



NGS: technologies and challenges

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Today we will talk about



- Genomics Platforms and sequencing services at NGI, SciLifeLab
- History and current status of technologies for sequencing
- NGS applications and technologies
- NGS challenges and sample requirements
- Data analysis pipelines, R&D and strategic projects



Service areas of SciLifeLab

Bioinformatics

Bioimaging and Molecular Structure

Chemical Biology and
Genome Engineering

Drug Discovery

Diagnostics

Genomics

Metabolomics

Single Cell Biology

Spatial Omics

Proteomics

Across all service areas: dedicated staff scientists that can offer support **throughout the experimental process** – from study design to data handling

SciLifeLab Genomics



RELEVANT UNITS / GENOMICS

National Genomics Infrastructure (NGI)

The National Genomics Infrastructure (NGI) provides services for next generation sequencing and SNP genotyping on all scales using a comprehensive range of modern (...)

[Learn More →](#)

Ancient DNA

Use cleanroom labs and specialized molecular genetics techniques to extract, make libraries, sequence and analyze DNA in ancient and/or degraded biological material.

[Learn More →](#)

Clinical Genomics

Develops and provides clinical genetic tests using state-of-the-art genomic methods, such as next-generation sequencing, for translational research and healthcare.

[Learn More →](#)

Eukaryotic Single Cell Genomics

Provides service for high-throughput single cell genomics analysis

[Learn More →](#)

Microbial Single Cell Genomics

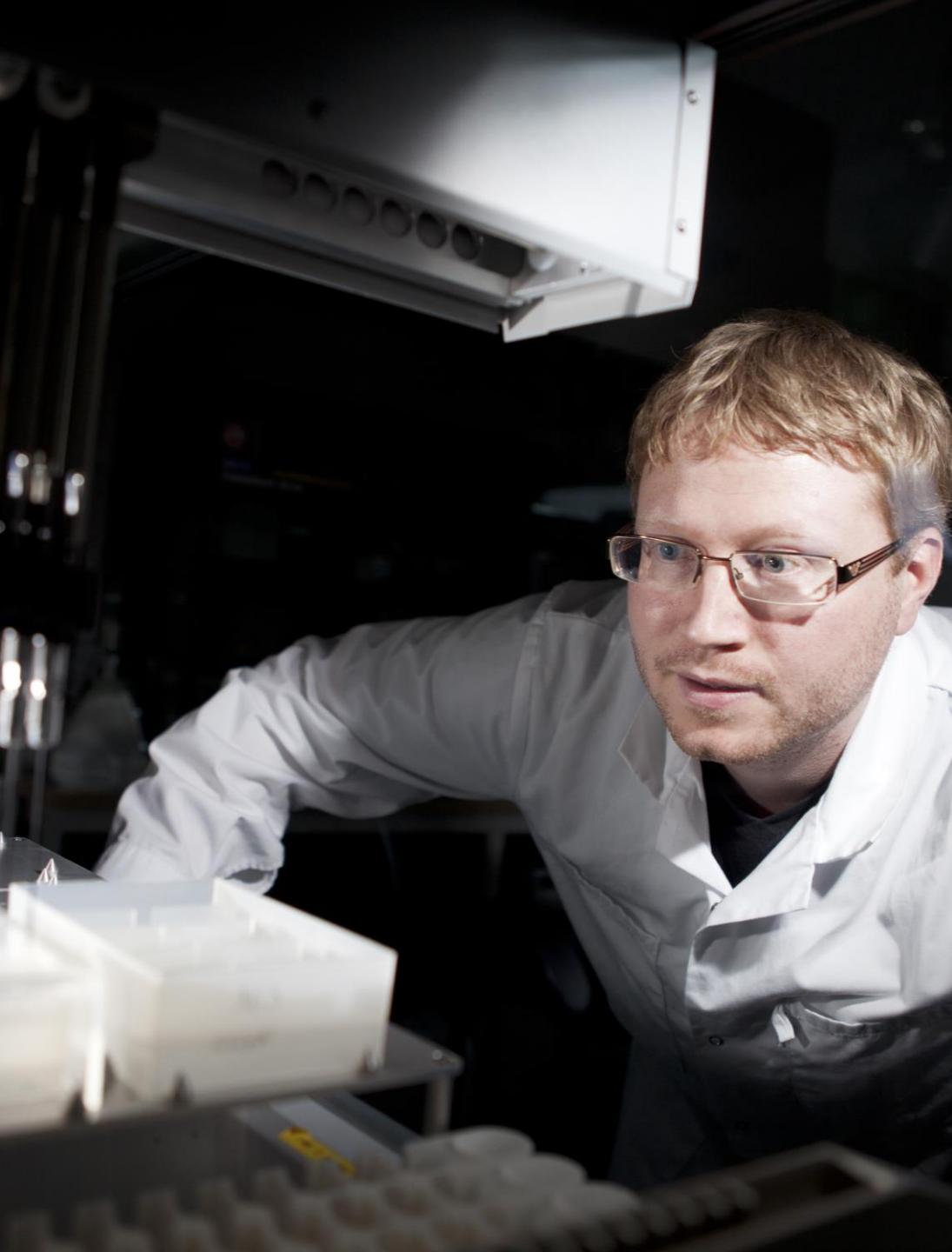
Provides streamlined single-cell sorting, lysis, whole-genome amplification and screening of individual microbial cells, as well as whole genome and targeted gene sequencing (...)

[Learn More →](#)

National Bioinformatics Infrastructure (NBIS)

Provides custom-tailored support with data analysis, computational tools, systems development and training.

[Learn More →](#)



What is NGI?

NGI provides access to technology for massively parallel/next generation DNA sequencing, genotyping and associated bioinformatics support

NGI Platform organisation



Tuuli Lappainen
Platform Director
Professor KTH



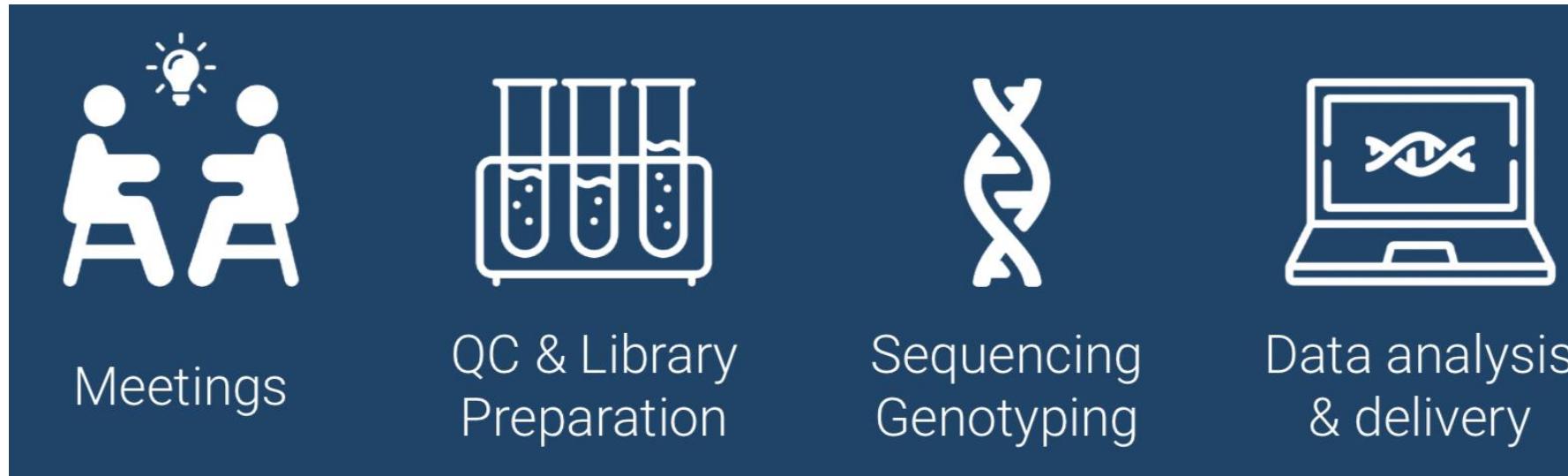
Lars Feuk
Platform Co-Director
Professor UU

**NGI-Uppsala
SNP&SEQ
Technology
platform**

**NGI-Uppsala
Uppsala
Genome Center**

NGI-Stockholm

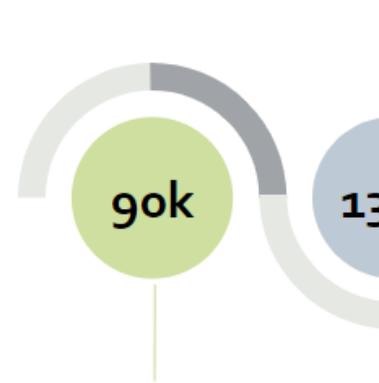
Project workflow





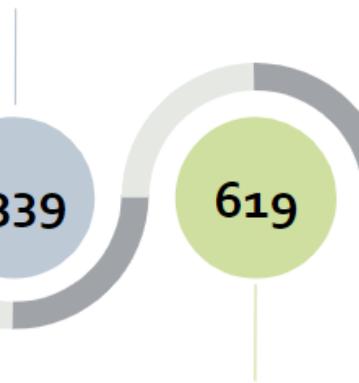
Projects

- Assemblies of high-quality reference genomes
- Human genome variation analyses
- Transcriptome profiling
- Single-cell sequencing and much more



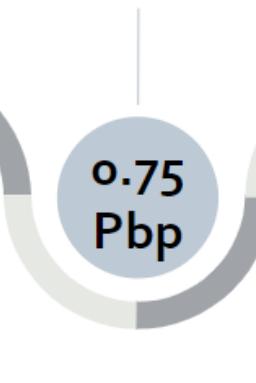
Samples

- All types of sample sources: from environment, lab cultured, biobank, etc
- All types of organisms: microbes, plants, insects, mammals, ...



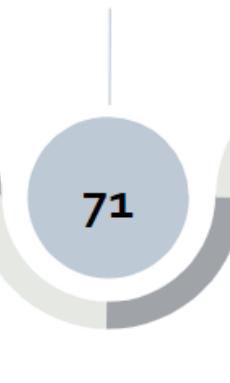
Support meetings

- Experimental design
- Advising on sample preparations
- Optimizing sequencing setup
- Guidelines for further data analysis



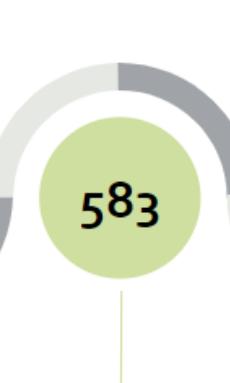
Publications

- Contribution to a number of articles in high impact journals such as Nature, Cell, Science, Nature Biotechnology, Nature Genetics, Nature Neuroscience, etc.



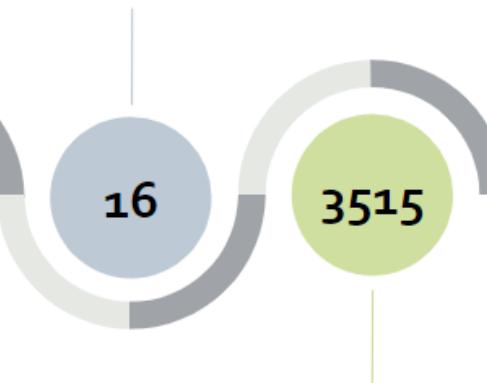
Technology development

- Evaluation of new protocols, applications, bioinformatics tools and sequencing methods
- Methodological developments in spatial and single-cell transcriptomics technologies



Education and Outreach

- Teaching at courses from undergraduate to PhD level
- Participating in national and international conferences
- Webinars, workshops and hackathons



Users

- Unique project PIs from more than 18 different universities, institutes, healthcare and industry companies used NGI services in 2023



Communication tickets

- 44865 ticket updates
- 98.6% satisfaction score

NGS technologies at NGI



Short-reads
illumina



Short-reads
ion torrent
by life technologies™



Long -reads
PACBIO®



Oxford
NANOPORE
Technologies

Sequencing instruments at NGI



Short read NGS

High throughput, low cost per base

3x NovaSeq X Plus – **New!**

5 x Illumina NovaSeq

4 x Illumina MiSeq

1 x Illumina NextSeq

1 x Illumina iSeq

1 x Thermo Fisher IonS5



Long read NGS

Very long reads, lower throughput

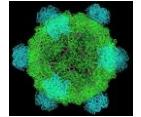
1 x PacBio Revio – **New!**

1 x PacBio Sequel IIe

1 x Oxford Nanopore-PromethION



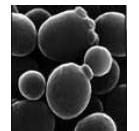
History and current status of sequencing



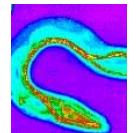
First genome: virus ϕ X 174 - 5 368 bp (1977)



First organism: *Haemophilus influenzae* - 1.5 Mb (1995)



First eukaryote: *Saccharomyces cerevisiae* - 12.4 Mb (1996)



First multicellular organism: *Cenorhabditis elegans* - 100 Mb (1998-2002)



First plant: *Arabidopsis thaliana* - 157 Mb (2000)

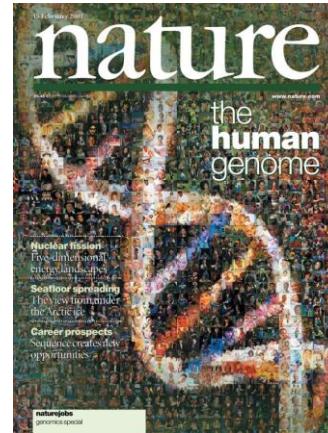


First human genome- 3Gb (2003)

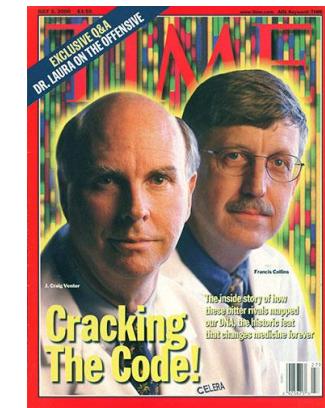


An interesssting comparison

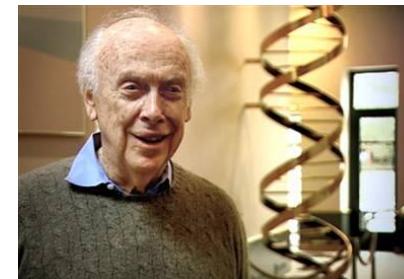
Human genome project (HUGO, 2003)
Sanger Sequencing
2.7 Billion USD



Craig Venter's Genome
Sanger Sequencing
70 Million USD



James Watson's Genome
454 pyro sequencing (Roche)
2 Million USD



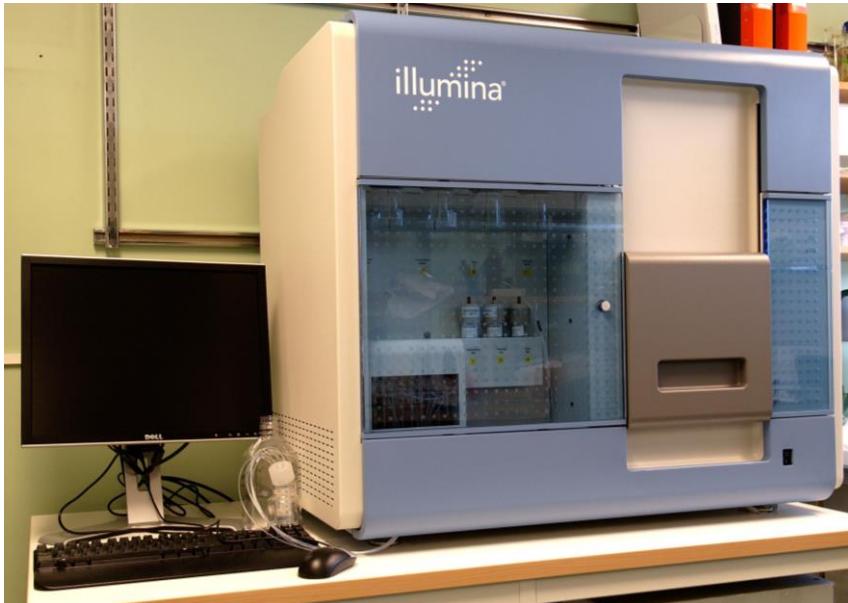
Yesterday's genome
NovaSeq 6000 (Illumina)
~1 000 USD

Today's genome
NovaSeq X (Illumina)
~600 USD

15 years of Illumina sequencing at NGI



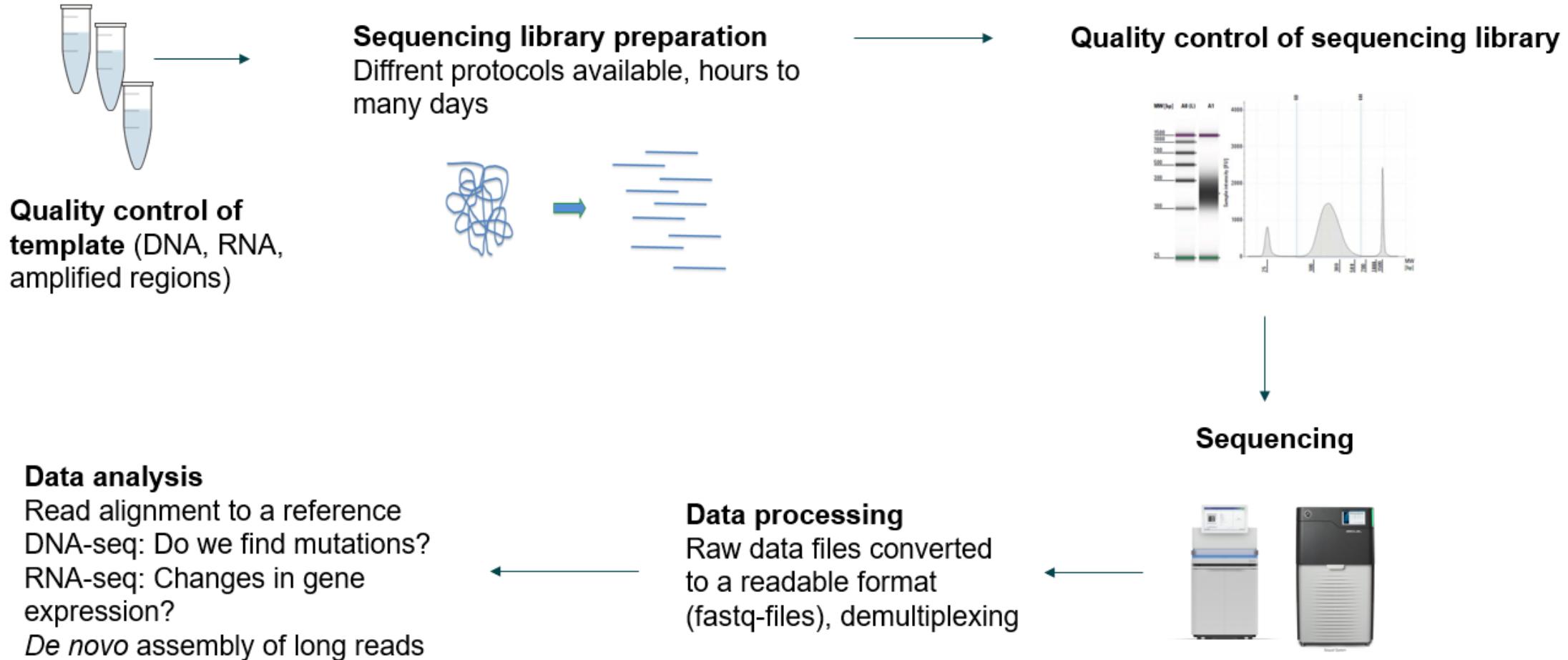
2007: Installation of Illumina GA



2023: Arrival of NovaSeq X Plus



Workflow, Illumina sequencing



Short reads, Illumina sequencing



illumina®



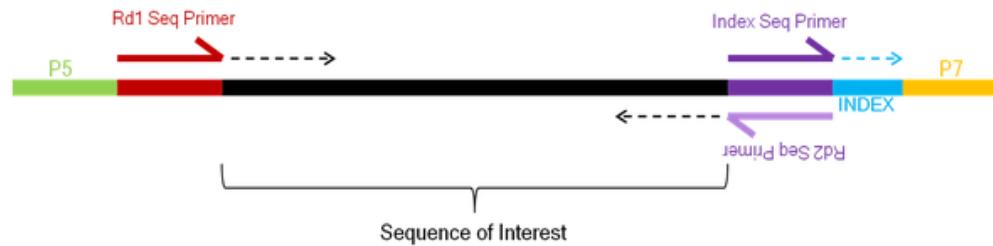
**36-300 bp, paired end sequencing
150 Mb-16 Tb per run
12 hours - 3 days**

