Epigenomics methods at NGI

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NATIONAL GENOMICS INFRASTRUCTURE

Overview

The **N**ational **G**enomics **I**nfrastructure (NGI)

DNA methylation

Omni-C and Micro-C

Omni-ATAC

Nanopore Sequencing

NGI

SciLifeLab

NGI Sweden Nodes

NGI stockholm

The **SNP&SEQ**
Technology Platform

Uppsala Genome
Center - **UGC**

Umeå (UmU, SLU)

Uppsala (UU, SLU)
Örebro (ÖRU)   Stockholm (KI, KTH, SU)
Linköping (LiU)
Gothenburg
(GU, Chalmers)

Lund (LU)

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

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SciLifeLab

NGI Stockholm

663 projects

32 185 samples

2023

11 Human genomes at 30X per day

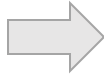**1 093 Gbp per day**

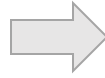https://ngisweden.scilifelab.se/resources/ngi-stockholm-status/
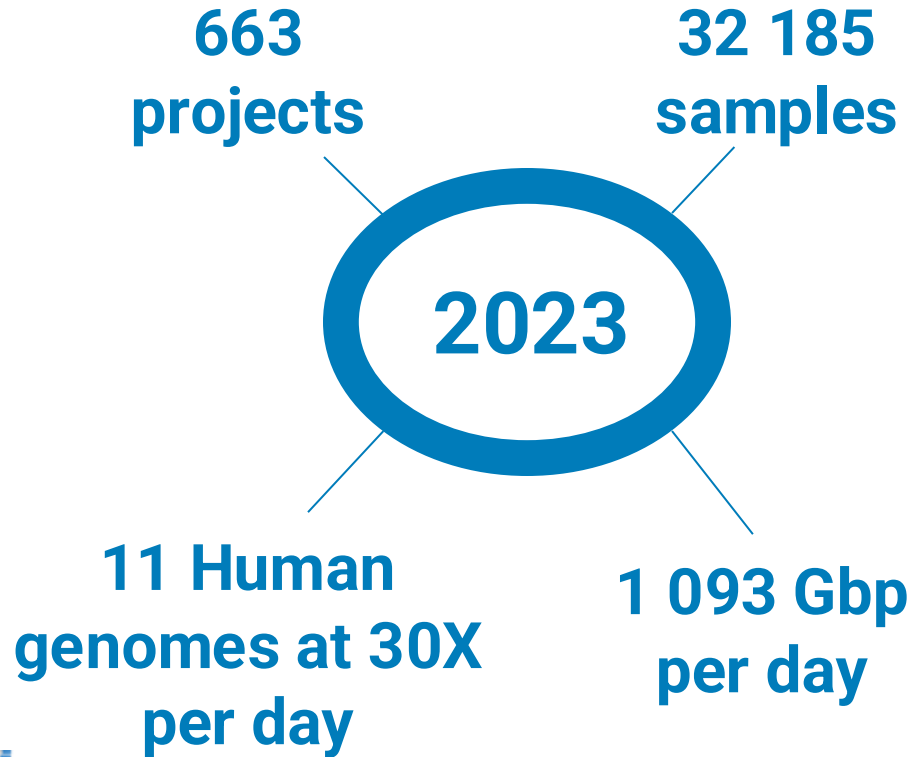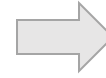
NGI Workflow

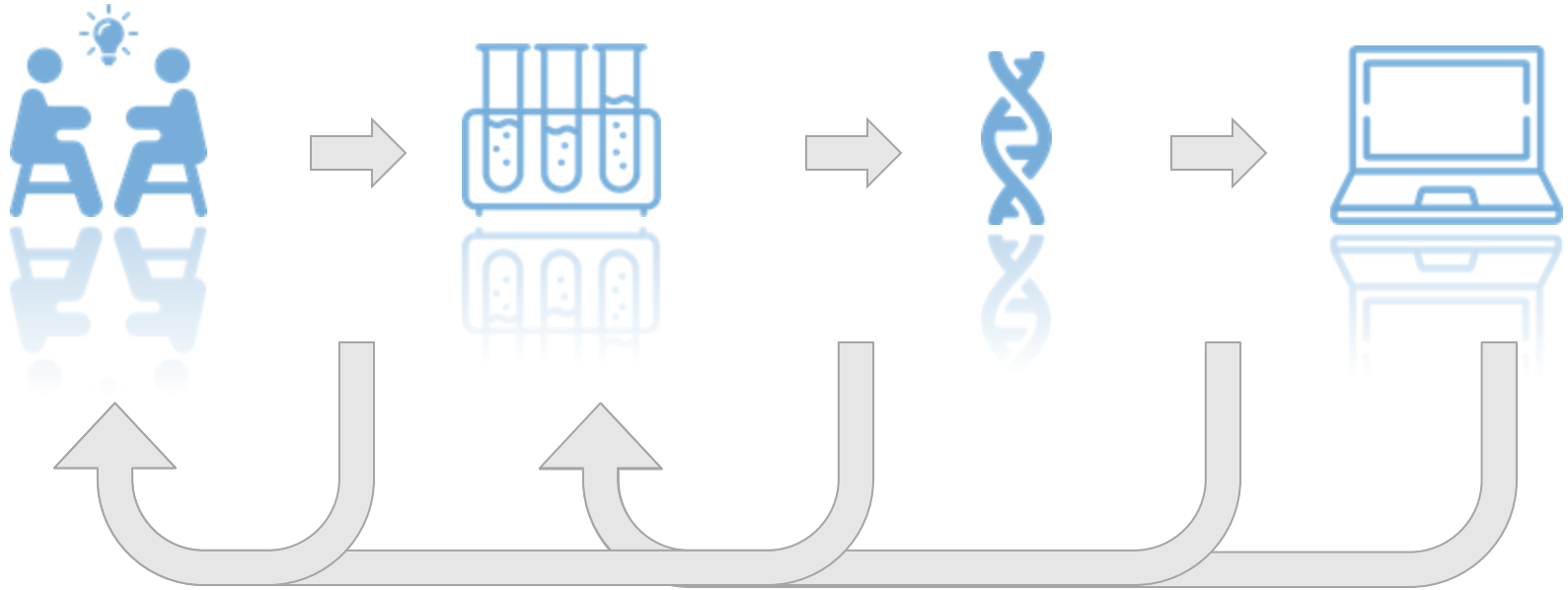Meetings → QC & Library Preparation → Sequencing → Data analysis & delivery

