantity ↑

I have bacteria
I want a whole transcriptome analysis

Total RNA

Illumina TruSeq stranded total RNA prep with RiboZero Plus
100ng
Ideally RIN ≥ 5
rRNA depletion using RiboZero
Samples: Human/rat/mouse
Blood (globin mRNA depletion)
Plants
Bacteria
Yeast
192 barcodes

Total RNA

For RNA from all species
100ng
Ideally RIN ≥ 5
High abundant transcripts depleted at cDNA level
96 barcodes

mRNA

Illumina TruSeq stranded mRNA kit
200 ng-1µg
RIN ≥ 8
Needs polyA tail ☺
96 barcodes

I can't get highly intact RNA
I have many samples, DGE project and don't need full transcript info
I am on a tight budget

Small RNA

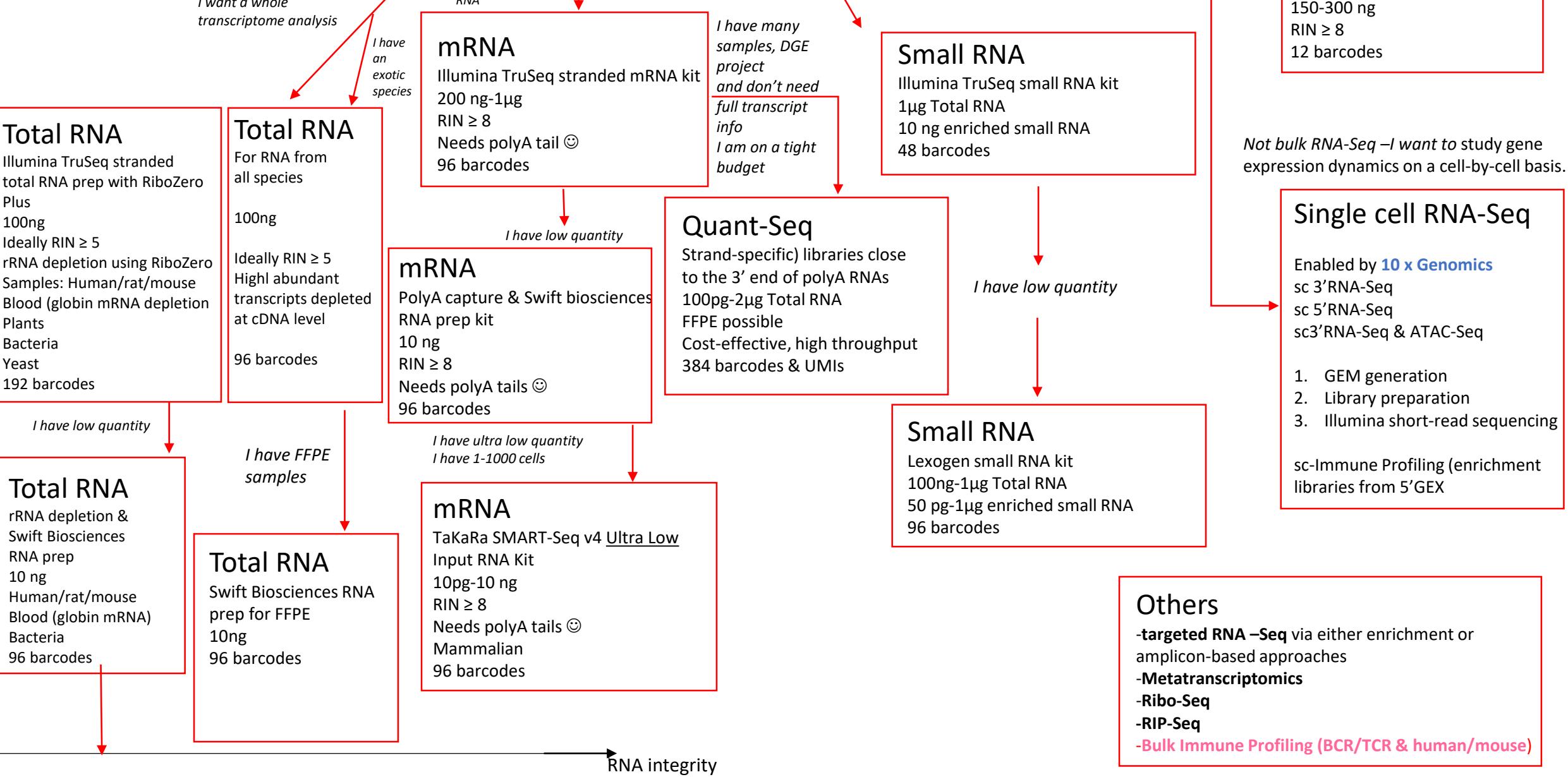
Illumina TruSeq small RNA kit
1µg Total RNA
10 ng enriched small RNA
48 barcodes

Total RNA

rRNA depletion & Swift Biosciences
RNA prep
10 ng
Human/rat/mouse
Blood (globin mRNA)
Bacteria
96 barcodes

Total RNA

Swift Biosciences RNA prep for FFPE
10ng
96 barcodes



I would like full-length sequencing of isoforms and examination of splicing

PacBio Iso-Seq

Full-length transcripts up to 10kb
150-300 ng
RIN ≥ 8
12 barcodes

Not bulk RNA-Seq – I want to study gene expression dynamics on a cell-by-cell basis.

Single cell RNA-Seq

Enabled by **10 x Genomics**
sc 3'RNA-Seq
sc 5'RNA-Seq
sc3'RNA-Seq & ATAC-Seq

1. GEM generation
2. Library preparation
3. Illumina short-read sequencing

sc-Immune Profiling (enrichment libraries from 5'GEX)

Others

- targeted RNA -Seq via either enrichment or amplicon-based approaches
- Metatranscriptomics
- Ribo-Seq
- RIP-Seq
- Bulk Immune Profiling (BCR/TCR & human/mouse)

Developmental Projects- ***NGSP***

2019-2020 –Testing WGBS protocol

2019-2020 –Testing/comparing different ChIP-Seq protocols

Zhou, L., Ng, H.K., Drautz-Moses, D.I. et al. Systematic evaluation of library preparation methods and sequencing platforms for high-throughput whole genome bisulfite sequencing. *Sci Rep* **9**, 10383 (2019).
<https://doi.org/10.1038/s41598-019-46875-5>

Sundaram AY, Hughes T, Biondi S, et al. A comparative study of ChIP-seq sequencing library preparation methods. *BMC Genomics*. 2016;17(1):816. Published 2016 Oct 21. doi:10.1186/s12864-016-3135-y

2019-2020 –Testing/comparing different small RNA-Seq protocols

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2020-2021 – 10 x Genomics -Linked short sequencing reads from cDNA molecules

2020-2021 – 10 x genomics “Scifi protocol”

RNA-Seq of human whole blood: Evaluation of globin RNA depletion on Ribo-Zero library method

Christina A. Harrington¹, Suzanne S. Fei, Jessica Minnier, Lucia Carbone, Robert Searles, Brett A. Davis, Kimberly Ogle, Stephen R. Planck, James T. Rosenbaum & Dongseok Choi

Scientific Reports | 10, Article number: 6271 (2020) | Cite this article

3110 Accesses | 1 Citations | 10 Altmetric | Metrics

Abstract

Peripheral blood is a highly accessible biofluid providing a rich source of information about human physiology and health status. However, for studies of the blood transcriptome with RNA sequencing (RNA-Seq) techniques, high levels of hemoglobin mRNAs (hgbRNA) present

Research | Open Access | Published: 21 November 2019

Whole blood vs PBMC: compartmental differences in gene expression profiling exemplified in asthma

Daniel He, Chen Xi Yang, Basak Sahin, Amrit Singh, Casey P. Shannon, John-Paul Oliveria, Gail M. Gauvreau & Scott J. Tebbutt¹

Allergy, Asthma & Clinical Immunology | 15, Article number: 67 (2019) | Cite this article

5935 Accesses | 1 Citations | 6 Altmetric | Metrics

Abstract

Background

Blood has proven to be a useful resource for molecular analysis in numerous biomedical studies, with peripheral blood mononuclear cells (PBMCs) and whole blood being the major specimen types. However, comparative analyses between these two major compartments (PBMCs and whole blood) are few and far between. In this study, we compared gene

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in Genetics | Livestock Genomics

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Articles

THIS ARTICLE IS PART OF THE RESEARCH TOPIC
Animal Genetics and Diseases: Advances in Farming and Livestock Genomics

ORIGINAL RESEARCH ARTICLE

Front. Genet. 14 August 2018 | https://doi.org/10.3389/fgene.2018.00278

RNA Sequencing (RNA-Seq) Reveals Extremely Low Levels of Reticulocyte-Derived Globin Gene Transcripts in Peripheral Blood From Horses (*Equus caballus*) and Cattle (*Bos taurus*)

Carolina N. Correia¹, Kirsten E. McLoughlin¹, Nicolas C. Nalpas¹, David A. Magee¹, John A. Browne¹, Kevin Rue-Albrecht¹, Stephen V. Gordon^{1,2} and David E. MacHugh^{1,3*}

¹ Animal Genomics Laboratory, UCD School of Agriculture and Food Science, UCD College of Health and Agricultural Sciences, University

Journal List | PLoS One | v 14(12); 2019 | PMC6887427

PLoS ONE

PMC6887427
Published online 2019 Dec 6. doi: 10.1371/journal.pone.0225137

Investigating gene expression profiles of whole blood and peripheral blood mononuclear cells using multiple collection and processing methods

Aarti Gautam, Conceptualization, Investigation, Supervision, Writing – original draft,¹ Duncan Donohue, Formal analysis, Visualization,^{1,2} Alison Hale, Data curation, Methodology, Writing – review & editing,^{1,3} Stacy Ann Miller, Data curation, Methodology,^{1,3} Suganthini Srikanthan, Data curation,^{1,2} Bintu Sooini, Data curation,^{1,3} Leanne Detter, Methodology,^{1,2} Jesse Lynch, Methodology,^{1,3} Michael Levante, Methodology,^{1,2} Radha Hammami, Project administration,¹ and Mark Jon, Funding acquisition, Supervision,¹

