Epigenomics methods at NGI

GANATIONALCTAC
ATCAGENOMICSGT
INFRASTRUCTURE

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Overview

- The **N**ational **G**enomics **I**nfrastructure (NGI)
- **DNA methylation**
- **Omni-C (HiC)**
- **Omni-ATAC**
- **Oxford Nanopore (ONT) Sequencing**

What word comes to mind when you think of epigenetics?

epigenomics

outside genome

chromatin modifications

changes

regulation

three dimensional

histones

hi stone modification

methylation

complexity

dna methylation

modification

chromatin

gene expression

midification

not dna sequence

chromatin accessibility

modifications

control of gene expressio

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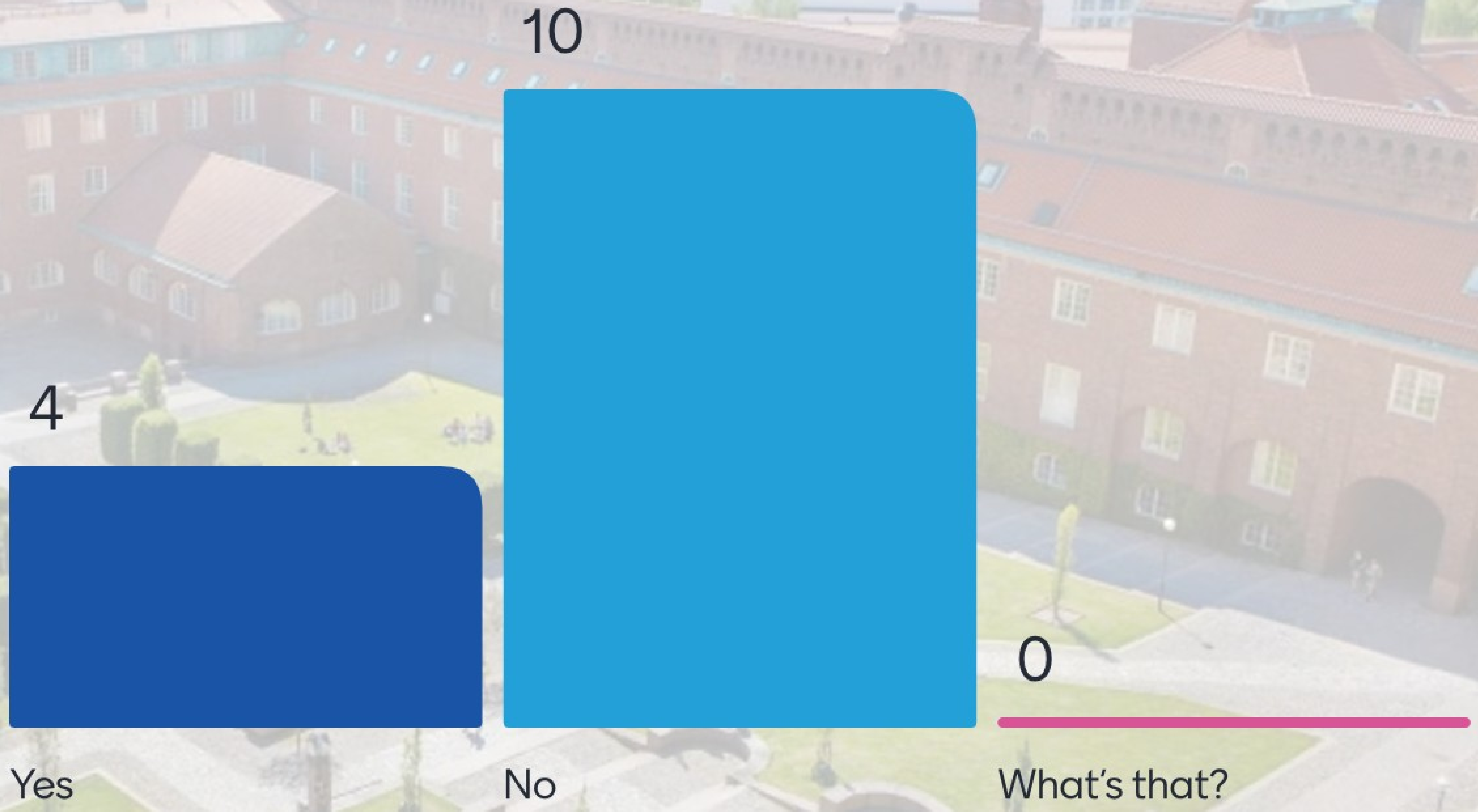
Have you ever submitted a project to NGI?