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:
    o Not knowing enough about their own sample
    o Lacking the necessary tools for quality control
    o Needing more bioinformatics capabilities
    o Feeling lost in communication/literature

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

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De novo sequencing

DNA extractions

DNA resequencing

Environmental sequencing

Epigenetics

Genotyping

Single-cell

Spatial

Transcriptomics

User-prepared libraries

Proteomics with NGS readout

Overview

SciLifeLab

The National Genomics Infrastructure

DNA methylation

Omni-C and Micro-C

Omni-ATAC

Nanopore Sequencing

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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

Cytosine → (Deamination) → Uracil

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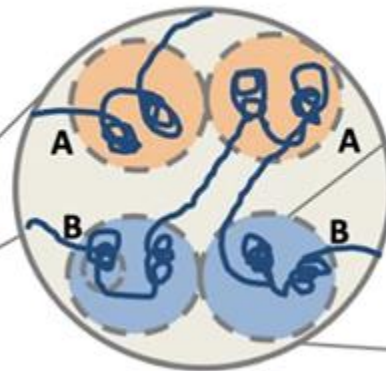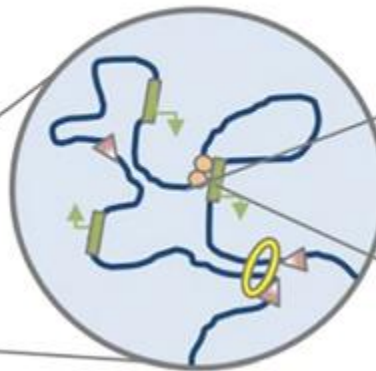
Epigenetics - HiC

Chromosome territories   A/B compartments   TADs   Loops
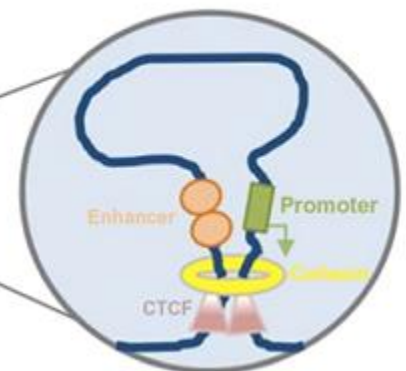
**Resolution
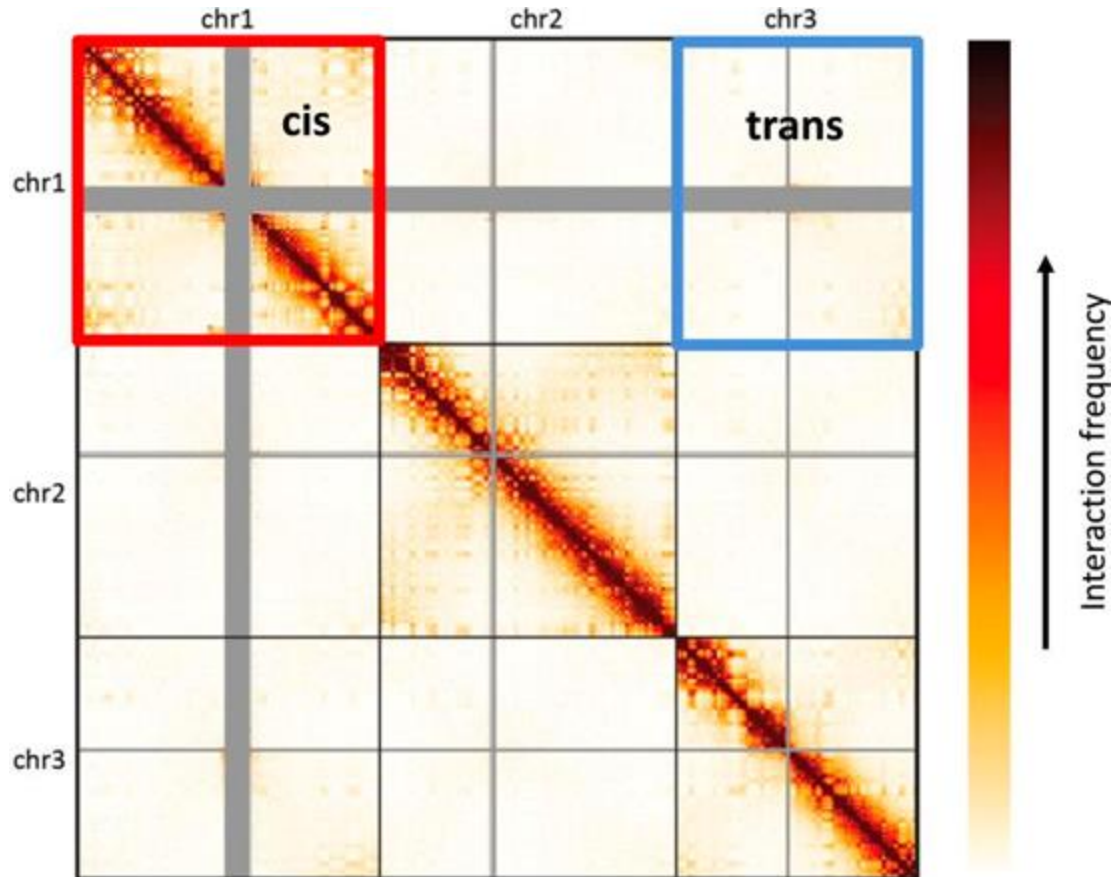(Seq Depth)**