Jeffrey Charnier, Editor

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PLOS ONE

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OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

Variation in RNA-Seq Transcriptome Profiles of Peripheral Whole Blood from Healthy Individuals with and without Globin Depletion

Hannah Shin¹, Casey P. Shannon¹, Nick Fakhraie, Jan Russ, Mi Zhou, Robert Belknap, Janet E. Wilson-McGinnis, Raymond T. Ng, Bruce M. McManus, Scott J. Tebbutt¹, for the PROOF Centre of Excellence Team

Published: March 7, 2014 • https://doi.org/10.1371/journal.pone.0091041

Article

Authors

Metrics

Comments

Media Coverage

Abstract

Background

The molecular profile of circulating blood can reflect physiological and pathological events occurring in other tissues and regions of the body and delivers a comprehensive view of the state of the immune system. Blood has been useful in studying the pathobiology of many diseases, including asthma, and has been used in the development of a variety of diagnostic biomarker tests. The blood transcriptome has a high content of globin RNA that could potentially saturate next-generation sequencing platforms, masking lower abundance transcripts. Methods to deplete globin mRNA are available, but their effect has not been comprehensively studied in peripheral whole blood RNA-seq data. In this study we aimed to

PLOS ONE

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RESEARCH ARTICLE

Gene expression profiling of whole blood: A comparative assessment of RNA-stabilizing collection methods

Duncan E. Donohue¹, Aarti Gautam¹, Stacy Ann Miller, Seethanarai Srikanthan, Dura AbuAmera, Ross Campbell, Charles R. Murrat, Radha Hammami, Mark Jon¹

Published: October 10, 2019 • https://doi.org/10.1371/journal.pone.0223065

Article

Authors

Metrics

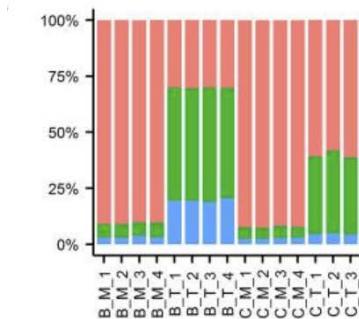
Comments

Media Coverage

Abstract

Introduction

Peripheral Blood gene expression is widely used in the discovery of biomarkers and development of therapeutics. Recently, a range of commercial blood collection and preservation systems have been introduced with proprietary variations that may differentially impact the transcriptome profiles. Comparative analysis of these collection platforms will help optimize protocols to detect, identify, and reproducibly validate true biological variance among subjects.



Sample	Replicate ^a	Source	Protocol ^b	Total_reads	Unique_Mapped	Exonic_Reads ^c	Exonic_Reads (%)
Blood_PolyA	B_M_1	blood	polyA+	50,000,000	43,215,282	38,254,168	76.51
	B_M_2	blood	polyA+	50,000,000	42,476,355	37,646,793	75.29
	B_M_3	blood	polyA+	50,000,000	42,185,029	37,072,203	74.14
	B_M_4	blood	polyA+	50,000,000	42,954,993	37,813,280	75.63
Blood_RiboZ	B_T_1	blood	RiboZ	50,000,000	41,513,424	11,665,272	23.33
	B_T_2	blood	RiboZ	50,000,000	41,614,111	11,714,372	23.43
	B_T_3	blood	RiboZ	50,000,000	41,637,819	11,641,934	23.28
	B_T_4	blood	RiboZ	50,000,000	41,265,342	11,405,741	22.81
Colon_PolyA	C_M_1	colon	polyA+	50,000,000	43,281,953	38,971,070	77.94
	C_M_2	colon	polyA+	50,000,000	44,375,310	40,053,155	80.11
	C_M_3	colon	polyA+	50,000,000	42,908,688	38,368,949	76.74
	C_M_4	colon	polyA+	50,000,000	44,432,717	39,891,693	79.78
Colon_RiboZ	C_T_1	colon	RiboZ	50,000,000	42,294,547	23,989,467	47.98
	C_T_2	colon	RiboZ	50,000,000	41,719,947	22,908,423	45.82
	C_T_3	colon	RiboZ	50,000,000	42,491,691	24,288,251	48.58
	C_T_4	colon	RiboZ	50,000,000	42,028,918	23,779,962	47.56

Sci Rep. 2018; 8: 4781.

Published online 2018 Mar 19. doi: 10.1038/s41598-018-23226-4

PMCID: PMC5859127

PMID: 29556074

SCIENTIFIC REPORTS

natureresearch

Evaluation of two main RNA-seq approaches for gene quantification in clinical RNA sequencing: polyA+ selection versus rRNA depletion

Shanrong Zhao,^{1,4} Ying Zhang,^{2,2} Ramya Gamini,¹ Baohong Zhang,¹ and David von Schack²

► Author information ► Article notes ► Copyright and License information Disclaimer

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2019-2020 –Testing WGBS protocol

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2020 –Testing and comparing various 16S-Seq protocols –*results pending*

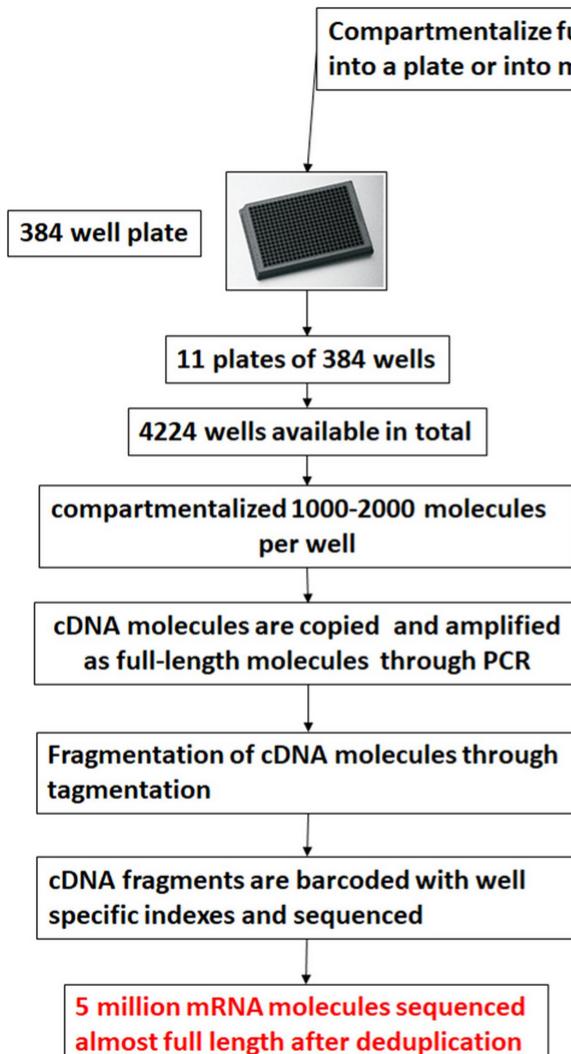
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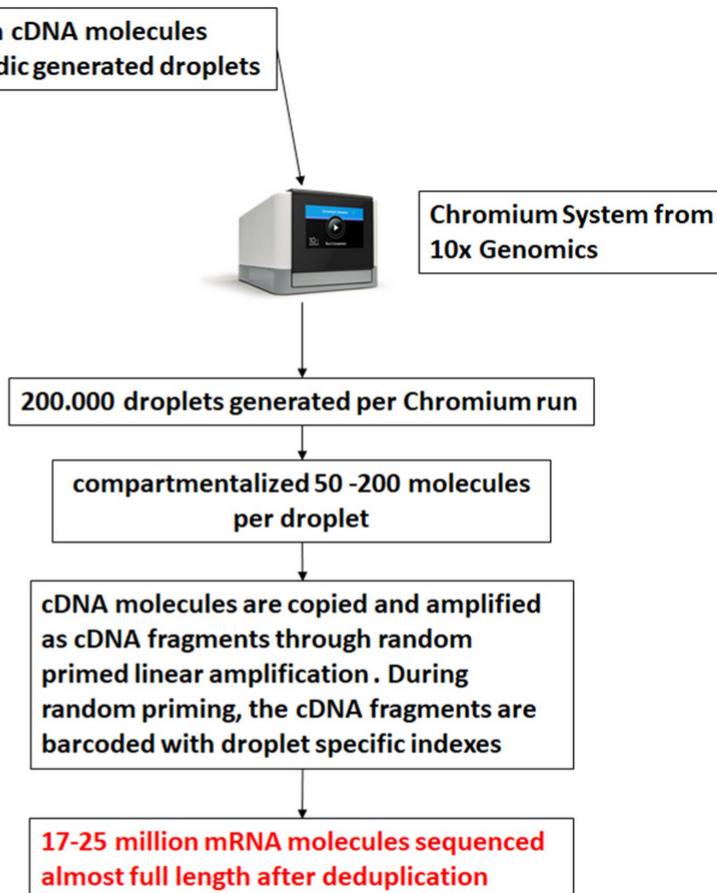
2020-2021 – 10 x Genomics -Linked short sequencing reads from cDNA molecules

2020-2021 – 10 x genomics “Scifi protocol”

SLR-RNA-seq method



spIISO-seq method



linked exons from short-reads

Tilgner, H., Jahanbani, F., Gupta, I., Collier, P., Wei, E., Rasmussen, M., et al. (2018). Microfluidic isoform sequencing shows widespread splicing coordination in the human transcriptome. *Genome Res.* 28, 231–242. doi: 10.1101/gr.230516.117

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2020-2021 – 10 x Genomics -Linked short sequencing reads from cDNA molecules

2020-2021 – 10 x genomics “Scifi protocol”