NGI Sweden Nodes

NGI stockholm

The **SNP&SEQ** Technology Platform

Uppsala Genome Center - **UGC**

http://snpseq.medsci.uu.se/
http://ugc.igp.uu.se/

NGI Stockholm

1331 projects

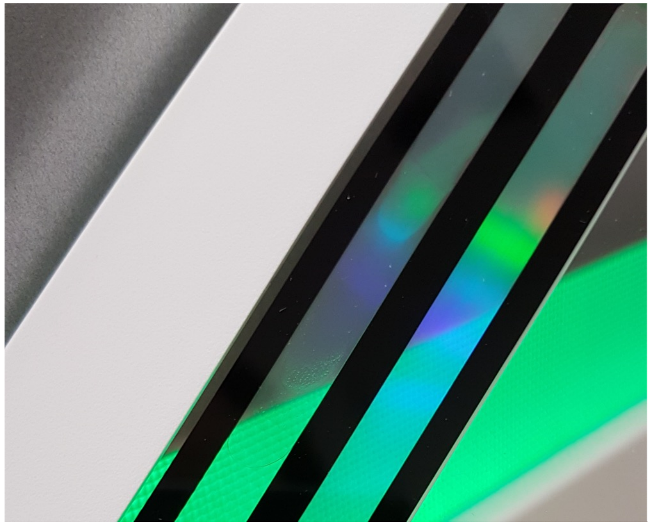
133 000 samples

2024

4,500 human genomes at 30X

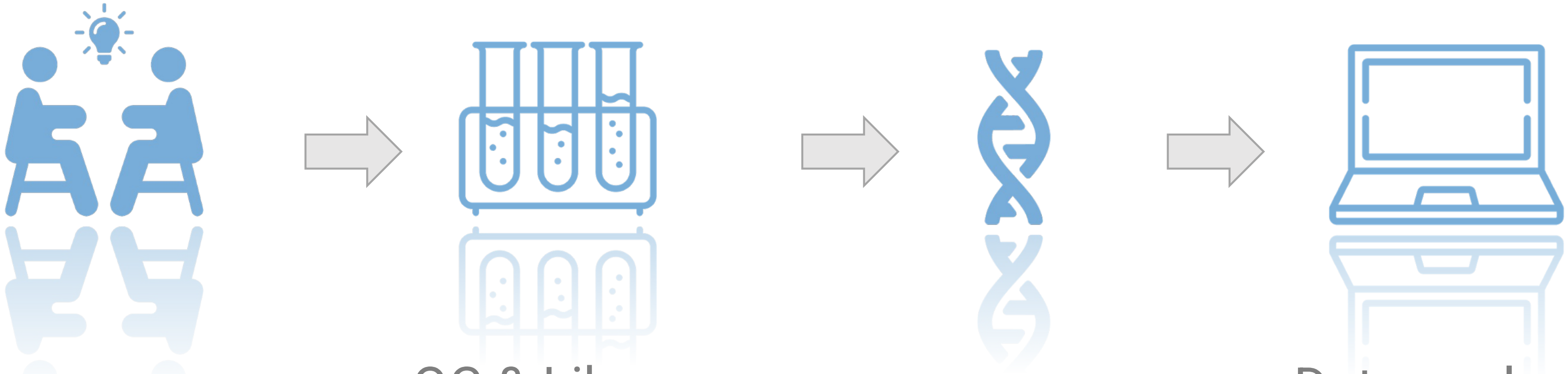
833 Tbp

NGI stockholm



https://ngisweden.scilifelab.se/resources/ngi-stockholm-status/

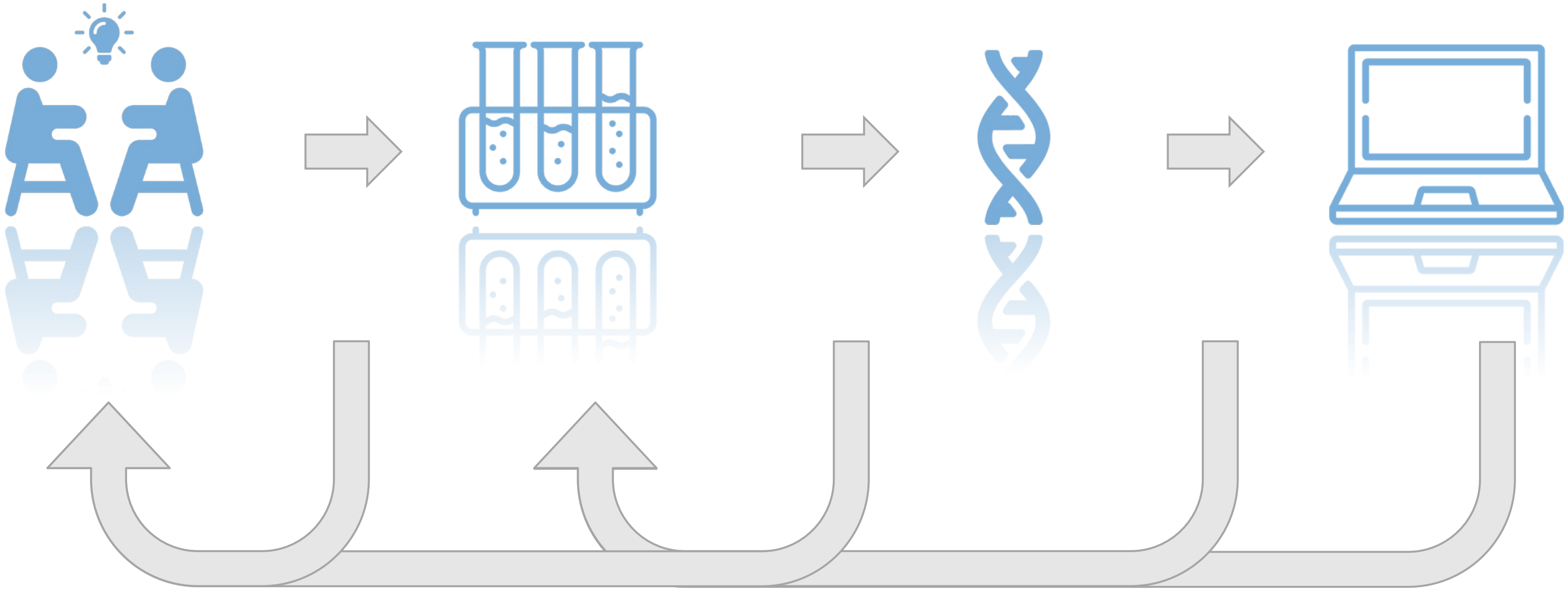
NGI Workflow



Meetings → QC & Library Preparation → Sequencing → Data analysis & delivery

We provide **guidelines and support** for sample collection, study design, protocol selection and bioinformatics analysis

NGI Workflow



NGI Workflow

Meetings

1) Here are a few challenges that users often face:
   - Not knowing enough about their own sample
   - Lacking the necessary tools for quality control
   - Needing more bioinformatics capabilities
   - Feeling lost in communication/literature

2) NGI's tools
   - Knowledge gathered from diverse sample types
   - Providing guidance and sample QC service, clear thresholds and requirements.
   - Offering basic data QC and connecting to NBIS