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HiC: Restriction Enzyme

Endogenous chromatin

Chromatin fixation

Lysis

Chromatin digestion Restriction Enzyme

End-repair & biotin-labeling

Intra-aggregate ligation

Cross Link Reversal

Fragmentation

Library generation and biotin pull-down

Dovetail Genomics

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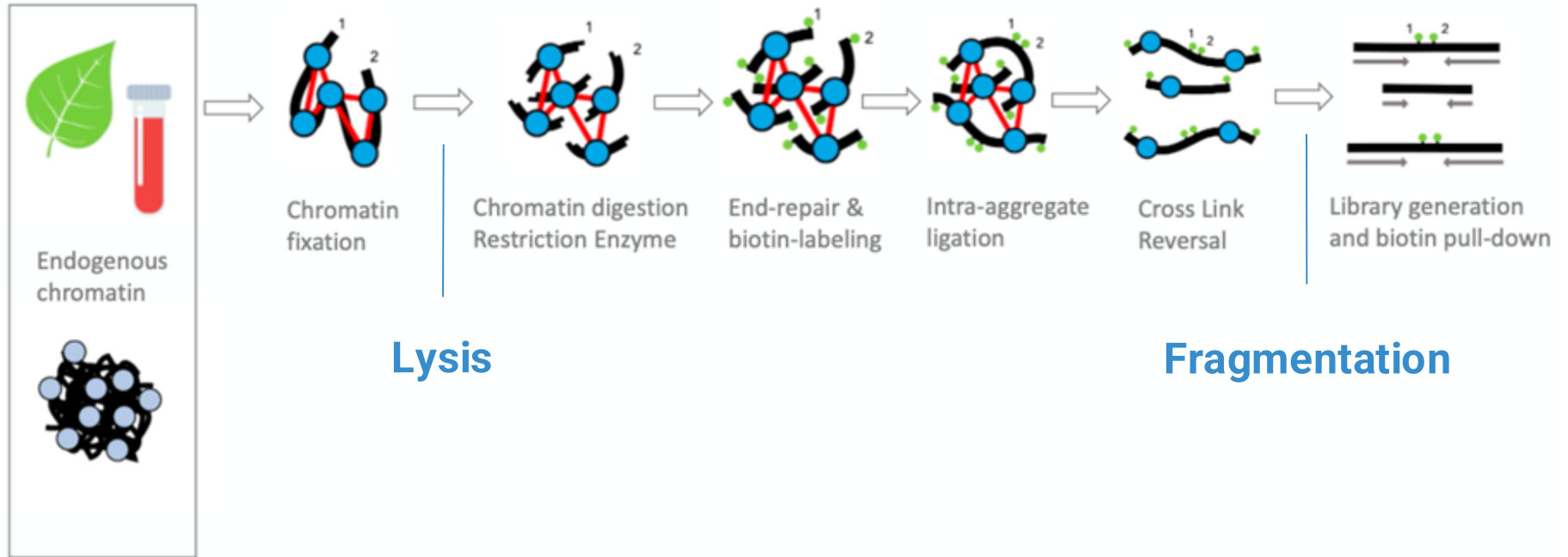
Epigenetics - HiC

Epigenetics - HiC
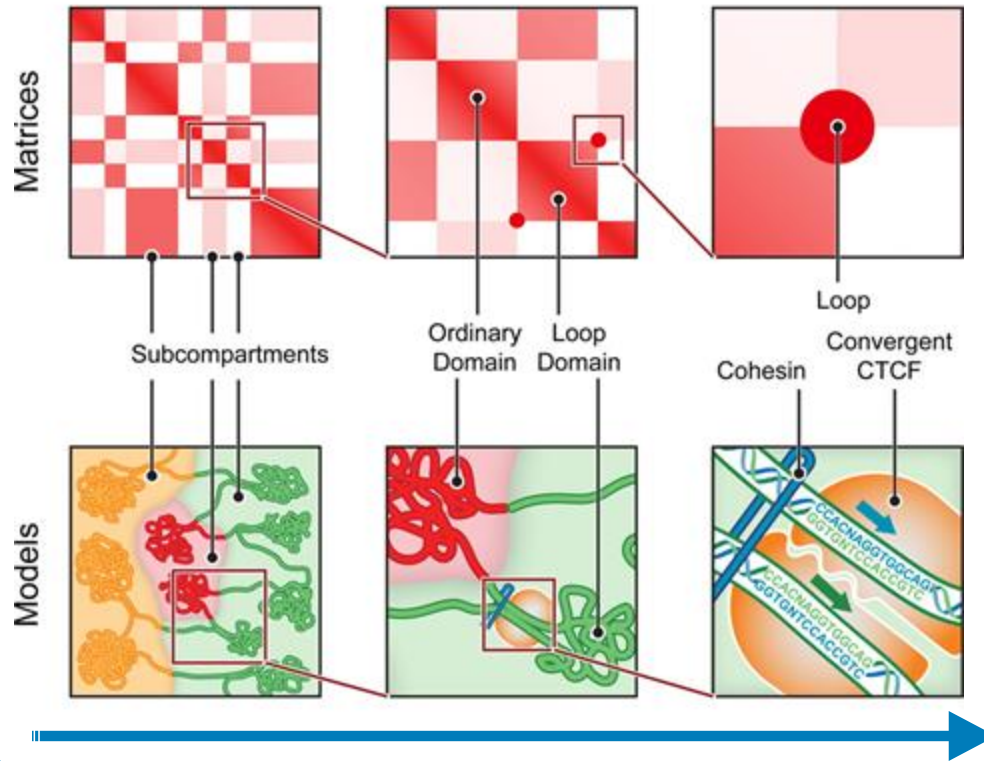
Resolution (Seq Depth)