1 **Ultra-high throughput single-cell RNA sequencing by combinatorial fluidic indexing**

2 Paul Datlinger^{1*}, André F Rendeiro^{1*}, Thorina Boenke¹, Thomas Krausgruber¹, Daniele Barreca¹, Christoph Bock^{1,2}

3 ¹ CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

4 ² Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria

5 * These authors contributed equally to this work

6 Correspondence: Christoph Bock (cbock@cemm.oeaw.ac.at)

7 **Keywords:** Single-cell RNA-seq, transcriptome profiling, combinatorial indexing, droplet microfluidics, next-gen-
8 eration sequencing, high-throughput biology

9

10 **Abstract**

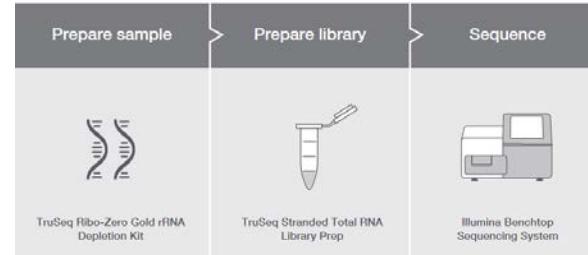
11 Cell atlas projects and single-cell CRISPR screens hit the limits of current technology, as they require cost-effective
12 profiling for millions of individual cells. To satisfy these enormous throughput requirements, we developed “sin-
13 gle-cell combinatorial fluidic indexing” (scifi) and applied it to single-cell RNA sequencing. The resulting scifi-
14 RNA-seq assay combines one-step combinatorial pre-indexing of single-cell transcriptomes with subsequent sin-
15 gle-cell RNA-seq using widely available droplet microfluidics. Pre-indexing allows us to load multiple cells per
16 droplet, which increases the throughput of droplet-based single-cell RNA-seq up to 15-fold, and it provides a
17 straightforward way of multiplexing hundreds of samples in a single scifi-RNA-seq experiment. Compared to
18 multi-round combinatorial indexing, scifi-RNA-seq provides an easier, faster, and more efficient workflow,
19 thereby enabling massive-scale scRNA-seq experiments for a broad range of applications ranging from population
20 genomics to drug screens with scRNA-seq readout. We benchmarked scifi-RNA-seq on various human and mouse
21 cell lines, and we demonstrated its feasibility for human primary material by profiling TCR activation in T cells.

22

Covid-19- *at the NGSP*

- Shotgun sequencing approach using illumina -a shotgun metagenomic workflow for detecting coronavirus

10M reads per sample

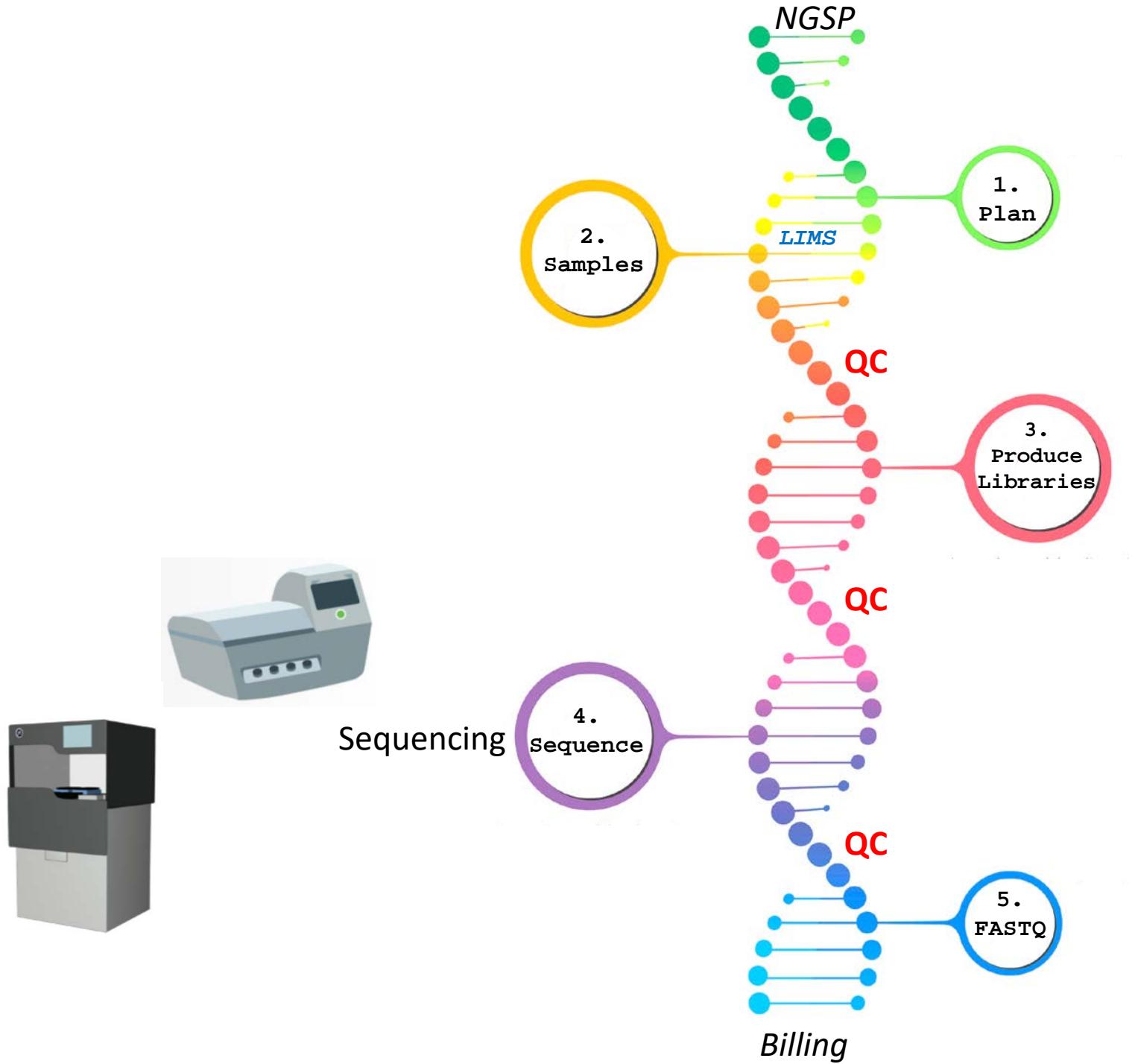


- Library Hybridisation approach –cDNA into a NGS library or RNA made into cDNA library –myBaits Expert Virus SARS-CoV-2 panel

The flexible nature of hybridization capture allows these probes to enrich for even novel variants, including point mutations, small or large indels, or other genomic changes. The design of the probes is compatible with enriching both long and short NGS library molecules (even from degraded targets)

- Swift Amplicon SARS-CoV-2 Panel

Multiplex PCR technology, enabling library construction from 1 st-strand or 2nd-strand cDNA using tiled primer pairs to target the entire 29.9 kb viral genome with a single pool of multiplexed primer pairs. Primers were designed against the NCBI Reference Sequence NC_045512.2 (Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome)

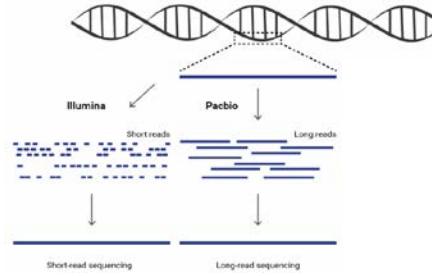


4. Sequencing of Libraries

Things to consider:

- Coverage x
- No. of reads/sample
- Read-length -what makes sense?
- For RNA-Seq, the ENCODE project provides good guidelines
- Barcoding limitations
- Costs

MiSeq



NovaSeq6000

650 M-10 B reads
passing filter

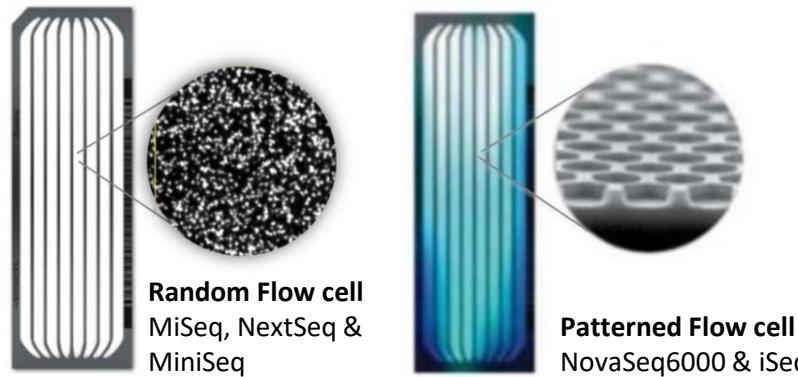
	NovaSeq 6000 System			
Flow Cell Type	SP	S1	S2	S4
Single-end Reads	650–800 M	1.3–1.6 B	3.3 B–4.1 B	8–10 B