   - Accessing the website and scheduling one-to-one meetings

NGI stockholm

- De novo sequencing
- DNA resequencing
- Epigenetics
- Single-cell
- Transcriptomics
- Proteomics with NGS readout

- DNA extractions
- Environmental sequencing
- Genotyping
- Spatial
- User-prepared libraries

Overview

- SciLifeLab
- The National Genomics Infrastructure
- DNA methylation
- Omni-C (HiC)
- Omni-ATAC
- ONT Sequencing

DNA methylation analysis

Library preparation for whole genome methylation sequencing

1. NEBNext® Enzymatic Methyl-seq (EM-Seq)
2. SPlinted Ligation Adapter Tagging (SPLAT) for Whole Genome Bisulphite sequencing

   *Developed at NGI!**

1. Illumina EPIC Arrays.

*Amanda Raine, Erika Manlig, Per Wahlberg, Ann-Christine Syvänen, Jessica Nordlund, SPlinted Ligation Adapter Tagging (SPLAT), a novel library preparation method for whole genome bisulphite sequencing, *Nucleic Acids Research*, Volume 45, Issue 6, 7 April 2017, Page e36

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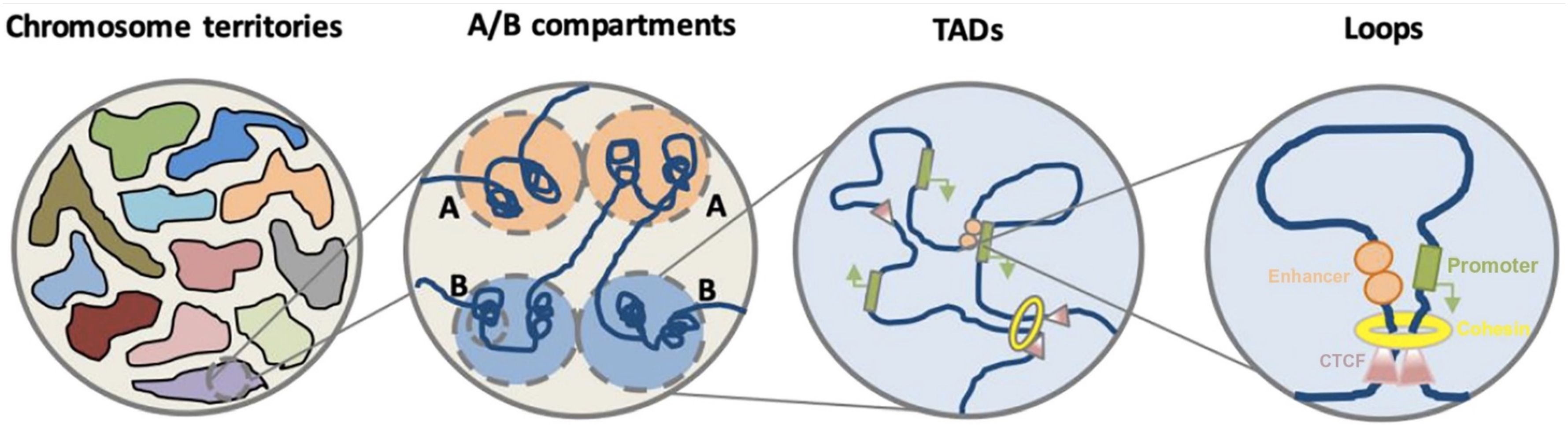
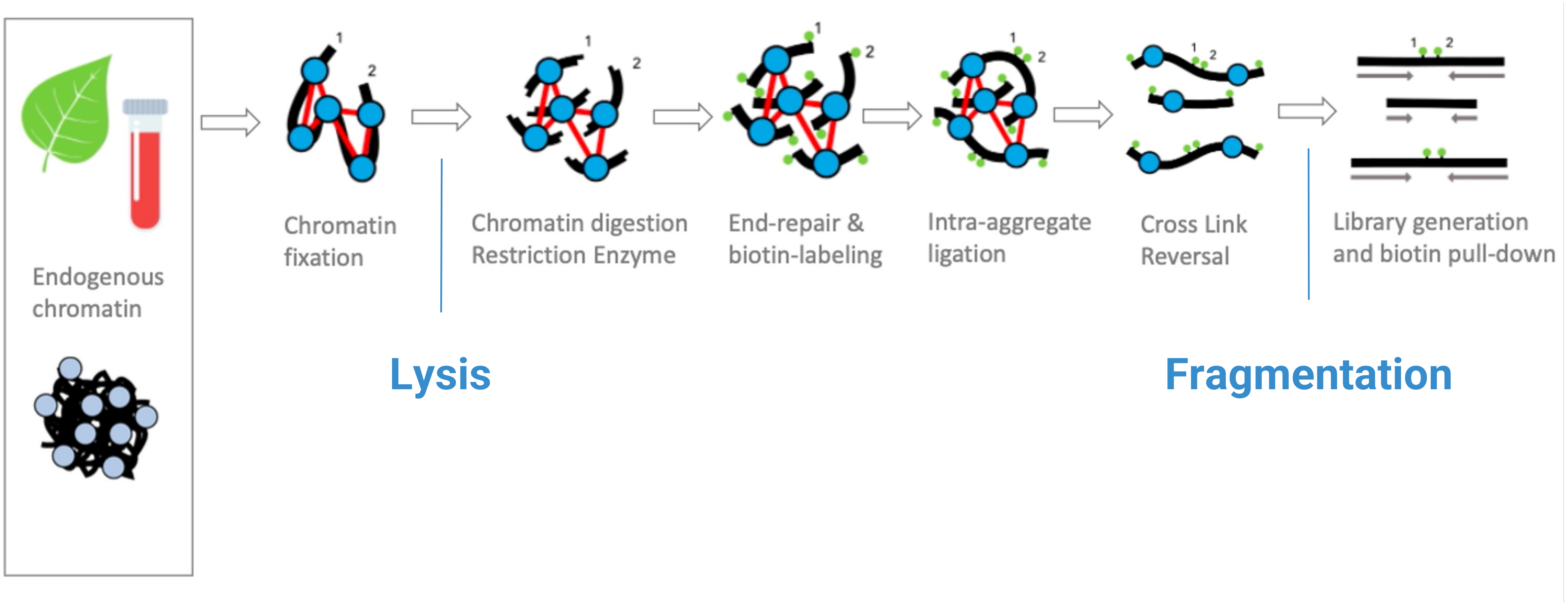
Epigenetics - HiC