Whole genome sequencing, any size
Whole genome sequencing, human
Exome
Transcriptomes
Target genes and panels
Amplicons (up to 500 bp)
ChIP-sequencing
Methylome
RAD-sequencing
Metagenomes and metatranscriptomes
Ultra-low input samples

Library preparation



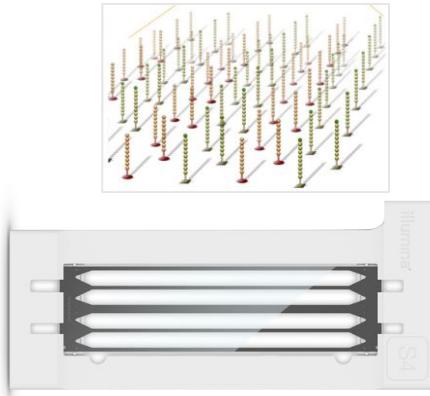
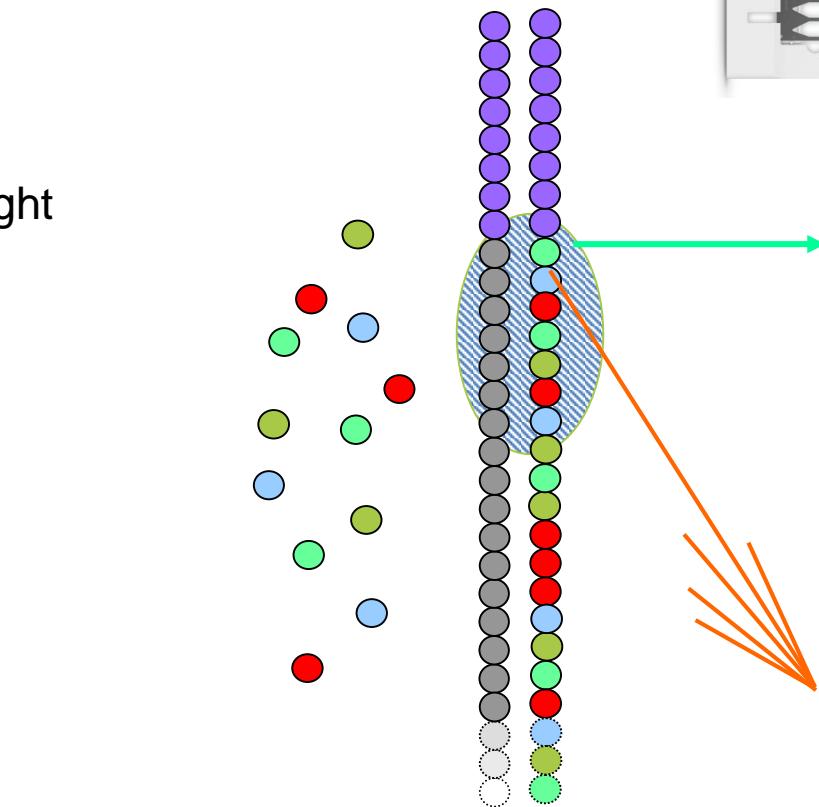
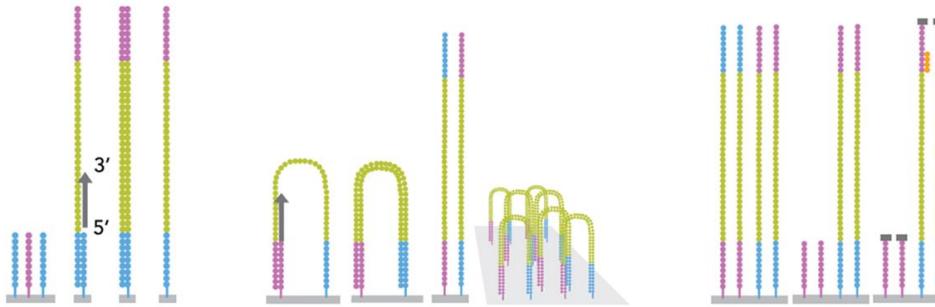
- A sequencing library is a pool of DNA fragments with adapters attached to both ends of the fragments
- Approx. 20 protocols for Illumina library prep at NGI



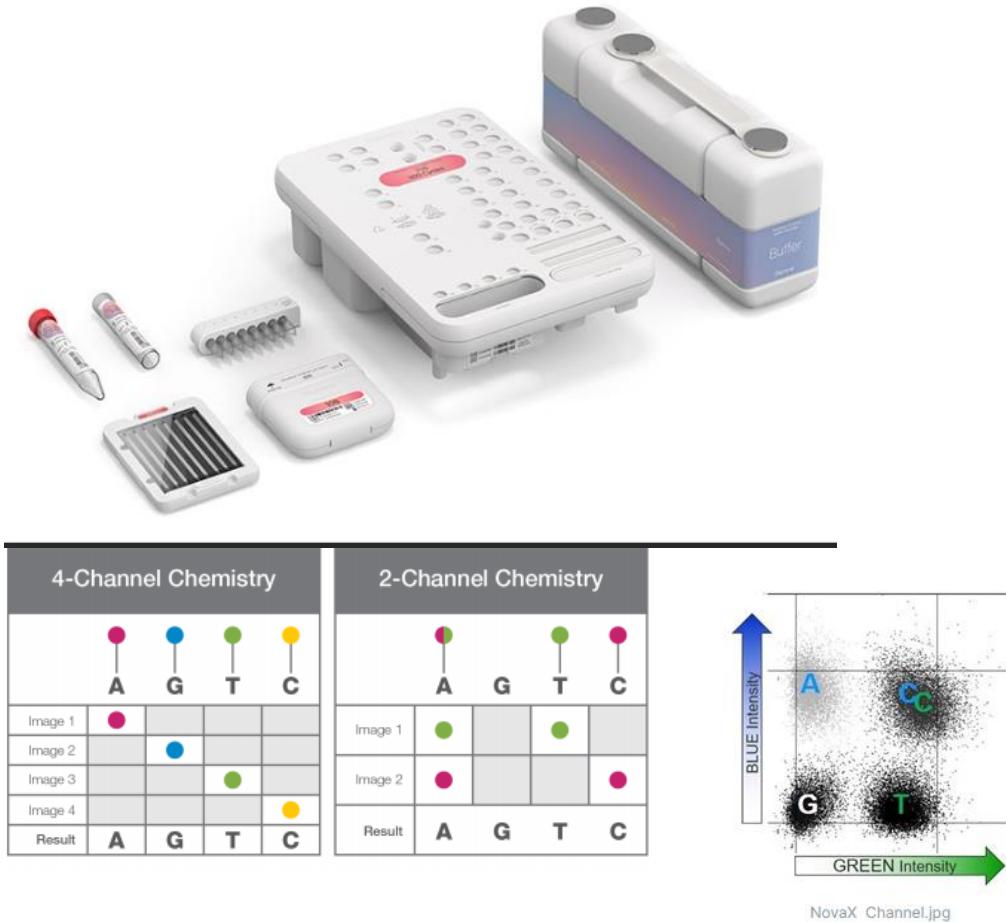
Illumina cluster generation & sequencing



- The sequencing library is hybridized to a flowcell ("cluster generation")
 - - A flowcell is a slide that is coated with oligos
- Rapid bridge amplification
- Hybridization of sequencing primers
- Sequencing by synthesis
 - fluorophore labeled nucleotides emitting light



Illumina sequencing by synthesis



Youtube:
<https://www.youtube.com/watch?v=fCd6B5HRaZ8>

New instrument - NovaSeq X Plus



| Flowcell Type | 1.5 B | 10 B | 25 B |
|--|--------|------|------|
| Output per flowcell (paired end150 bp) | 500 Gb | 3 Tb | 8 Tb |
| Number of human genomes per flowcell | ~ 4 | ~ 24 | ~ 64 |
| Run time (paired end150 bp) | 21 h | 24 h | 48 h |

| Run ID - Lane | Mb Total Yield | M Total Clusters | % bases ≥ Q30 |
|---------------------------------------|----------------|------------------|---------------|
| 20230612_LH00179_0005_A2255M2LT3 - L1 | 295 764.0 | 979.4 | 95.4% |
| 20230612_LH00179_0005_A2255M2LT3 - L2 | 323 896.8 | 1 072.5 | 95.3% |
| 20230612_LH00179_0005_A2255M2LT3 - L3 | 366 557.1 | 1 213.8 | 95.6% |
| 20230612_LH00179_0005_A2255M2LT3 - L4 | 383 028.6 | 1 268.3 | 95.0% |
| 20230612_LH00179_0005_A2255M2LT3 - L5 | 251 454.3 | 832.6 | 97.3% |
| 20230612_LH00179_0005_A2255M2LT3 - L6 | 284 351.5 | 941.6 | 97.1% |
| 20230612_LH00179_0005_A2255M2LT3 - L7 | 388 065.2 | 1 285.0 | 94.0% |
| 20230612_LH00179_0005_A2255M2LT3 - L8 | 363 776.7 | 1 204.6 | 95.0% |

NovaSeq X results for our first 10 runs



| Runfolder | Number of reads (B read pairs) | Quality score Q30 (Average % >= Q30) | Error rate Phix (%) |
|----------------------------------|--------------------------------|---|---------------------|
| 20231019_LH00179_0007_B22CT72LT3 | 10,16 B/FC (1034-1426 M/lane) | 84.6 | 0.90 |
| 20231027_LH00179_0008_A22CT5YLT3 | 8,84 B/FC (881-1245 M/lane) | 87.4 | 0.40 |
| 20231030_LH00179_0009_A22FGLHLT3 | 11,78 B/FC (1304-1610 M/lane) | 93.5 | 0.11 |
| 20231110_LH00179_0012_A22FMKMLT3 | 8,95 B/FC (773-1423 M/lane) | 83.8 | 1.59 |
| 20231121_LH00179_0013_B22FMWLLT3 | 12,05 B/FC (1252-1612 M/lane) | 89.5 | 0.23 |
| 20231201_LH00179_0015_A22FY3TLT3 | 10,9 B/FC (774-1683 M /lane) | 92.56 | 0.32 |
| 20231205_LH00179_0016_A22FWYLLT3 | 12,3 B/FC (1527-1560 M /lane) | 92.20 | 0.19 |
| 20231205_LH00179_0017_B22GHKLT3 | 11,9 B/FC (1453-1529 M/ lane) | 93.87 | 0.15 |
| 20231207_LH00179_0018_A22GHLKL3 | 10,7 B/FC (1248-1360 M lane) | 92.71 | 0.20 |
| 20231213_LH00179_0019_A22GTJ3LT3 | 11,7 B/FC (1408-1482 M/lane) | 94.5 | 0.14 |

Software
Upgrade



Advantages and challenges NovaSeqX



+

Cost per base is low

Quick data generation

Easy workflow in the lab

Reagents shipped in RT

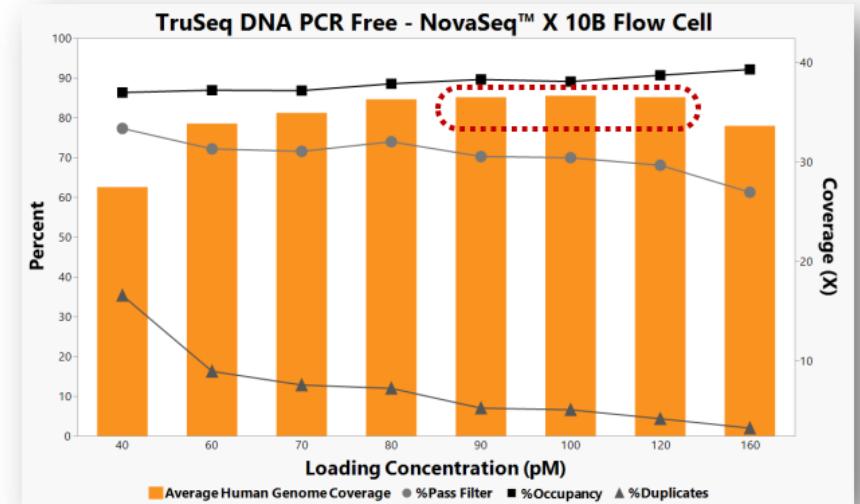
On-instrument analysis

-

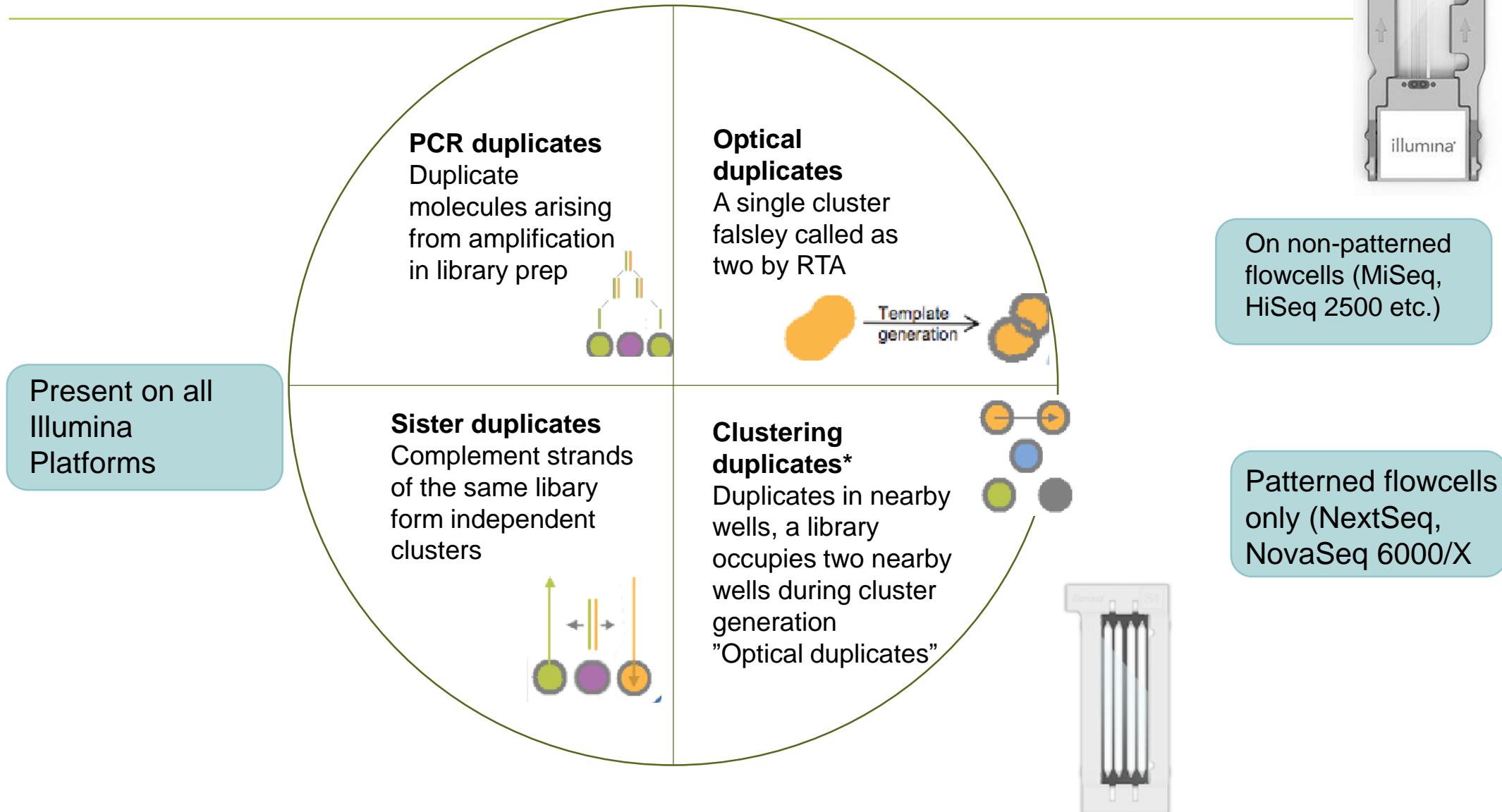
Yield vs duplicates

More sensitive to challenging samples and short inserts

Sensitive to colour balancing (C-A)



Duplicates, duplicates, duplicates....



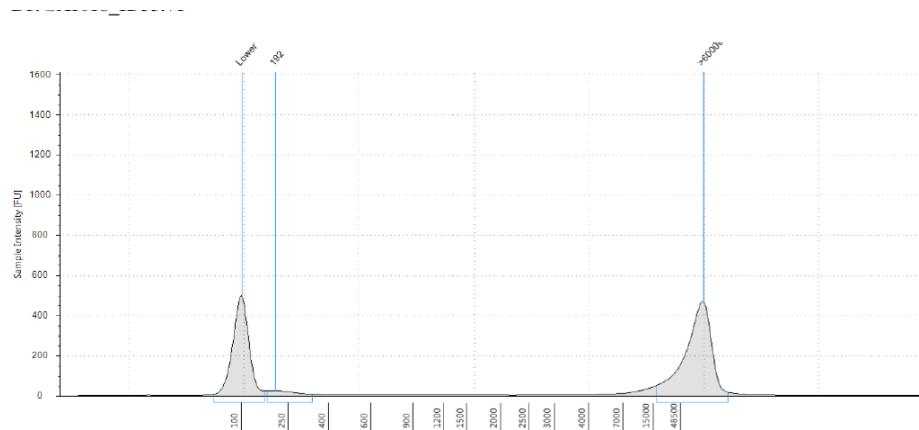
Quality control of RNA/DNA



DNA

Concentration: QuantIT

Degradation: Fragment Analyzer/TapeStation



Sample Table

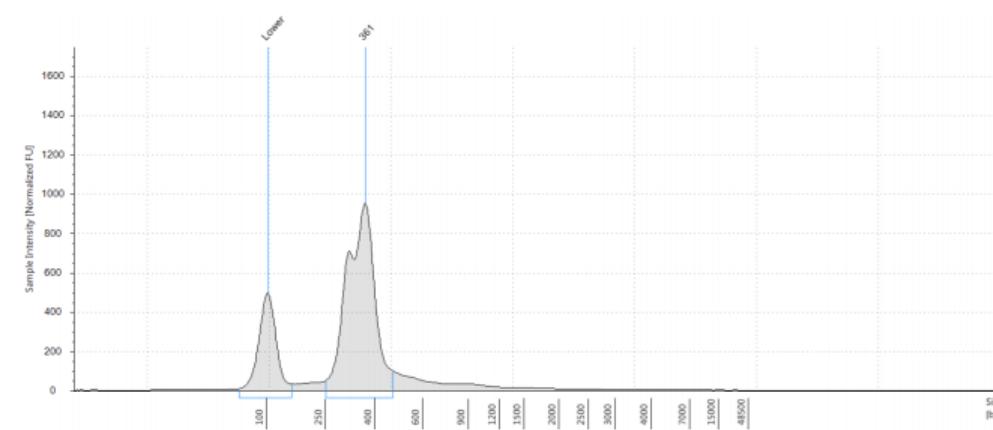
| Well | DIN | Conc. [ng/ μ l] | Sample Description | Alert | Observations |
|------|-----|---------------------|--------------------|-------|--------------|
| B1 | 9.6 | 16.0 | SXI018_ID33.v1 | | |

High quality DNA sample

RNA

Concentration + RIN-value:

Fragment Analyzer/TapeStation



Sample Table

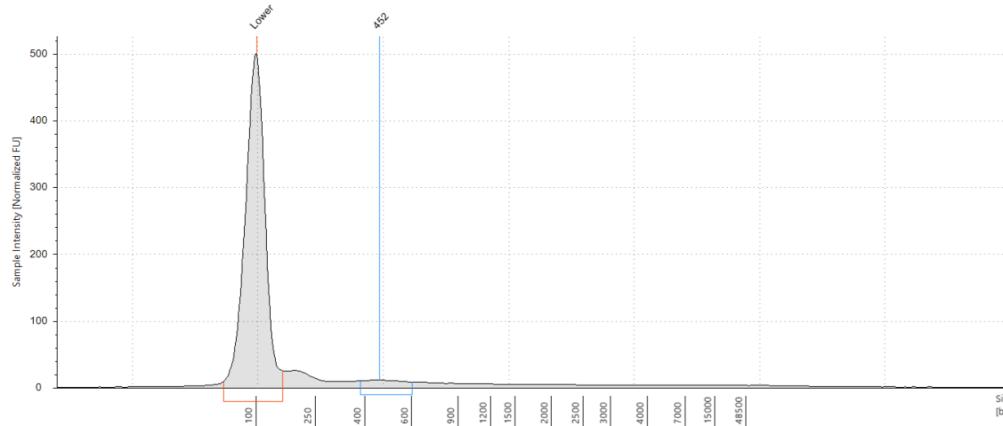
| Well | DIN | Conc. [ng/ μ l] | Sample Description | Alert | Observations |
|------|-----|---------------------|-------------------------|-------|--------------|
| E1 | 1.0 | 33.0 | 92-291039_RJ-1964-pool3 | | |

Degraded DNA sample

Quality of sample/library will affect sequencing result!



