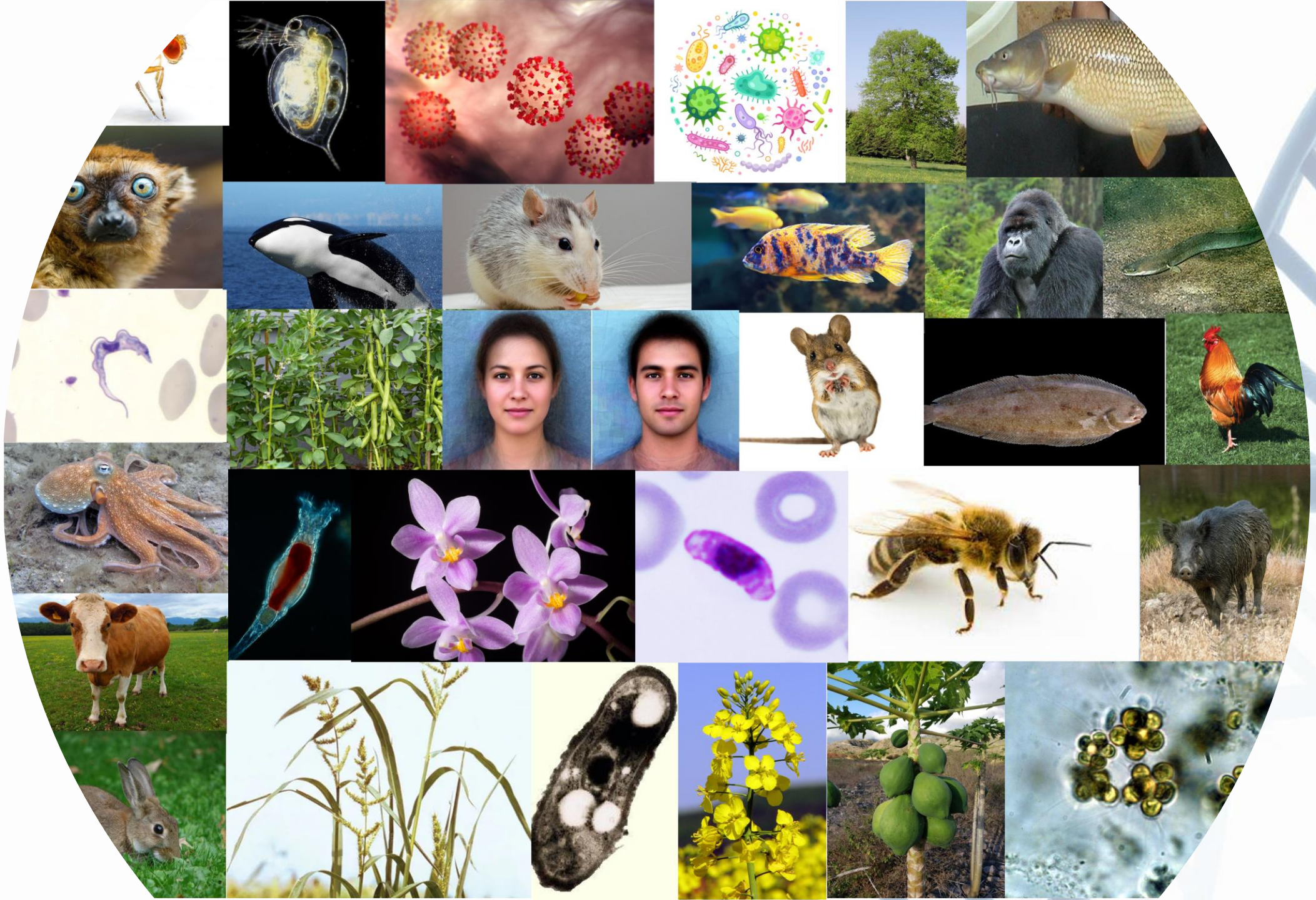
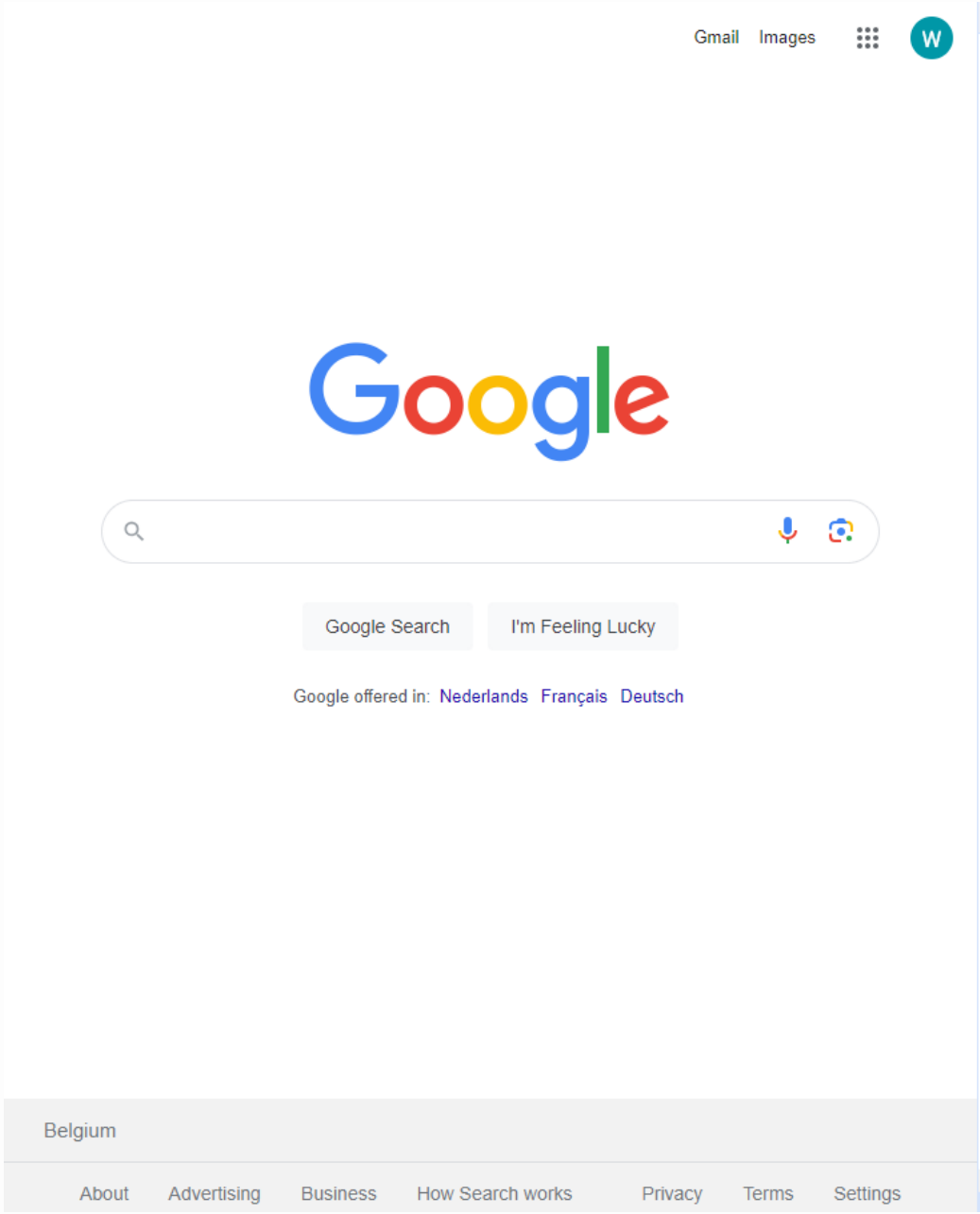
Long read sequencing

When to think about it and what to think about