doi.org/10.1016/j.tibs.2019.03.001

HiC: Restriction Enzyme



Endogenous chromatin

Chromatin fixation

Chromatin digestion Restriction Enzyme

End-repair & biotin-labeling

Intra-aggregate ligation

Cross Link Reversal

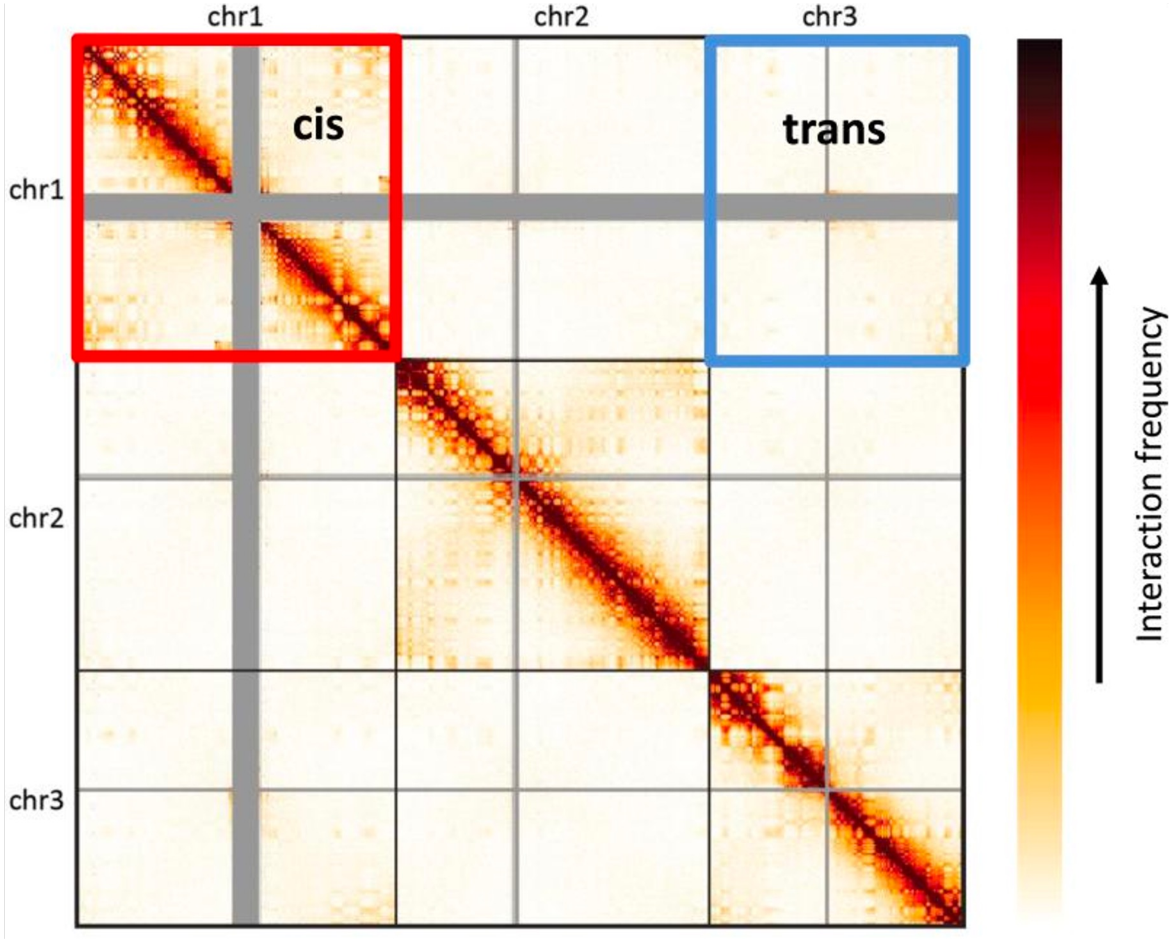
Library generation and biotin pull-down

Lysis

Fragmentation

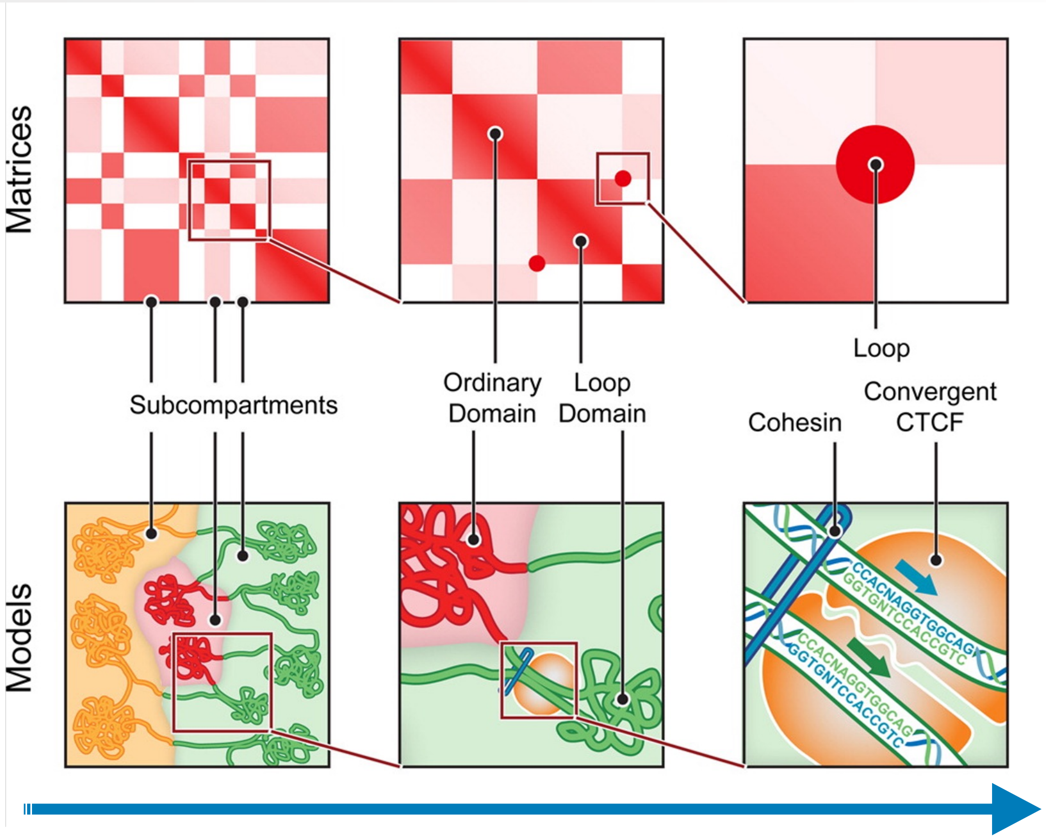
Dovetail Genomics

Epigenetics - HiC



doi.org/10.1016/j.tibs.2019.03.001

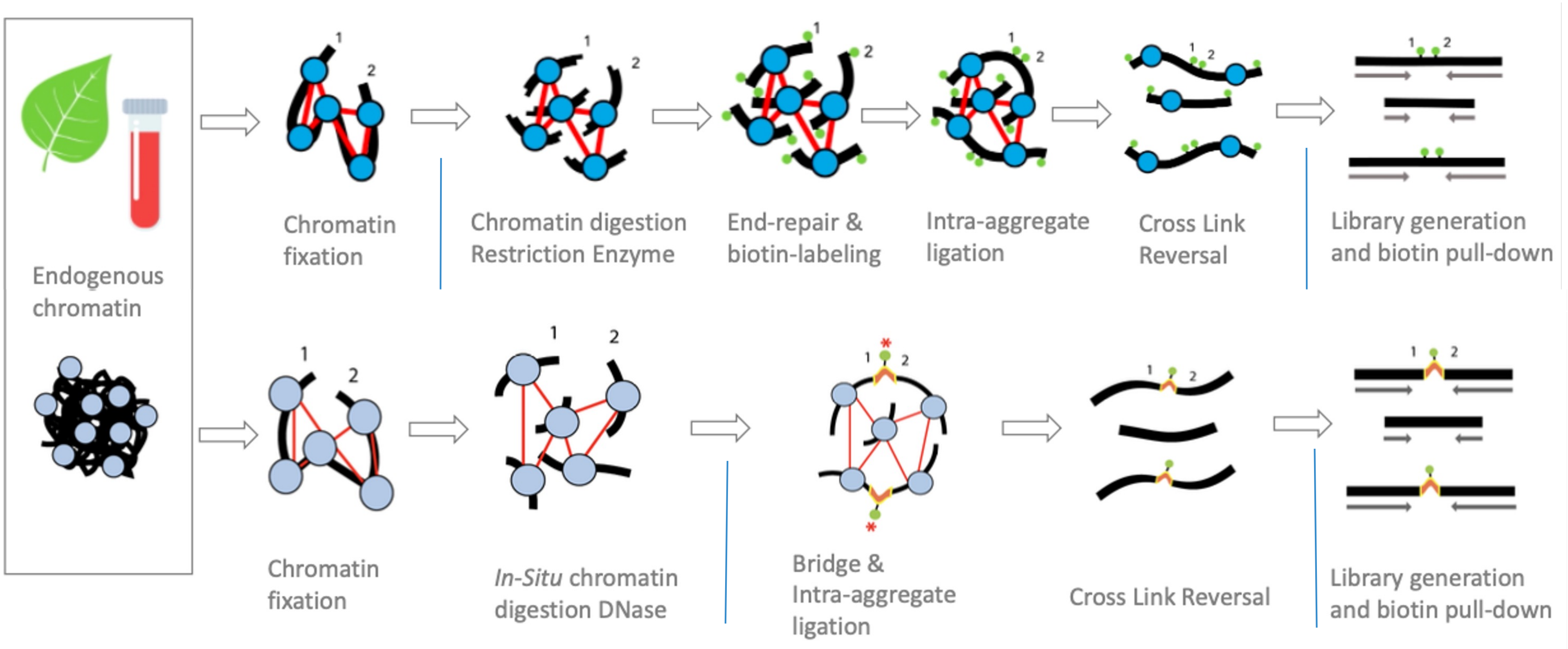
Epigenetics - HiC



Resolution
(Seq Depth)