DNA-sample: 2.5 ng/ul, DIN-value 0



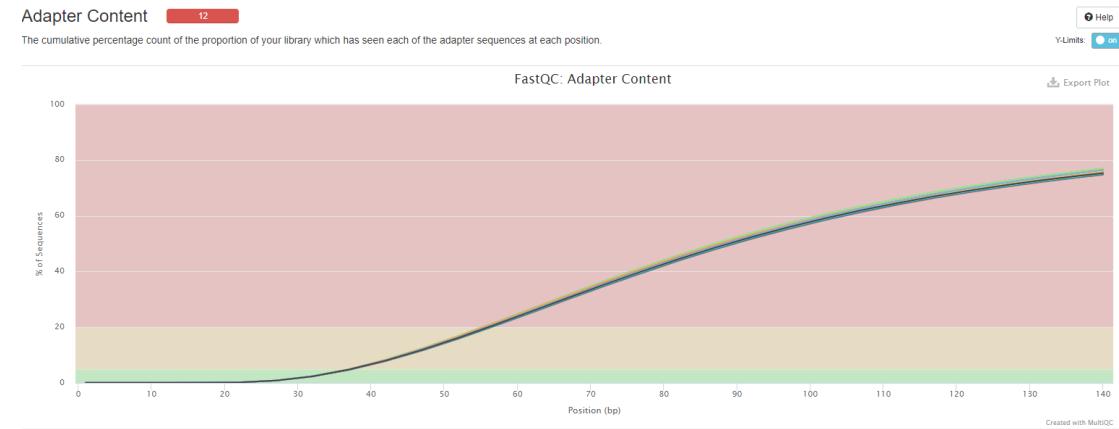
Sample Table

| Well | DIN | Cone. [ng/ul] | Sample Description | Alert | Observations |
|------|-----|---------------|--------------------|-------|---|
| A1 | - | 2.46 | SXI162_Sl.v1 | ⚠ | Sample concentration outside functional range for DIN |

20 ng of DNA, Thurplex Low-input library prep, 3 libraries

Amount of data generated: 800 M read pairs (aiming for $\geq 60x$ coverage)

Result: 12x coverage



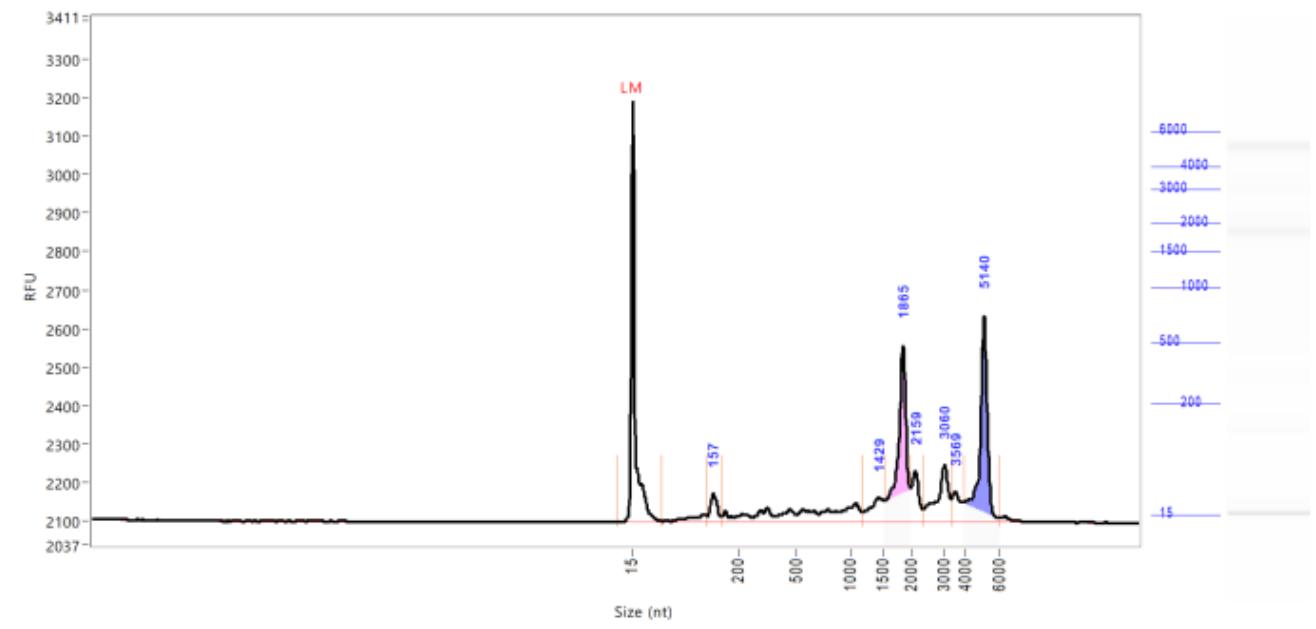
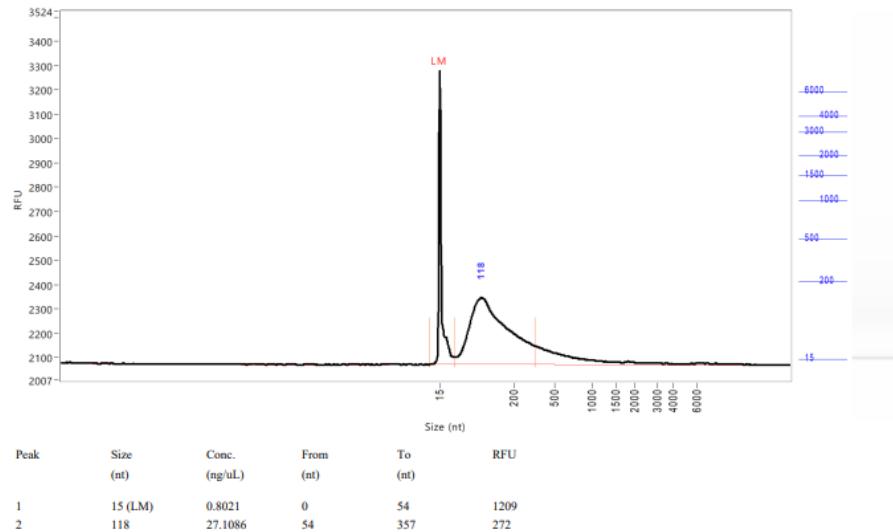
Copy table Configure Columns Plot Showing 7/7 rows and 14/23 columns.

| Sample Name | % GC | Ins. size | $\geq 30X$ | Coverage | % Aligned | Change rate | Ts/Tv | M Variants | TiTV ratio (known) | TiTV ratio (novel) | % Dups | % Dups | % GC | M Seqs |
|-------------|------|-----------|------------|----------|-----------|-------------|-------|------------|--------------------|--------------------|--------|--------|-------|--------|
| S1 | 46% | 55 | 11.1% | 2.0X | 98.2% | 893 | 1.645 | 3.47 | 2.0 | 1.6 | 76.6% | 1.1% | 46.6% | 1 |

Quality of sample/library will affect sequencing result!



- RNA samples, RIN-values between 1-9,6
- Library prep Illumina Ligation Ribo-Zero Plus



Results on next page...

Continued... Quality of sample/library will affect sequencing result



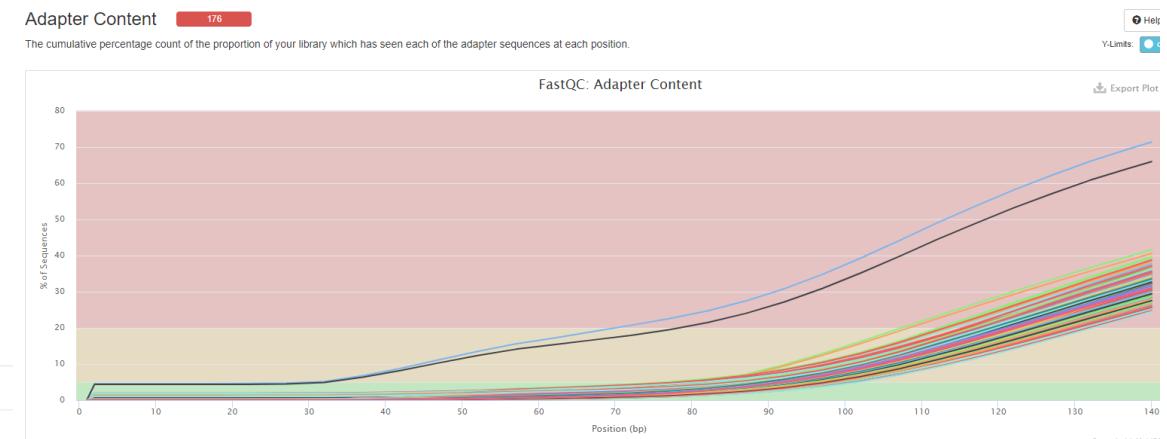
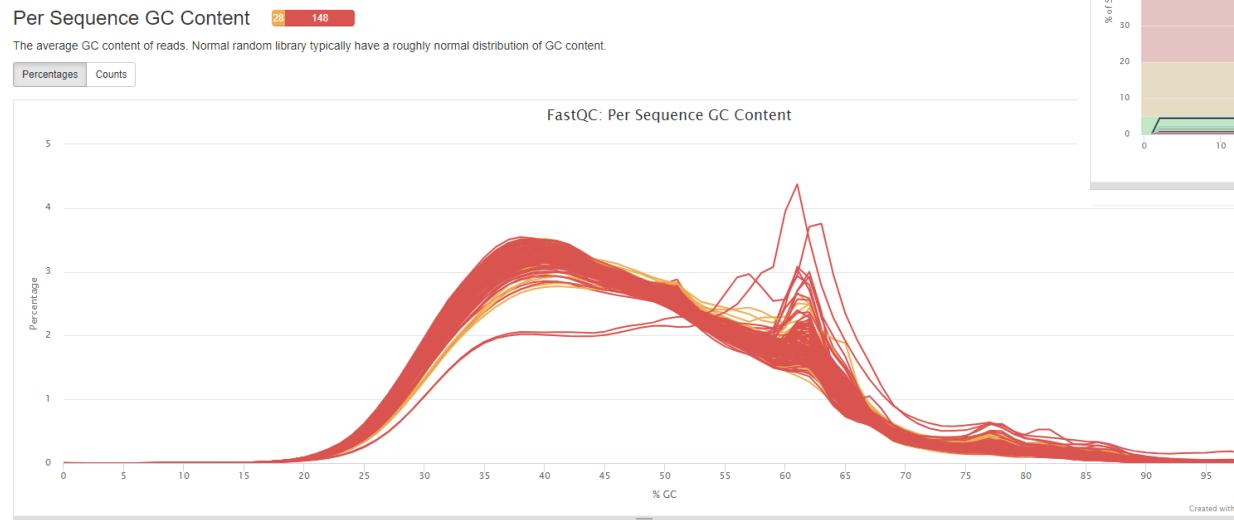
QC-results RNA-seq

Uneven amounts of data (17-100 M reads per sample)

A lot of duplicates

High rRNA content

High adapter content

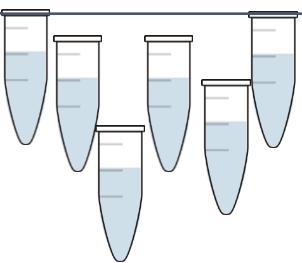


Some of the applications offered



Templates:

DNA, RNA, cells,
serum or plasma



Whole Genome Sequencing (WGS)

- *De novo* sequencing (PacBio, ONT)
- Re-sequencing (PCR-Free, low input)

Transcriptome Sequencing

- mRNA-Seq (poly-A selection)
- Total RNA-seq (ribosomal depletion)
- miRNA & small RNAs
- Full-length transcriptomes

Targeted re-sequencing

- Exome
- Gene panels
- Amplicons (including bacterial 16S for metagenomics)
- RAD-seq

Epigenetics

- Chromatin (HiC, ATAC-Seq)
- WGBS
- ChIP Sequencing

Ready-made libraries

- User-made libraries
- High throughput
- Fast turn around time

Single-cell applications

- 10x Genomics
- Dolomite Nadia
- Single-cell WGBS

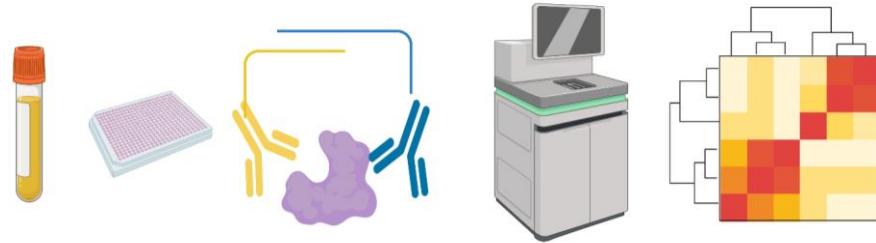
Spatial transcriptomics

- 10x Genomics Visium

Proteomics with NGS readout

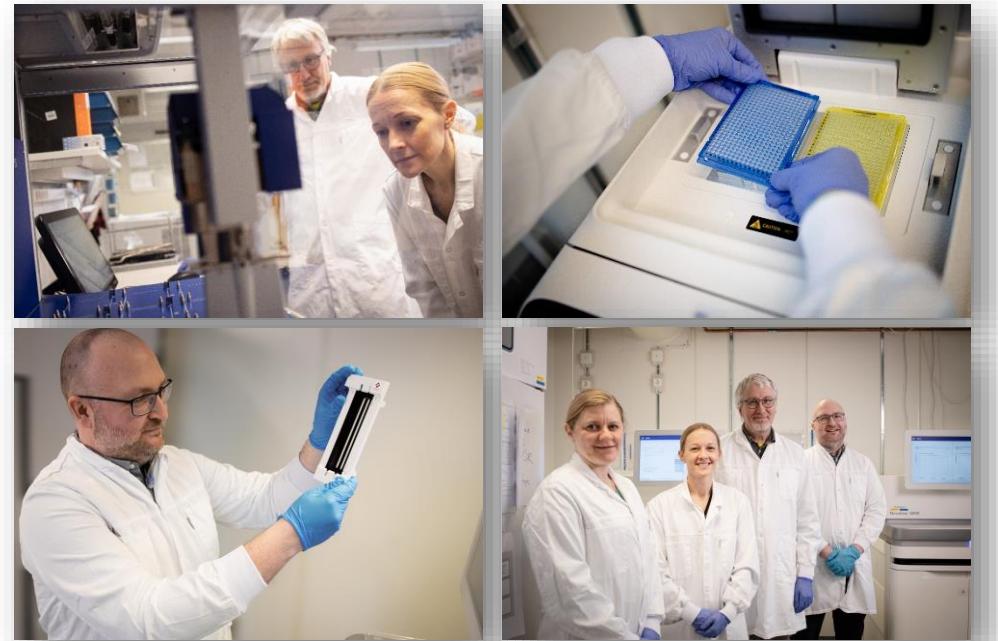
- Olink Explore 1536/3072/5300

Protein analysis, Olink Explore with NGS readout



- Highly multiplex protein biomarker analysis:
 - Olink Explore 384-5300 protein assays available
 - Cardio-metabolic
 - Inflammation
 - Neurology
 - Oncology
- Stats
 - >25 000 samples analyzed since the method was set up in the spring of 2021

SciLifeLab Explore Lab: NGI in collaboration with the Affinity Proteomics Uppsala unit and Olink Proteomics AB



Examples, recent successful projects



Forensic Science International: Genetics 53 (2021) 102525

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Check for updates

Research paper

Getting the conclusive lead with investigative genetic genealogy – A successful case study of a 16 year old double murder in Sweden

Andreas Tillmar ^{a,b,*}, Siri Aili Fagerholm ^c, Jan Staaf ^d, Peter Sjölund ^e, Ricky Ansell ^{c,f,**}

^a Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden

^b Department of Biomedical and Clinical Sciences, Faculty of Medicine and Health Sciences, Linköping University, Linköping, Sweden

^c National Forensic Centre, Swedish Police Authority, Linköping, Sweden

^d Polisregion Ost, Swedish Police Authority, Linköping, Sweden

^e Peter Sjölund AB, Härnösand, Sweden

^f Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden

Article | Published: 17 February 2021

Million-year-old DNA sheds light on the genomic history of mammoths

Tom van der Valk , Patrícia Pečnerová , David Díez-del-Molino , Anders Bergström , Jonas Oppenheimer , Stefanie Hartmann , Georgios Xenikoudakis , Jessica A. Thomas , Marianne Dehasque , Ekin Sağlınca , Fatma Rabia Fidan , Ian Barnes , Shannin Liu , Mehmet Somel , Peter D. Heintzman , Pavel Nikolskiy , Beth Shapiro , Pontus Skoglund , Michael Hofreiter , Adrian M. Lister , Anders Götherström , Love Dalén

Nature 591, 265–269 (2021) | [Cite this article](#)

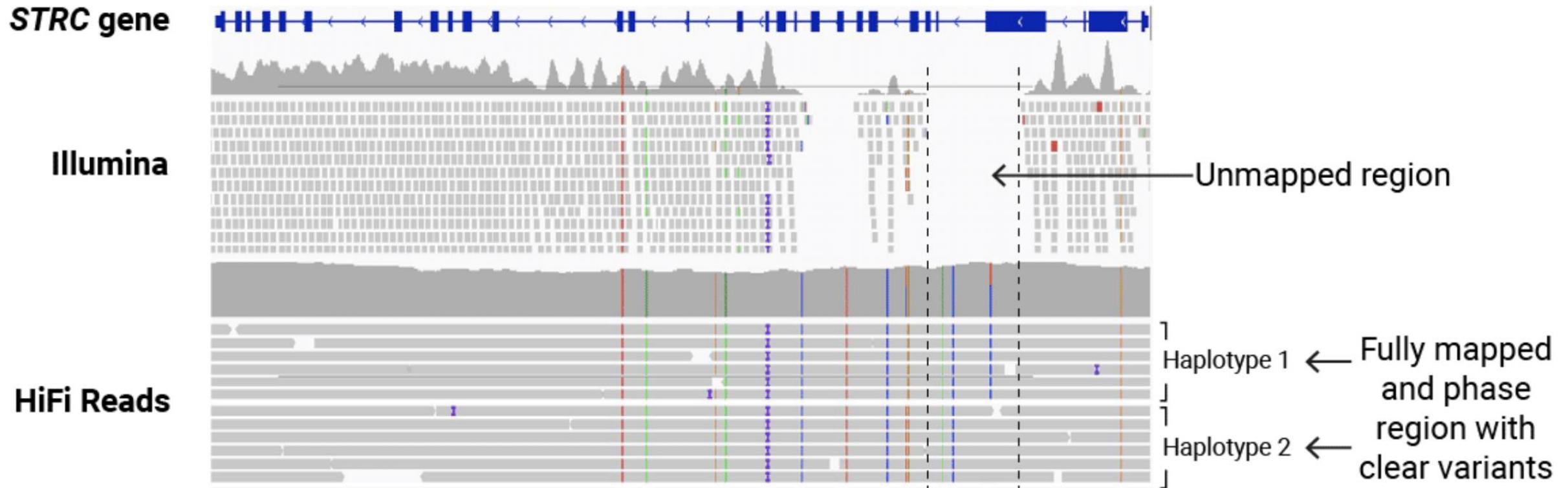
30k Accesses | 89 Citations | 2528 Altmetric | [Metrics](#)





Limitations with short reads

- You don't get complete genome information!



Long-read sequencing

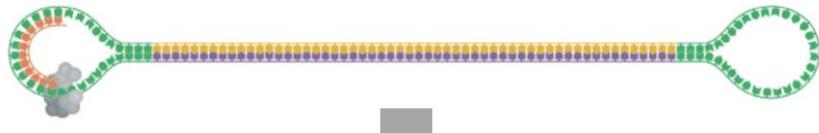


No longer a niche technology!