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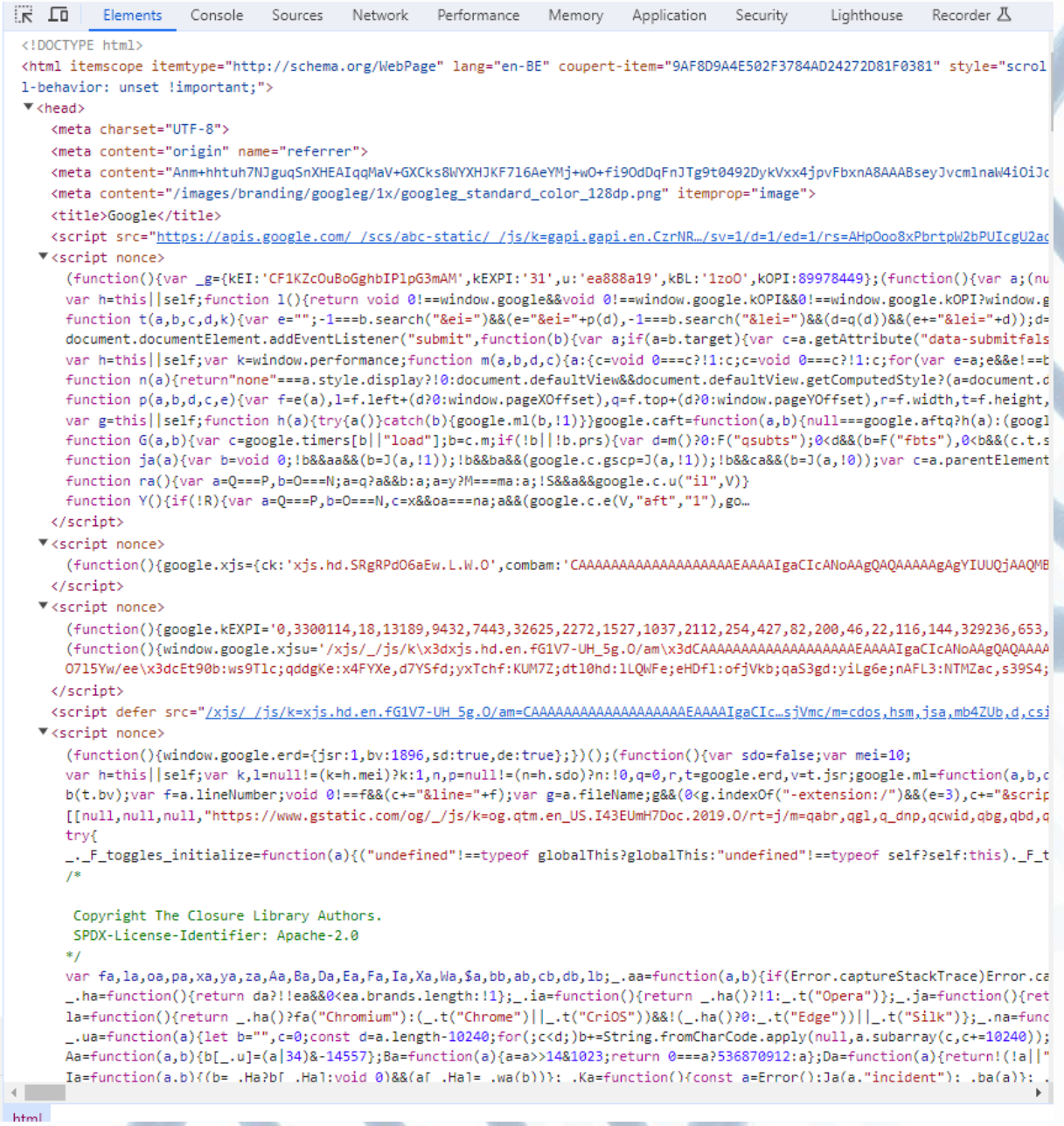
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