doi.org/10.1016/j.cell.2014.11.021

HiC: Restriction Enzyme



Endogenous chromatin

Chromatin fixation

Chromatin digestion Restriction Enzyme

End-repair & biotin-labeling

Intra-aggregate ligation

Cross Link Reversal

Library generation and biotin pull-down

Chromatin fixation

In-Situ chromatin digestion DNase

Bridge & Intra-aggregate ligation

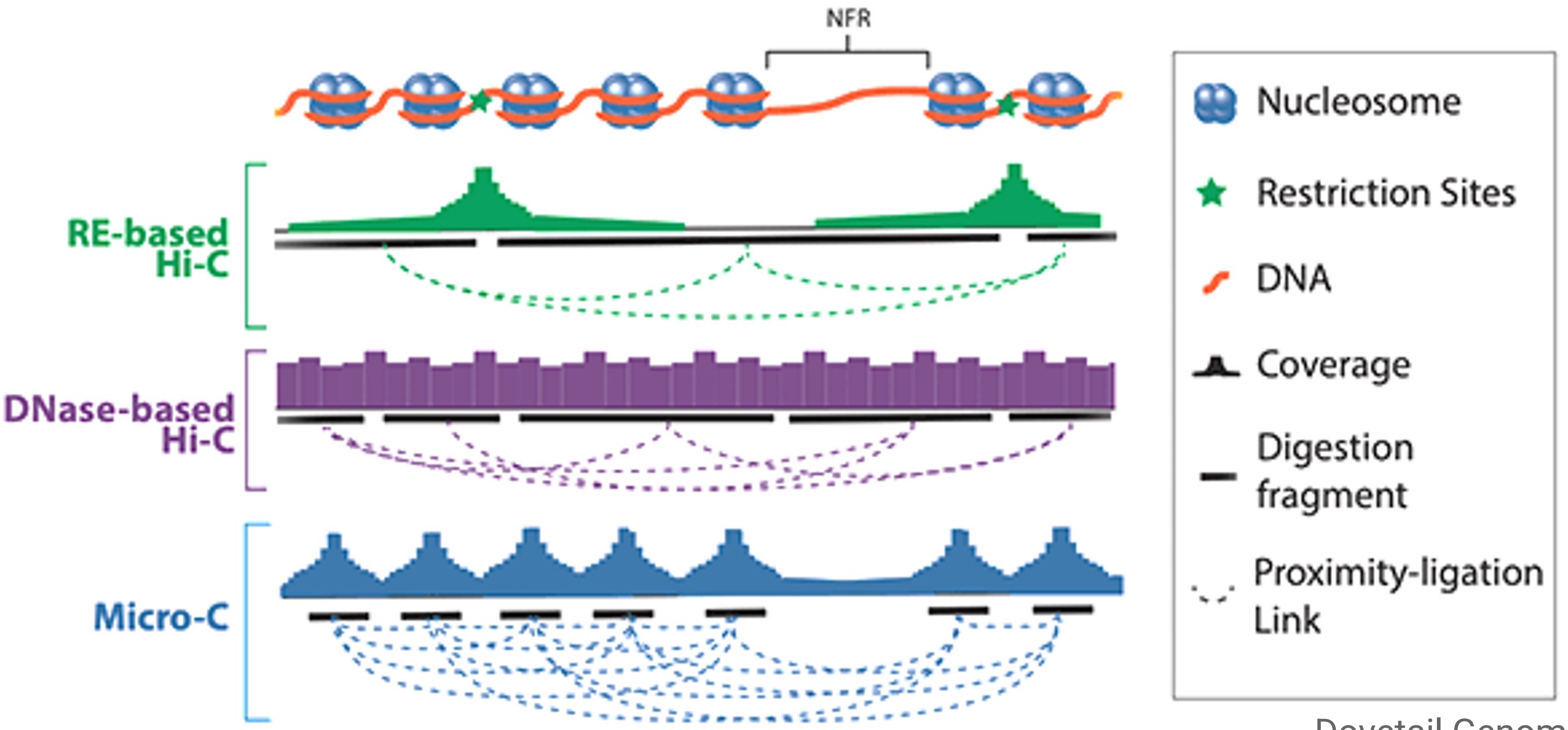
Cross Link Reversal

Library generation and biotin pull-down

Lysis

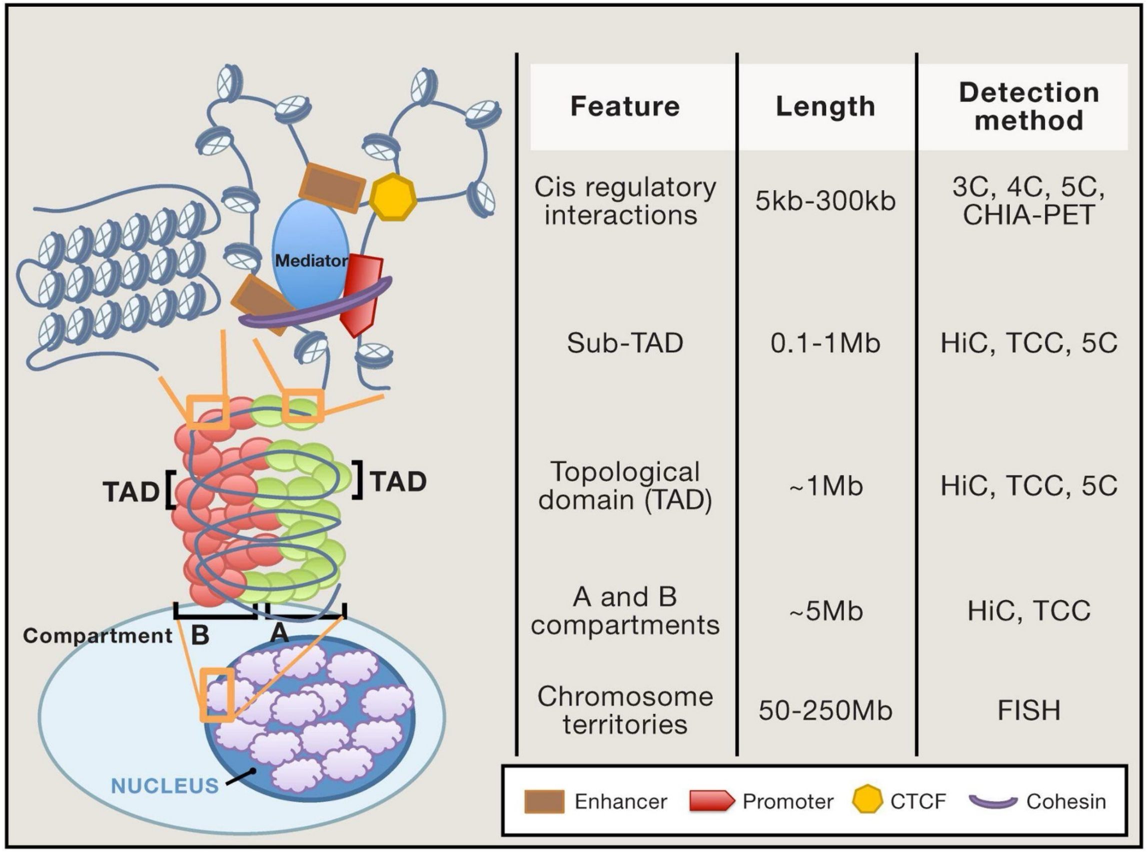
No Fragmentation

DNase/RE digestion



Dovetail Genomics

Omni-C features



Feature	Length	Detection method
Cis regulatory interactions	5kb-300kb	3C, 4C, 5C, CHIA-PET
Sub-TAD	0.1-1Mb	HiC, TCC, 5C
Topological domain (TAD)	~1Mb	HiC, TCC, 5C
A and B compartments	~5Mb	HiC, TCC
Chromosome territories	50-250Mb	FISH

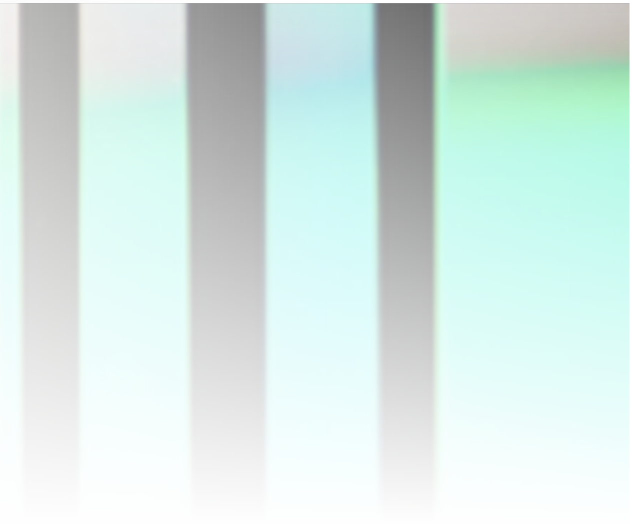
- Enzymatic titration
- Sample quality

- Frozen cells
- Frozen nuclei

- Genome Assembly
- Frozen Animal tissues
- Frozen Plant tissues/nuclei

10.1016/j.cell.2013.09.011

Steps at NGI



- → **Contact and Planning**
  - ◆ Study design
  - ◆ Aims and Biological Question.
  - ◆ Expectations and Limitations
  - ◆ Turnaround Times
  - ◆ Paperwork and Sample delivery

→ **300M reads / 1Gb genome to start with**

→ **Around 6500 kr / Sample**

Overview

- SciLifeLab
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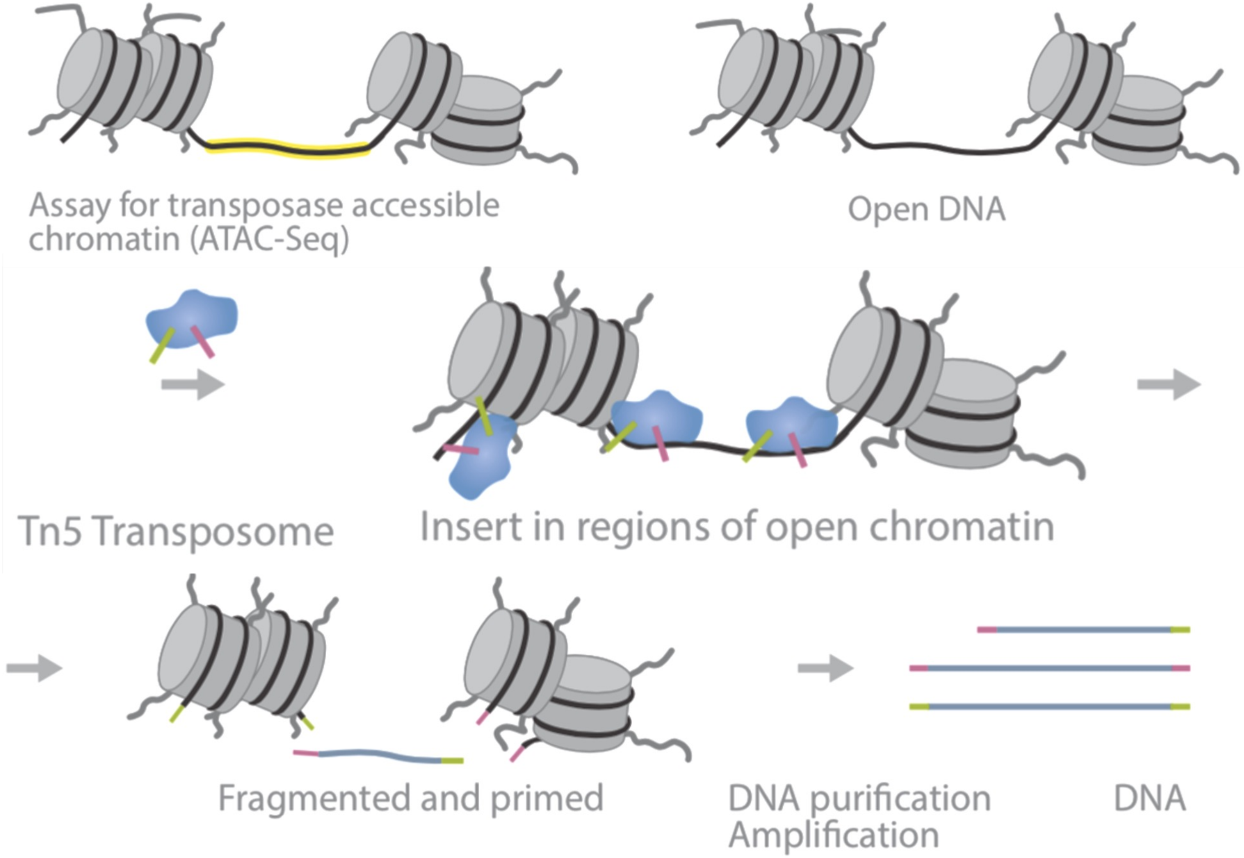
- Omni-C and Micro-C
- **Omni-ATAC**
- **Nanopore Sequencing**

Epigenetics - ATAC-seq



- Nucleosome mapping
- Transcription factor binding analysis
- Novel enhancer identification
- Evolutionary studies
- Comparative epigenomics