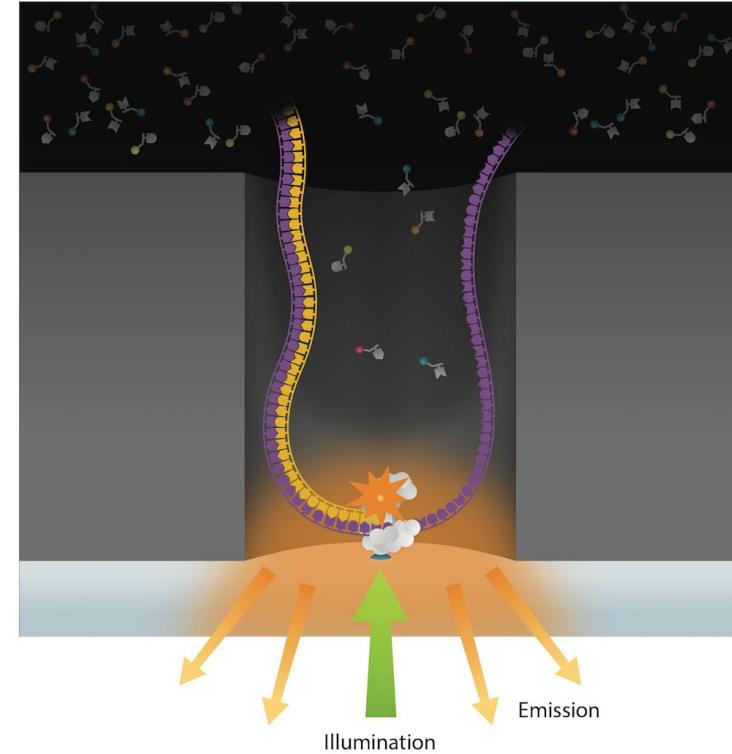
- Assemble complete genomes
- Find all genetic variants
- Detect epigenetic modifications
- At a “reasonable” cost



PacBio Sequencing



Multiplexed
ZMWs



PacBio RSII



PacBio Sequel
(Sequel I & II)

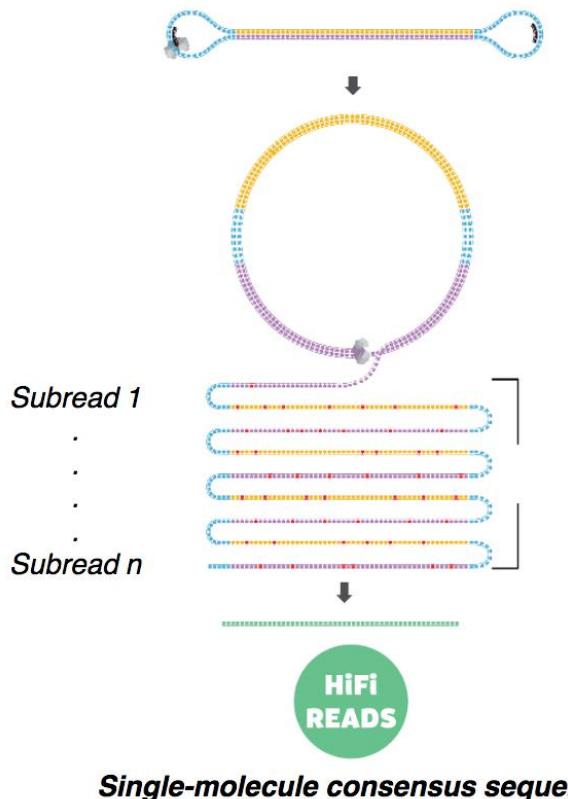
PacBio Sequencing



TWO MODES OF SMRT SEQUENCING

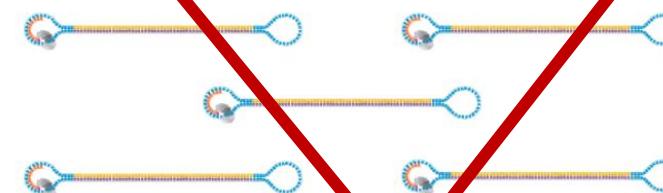
Circular Consensus Sequencing (CCS) Mode

Inserts 10-20 kb



Continuous Long Read (CLR) Sequencing Mode

Inserts >25 kb, up to 175 kb

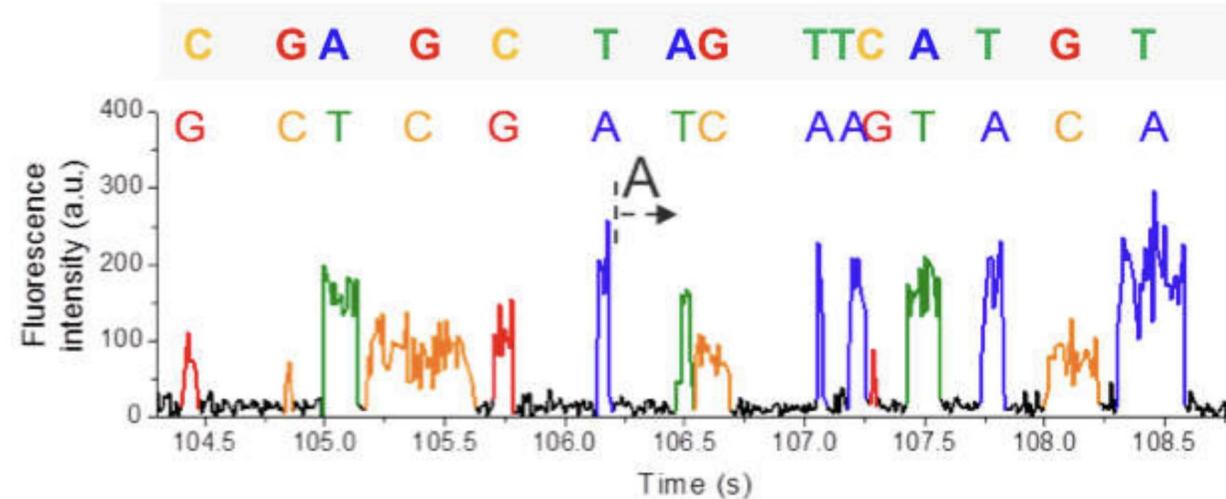
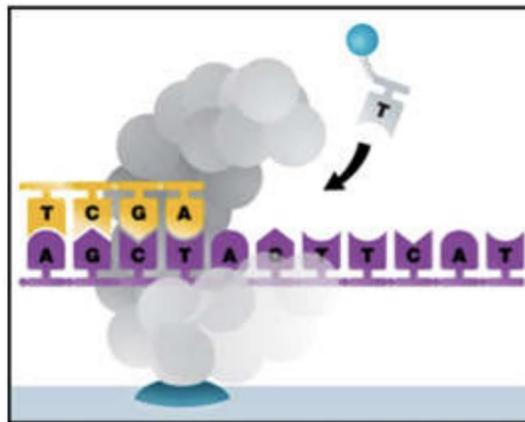
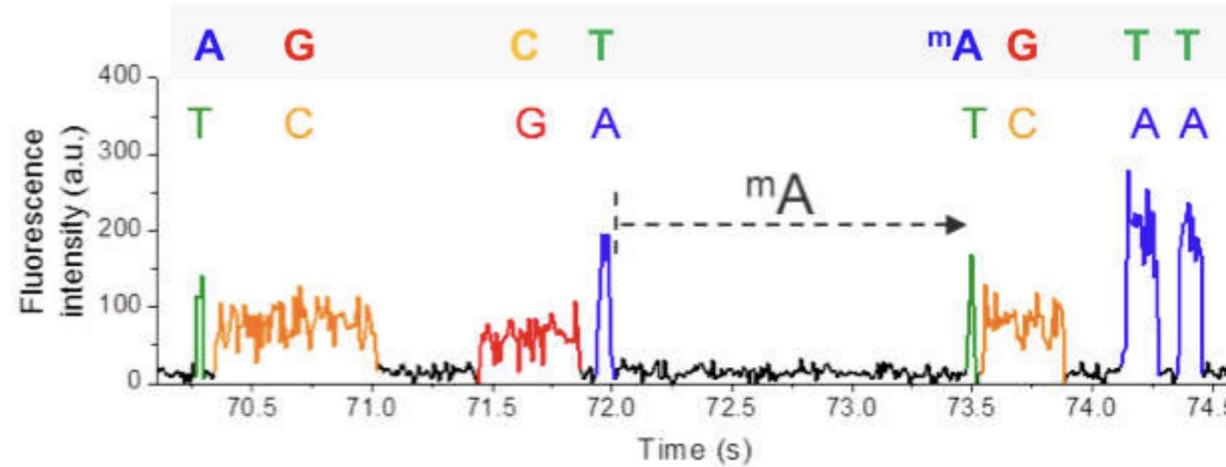
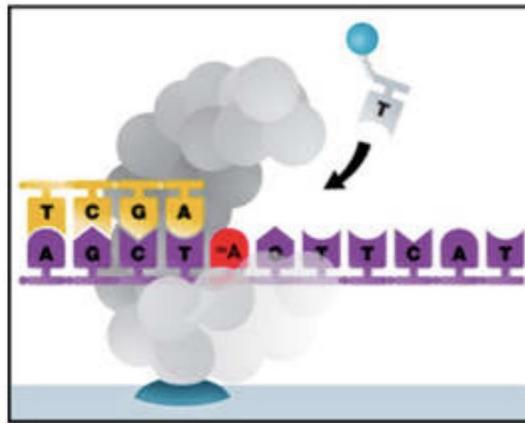


**CLR sequencing
no longer supported**

PacBio – Methylation detection



- Base modifications on native DNA molecules can be detected!



A decade of PacBio sequencing at NGI



2013: Installation of PacBio RSII



2023: Arrival of PacBio Revio



The PacBio Revio System



- Up to 90Gb data from one SMRT cell
- Read lengths: 15-20kb
- >QV20 quality (>99% read accuracy)
- Can run 1,300 human genomes/year!
- We installed PacBio Revio in March 2023



Revio – results for our first 16 runs



| Sample/Species/Proj | Number of reads | Total yield (Gbp) | Average read length (kb) | Size selection method | Comment |
|---------------------|-----------------|-------------------|--------------------------|---------------------------|---------------------|
| Human 1_1 | 6,873,030 | 84.7 | 12.3 | Ampure beads | Also Sequel II data |
| Human 1_2 | 6,846,419 | 102.2 | 15.0 | Ampure beads | Also Sequel II data |
| Human 1_3 | 7,170,075 | 90.3 | 12.6 | Ampure beads | Also Sequel II data |
| Human 1_4 | 6,015,366 | 67.6 | 11.2 | Ampure beads | Also Sequel II data |
| Human 2_1 | 6,895,775 | 104.2 | 15.1 | SageELF (2 fract. pooled) | |
| Human 2_2 | 5,684,755 | 100.3 | 17.6 | SageELF (2 fract. pooled) | |
| Human 2_3 | 6,022,465 | 111.5 | 18.5 | SageELF (2 fract. pooled) | |
| Human 3_1 | 7,544,871 | 72.3 | 9.6 | Ampure beads | |
| Human 3_2 | 7,857,802 | 65.6 | 8.3 | Ampure beads | |
| Human 3_3 | 7,164,744 | 102.3 | 14.3 | Ampure beads | |
| Human 3_4 | 6,695,524 | 82.4 | 12.3 | Ampure beads | |
| Human 3_5 | 6,541,509 | 80.4 | 12.3 | Ampure beads | |
| Plant 1_1 | 7,683,014 | 70.1 | 9.1 | Ampure beads | Also Sequel II data |
| Amphibian 1_1 | 2,700,447 | 23.5 | 8.7 | Ampure beads | 225 pM loading |
| Amphibian 1_1 | 5,219,472 | 42.3 | 8.1 | Ampure beads | 350 pM loading |
| Bird 1_1 | 6,812,139 | 90.2 | 13.2 | Ampure beads | |



Our best run so far > 114 Gb

| Value | Analysis Metric |
|-----------|--------------------------------|
| 6.6 M | HiFi reads |
| 114.17 Gb | HiFi reads yield |
| 17.21 kb | HiFi reads length (mean) |
| 16,564 | HiFi reads length (median, bp) |
| 17,585 | HiFi Read Length N50 (bp) |
| Q34 | HiFi Read Quality (median) |
| 92.36% | Base Quality \geq Q30 (%) |
| 8 | HiFi Number of Passes (mean) |

HiFi Read Length Distribution m84045_240305_200948_s3

The histogram displays the frequency of reads across different length bins. The peak of the distribution is at approximately 15,000 bp, with over 560,000 reads. The distribution tapers off as the read length increases beyond 20,000 bp.

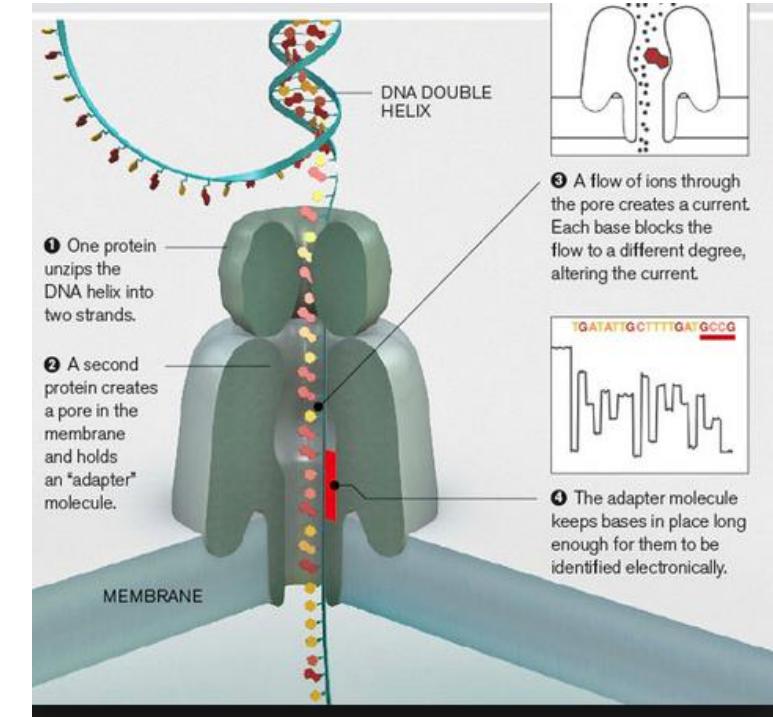
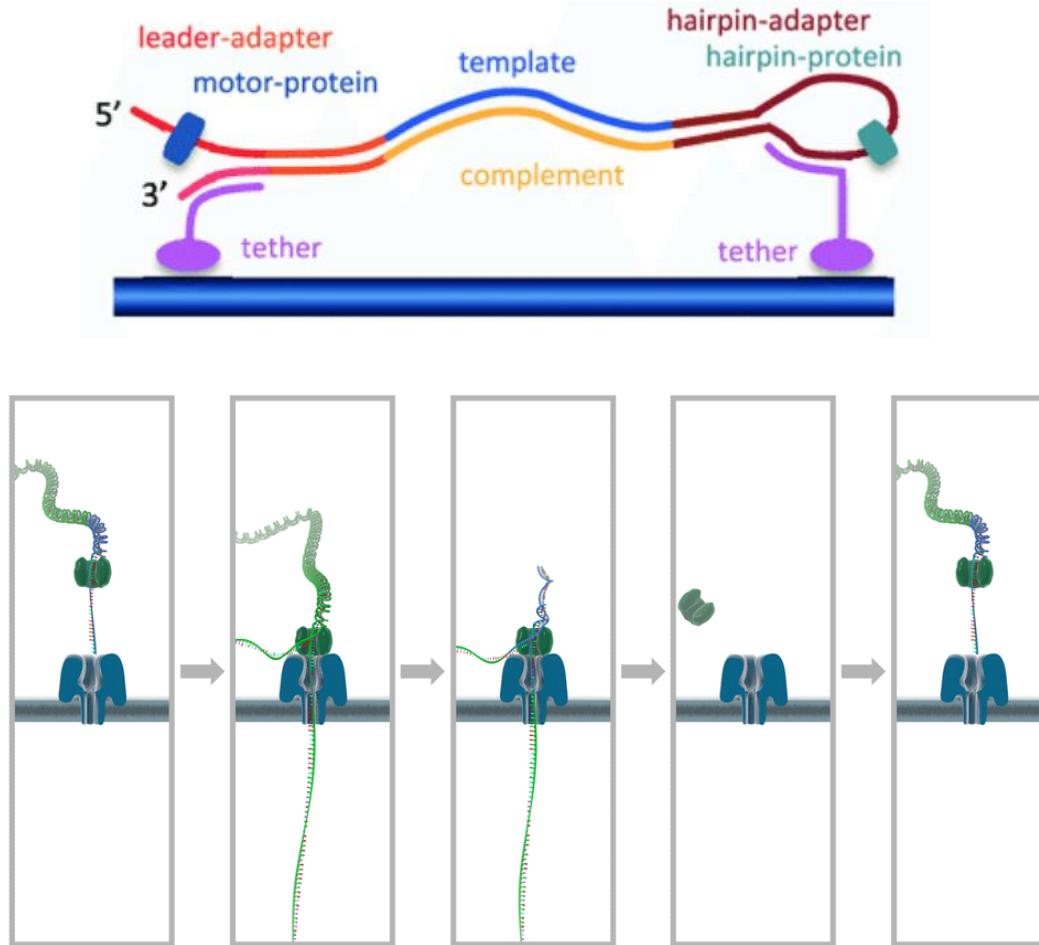
| HiFi Read Length (bp) | Number of Reads |
|-----------------------|-----------------|
| 10,000 | ~10,000 |
| 11,000 | ~70,000 |
| 12,000 | ~150,000 |
| 13,000 | ~240,000 |
| 14,000 | ~360,000 |
| 15,000 | ~560,000 |
| 16,000 | ~520,000 |
| 17,000 | ~480,000 |
| 18,000 | ~450,000 |
| 19,000 | ~420,000 |
| 20,000 | ~360,000 |
| 21,000 | ~280,000 |
| 22,000 | ~220,000 |
| 23,000 | ~180,000 |
| 24,000 | ~140,000 |
| 25,000 | ~110,000 |
| 26,000 | ~90,000 |
| 27,000 | ~70,000 |
| 28,000 | ~50,000 |
| 29,000 | ~30,000 |
| 30,000 | ~10,000 |
| 31,000 | ~5,000 |
| 32,000 | ~2,000 |
| 33,000 | ~1,000 |
| 34,000 | ~500 |
| 35,000 | ~200 |
| 36,000 | ~100 |
| 37,000 | ~50 |
| 38,000 | ~20 |
| 39,000 | ~10 |
| 40,000 | ~5 |



Example: Data at a translocation site



Oxford Nanopore sequencing



Base modification info is retained

Oxford Nanopore sequencing



| Instrument | Run time /FC | Output / FC | Nr of pores | Max read length |
|------------|--------------|-------------|-------------|-----------------|
| Flongle | 16 hrs | 1 Gb | 126 | 1 Mb |
| MinION | 24 hrs | 2-15 Gb | 512 | 1 Mb |
| GridION | 24 hrs | 2-15 Gb | 512 | 1 Mb |
| PromethION | 72 hrs | 10 – 150 Gb | 3 000 | 2 Mb |

ONT - Portability



The International Space Station

In 2016, MinION was used to conduct the first ever DNA sequencing in space. MinION performance was unaffected by the flight to the International Space Station (ISS) or microgravity conditions. The team stated that '*these findings illustrate the potential for sequencing applications including disease diagnosis, environmental monitoring, and elucidating the molecular basis for how organisms respond to spaceflight.*' Further to this, in 2020, an end-to-end sample-to-sequencer workflow conducted entirely aboard the ISS resulted in off-Earth identification of microbes for the first time.

Photograph: NASA ©

[Read more >](#)



Entirely off-grid, solar-powered sequencing

In 2019, Gowers *et al.* used MinION to demonstrate '*the ability to conduct DNA sequencing in remote locations, far from civilised resources (mechanised transport, external power supply, internet connection, etc.), whilst greatly reducing the time from sample collection to data acquisition.*' The team transported their portable lab for 11 days using only skis and sledges across Europe's largest ice cap (Vatnajökull, Iceland), before carrying out a tent-based study, resulting in 24 hours of sequencing data using solar power alone.