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
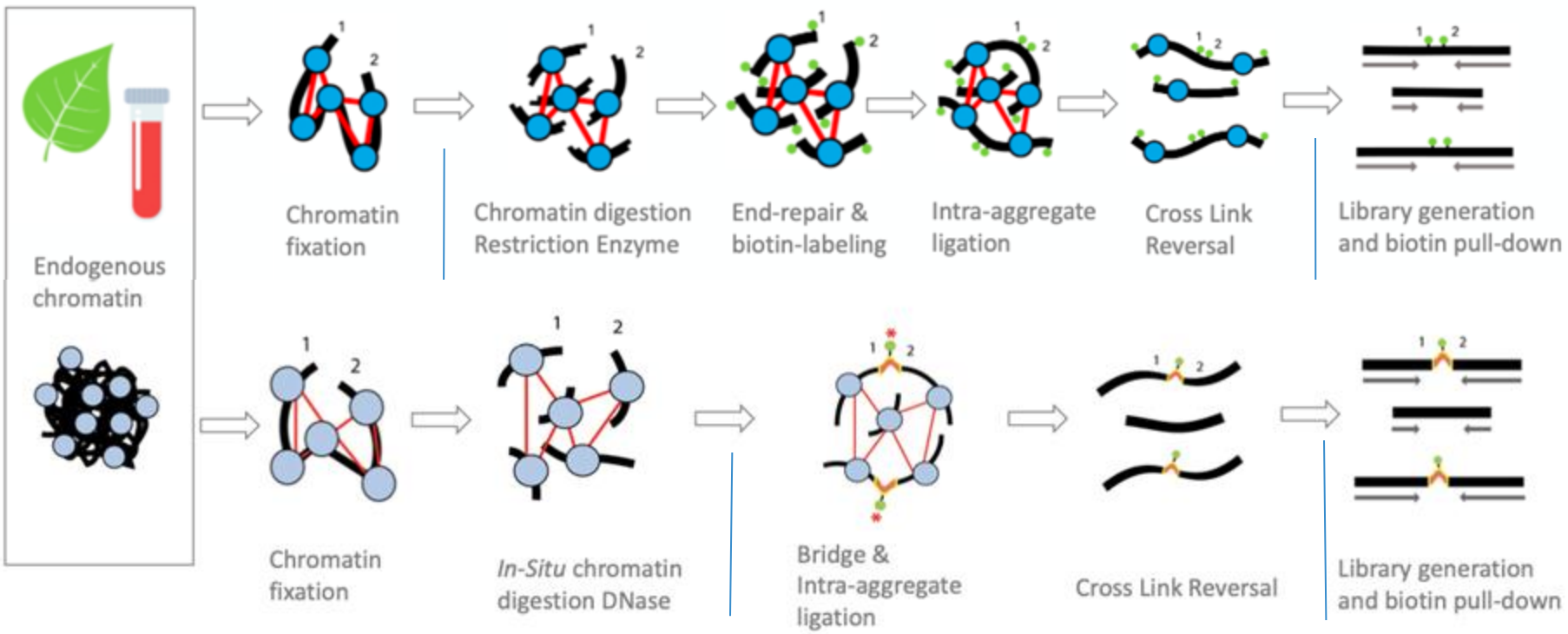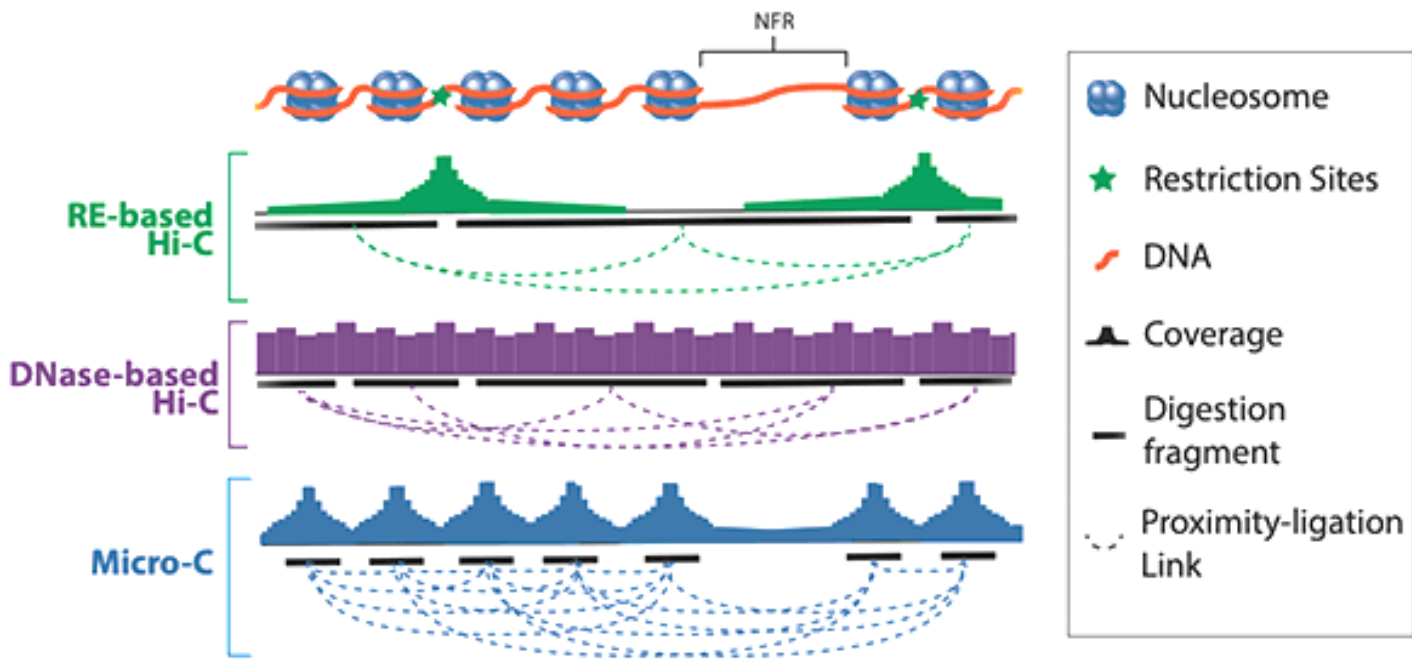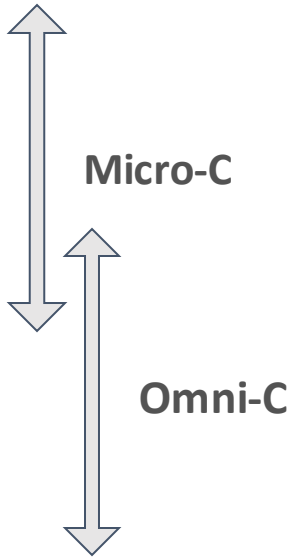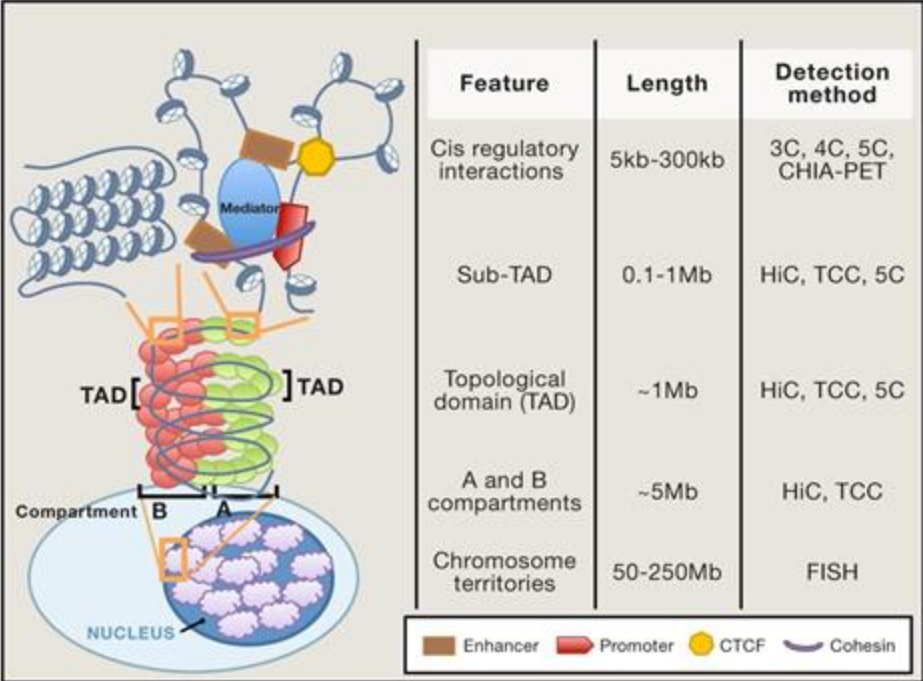
Lysis   **No Fragmentation**