Illumina

Epigenetics - Omni ATAC

Cell preparation for ATAC-seq

1. Frozen cryopreserved cells
2. Thaw cells in 37°C culture media
3. DNase digestion
4. PBS washes and cell counting

DNA transposition

1. Lysis of 50,000 cells
2. Transposition
3. DNA extraction

Library construction

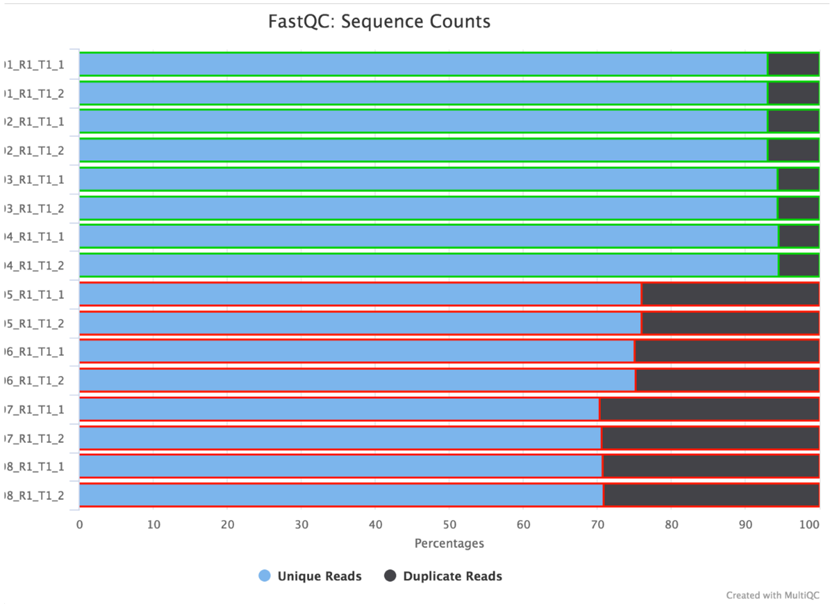
1. PCR amplification
2. First bead purification
3. Second bead purification

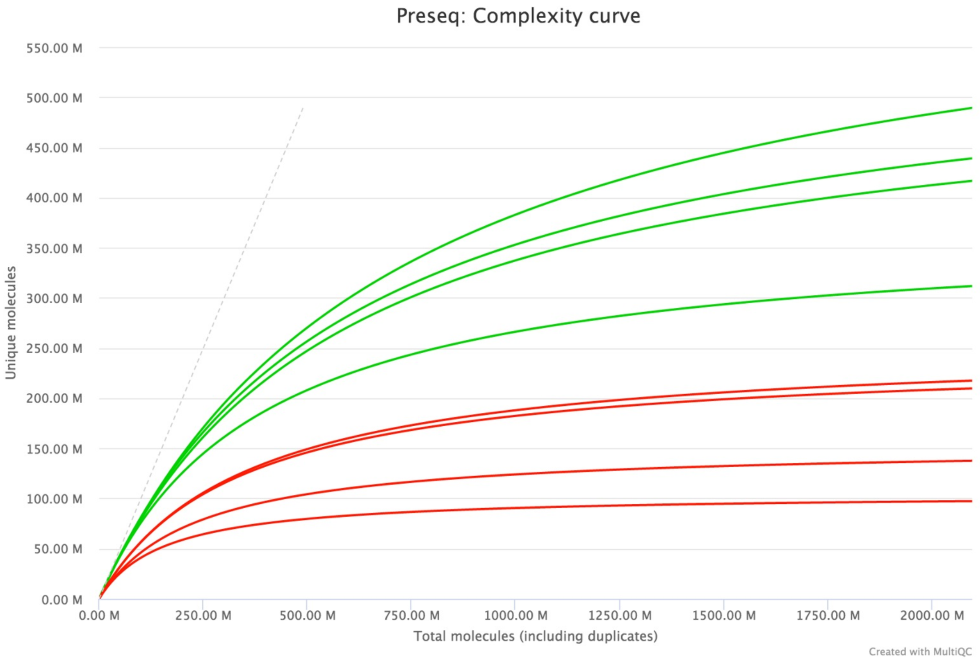
SEQUENCING

This parts can be done by the user

corceslab.com/pages/Resources/
10.21769/BioProtoc.4294s/

Epigenetics - Omni ATAC


FastQC: Sequence Counts

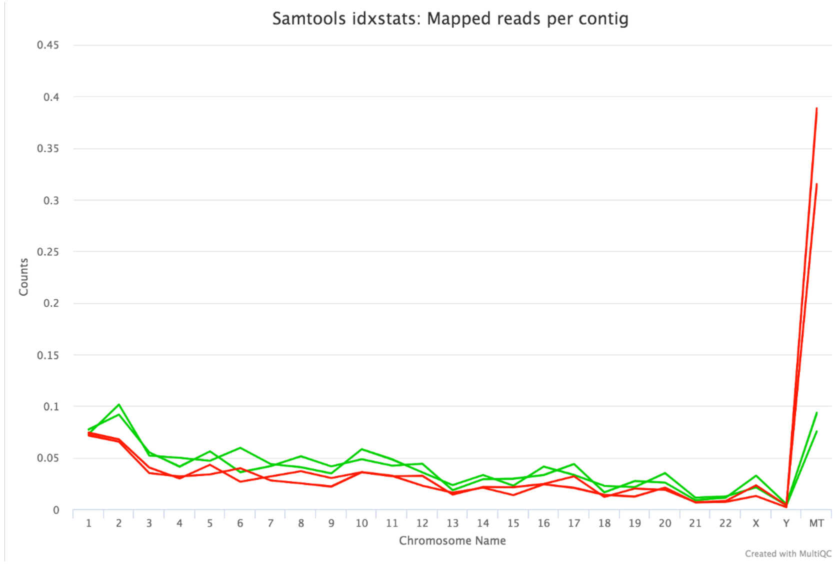

Preseq: Complexity curve

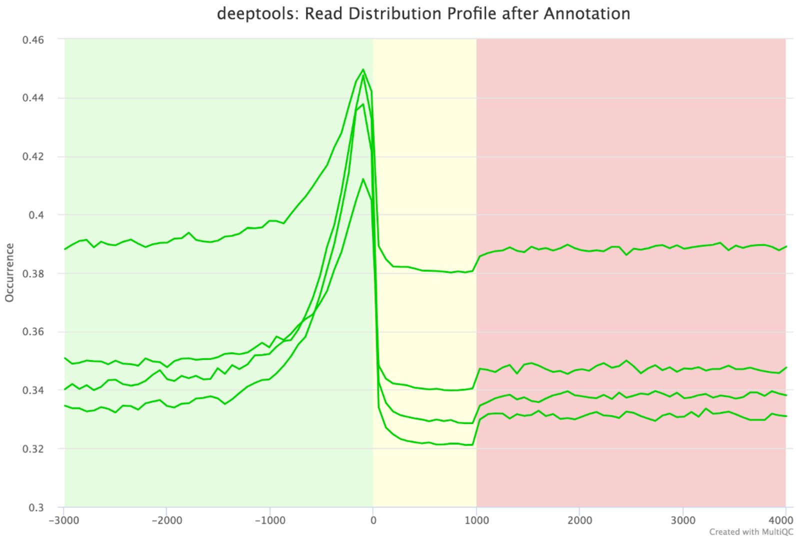
Omni ATAC has a higher number of unique reads, compared to regular ATACseq

NGI

SciLifeLab

Epigenetics - Omni ATAC





TSS enrichment between comparable for all samples

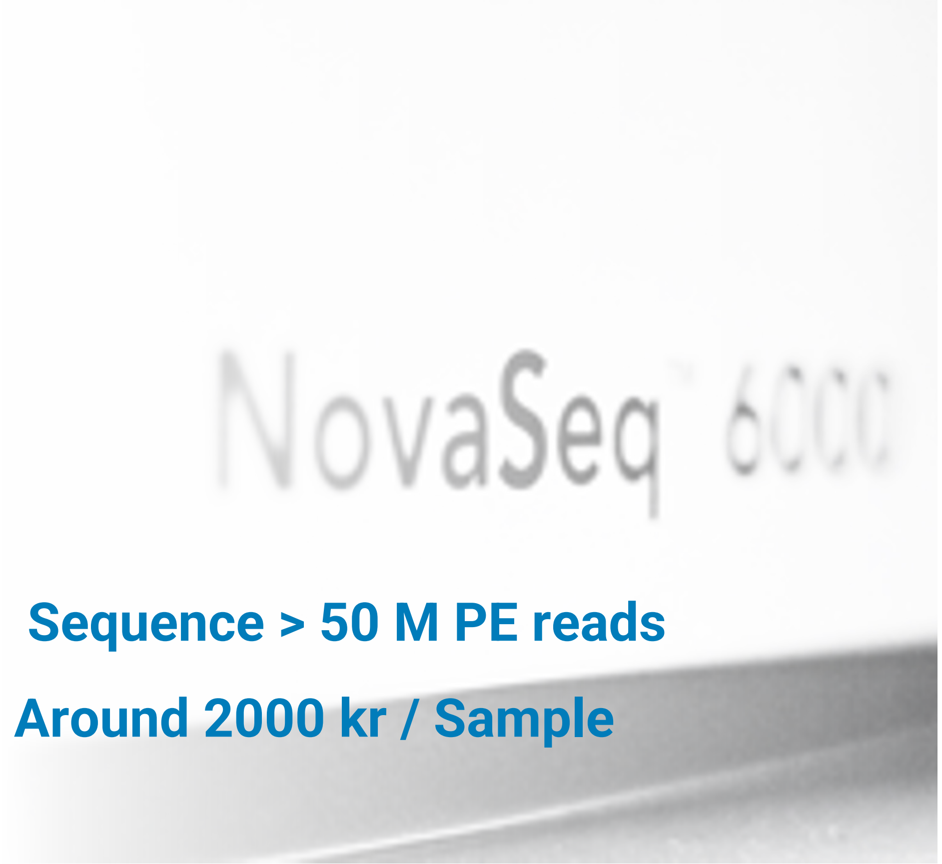
All annotated genes have been normalized to the same size.