	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

1-25M reads passing filter

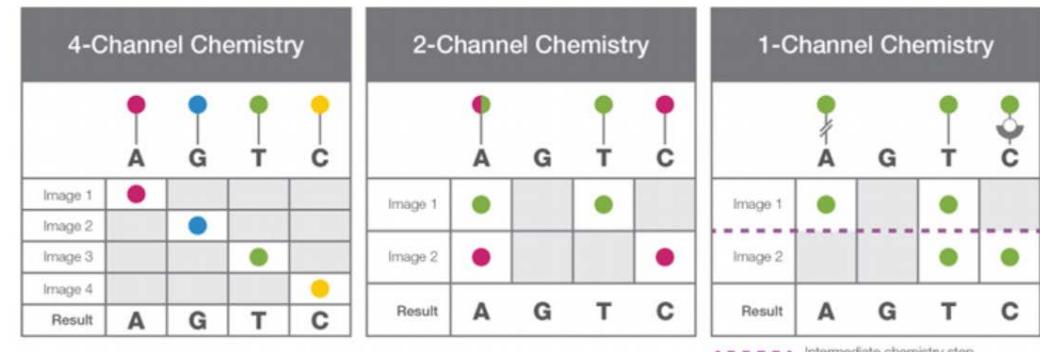


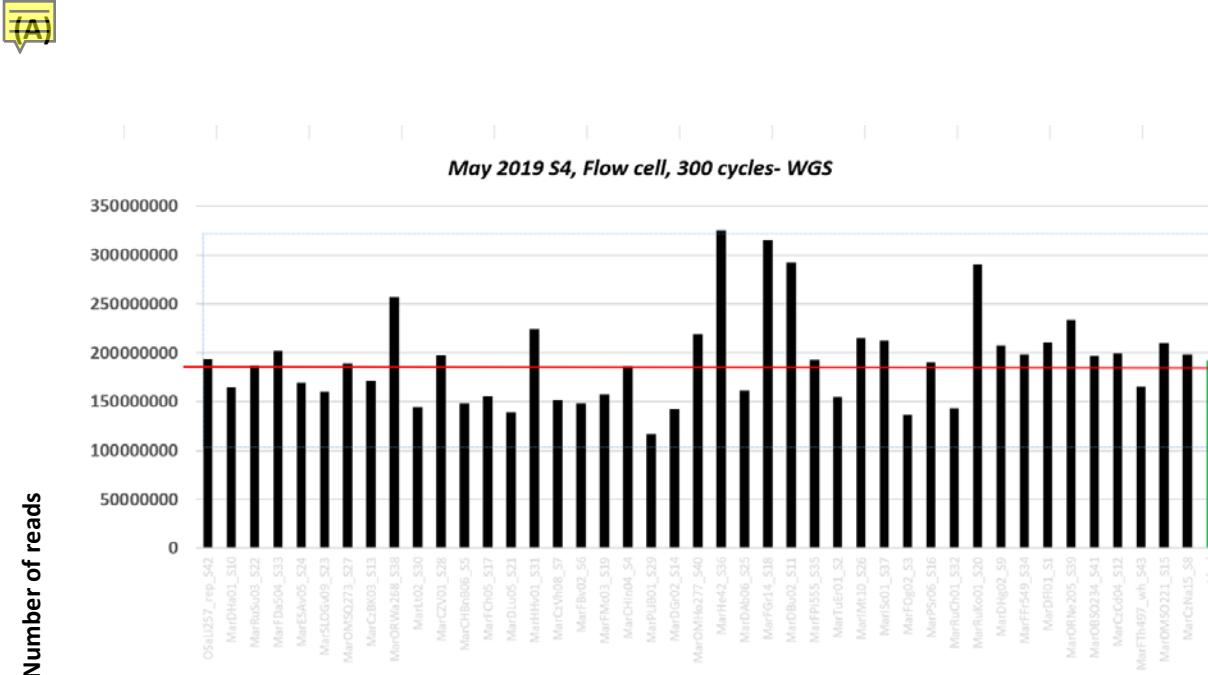
iSeq 100 4M clusters



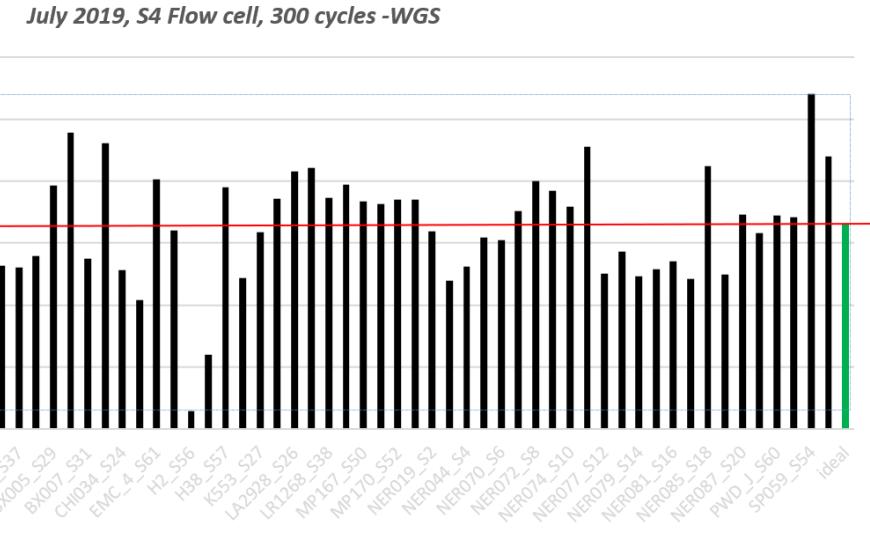
Flowcell	MiSeq				NextSeq		NovaSeq 6000				
	v2 Nano	v2 Micro	v2	v3	Mid-Output	High-Output	SP	S1	S2	S4	
	Clusters	1 M	4 M	15 M	25 M	130 M	400 M	800 M	1600 M	4100 M	10000 M
Read length	1x36		0.54								
	1x75										
	2x25		0.75								
	2x50										
	2x75				3.8						
	2x100										
	2x150	0.3	1.2	4.5							
	2x250	0.5		7.5							
	2x300				15						
Expected output (gigabases)											

Read Length	Total Time	Output
1 x 36 bp	9.5 hrs	144 Mb
1 x 50 bp	10 hrs	200 Mb
1 x 75 bp	11 hrs	300 Mb
2 x 75 bp	14 hrs	600 Mb
2 x 150 bp	19 hrs	1.2 Gb

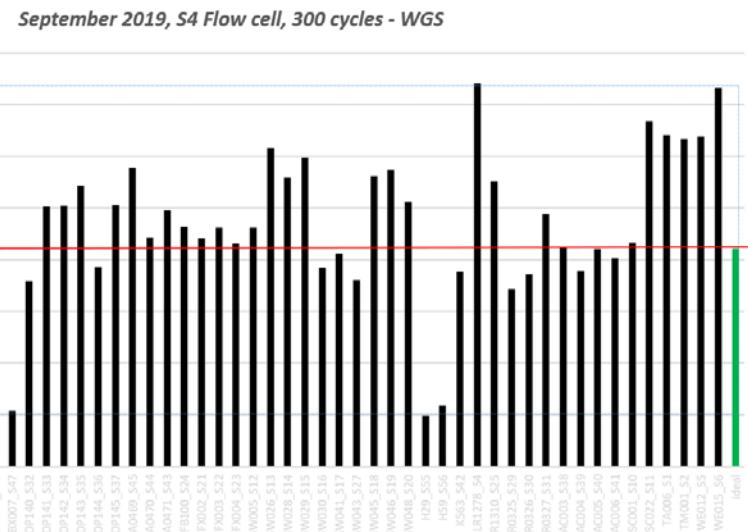




(B)

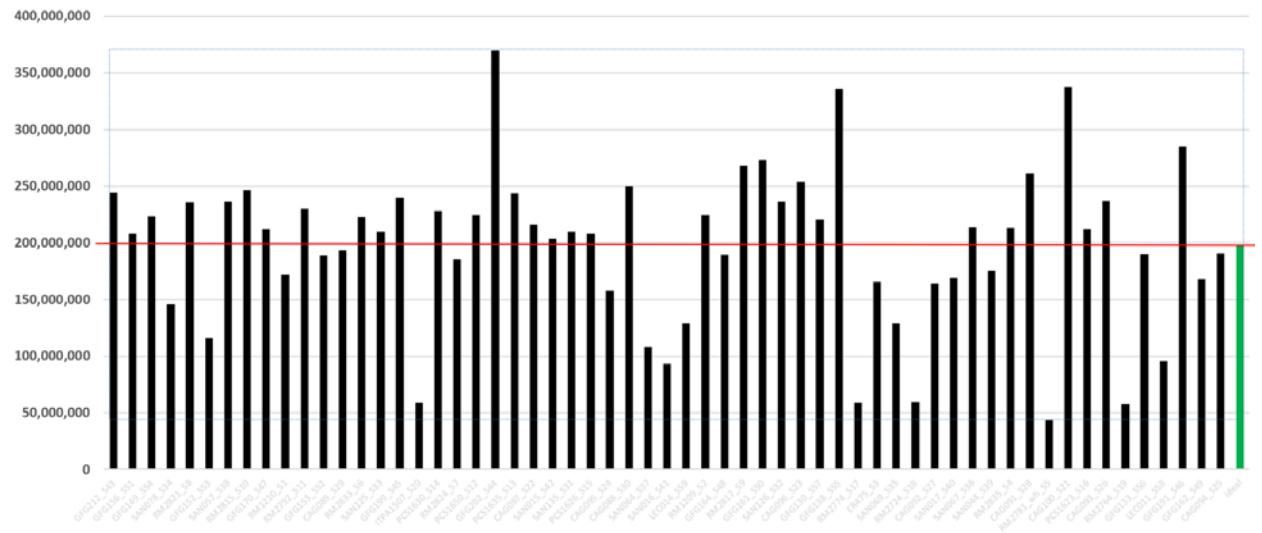


(C)



Sample name

December 2019, S4 Flow cell, 300 cycles -WGS





Pilot Study Workflow



Protocol Details

54 gDNA samples isolated from diverse large genomes species were submitted to the NGSP for WGS from the Science and Vetsuisse faculties. illumina TruSeq DNA PCR-free libraries were prepared using a pipetting robot.

The libraries underwent usual QC2 tests for quantity (Qubit 4.0) and size (Fragment Analyzer). This information allowed all libraries to be normalised to 1nM and equimolar pooled by combining 1 µL of each 1 nM library.