[Read more >](#)

Uncovering cryptic transmission of Zika virus

The origin and epidemic history of Zika virus (ZIKV) in Brazil and the Americas remained poorly understood despite observed trends in reported microcephaly. Using a mobile genomics lab to conduct genomic surveillance of ZIKV, the team identified the earliest confirmed ZIKV infection in Brazil. Analysis of these genomes estimated that ZIKV is likely to have disseminated from north-east Brazil in 2014, before the first detection in 2015, indicating a period of pre-detection cryptic transmission that would not have been identified without genomic sequencing.

[Read more >](#)



Credit: Nuno R. Faria



ONT - Speed



New DNA Sequencing Tech

January 17, 2022

[Tweet](#) [Share 1](#) [Share](#) [Email](#)

A new ultra-rapid genome sequencing approach developed by a team of international collaborators was used to diagnose rare genetic conditions in newborns, many of which were unheard of in standard clinical care.

"A few weeks is what most clinicians call 'rapid' when they receive genetic test results," said Euan Ashley, MB, professor of medicine at the University of California San Diego School of Medicine and senior author of the study.

Genome sequencing allows scientists to see a person's complete genetic code, revealing everything from eye color to inherited diseases. These traits are all rooted in their DNA: Once doctors know the specific sequence of a patient's genome, they can quickly identify abnormalities.

Now, a mega-sequencing approach devised by a team of researchers at the University of California San Diego and the Netherlands Cancer Institute offers a new way to speed up diagnostics: Their fastest diagnosis was made in just 10 hours. Such rapid diagnoses, which often require days or weeks in less time in critical care units, require fewer tests and less time for analysis.

A paper describing the researchers' work is published online in *Nature*.

nature

Explore content ▾ About the journal ▾ Publish with us ▾

nature > articles > article

Article | [Open access](#) | Published: 11 October 2023

Ultra-fast deep-learned CNS tumour classification during surgery

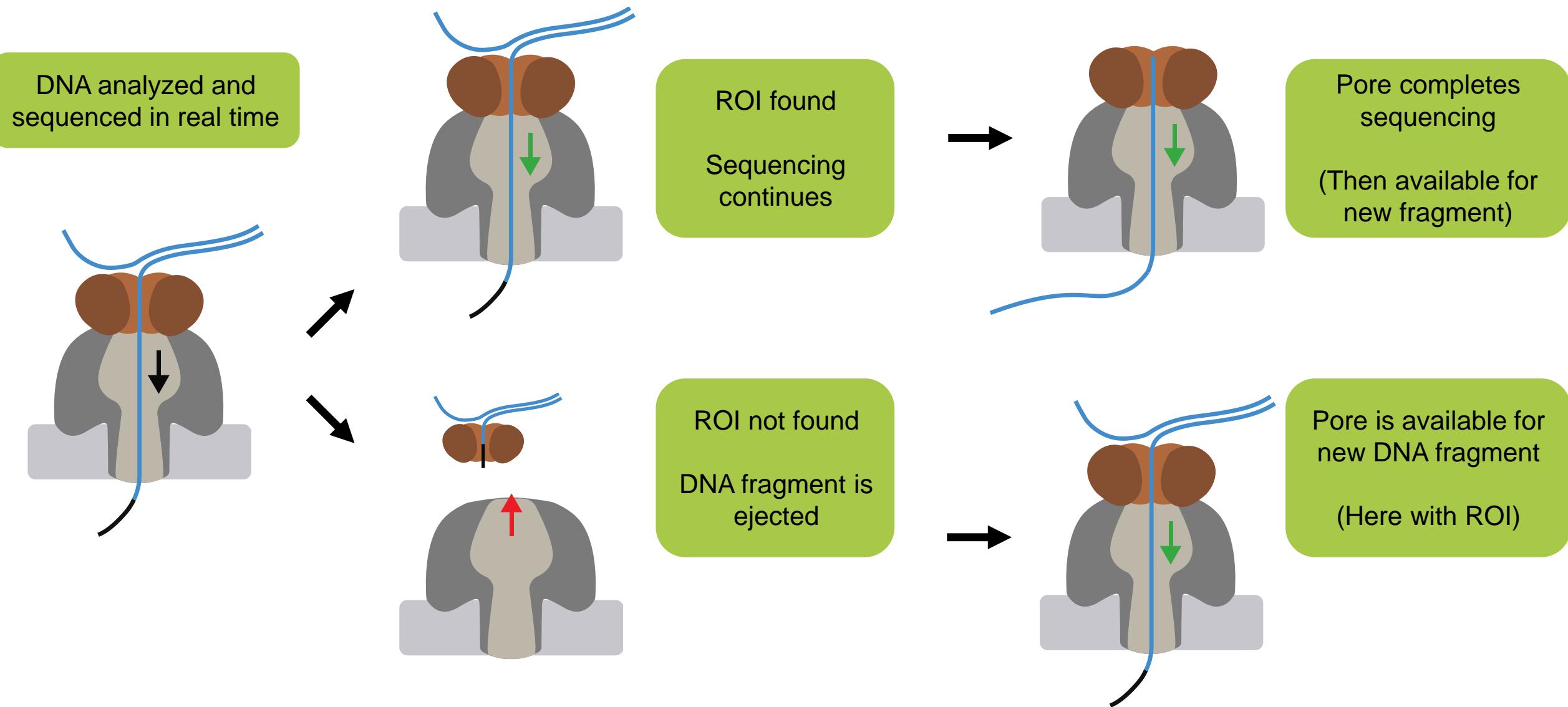
[C. Vermeulen](#), [M. Pagès-Gallego](#), [L. Kester](#), [M. E. G. Kranendonk](#), [P. Wesseling](#), [N. Verburg](#), [P. de Witt Hamer](#), [E. J. Kooi](#), [L. Dankmeijer](#), [J. van der Lugt](#), [K. van Baarsen](#), [E. W. Hoving](#), [B. B. J. Tops](#)✉ & [J. de Ridder](#)✉

Nature **622**, 842–849 (2023) | [Cite this article](#)

34k Accesses | **563** Altmetric | [Metrics](#)

Burnell Professor in Genomics and Precision Health, is the senior author of the paper. Postdoctoral scholar John Gorzynski, DVM, PhD, is the lead author.

ONT target sequencing - adaptive sampling

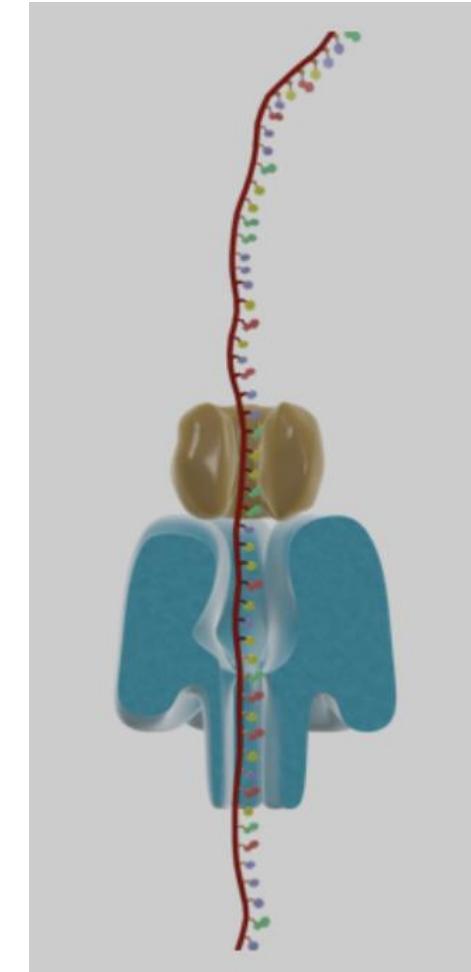


ONT direct RNA sequencing



ONT can sequence native RNA molecules!

- No bias due to cDNA conversion
- Allows to study RNA modifications
- Higher error rate
- Lower throughput



What people are using long reads for...

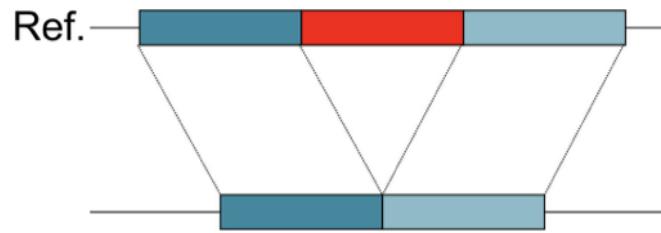


Example 1: Detect all genetic variants

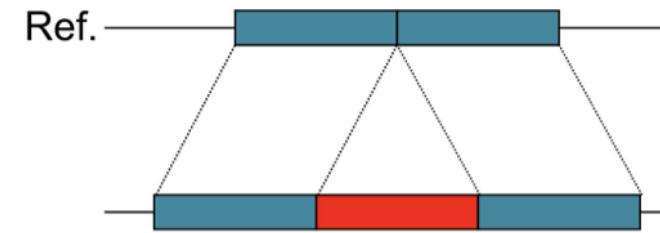


Long-read sequencing can detect more genetic variants than with short reads:

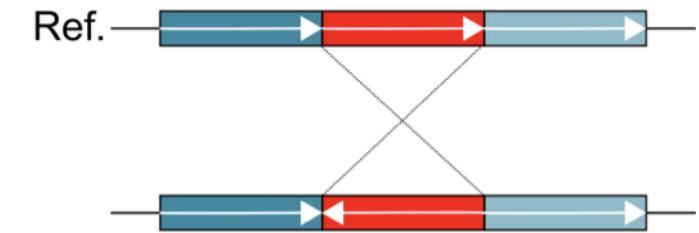
a) Deletion



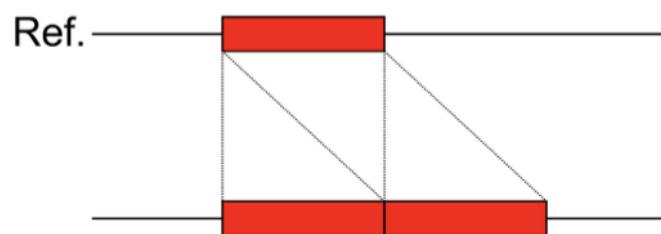
b) Insertion



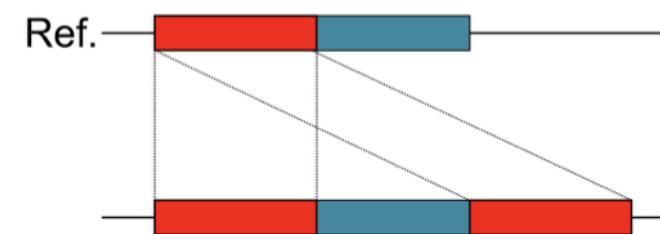
c) Inversion



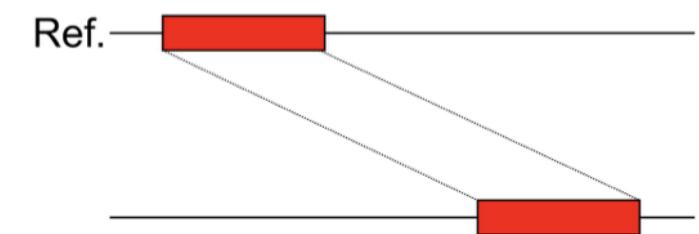
d) Tandem Duplication



e) Interspersed Duplication



f) Translocation



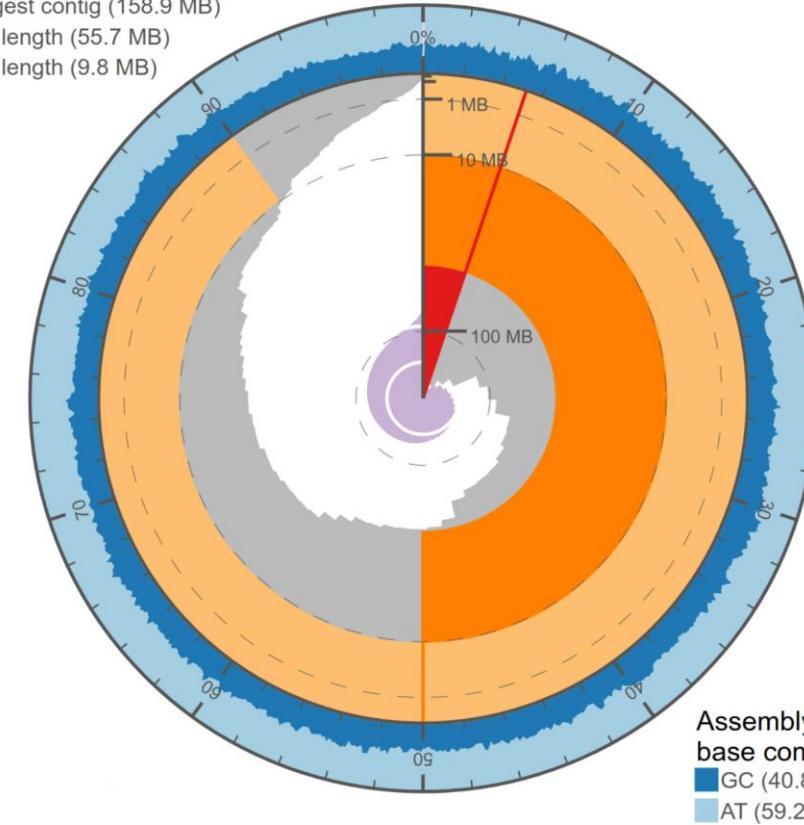
Example 2: Assemble complete genomes



It took just **3.5 h** on a **96** core compute node for *de novo* assembly of a human sample!

| | |
|----------------------------------|------------|
| span (Gbp) | 3.1 |
| GC (%) | 40.84 |
| AT (%) | 59.16 |
| longest contig (Mbp) | 159 |
| contig count | 373 |
| contig N50 length (Mbp) | 56 |
| contig N50 count | 17 |
| contig N90 length (Mbp) | 10 |
| contig N90 count | 59 |

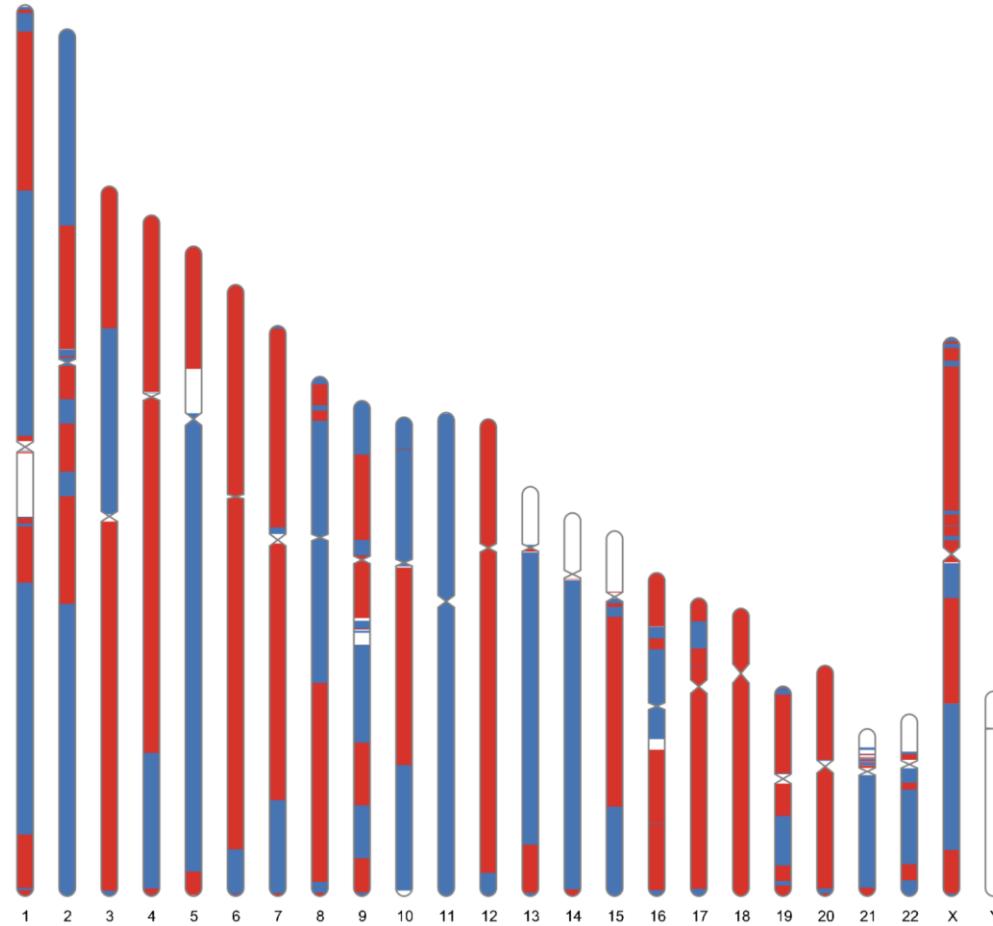
Contig statistics
Log₁₀ contig count (total 373)
Contig length (total 3 GB)
Longest contig (158.9 MB)
N50 length (55.7 MB)
N90 length (9.8 MB)



Assembly
base composition
GC (40.8%)
AT (59.2%)

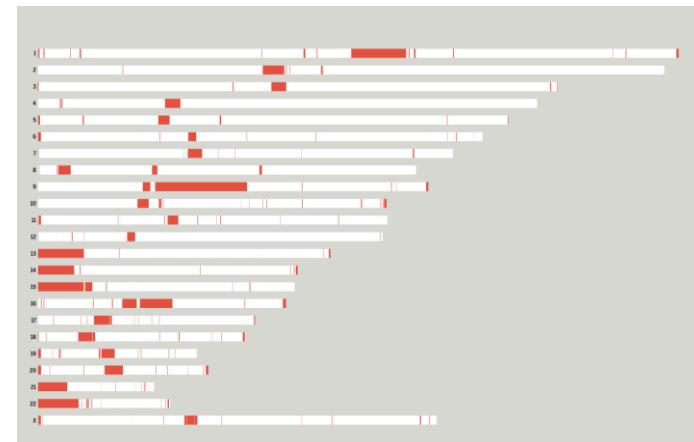
Ignas Bunikis

De novo assembly mapped to GRCh38



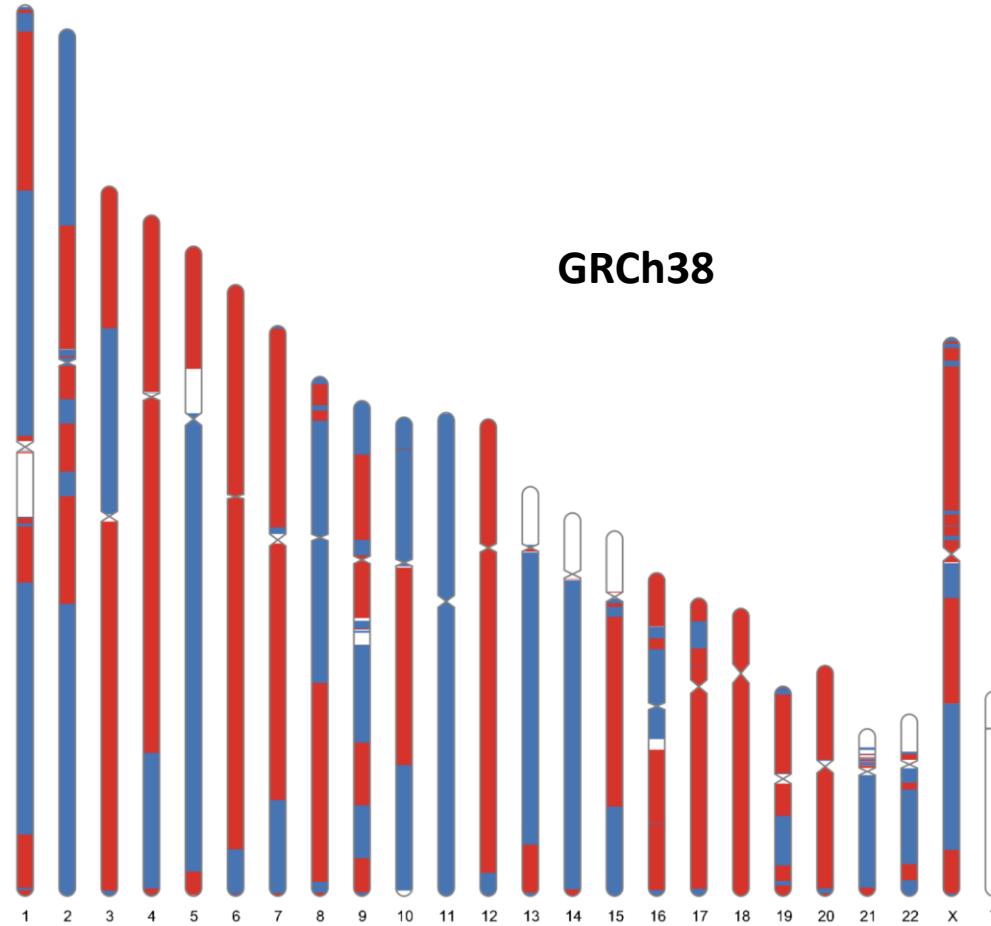
Chromosomes **11** and **18** were assembled in single contigs

...but GRCh38 is missing ~200Mbp of genetic information...