- Green: -3.0Kb upstream of gene to TSS
- Yellow: TSS to TES
- Pink: TES to 3.0Kb downstream of gene

Omni ATAC generates fewer reads that map to mitochondrial DNA, compared to regular ATACseq

Omni ATAC at NGI

- → **Contact and Planning**
  - ◆ Study design
  - ◆ Aims and Biological Question.
  - ◆ Expectations and Limitations
  - ◆ Turnaround Times
  - ◆ Paperwork and Sample delivery



→ **Sequence > 50 M PE reads**

→ **Around 2000 kr / Sample**

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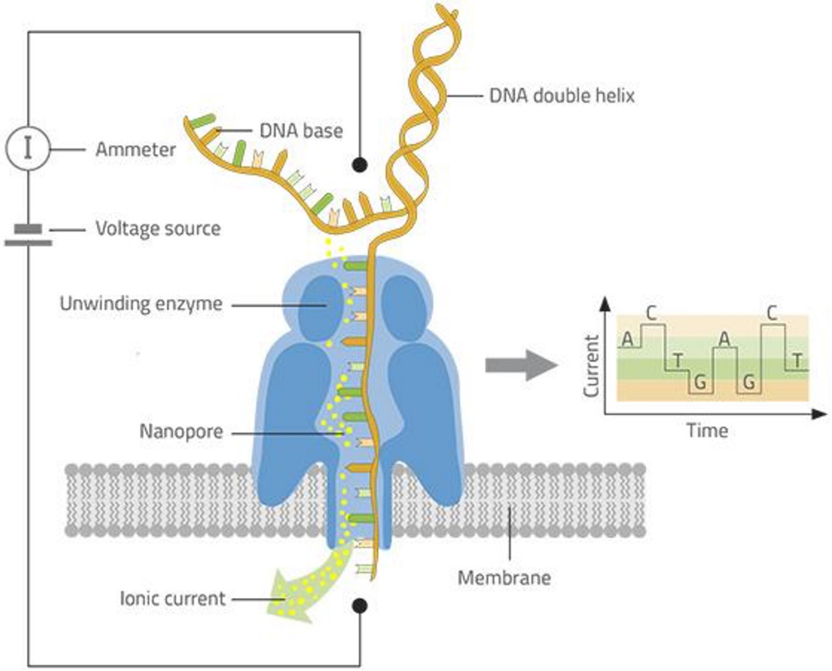
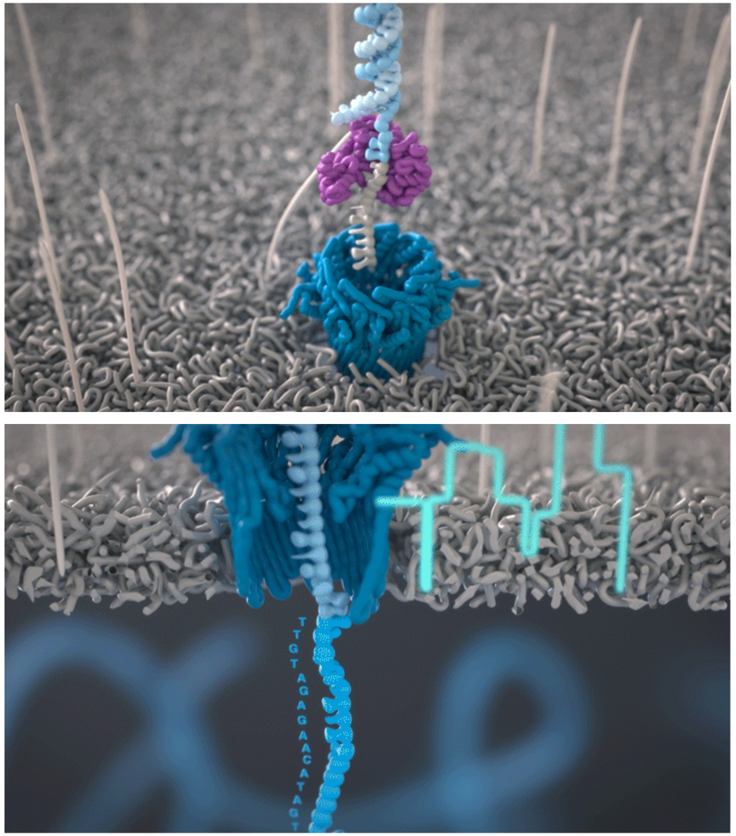
- **Nanopore Sequencing**

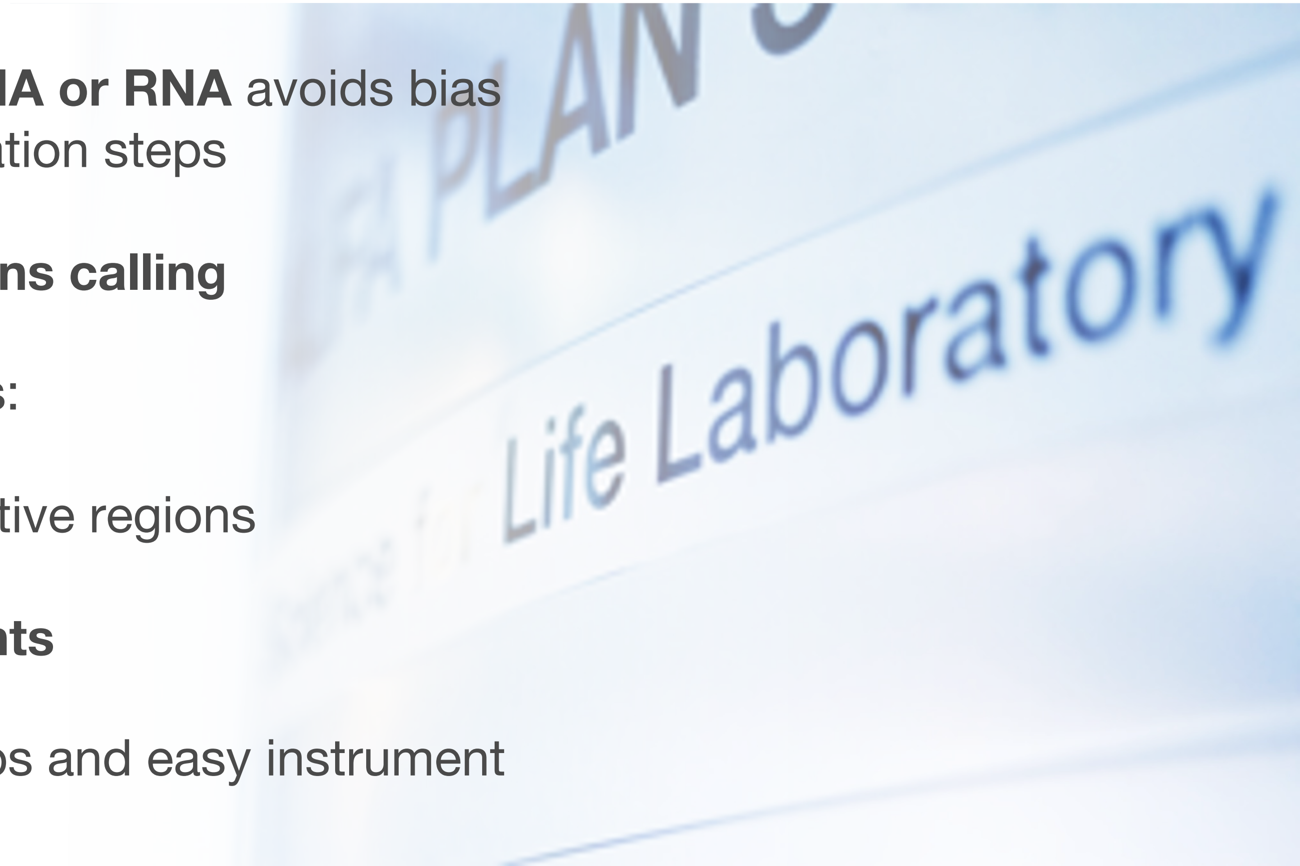
Oxford Nanopore Technologies (ONT)