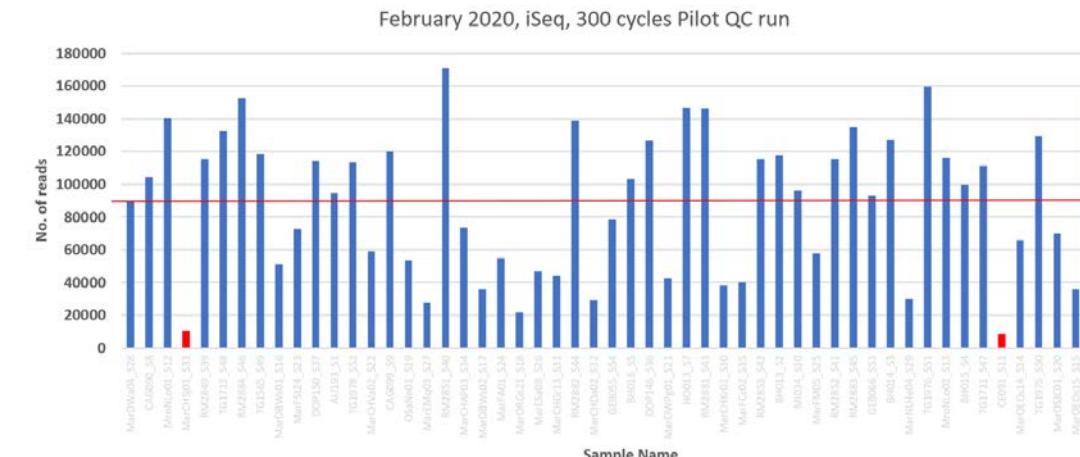
The library pool was diluted to 100 pM and a control library was also added to the pool, according to iSeq sequencing guidelines

The library pool was paired-end sequenced using 300 cycles on the iSeq system*. This run took 19 hours. The raw base call files were demultiplexed and converted to fastq files. More than 1.2 Gb of sequencing output was generated

The demultiplexed data enabled a read statistics table to be generate; the run generated 4.9M reads across 52 libraries. This data allowed us to identify drop-outs (2 libraries) and perform calculations to rebalance the library pool prior to NovaSeq 6000 sequencing.

The rebalanced pool was paired-end sequenced on a NovaSeq 6000 S4 flow cell, 300 cycles. This run took 44 h and gave a sequencing output of more than 3000 Gb. The raw base call files were demultiplexed and converted to fastq files; the run generated 1.1 B reads across 51 libraries

Results





The envisaged full utilisation of the iSeq sequencing device at the NGSP

NovaSeq6000 QC device

- For large NovaSeq6000 flow cells & /or large sets of multiplexed data
- Accurate pooling for 10 x Genomics single cell experiments

Teaching device

Pilot studies prior to grant applications or for riskier projects

Species identification **aiding diagnostic units** situated at the Vetsuisse and Medical Faculty It is a rapid and cost effective genetic analysis



Targeted Sequencing

- Amplicons (illumina Ampli-Seq), clone checking, Sanger sequencing projects migration to the iSeq)

Genomics for the Microbiology

Small genomes WGS, de novo sequencing, resequencing
e.g. 10 *E. coli* or 20 *S. aureus* at 30 x coverage or Covid-19 detection in 10 viral culture samples.

Transcriptional Sequencing

- RNA-Seq for microbiology
- As well as targeted RNA sequencing, digital gene expression (Quant-Seq), miRNA-Seq, small RNA-Seq which do not require many reads/sample.

Metagenomics

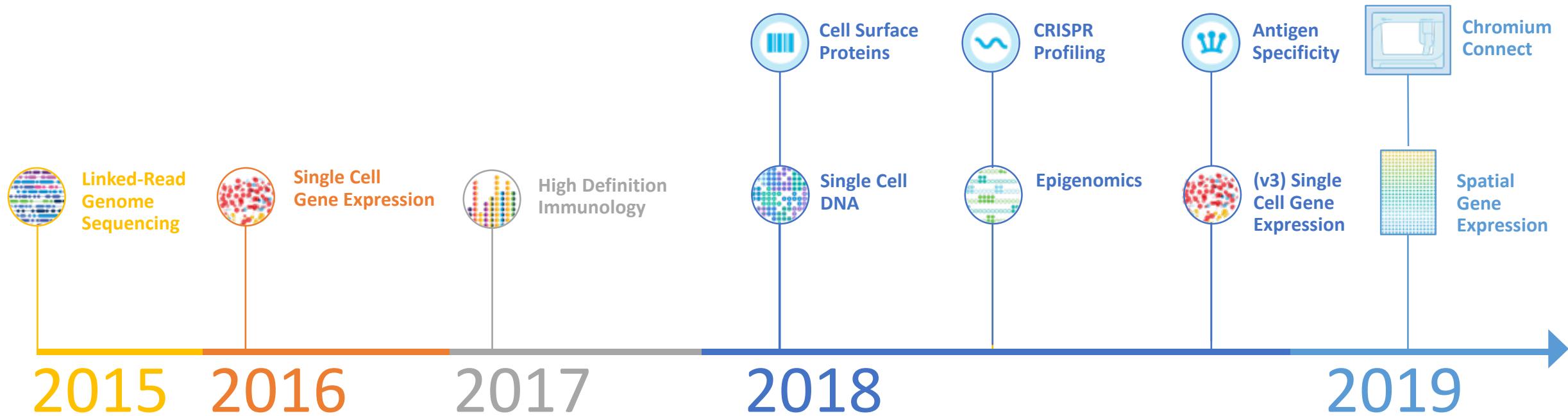
Shotgun and 16S &/ITS sequencing
40-50 samples/run

Plasmid sequencing

- **Mitochondrial sequencing**

The 10x Genomics Innovation Engine

Evolution Over Time



-10 x Genomics	Target recovery
Chromium Single Cell Gene Expression (3')	500-10'000 cells
Cells to 3' gene expression library + Cell surface protein library	
Chromium Single Cell ATAC	500-10'000 nuclei
Nuclei to ATAC library	
Chromium Single Cell Multiome ATAC + Gene Expression (3')	500-10'000 nuclei
Nuclei to ATAC library and gene expression library	
Chromium Single Cell Immune Profiling -cells to ds cDNA	500-10'000 cells
+ 5' gene expression library + VDJ enrichment library + Cell surface protein library	
Visium Spatial Gene Expression	Capture area in %

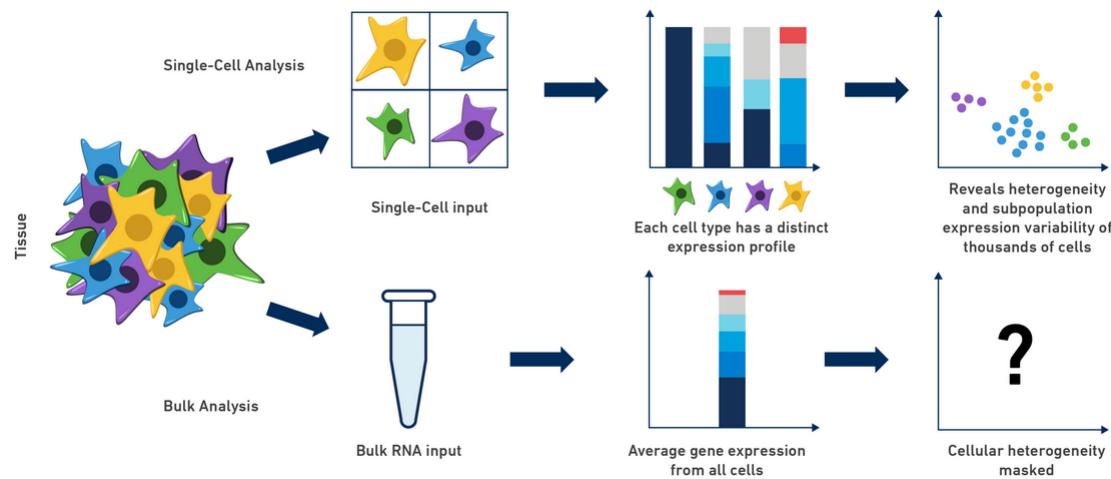
→ Now v3.1 single indexed, in 2021-V3.1, dual indexing