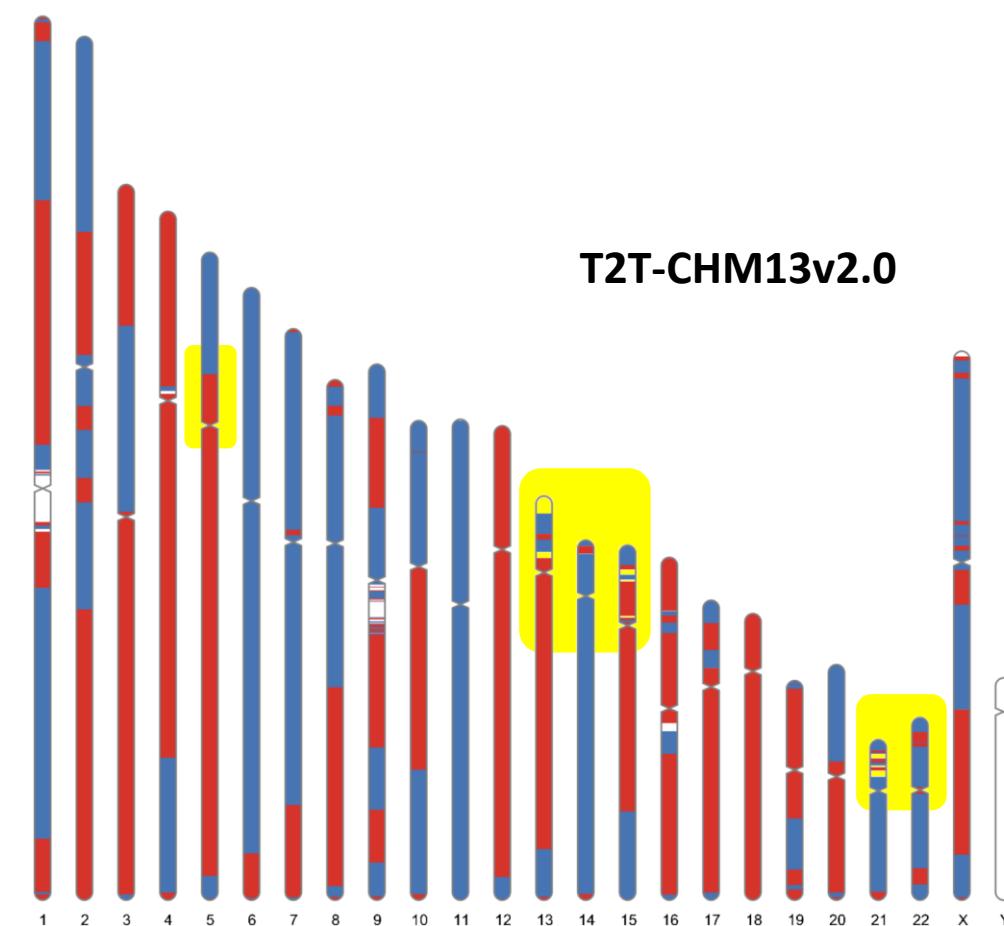


Red segments resolved by T2T Consortium
DOI: [10.1126/science.abp8653](https://doi.org/10.1126/science.abp8653)

De novo assembly mapped to T2T



Colour change represents adjacent contigs

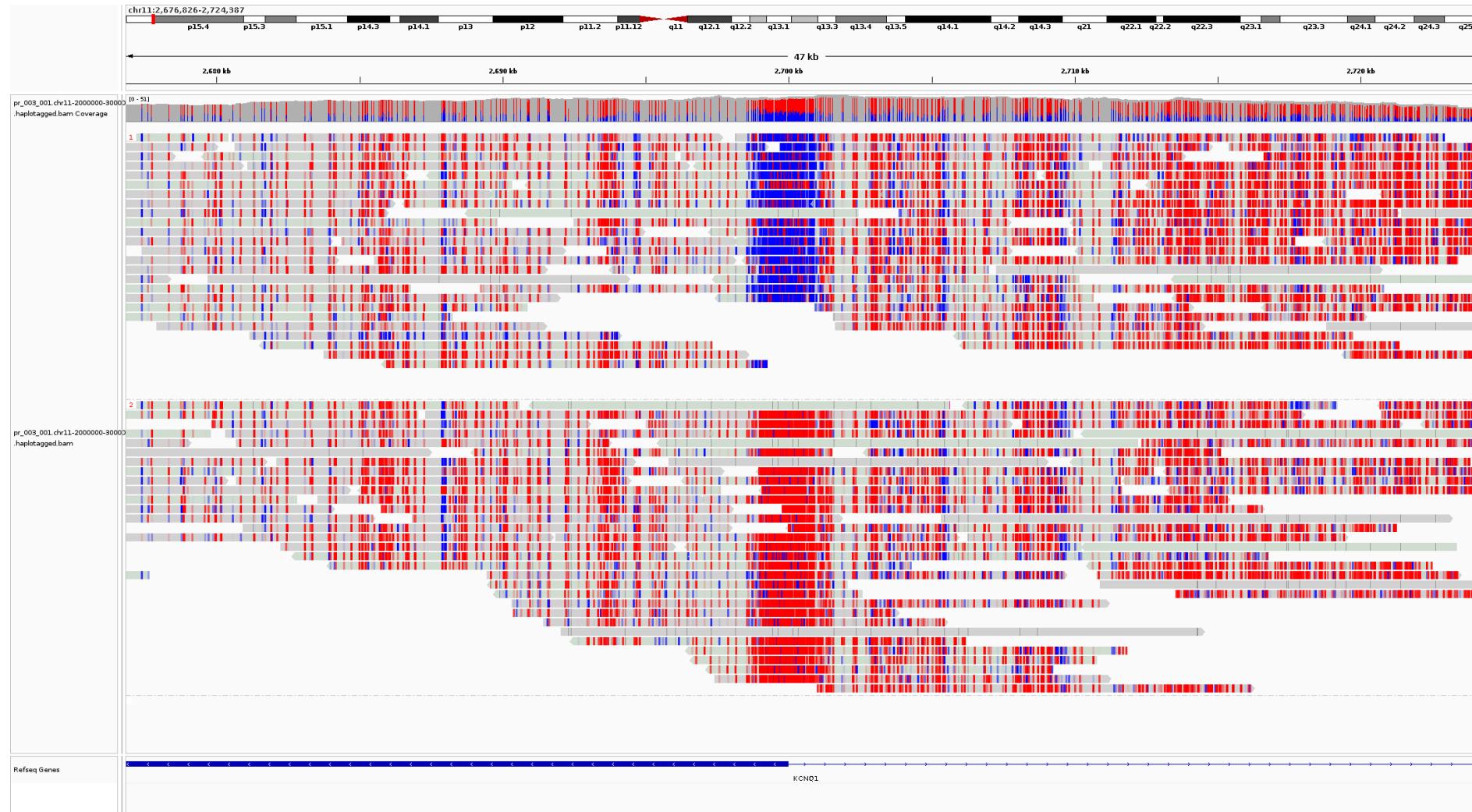


Ignas Bunikis

Example 3: Investigate methylation



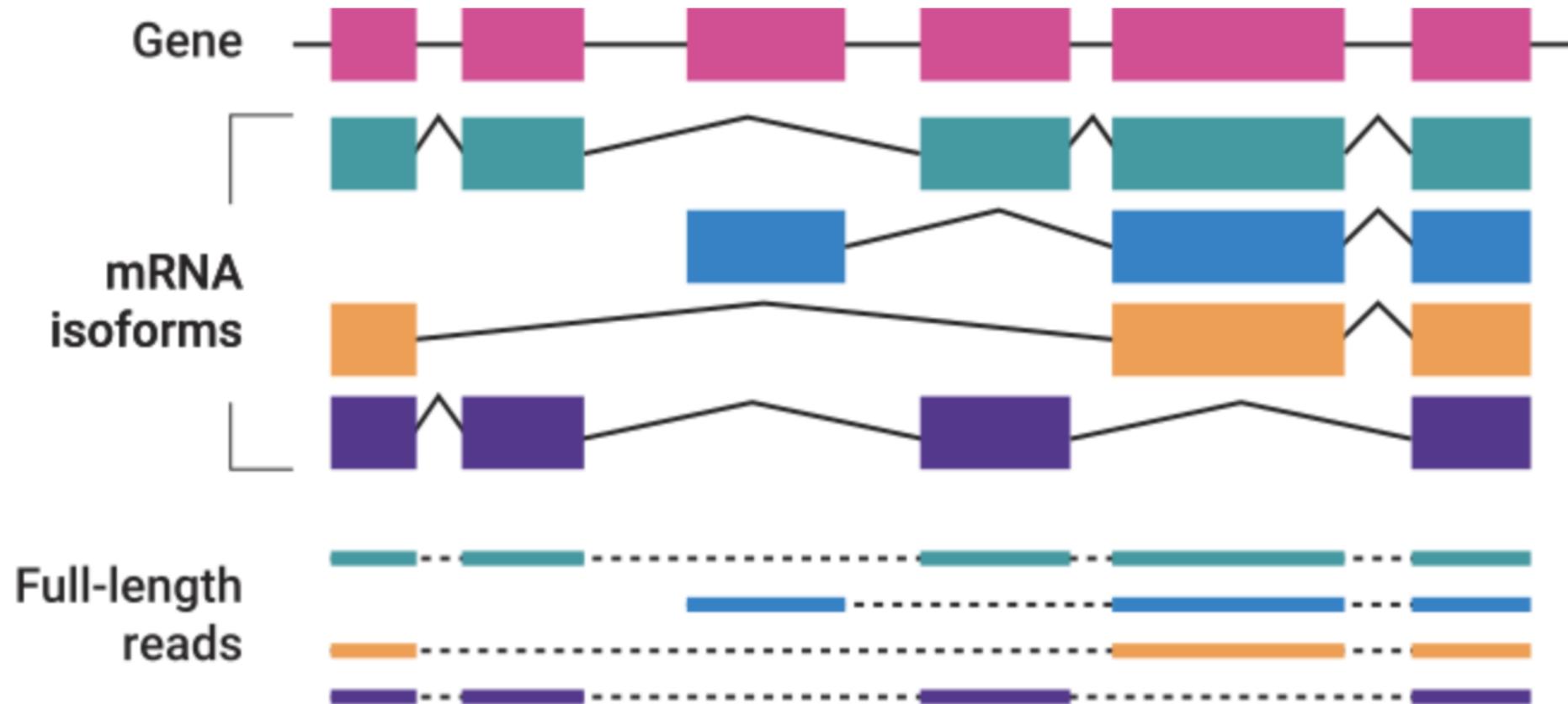
Obtain methylation patterns, phased with haplotypes (example for imprinted region)



Example 4: Full-length RNA sequencing



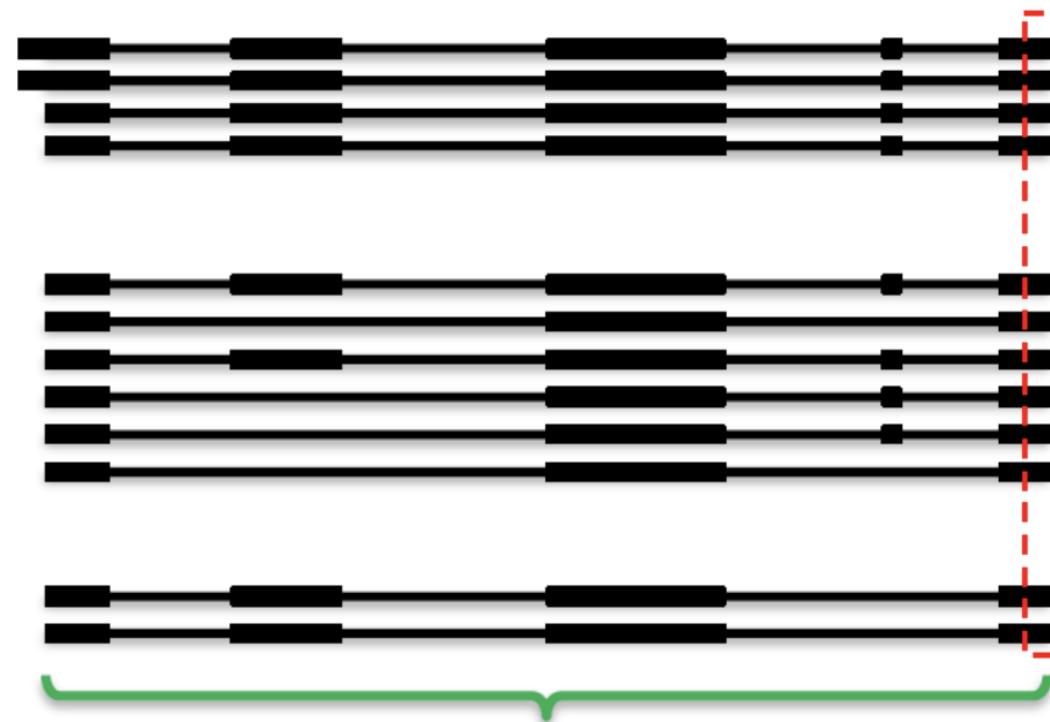
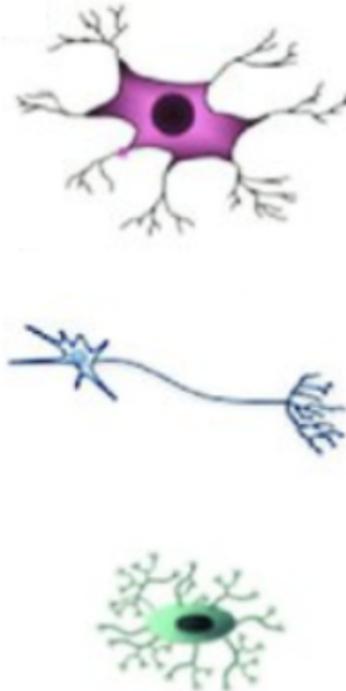
- Get complete information about RNA molecules!



Example 5: Single-cell long-read RNA



- It is possible to study RNA isoforms even in single cells!



Cell type specific
mRNA splicing

Not captured with 3'-end
short-read scRNA seq

Resolved with single-cell full-length RNA seq

Challenge: good sample quality required!



<https://www.qiagen.com/ja-us/applications/molecular-biology-research/hmw-dna>

HMW-DNA Extraction – Options at NGI



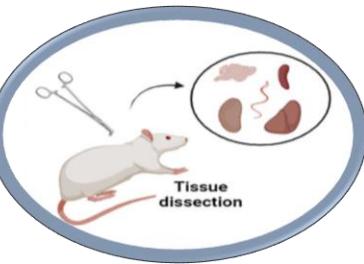
Cells/Blood

$1 \times 10^6 - 5 \times 10^6$



Tissue

25-100 mg



Insects/Mollusc/Crustaceans

25-200 mg



Plants

1-3 g



Fungi

100-600 mg



Commercial Kits

MONARCH

High input quality required
Few special protocols

Top choice for high quality
samples with low amount of
contaminants

NANOBIND

Lower input quality tolerated
Many special protocols

Supplemental buffers for insects
Top choice for most non-standard
samples except for low input and
high polysaccharide samples

Phenol/Chloroform

SDS Lysis

High polyphenol
High recovery for low input

Top choice for samples high
in polyphenols without
polysaccharides

CTAB Lysis

High polysaccharide
Also handles polyphenols

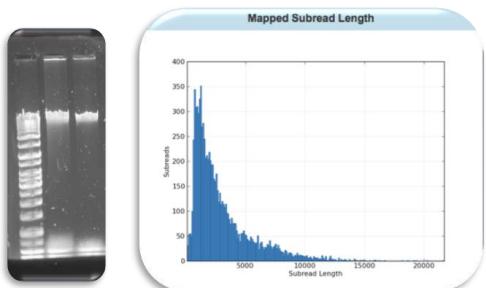
Top choice for plants, fungi,
and other samples high in
polysaccharides

HMW-DNA Extraction – Contaminants

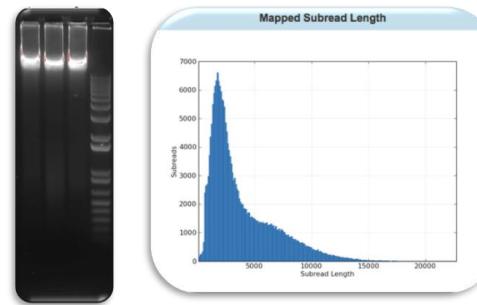


Importance of purity – even for model organisms

Impurities can originate from both host tissue and extraction chemicals.



Same yeast -
different
extractions!



| | | | |
|-------------------|-------|-----------------------|---------|
| Polished Contigs | 223 | Max Contig Length | 36,298 |
| N50 Contig Length | 2,932 | Sum of Contig Lengths | 480,087 |

| | | | |
|-------------------|-----------|-----------------------|-----------|
| Polished Contigs | 9 | Max Contig Length | 1,508,929 |
| N50 Contig Length | 1,353,702 | Sum of Contig Lengths | 7,813,244 |

We extract what we get!



Sequencing of the last supper?

Which would you expect to have less contaminants?



VS



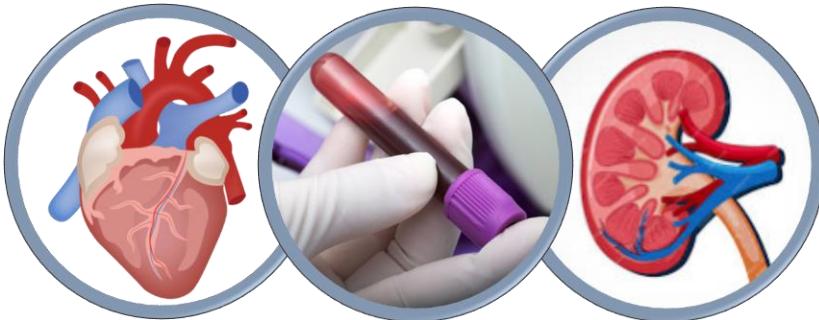
VS



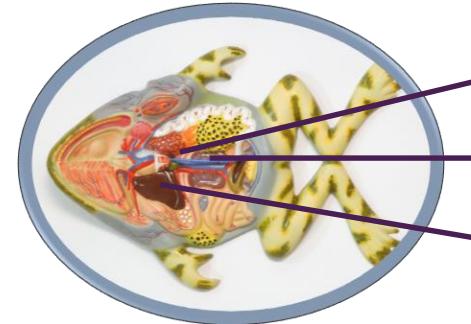
HMW-DNA Extraction – Best Options



- ❑ Plan ahead and divide according to what you plan to do



- ❑ Freeze as fast and cold as possible to minimize fragmentation



- ❑ Choose tissue high in DNA and low in contaminants when possible

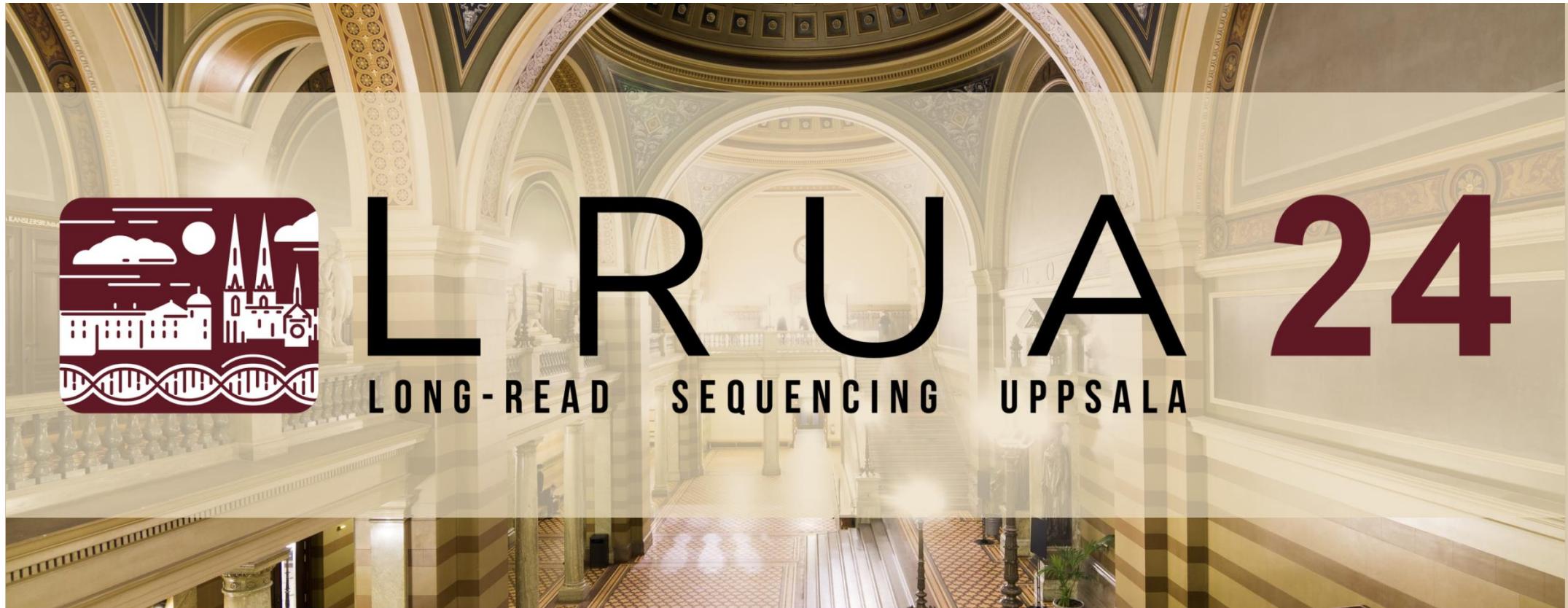


- ❑ All samples are different – Investigate what are best options for your samples!