DNase/MNase digestion

Dovetail Genomics

Omni-C vs Micro-C

- **Enzymatic titration**
- **Sample quality**

- **Frozen cells**
- **Frozen nuclei**

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

10.1016/j.cell.2013.09.011

-C steps at NGI

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

➔ **200M reads / 1Gb genome to start with**

➔ **Around 6500 kr / Sample**

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Overview

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Epigenetics - ATAC-seq

Assay for transposase accessible chromatin (ATAC-Seq)

Open DNA

Tn5 Transposome

Insert in regions of open chromatin

Fragmented and primed

DNA purification Amplification

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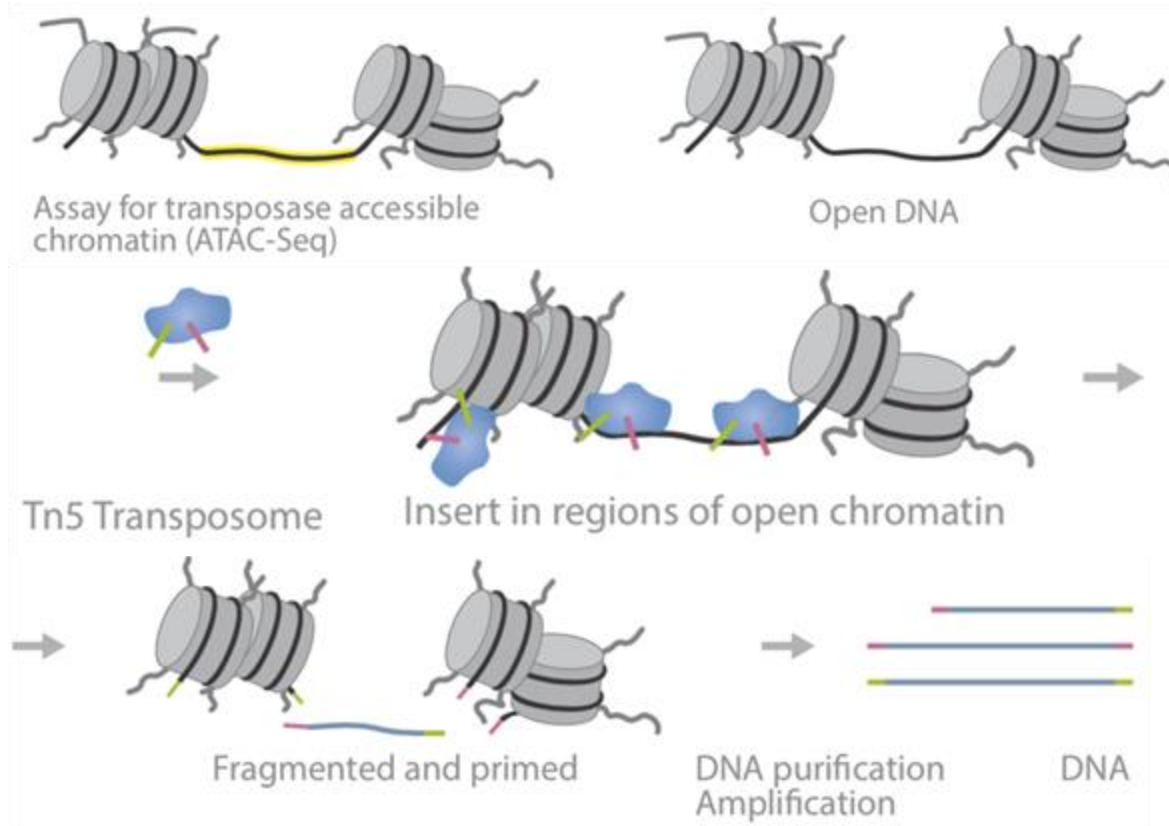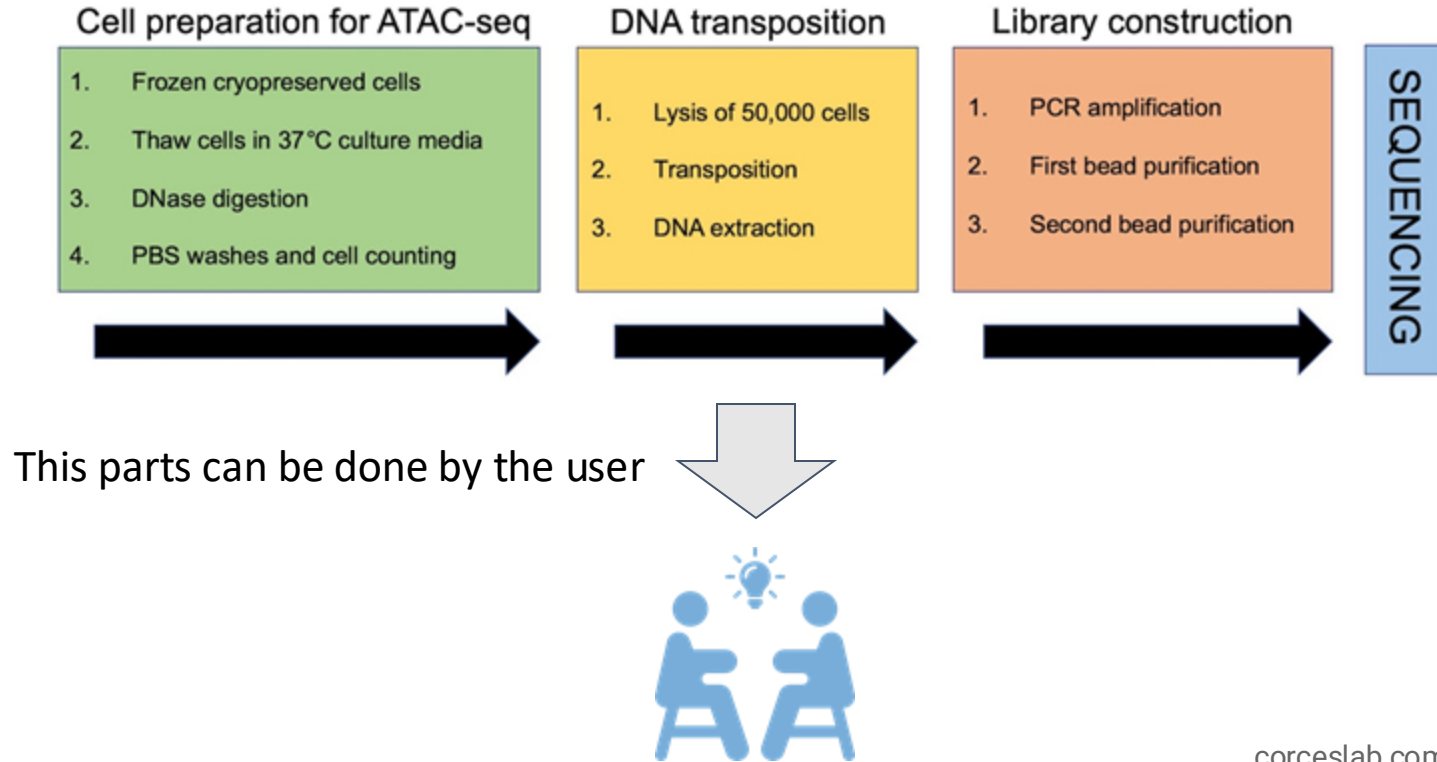
- Nucleosome mapping
- Transcription factor binding analysis
- Novel enhancer identification
- Evolutionary studies
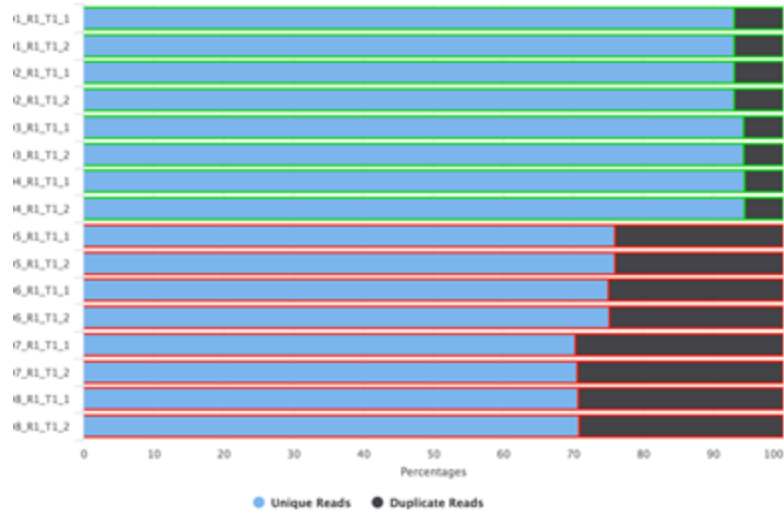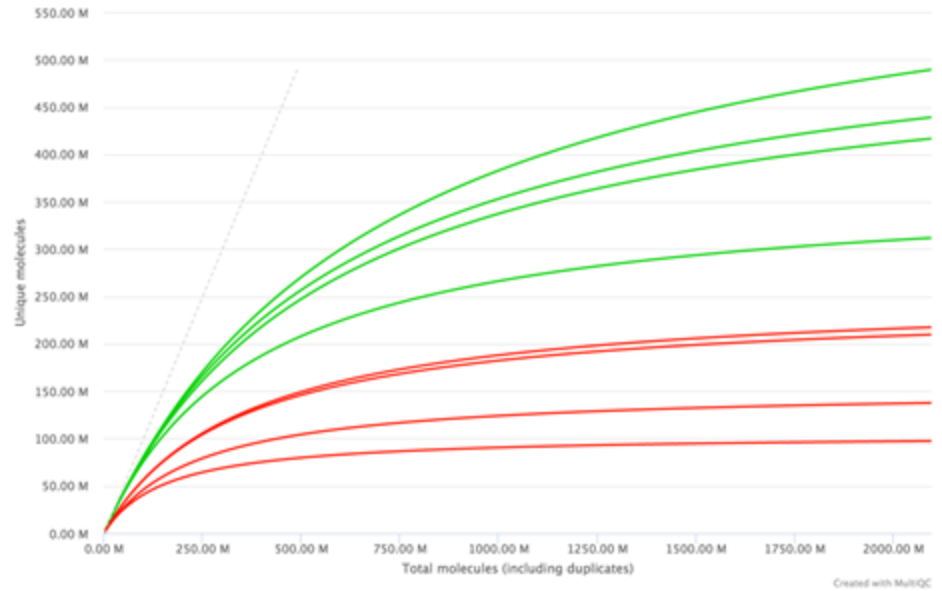- Comparative epigenomics