→ V1.1

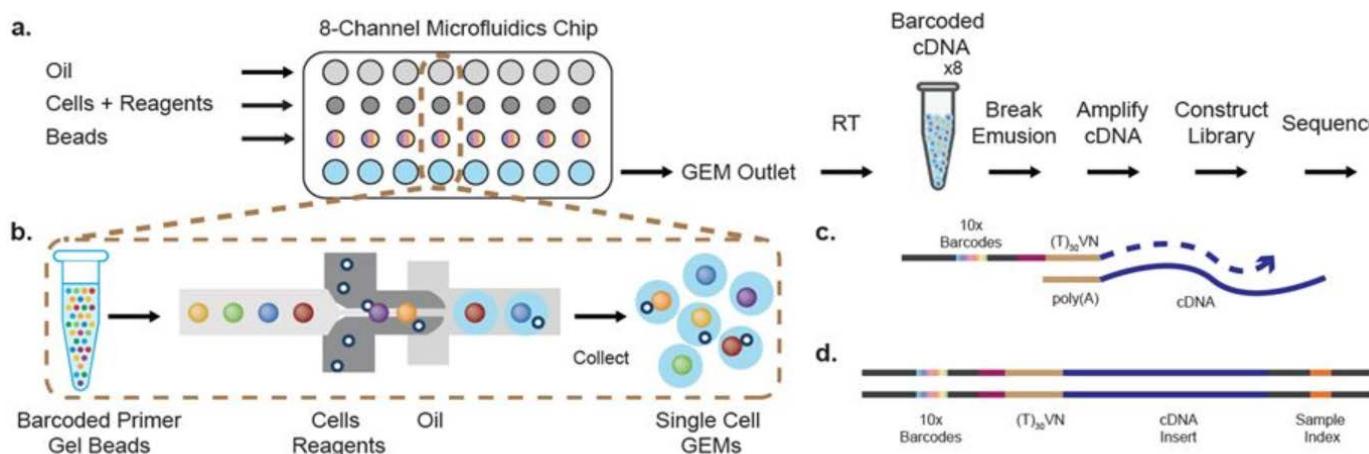
→ New as of Autumn 2020

→ Now v1.1, in 2021, V2, dual indexing

→ First pilot project to be sequenced before Christmas



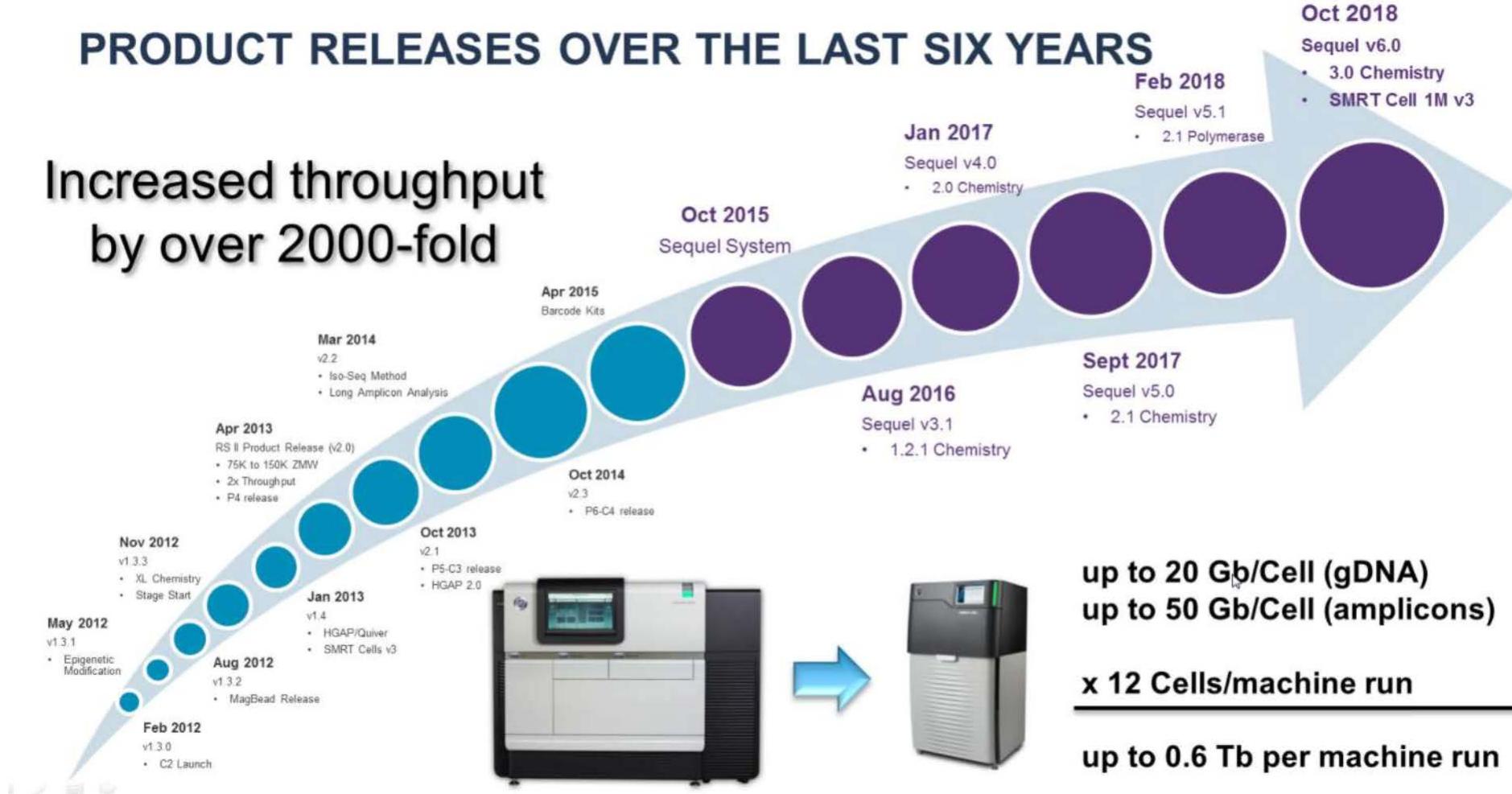
scRNA-seq allows researchers to explore the true diversity of gene expression at the single-cell level, providing access to critical data that is often masked by bulk RNA-seq methods



Stuart et al., *Nat. Rev.*, 2019
 Lafzi et. al, *Nat. Protocols*, 2018
 Nguyen et al., *Front. Cell Dev. Biol.*, 2018
 See et al., *Front Immunol.*, 2019