Long-read Uppsala Meeting 2024!

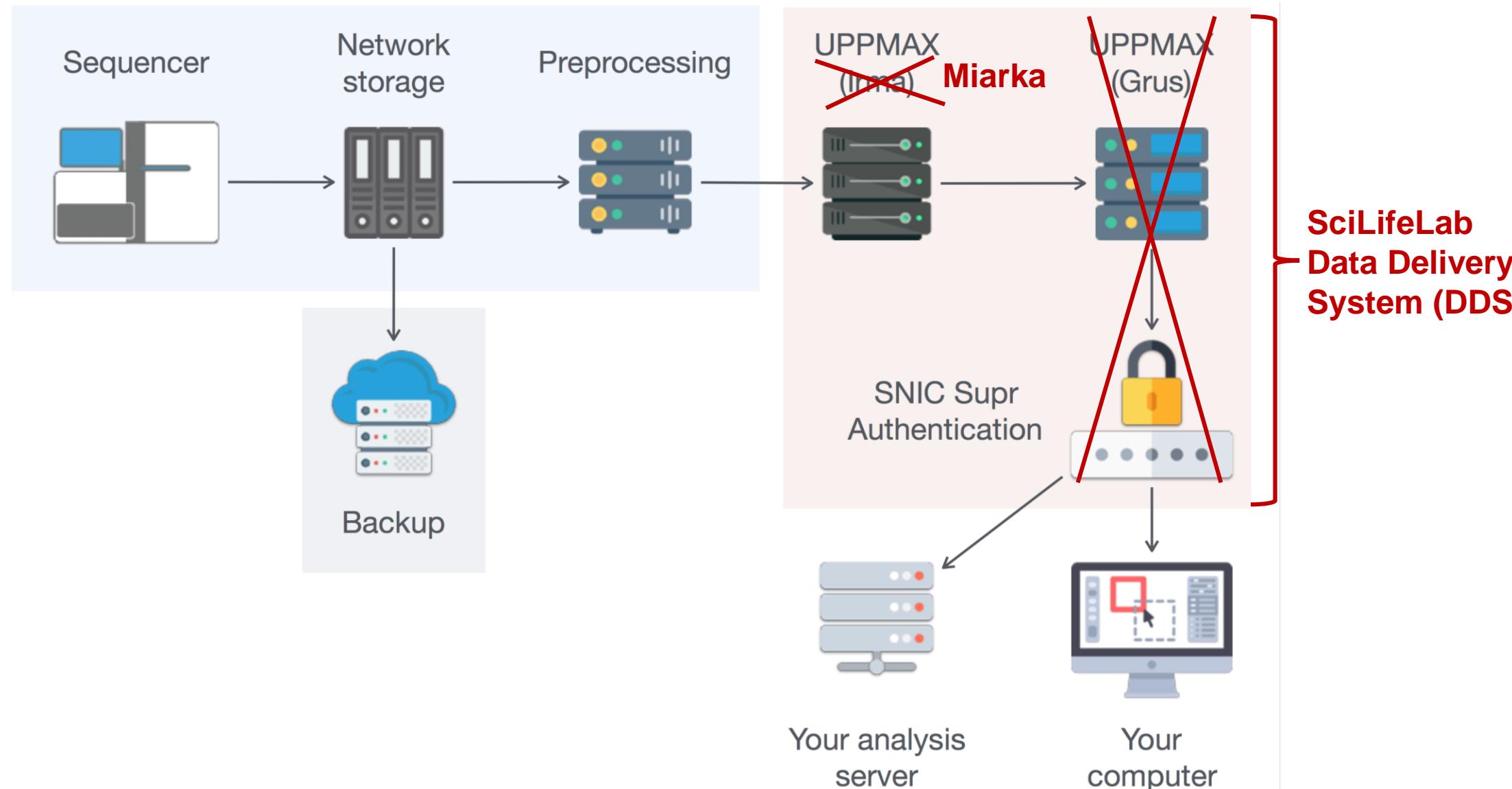


- October 21-23 2024, more information at: www.lrua2024.se



NGI Data Handling and Analysis Pipelines

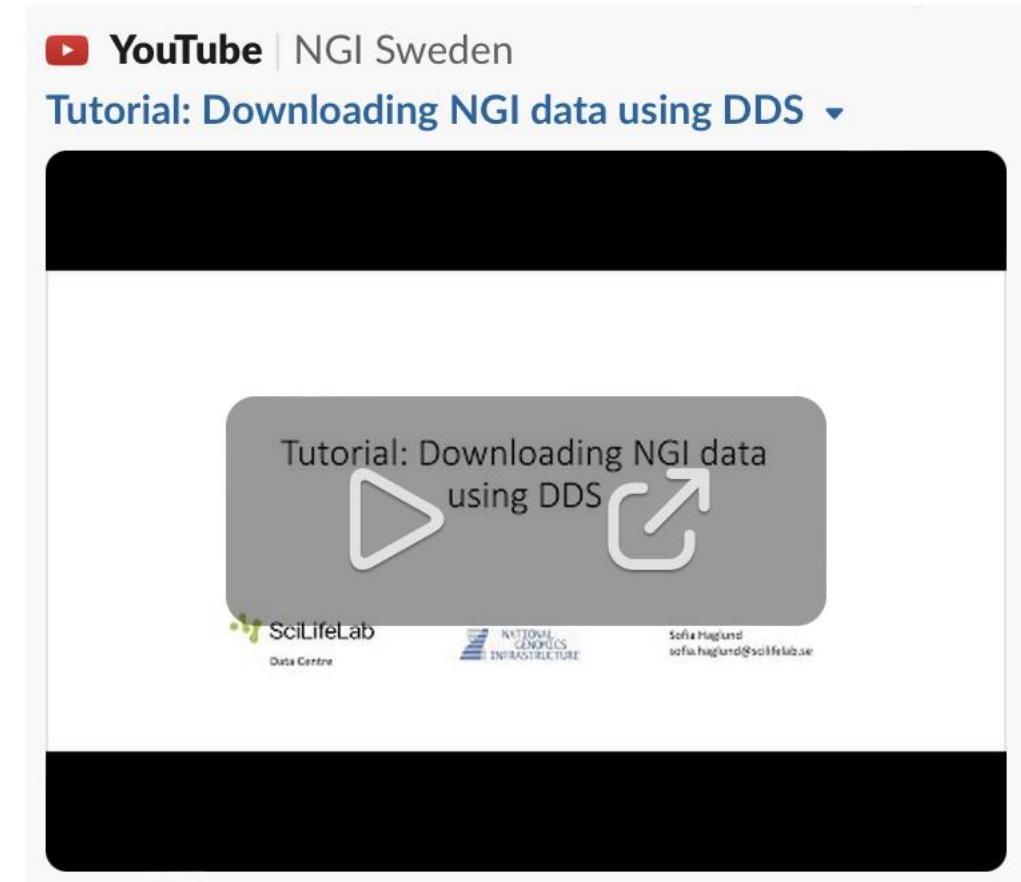
NGI Data Handling



Data delivery via DDS



- DDS is a system for delivery of data from SciLifeLab platforms
 - Cloud-based solution
 - Command line and web interface
 - Can handle also sensitive data
- Instruction video available on Youtube!



Quality control



- Every project has some level of quality control checks
 - Technical run performance
 - Read length distribution
 - Sequencing quality
- Analysis pipelines give application-specific QC
- Reporting done using MultiQC (Illumina projects)



Multi QC example



MultiQC
v1.0

P1234: Test_NGI_Project

- General Stats
- NGI-RNAseq
- Sample Similarity
- MDS Plot
- STAR**
- Cutadapt
- FastQC
- Sequence Quality Histograms
- Per Sequence Quality Scores
- Per Base Sequence Content
- Per Sequence GC Content
- Per Base N Content
- Sequence Length Distribution
- Sequence Duplication Levels
- Overrepresented sequences
- Adapter Content

MultiQC

P1234: Test_NGI_Project

This is an example project. All identifying data has been removed.

Contact E-mail: phil.ewels@scilifelab.se
Application Type: RNA-seq
Sequencing Platform: HiSeq 2500 High Output V4
Sequencing Setup: 2x125
Reference Genome: hg19

Report generated on 2017-05-17, 18:43 based on data in:
/Users/philewels/GitHub/MultiQC_website/public_html/examples/ngi-rna/data

NGI names User supplied names

General Statistics

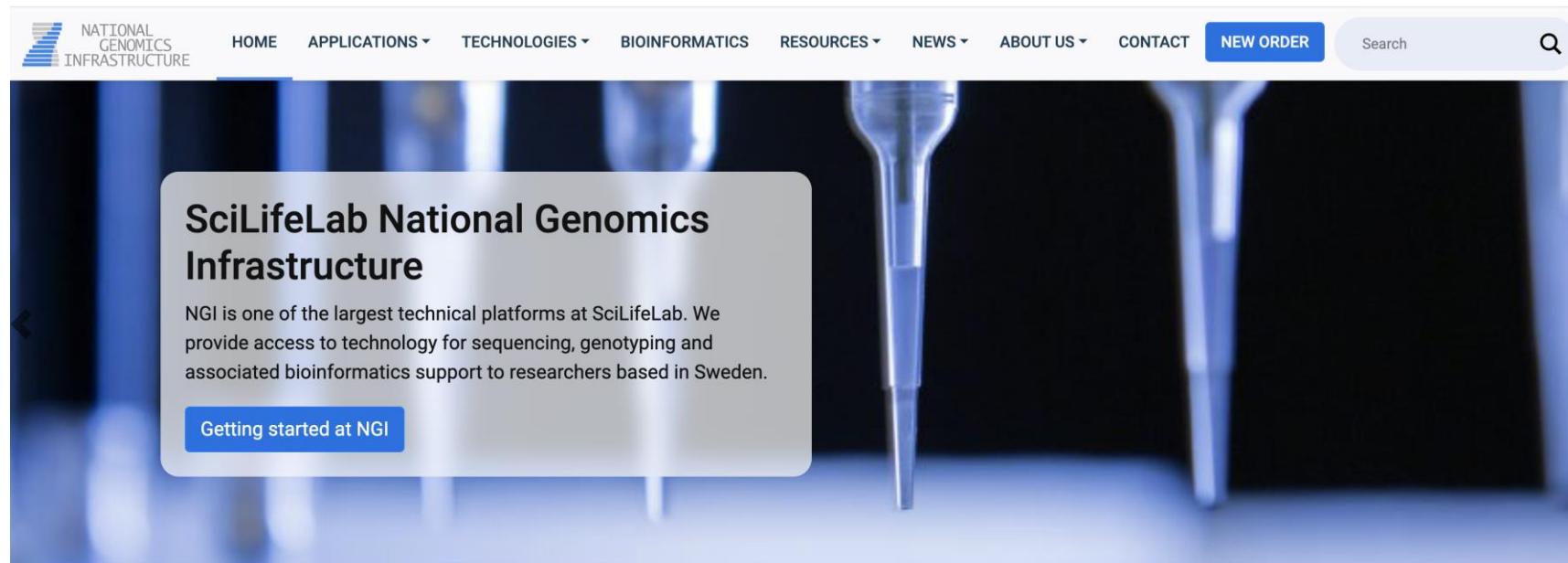
Copy table Configure Columns Plot Showing 22/22 rows and 6/9 columns.

| Sample Name | % Aligned | M Aligned | % Trimmed | % Dups | % GC | M Seqs |
|-------------|-----------|-----------|-----------|--------|------|--------|
| P1234_1001 | 68.2% | 22.8 | 10.3% | 71.3% | 49% | 33.7 |
| P1234_1002 | 67.9% | 20.9 | 10.7% | 70.1% | 50% | 31.1 |
| P1234_1003 | 64.7% | 21.7 | 11.0% | 72.3% | 50% | 33.7 |
| P1234_1004 | 55.2% | 17.0 | 13.2% | 73.4% | 51% | 31.2 |
| P1234_1005 | 53.0% | 17.7 | 15.9% | 75.8% | 52% | 33.8 |
| P1234_1006 | 52.7% | 16.1 | 14.1% | 73.8% | 52% | 30.8 |
| P1234_1007 | 33.0% | 7.0 | 32.0% | 60.5% | 52% | 21.8 |
| P1234_1008 | 27.5% | 4.3 | 44.2% | 79.1% | 50% | 16.7 |
| P1234_1009 | 52.3% | 10.5 | 20.9% | 64.2% | 48% | 20.5 |

Analysis pipelines



- NGI provides data analysis for most applications
- Analysis requirements: Automated, reliable, easy to run, reproducible



nf-core: a popular pipeline system



- A community effort to collect a curated set of Nextflow analysis pipelines
- GitHub organisation to collect pipelines in one place
- No institute-specific branding
- Strict set of guideline requirements

nature biotechnology

Correspondence | Published: 13 February 2020

The nf-core framework for community-curated bioinformatics pipelines

Philip A. Ewels, Alexander Peltzer, Sven Fillinger, Harshil Patel, Johannes Alneberg, Andreas Wilm,
Maxime Ulysse Garcia, Paolo Di Tommaso & Sven Nahnsen 

nf-core 
<https://nf-co.re>

Available pipelines at NGI



- All information available on our website: <https://ngisweden.scilifelab.se>

Amplicon-seq analysis



ATAC-seq analysis

Methylation-seq analysis



ChIP-seq analysis

Genome assemblies with HiFi data



Ion Torrent secondary analysis

Nanopore analysis



PacBio Iso-Seq Analysis

PromethION secondary analysis



Illumina QC analysis

RAD-seq analysis



RNA-fusion analysis

RNA-seq analysis



Small-RNA analysis

Spatial Transcriptomics analysis



WGS and WES germline / somatic analysis

WES and WGS analysis



WGS and WES germline / somatic analysis

Runs with illumina DNA-sequencing data, WGS or targeted sequencing e.g. WES. Aligns to the reference genome, gives QC metrics, does variant-calling and finishes with annotation.

[nf-core/sarek \(paper\)](#) is an analysis pipeline for WGS and targeted sequencing data e.g WES. Previously known as the Cancer Analysis Workflow (CAW), Sarek can handle regular samples or tumour/normal pairs, including relapse samples if required. Sarek was co-developed by NGI.

Sarek analysis can be divided into two different use cases: germline analysis and somatic analysis. These two use cases share the same main steps: mapping, variant calling and annotation.

The screenshot shows the GitHub repository page for nf-core/sarek. It features a green circular icon with a white geometric shape inside. The repository name "nf-core/sarek" is at the top, followed by the URL "https://github.com/nf-core/sarek". A brief description below states: "Analysis pipeline to detect germline or somatic variants (pre-processing, variant calling and annotation) from WGS / targeted sequencing - https://nf-co.re/sarek". At the bottom, there are statistics: 324 forks, 324 stars, and 354 issues.

When we run analysis

We routinely run Sarek germline analysis upon request for human WGS and WES projects. For the Sarek somatic analysis, the decision to run the analysis is made on a case by case basis. If you're interested, please get in touch with us and mention that you would like us to run this analysis.

The analysis currently works with the human reference genomes available in AWS-iGenomes ([GRCh37/GRCh38](#)). If in doubt, please ask whether we can run the pipeline for you.

Input data

Sarek can start from the unprocessed demultiplexed FastQ files from the sequencer together with a small bit of contextual data in the form of a TSV-file. For each sample, the TSV-file should denote the sex of the subject and whether the sample is tumour or normal. In most cases, this information needs to be submitted to NGI by the user.

Results

The pipeline generates BAM alignment files and variant-calling VCF files, along with numerous quality control metrics. For more information, please see the [official documentation](#).

Available pipelines at NGI



Amplicon-seq analysis



ATAC-seq analysis

Methylation-seq analysis



ChIP-seq analysis

Genome assemblies with HiFi data



ion

Ion Torrent secondary analysis

Nanopore analysis



PacBio Iso-Seq Analysis

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Illumina QC analysis

RAD-seq analysis



RNA-fusion analysis

RNA-seq analysis



Small-RNA analysis

Spatial Transcriptomics analysis



WGS and WES germline / somatic analysis

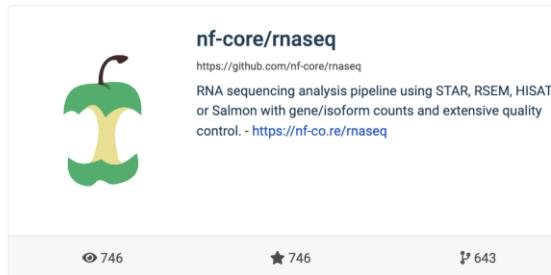


Example: RNA-seq analysis

RNA-seq analysis

Runs with illumina total RNA-sequencing data. Aligns to the reference genome, gives QC metrics and finishes with gene count matrices.

RNA-Seq is a bioinformatics analysis pipeline used for RNA sequencing data. The pipeline is built using [Nextflow](#), a workflow tool to run tasks across multiple compute infrastructures in a very portable manner. It processes raw data from FastQ inputs, aligns the reads, generates counts relative to genes or transcripts and performs extensive quality control on the results.



When we run analysis

We run this analysis routinely for all RNA-seq projects where we have prepared the sequencing library in-house. If you have prepared a library yourself and we are just sequencing, please get in touch and mention that you would like us to run this analysis.

The analysis works with any of the species that have a reference genome available in [AWS-iGenomes](#). If in doubt, please ask whether we can run the pipeline for you.

Input data

bcl2fastq demultiplexed FastQ files and a genome reference.

Results

The pipeline generates aligned BAM-files, gene count matrices and FPKM metrics for genes and transcripts, along with numerous quality control metrics. For more information, please see [https://nf-co.re/rnaseq/\[release\]/docs/output](https://nf-co.re/rnaseq/[release]/docs/output)

Available pipelines at NGI



Amplicon-seq analysis



ATAC-seq analysis



Methylation-seq analysis



ChIP-seq analysis



Genome assemblies with HiFi data



ion

Ion Torrent secondary analysis

Nanopore analysis



PacBio

PacBio Iso-Seq Analysis

PromethION secondary analysis



illumina

Illumina QC analysis

RAD-seq analysis



RNA-fusion analysis

RNA-seq analysis



Small-RNA analysis

Spatial Transcriptomics analysis



WGS and WES germline / somatic analysis



ChIP-seq analysis



ChIP-seq analysis

Runs with ChIP sequencing data. Pre-processes raw data from FastQ inputs, aligns the reads and performs peak calling and extensive quality-control on the results.

ChIP-Seq is a bioinformatics best-practice analysis pipeline used for chromatin immunoprecipitation (ChIP-seq) data analysis. The pipeline uses [Nextflow](#), a bioinformatics workflow tool. It pre-processes raw data from FastQ inputs, aligns the reads and performs peak calling and extensive quality-control on the results.