- A protein channel (nanopore) anchored to a non-conductive membrane
- A voltage can be applied across the membrane
- This causes ions to travel through the protein channel, creating an electrical current

ONT new possibilities



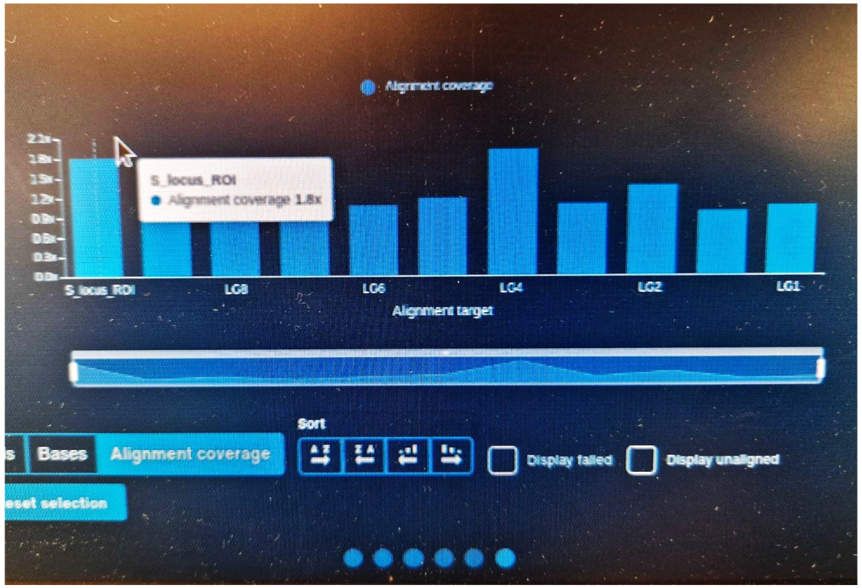
- **Sequencing native DNA or RNA** avoids bias introduced by amplification steps

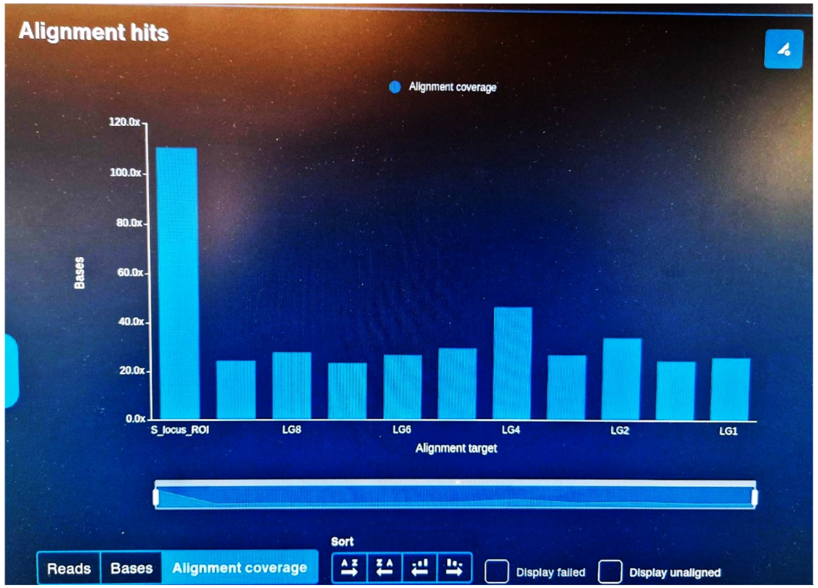
- **RNA/DNA modifications calling**

- Long reads advantages:
    - **haplotyping**
    - analysis of repetitive regions
    - phasing
    - **structural variants**

- Very simple library preps and easy instrument operation

ONT Adaptive Sampling





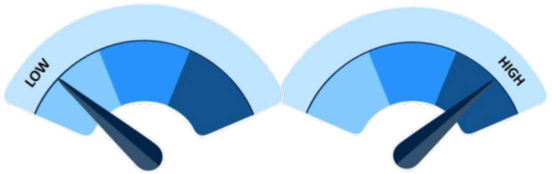
- ~150X coverage of the ROI (2Mb)
- 20-40X coverage for the rest of the genome
- No special Lib Prep
- Dependent on .bed file design

https://ngisweden.scilifelab.se/2024/02/tech-note-adaptive-sampling-targeted-oxford-nanopore-long-read-sequencing/

Other ONT methods

Other current methods:

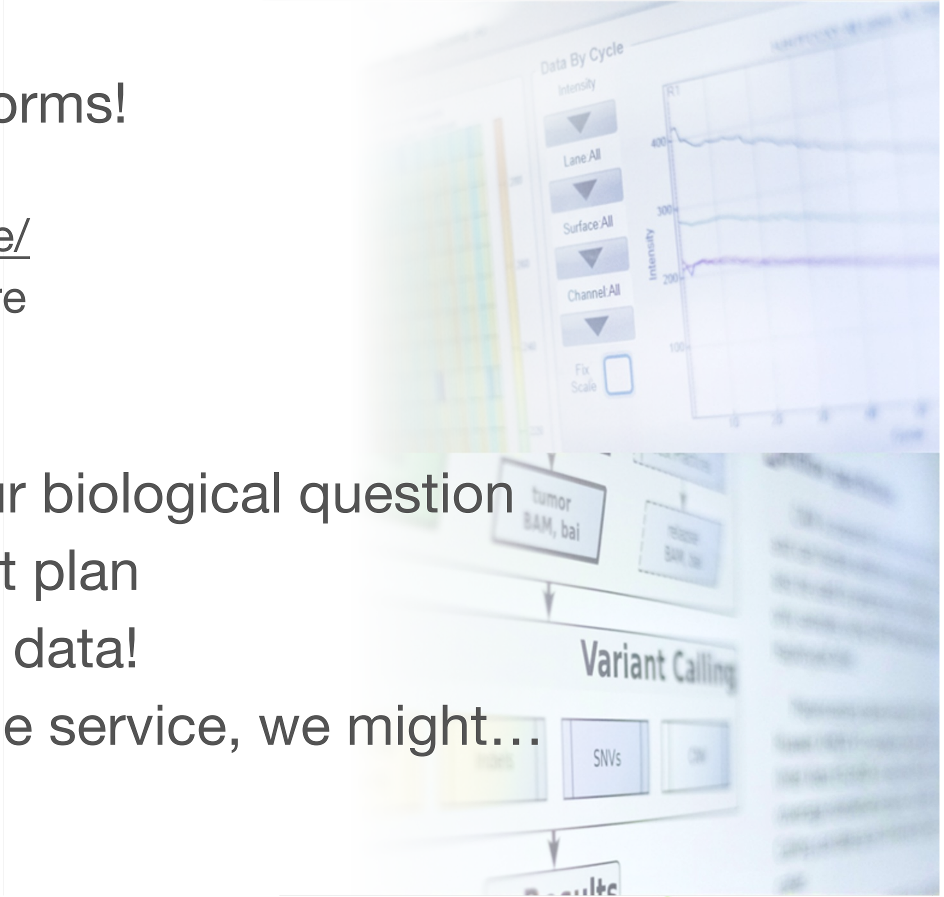
- **Direct RNA-seq**
- **cDNA-PCR**
- **16S/18S and ITS**
- **HLA typing**
- **Single Cell 10X Genomics**
    - 3’ Gene Expression
- **Spatial Transcriptomics 10X Genomics**
    - Visium Spatial



N50 (Kb)	**0.41**	**32.4**
Output (Gb)	44.8	34.8
Reads (M)	111.2	3.6

→ Around 2300 kr / Sample

Takeaway



- Make use of the SciLifeLab platforms!
  - National Genomics Infrastructure
    - https://ngisweden.scilifelab.se/
  - National Bioinformatics Infrastructure
    - http://nbis.se/

- Pick a protocol that matches your biological question
- Think carefully about your project plan
- Ensure that you understand your data!
- **Ask us!** -even if we don't offer the service, we might…