PRODUCT RELEASES OVER THE LAST SIX YEARS

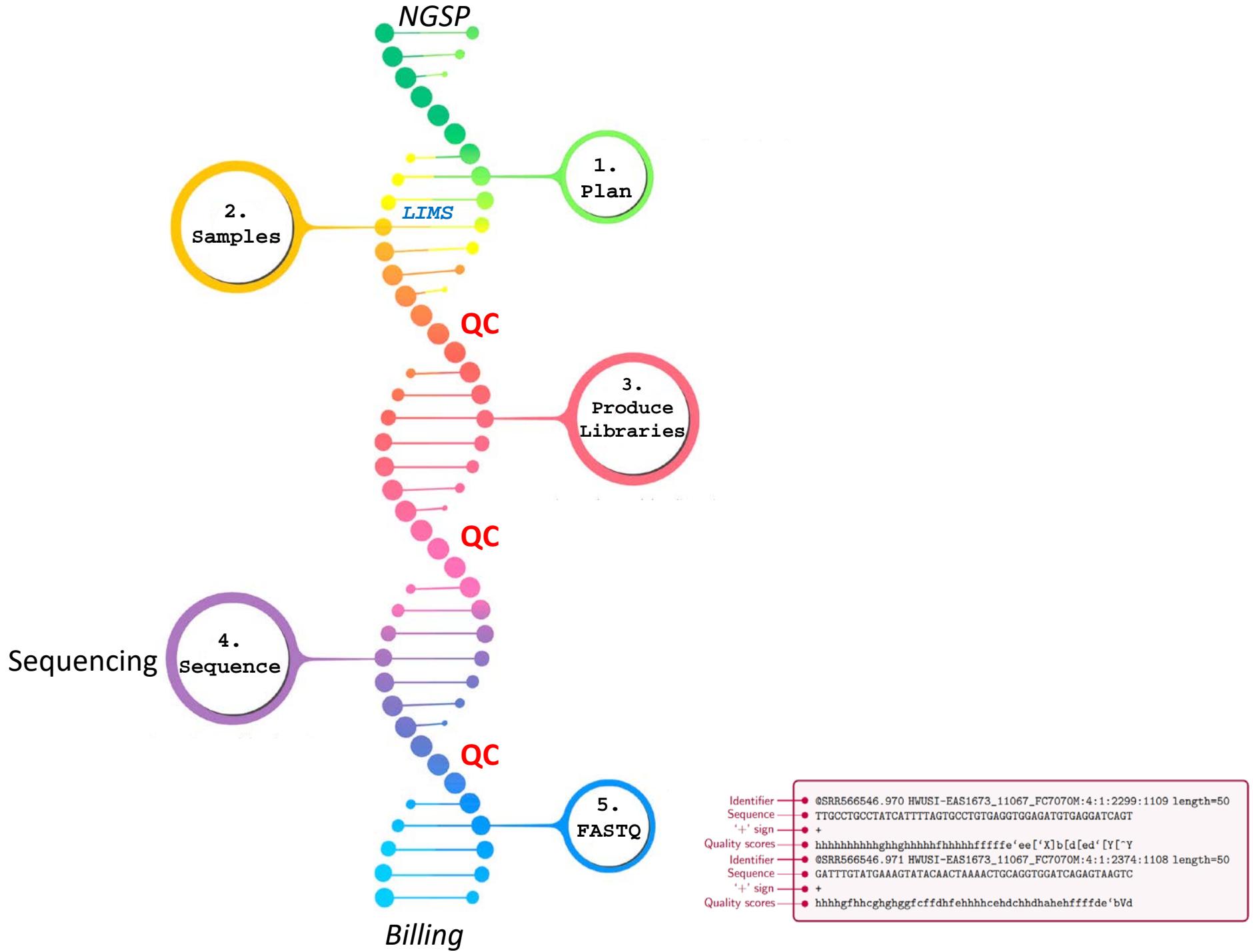
Increased throughput
by over 2000-fold



Application	Whole Genome Sequencing						RNA Sequencing		Metagenomics		Targeted Sequencing	
	De Novo Assembly - HiFi Reads	De Novo Assembly - for Low DNA Input	De Novo Assembly and Variant Detection - for Ultra-Low DNA Input	Microbial De Novo Assembly	Variant Detection	Structural Variation Detection	Iso-Seq Method	Single-Cell Iso-Seq	Full-length 16S rRNA Sequencing	Shotgun Metagenomic Profiling or Assembly	Amplicon Sequencing	No-Amp Targeted Sequencing
Experimental Design												
With 1 SMRT Cell 8M you can:	Produce reference quality assemblies for genomes up to 2 Gb	Produce reference quality assemblies for genomes up to 1 Gb	Produce reference quality assemblies for genomes up to 500 Mb	Sequence up to 48 microbes	With 2 SMRT Cells 8M, Call SNVs, InDels, and SVs in a 3 Gb genome	Call SVs for up to 2 samples with ~3 Gb genomes	Characterize alternative splicing/annotate a genome with full length transcripts	Characterize alternative splicing with full length transcripts up to 3M full length reads with cell barcode and UMI information	Multiplex up to 96 samples to provide strain level resolution	Generate near-complete assemblies of high-complexity sample(s) (e.g. gut microbiome)	Sequence 384 barcoded amplicons	Sequence 5 targeted regions in a multiplex of 20 samples
	10- to 15-fold HiFi read coverage per haplotype	10- to 15-fold HiFi read coverage per haplotype	>30-fold HiFi read coverage per diploid sample for de novo assembly	≥30-fold Unique Molecular Coverage (UMC) per microbial genome	≥15-fold HiFi read coverage of a human genome	5- to 25-fold UMC per diploid sample depending on study goals	One human transcriptome per SMRT Cell 8M	1,000 unique reads/ single cell for 3000 cells 10,000 unique reads/ single cell for 300 cells	8,000 HiFi reads / 16S sample	See Best practices guide	≥50-fold HiFi read coverage per target locus for variant detection 6,000-fold HiFi read coverage per target locus for minor variant detection (1% sensitivity)	≥100-fold HiFi read coverage per target locus
Library Insert Size	15 - 20 kb	~15 kb	10 - 12 kb	10 - 15 kb	15 - 20 kb	>15 kb	<2 kb to >3 kb	<2 kb to >3 kb	1 - 2 kb	10 kb	500 bp - 15 kb	4-6 kb or larger
Sample Preparation												
Procedure and Checklist Reference	Preparing HiFi SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0	Preparing HiFi Libraries from Low DNA Input Using SMRTbell Express Template Prep Kit 2.0	Preparing HiFi SMRTbell Libraries from Ultra-Low DNA Input	Preparing Multiplexed Microbial Libraries Using SMRTbell Express Template Prep Kit 2.0	Preparing HiFi SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0 for Structural Variation Detection	Preparing SMRTbell Libraries Using the SMRTbell Express Template Preparation Kit	Iso-Seq Express Template	Preparing Single-Cell Iso-Seq Systems	Amplification of Full-Length 16S Gene with Barcoded Primers for Multiplexed SMRTbell Library Preparation and Sequencing	Preparing 10 kb Library Using SMRTbell Express Template Prep Kit 2.0 for Metagenomics Shotgun Sequencing	Preparing SMRTbell Libraries using PacBio Barcoded Overhang Adapters for Multiplex SMRT Sequencing	No-Amp-Targeted-Sequencing-Utilizing-the-CRISPR-Cas9 System
	15 µg	>400 ng per 1 Gb genome size (single-sample) >300 ng per 600 Mb genome size (2-plex)	5 - 20 ng per 500 Mb genome size	1 µg per microbe	15 µg	3 µg	300 ng total RNA for 1st Strand cDNA Synthesis	>160 ng cDNA AFTER reamplification	500 ng - 1 µg	1.5 µg	250-500 ng for 250-1000 bp 500-1000ng for 1-3 kb bp 1000-2000 ng for 3-10 kb 1500-3000 ng for 15kb	5 to 10 µg (represented by either a single sample or the total of multiple samples that will be multiplexed)
Recommended PacBio Template Prep Kit	Express TPK 2.0 + SMRTbell Enzyme Cleanup kit + Sequencing primer v2	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + SMRTbell Enzyme Cleanup kit + Sequencing primer v2	Express TPK 2.0 + Sequencing primer v2	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	No-Amp Accessory Kit
Multiplexing/SMRT Cell	N/A	N/A	N/A	Up to 48 microbes / SMRT Cell 8M	Up to 2 human samples/ SMRT Cell 8M	N/A	The protocol supports up to 12 barcodes available.	Detects cell barcodes and UMIs	Up to 96 samples/ SMRT Cell 8M	Profile up to 4 communities/ SMRT Cell 8M	Up to 1,000+ samples/ SMRT Cell 8M or SMRT Cell 1M	Up to 20 samples/SMRT Cell
<i>Outsource to Sequel II unless small genome size</i>				<i>Outsource to Sequel II unless small genome size</i>				<i>Perfect on Sequel -16 plexing</i>				
<i>Outsource to Sequel II unless small genome size</i>				<i>Sequel, no plexing</i>				<i>Sequel, 96 plexing</i>				
<i>Sequel, no plexing</i>				<i>Sequel, 96 plexing</i>				<i>Sequel, no plexing</i>				

Application	RNA-Seq	Metagenomics	Targeted Seq	WGS	
	Iso-Seq	Full-length 16S rRNA Sequencing	Sequence 96 barcoded amplicons	Microbial De Novo Assembly	De novo Assembly, All variants & SV of large genomes
Explanation	Characterize alternative splicing/annotate a genome with full length transcripts	Multiplex up to 96 samples to provide strain level resolution	30-fold ≥Q20 CCS read coverage for variant detection 6,000-fold ≥Q20 CCS read coverage for minor variant detection (1% sensitivity)	Sequence up to 16 microbes 30-fold unique molecular coverage per microbial genome	Produce reference quality assemblies Call SNVs, InDels, and SVs Call SVs for up to 2 samples with ~3 Gb genomes
Library Insert Size	<2 kb to >3 kb	1- 2 kb	250 - 15 kb	10 - 15 kb	>20 kb
Amount Required	300 ng total RNA	500 ng - 1 µg	250-500 ng for 250-1000 bp 500-1000ng for 1-3 kb bp 1000-2000 ng for 3-10 kb 3000 ng for 15kb	1 µg per microbe	150 ng – 15 µg
PacBio Template Prep. Kit	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v2 or v4
Barcodes	1-12	1-96	96 (384 is possible)	16	2 for SV
Multilexing/SMRT cell	1-12	1-50*	96 is standard but more is possible	1-16 until 30-40 Mb total microbial genome size is reached	1 Or 2 for SV
Sequel Binding Kit & Seq. Plate	3.0	3.0	3.0	3.0	2.0
Sequencing Mode	CCS	CCS	CCS	CLR	CCS or CLR
Movie Collection Time	20	10	Insert Size-Dependent	10	15-30
SMRT cell type	1M v3 LR	1M v3	1M v3 or 1M v3 LR	1M v3	8M
SMRT Analysis	Iso-Seq pipeline +/- reference	CCS	CCS or Long Amplicon Analysis	Demultiplexing followed by Microbial Assembly analysis	Many options via PacBio
Notes	All steps are performed at NGSP using Sequel I instrument				QC and Library prep. performed on-site, and sequencing is performed in another lab on Sequel II

SMRT Sequencing Applications		Number of SMRT Cells 8M*
 WHOLE GENOME SEQUENCING	De Novo Assembly: Produce reference-quality assemblies for genomes up to 2 Gb	1
	Microbial De Novo Assembly: Generate reference-quality assemblies for up to 48 microbial isolates	1
	Variant Detection: Call single nucleotide, indel, and structural variants in a ~3 Gb genome	2
	Structural Variant Detection: Call structural variants for up to 2 samples with ~3 Gb genomes	1
 RNA SEQUENCING	Whole Transcriptome: Characterize alternative splicing with full-length transcripts	1
	Genome Annotation: Sequence full-length transcripts and multiplex up to 8 tissues	1
 TARGETED SEQUENCING	Amplicon Sequencing: Detect variation in specific regions by multiplexing	1
	No-Amp Sequencing: Enrich hard-to-amplify targets and multiplex up to 10 samples	1
 COMPLEX POPULATIONS	Full-length 16S: Gain strain-level resolution by multiplexing up to 96 samples	1
	Metagenomic Functional Profiling: Examine up to 3 low-complexity samples with multiplexing	1
	Shotgun Metagenomic Assembly: Generate near-complete assemblies of high-complexity samples (e.g. gut microbiome)	1