The screenshot shows the GitHub repository page for 'nf-core/chipseq'. The repository name is 'nf-core/chipseq' with the URL 'https://github.com/nf-core/chipseq'. A brief description follows: 'ChIP-seq peak-calling, QC and differential analysis pipeline.' Below the description is a green icon of a DNA double helix. At the bottom of the screenshot, there are three metrics: 161 forks, 161 stars, and 130 issues.

When we run analysis

We run this analysis routinely for all ChIP-seq projects where we have prepared the sequencing library in-house. If you have prepared a library yourself and we are just sequencing, please get in touch and mention that you would like us to run this analysis.

The analysis works with any of the species that have a reference genome available in [AWS-iGenomes](#). If in doubt, please ask whether we can run the pipeline for you.

Input data

bcl2fastq demultiplexed FastQ files and a genome reference.

Results

The pipeline generates aligned BAM-files, files with information about called peaks, along with numerous quality control metrics. For more information, please see <https://nf-co.re/chipseq/docs/output>.

Available pipelines at NGI



Amplicon-seq analysis



ATAC-seq analysis

Methylation-seq analysis



ChIP-seq analysis

Genome assemblies with HiFi data



Ion Torrent secondary analysis

Nanopore analysis



PacBio Iso-Seq Analysis

PromethION secondary analysis



Illumina QC analysis

RAD-seq analysis



RNA-fusion analysis

RNA-seq analysis



Small-RNA analysis

Spatial Transcriptomics analysis



WGS and WES germline / somatic analysis

Genome assembly with HiFi data



Genome assemblies with HiFi data

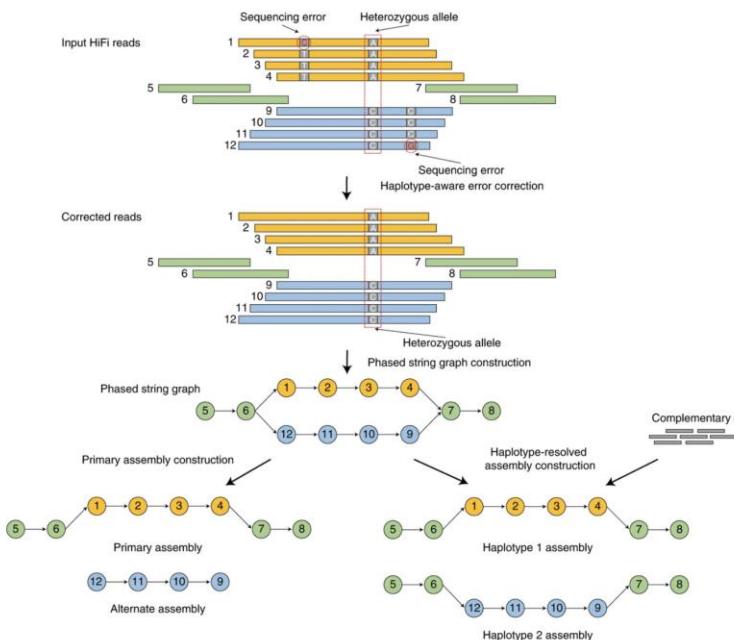
NGI can generate high quality assemblies using IPA and hifiasm assemblers

hifiasm &



Improved Phased Assembler (IPA) is the official PacBio software for HiFi genome assembly. IPA was designed to utilize the accuracy of PacBio HiFi reads to produce high-quality phased genome assemblies.

Hifiasm is a fast haplotype-resolved *de novo* assembler for PacBio HiFi reads. It emits partially phased assemblies of quality competitive with the best assemblers. Given parental short reads or Hi-C data, it produces arguably the best haplotype-resolved assemblies so far.



**Not yet implemented as a
nf-core pipeline!**



Trend: On-instrument analysis

More and more analyses being done on instrument GPUs

Illumina NovaSeqX

Mapping and variant calling (Dragen)



PacBio Revio

Onboard generation of HiFi reads



→ Can speed up and streamline the analysis process...

You can also get help from NBIS!

[About us](#)[Services](#)[Training](#)[Contact](#)

A distributed national bioinformatics infrastructure supporting life sciences in Sweden

[Get support](#)

- All solutions are not available from NGI, but NBIS has lots of experts!



Some tips for data analysis...

Think about analysis early on – already when planning the project!

- Which tools should be used?
- Can I run the analysis myself, or do I need assistance?
- Where should the analysis be run?
- Do I have enough storage space?
- Where should the data eventually be archived?

NGI strategic projects and collaborations



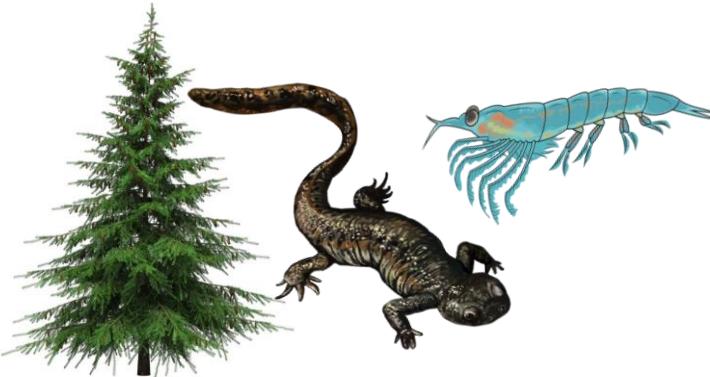
We are involved in some larger national and international projects...



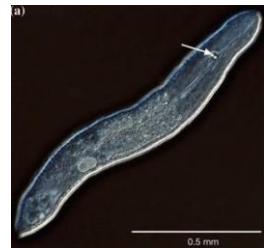
Biodiversity genomics



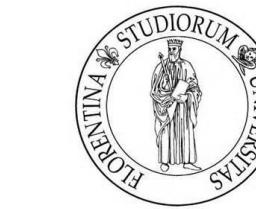
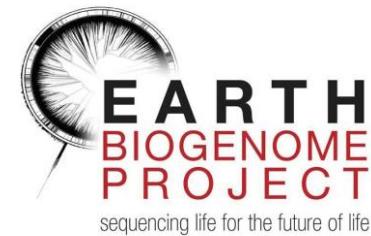
Reference genomes of **any** organism - a very challenging endeavour



Large genomes (18-22Gb)



Tiny organisms
with large genomes



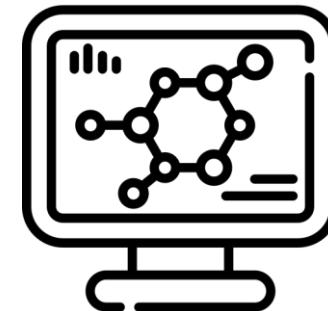
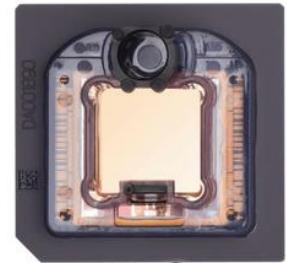
And many, many, ..., many other uncooperative

Reference genome sequencing



NGI & NBIS can help out with:

- DNA/RNA extractions
- Long-read sequencing
- Hi-C Illumina sequencing
- RNA sequencing
- De novo assembly
- Genome annotation



Human genome analysis



Photo: SVT

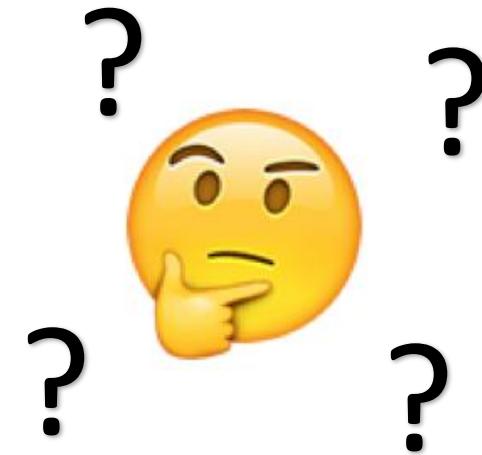
Human population-level sequencing



2017: 1,000 genomes sequenced on Illumina



2024: Time to do it again, with long reads?



How to build a long-read reference dataset



- Wishlist for a new Swedish population cohort (still in early planning)

| | Description | Priority |
|----------------------------------|---|------------|
| Consent for data sharing | It must be possible to share individual-level variant information (VCF files) on national level and ideally also internationally | Crucial |
| Amount and quality of DNA | At least 5ug of high-quality DNA per individual, ideally from fresh samples extracted for long-read sequencing | Crucial |
| Phenotype information | Detailed phenotype information available, that can be used for specific research projects (after approval) | Important |
| A cross-section of Sweden | The individuals should not be enriched for a specific disease or phenotype, and reflect the genetic variation in Sweden (ideally including ethnic minorities) | Important |
| Additional OMICS data | Possibility to perform other OMICs studies (RNA, protein, etc) on samples from the same individuals | Important |
| Available SNP array data | Data from SNP arrays, that can be used to infer the genetic background and select representative individuals for sequencing | Beneficial |
| Funding and resources | Possibility to get additional local funding and resources (for re-consent, sample collection, DNA extraction, etc.) | Beneficial |

We hope to do this as part of a EU project



“Genome of Europe” is a new EU initiative within the 1+MG project



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Beyond 1 Million Genomes

The Beyond 1 Million Genomes (B1MG) project is helping to create a network of genetic and clinical data across Europe. The project provides coordination and support to the 1+ Million Genomes Initiative (1+MG). This initiative is a commitment of 23 European countries to give cross-border access to one million sequenced genomes by 2022.

But B1MG will go ‘beyond’ the 1+MG Initiative by creating long-term means of sharing data beyond 2022, and enabling access to beyond 1 million genomes. See the [About page](#) for an overview of the project.

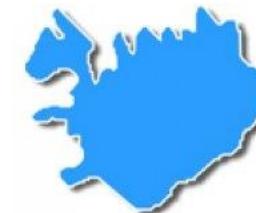
Collaborations on Rare Disease



We are collaborating with Genomic Medicine Sweden - Rare Disease Group

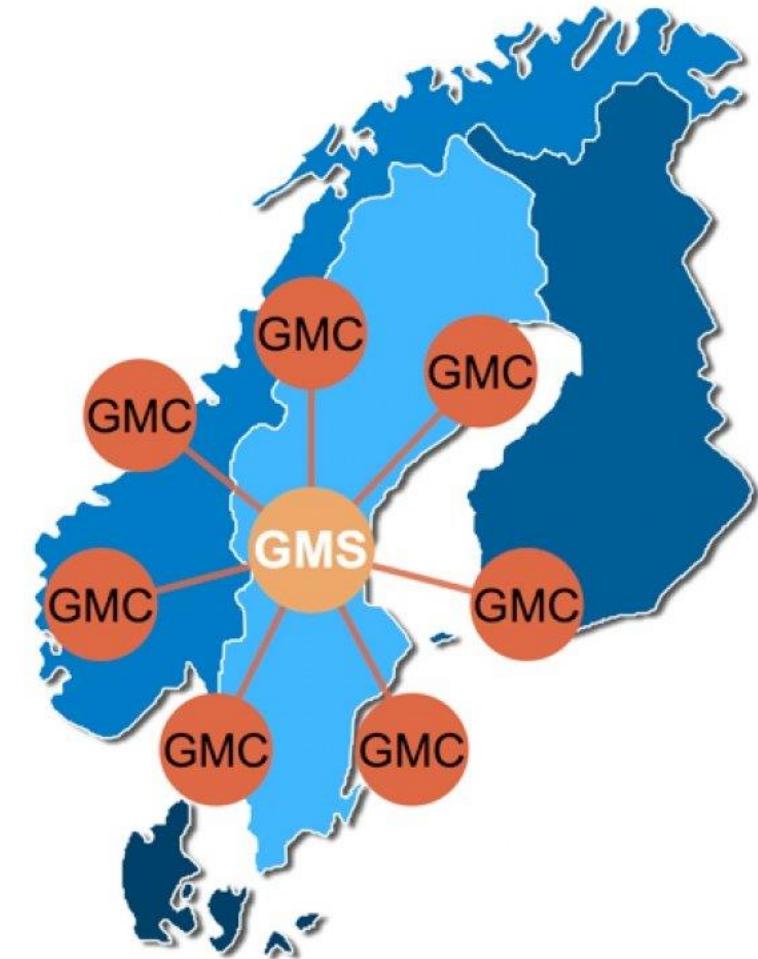
Long-Read Whole Genome Sequencing

- Improve diagnostics of rare disease patients
- Resolve complex SVs and other variants
- PacBio Revio and ONT PromethION



Long-Read Targeted Sequencing

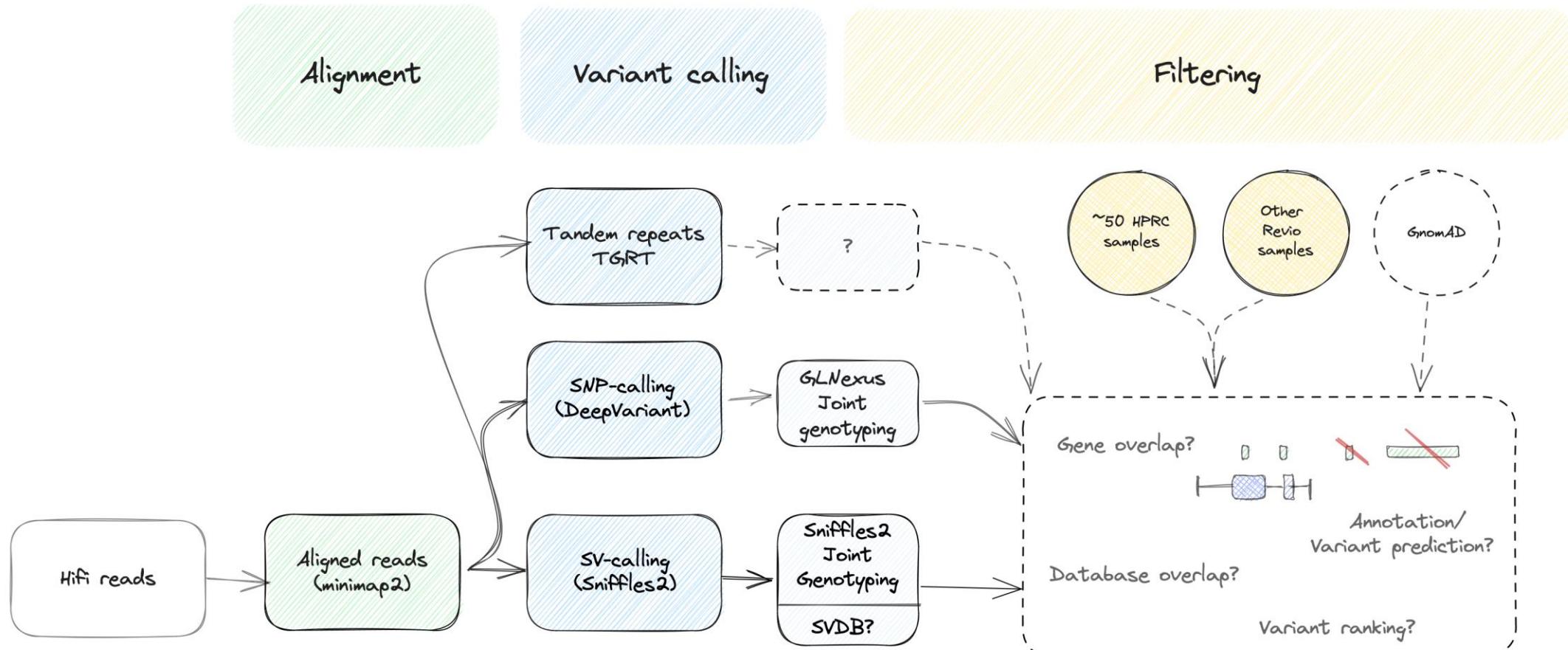
- Develop clinical assays for repeat expansions
- Cas9-based capture or adaptive sampling
- Aim: implementation at different hospital nodes



Analysis pipelines human WGS

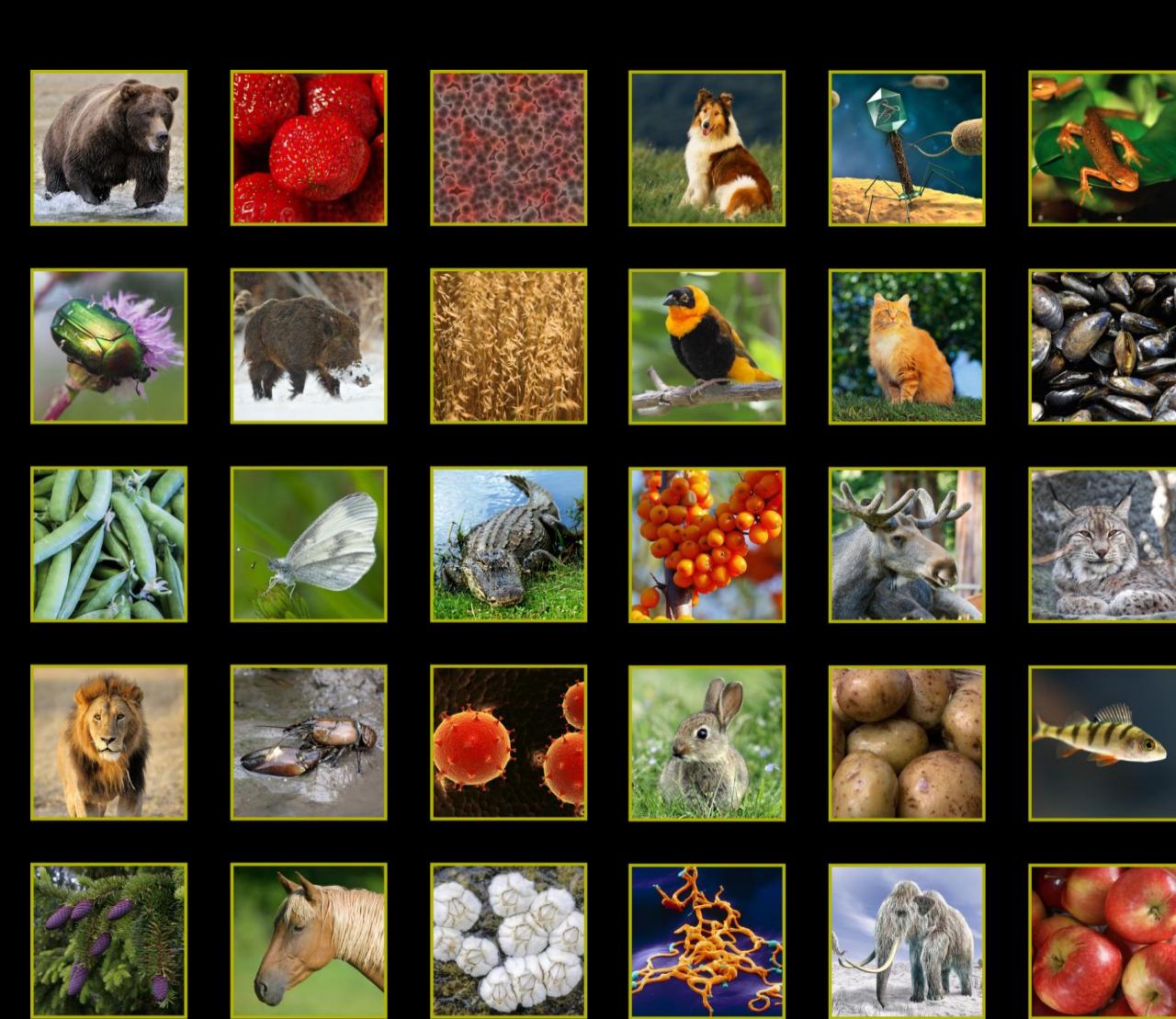


- New pipelines needed for human long-read sequencing



Thanks for your attention!







Diabetes
Alzheimer's disease
Whole-genome sequencing
Gene therapy
Infection screen
Whole-transcriptome sequencing
Target sequencing
Cancer prognosis
Gene regulation
Crohn's disease
Genomics of ageing
Exome sequencing
Schizophrenia
Cancer diagnostics
Organ donor matching
Gut microflora
Gene fusions
RNA editing
HIV
HPV
HCV
Scoliosis
Immune response
Monogenic disorders
Sudden infant death
Cervical cancer
Lynch syndrome
Leukemia
Scoliosis
HLA typing
Dyslexia
MRSA / BBBA screen
Sudden cardiac arrest
Transcriptional regulation
Prenatal diagnostics
Muscle dystrophy
Individualised cancer therapy
and much more...