Illumina

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Epigenetics - Omni ATAC

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

Epigenetics - Omni ATAC

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

Epigenetics - Omni ATAC

Samtools idxstats: Mapped reads per contig
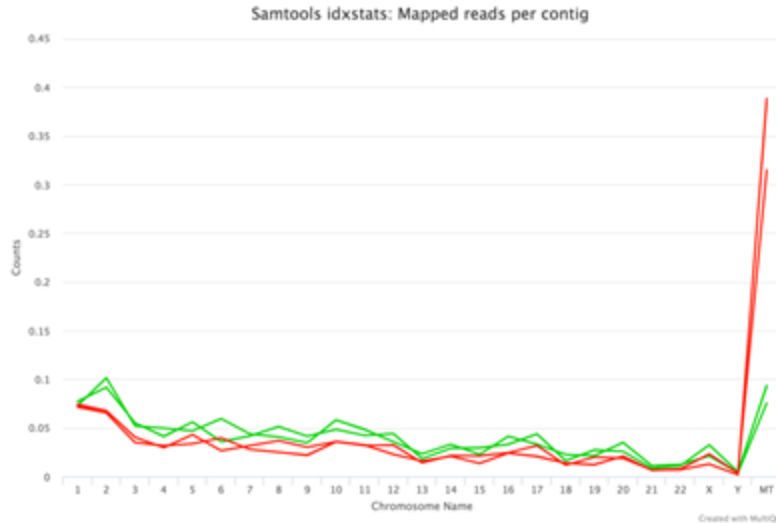
Omni ATAC generates fewer reads that map to mitochondrial DNA, compared to regular ATACseq

deeptools: Read Distribution Profile after Annotation
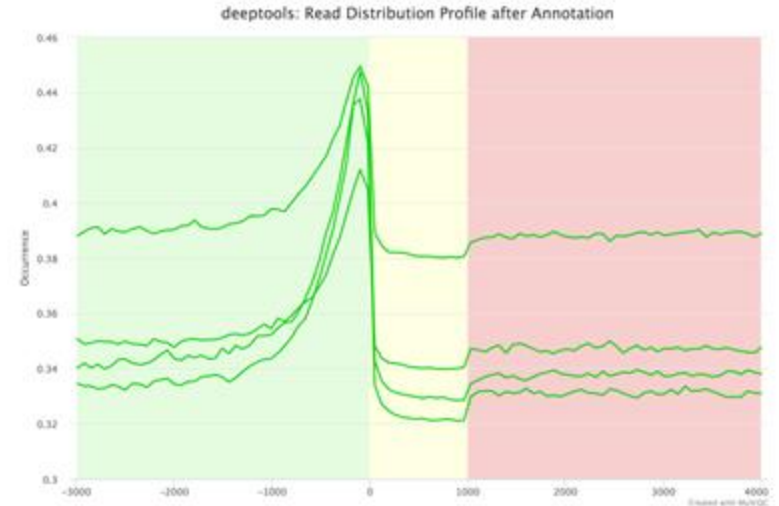
TSS enrichment between comparable for all samples