We QC the run

Samples are demultiplexed

Further QC steps –*read statistics*

Data is released –FASTQ files downloaded from LIMS

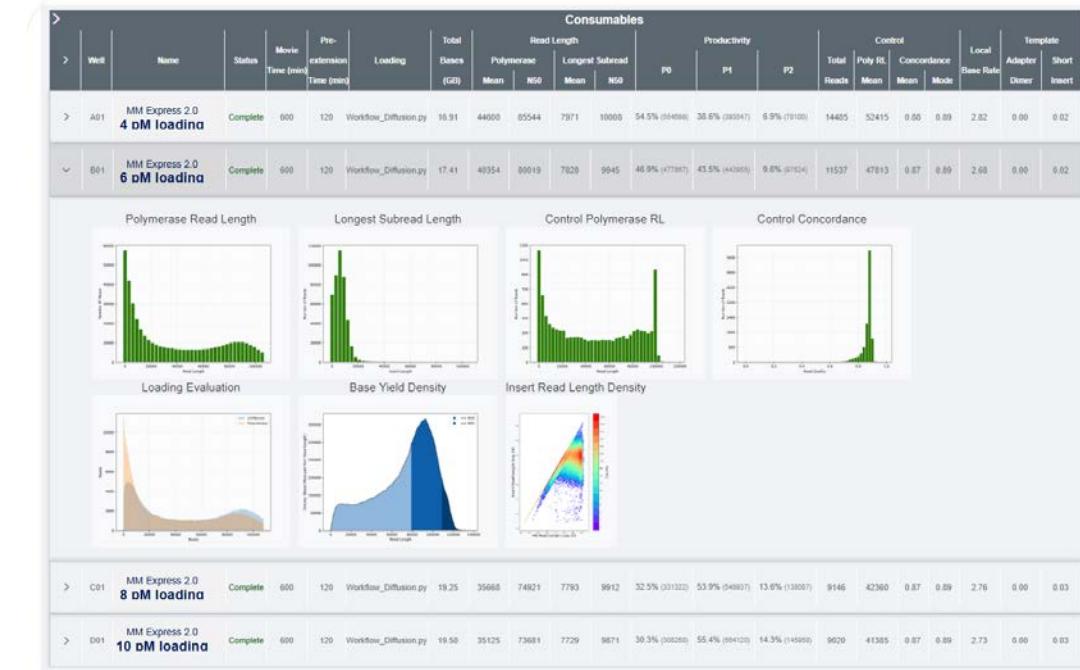
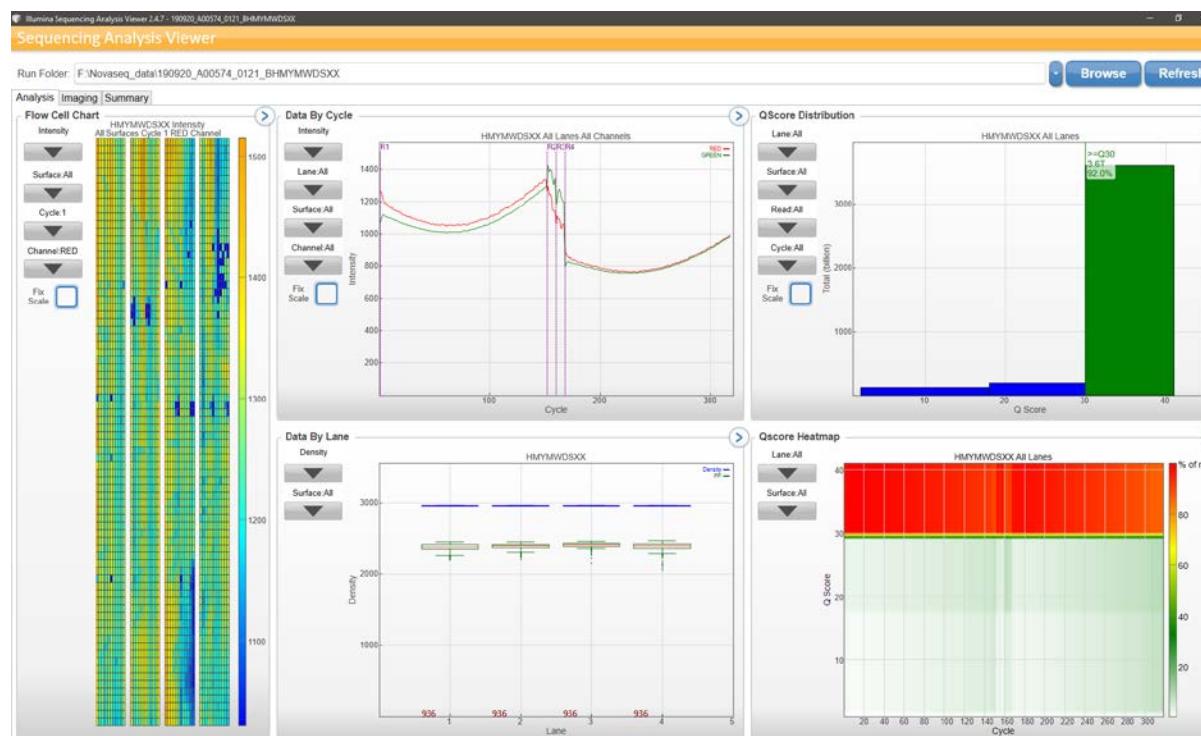
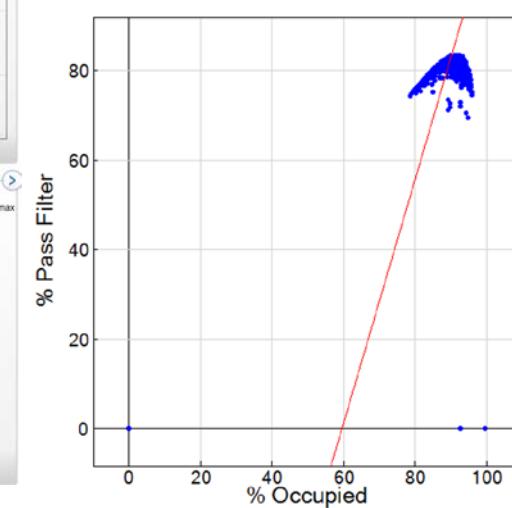


Figure 5 – Run QC from SMRT Link with a range of loading on-plate concentration from 4 to 10 pM which yield productive fraction loading of P1 from 38.6 to 55.4% respectively. Optimal loading at 6 to 8 pM shows good yield at approximately 18 Gb and average polymerase read length of 40 kb. Underloading may yield insufficient coverage for samples, whereas overloading may result in decrease sequencing quality which will also impact genome assembly results.



And at the very end....

Next Generation Sequencing Platform

Statement of Work

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3001 Bern
Phone: 031 631 22 85
nsg.lab@vetsuisse.unibe.ch

NGSP Bill#	DATE
2034	5/1/2014
CUSTOMER LIMS ID	TERMS
564	30 days from final bill

Please check the details below and inform us how to proceed with payment.

UniBe **internal** payment via eforms: we require an account number.

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BILL TO

Name	Fred Sanger
University	The Sanger Institute
Email	sanger.ac.uk

Project: Sequencing

If you have any questions about this invoice, please contact
[Pamela Nicholson, 031 631 22 85, pamela.nicholson@vetsuisse.unibe.ch]



We guarantee the storage of your samples, any generated libraries and your raw data:

Samples: up to 3 months

NGS Libraries: up to 12 months

Data: up to 6 months

And at the very very end....

<https://abrf.org/authorship-guidelines>



Next Generation Platform (NGSP)

Prof. Dr. Tosso Leeb

Marion Ernst

Catia Coito

Daniela Steiner

Nadine Bucher



Incoming Group Members:

Samia Imadjane

Sonja Gempeler

Interfaculty Bioinformatics Unit (IBU)

Dr. Rémy Bruggmann

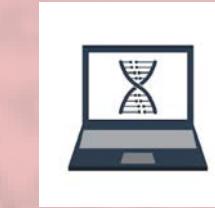
Dr. Irene Keller

Dr. Simone Oberhängsli

Dr. Heidi Tschanz-Lischer

Dr. Marco Kreuzer

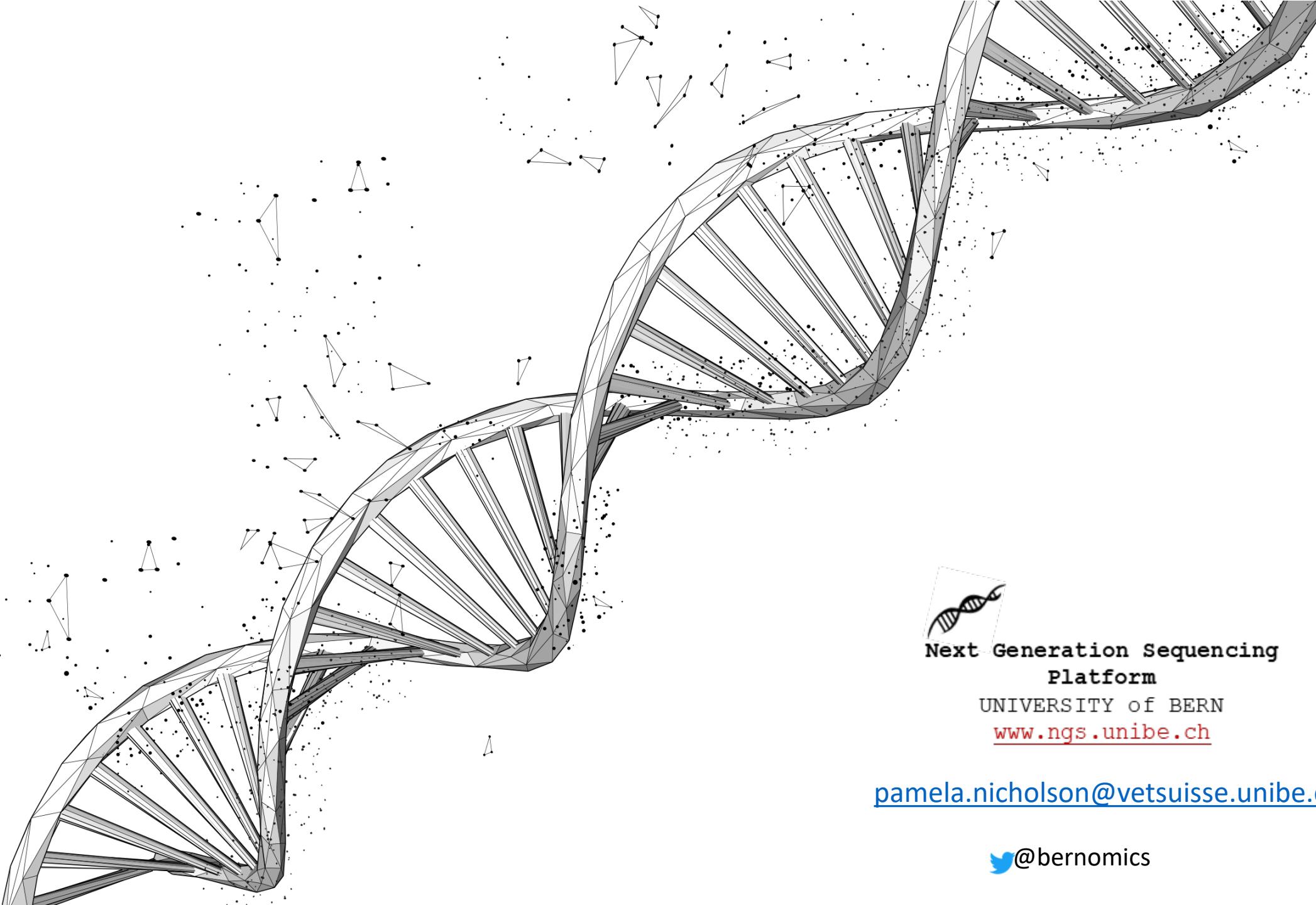
Dr. Geert van Geest



Clinical Genomics Lab (CGL)

**Cytometry Laboratory
/ FACS lab**

Dr. Stefan Müller



**Next Generation Sequencing
Platform**

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 @bernomics

Please Note:

- Website will be upgraded in early 2021
- Quarterly newsletter via email in 2021
- PacBio webinar from NGSP** – February 24th 2021 –save the date, more to follow
- 10xGenomics Day, Bern** in Summer 2021 – more to follow