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

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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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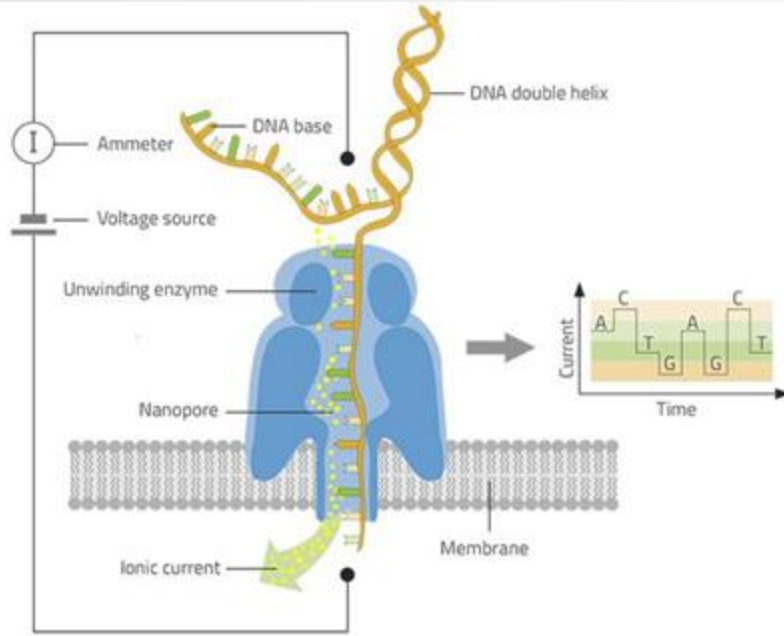The National Genomics Infrastructure

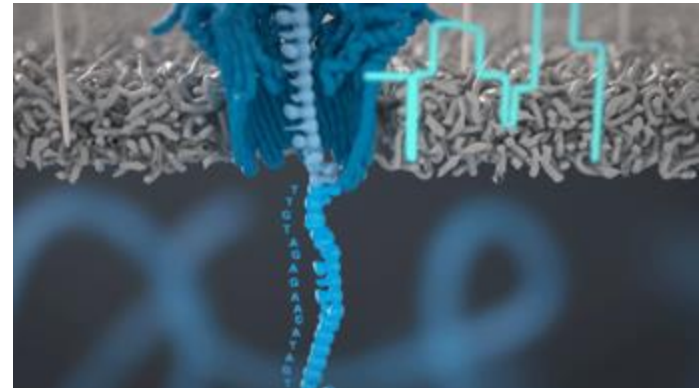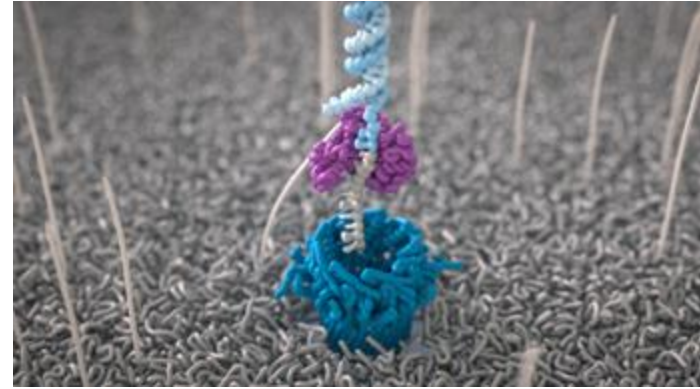DNA methylation

Omni-C and Micro-C

Omni-ATAC

**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

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ONT new possibilities

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

- **RNA/DNA Methylation calling**

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

- Very simple library preps and easy instrument operation

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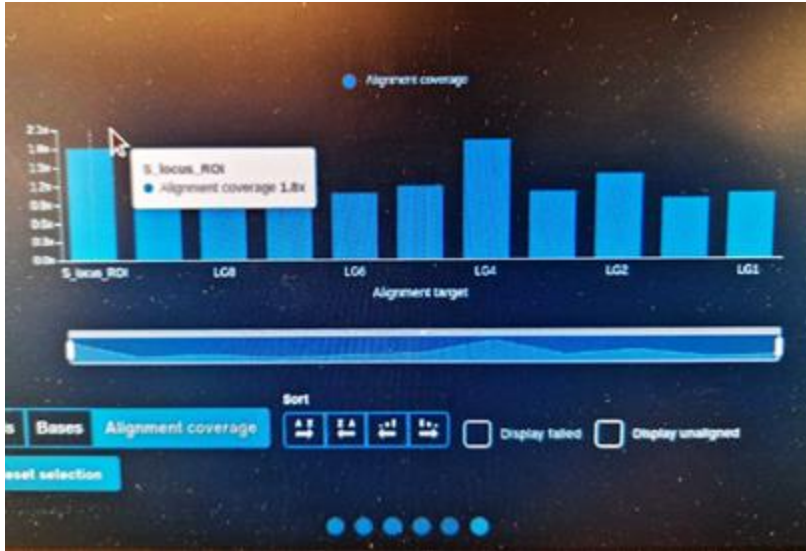
- ~150X coverage of the ROI (2Mb)
- 20-40X coverage for the rest of the genome
- No special Lib Prep
- Dependent on .bed file design

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

ONT coming soon

Future methods:

- **Direct RNA-seq**

- **Single Cell 10X Genomics**
  - 3' Gene Expression

- **Spatial Transcriptomics 10X Genomics**
  - Visium Spatial

- Pore-C: HiC + ONT

➔ **Around 2300 kr / Sample**

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

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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…