SPDX-License-Identifier: Apache-2.0
*/
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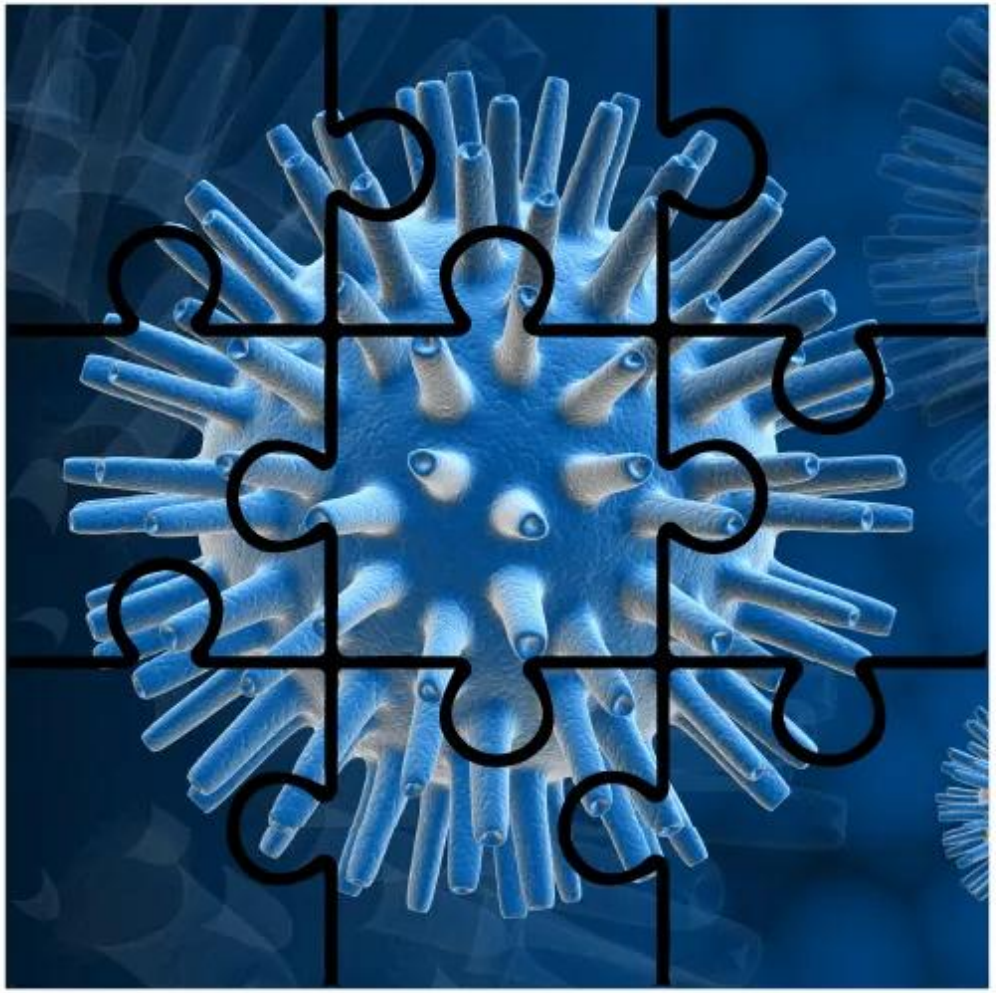
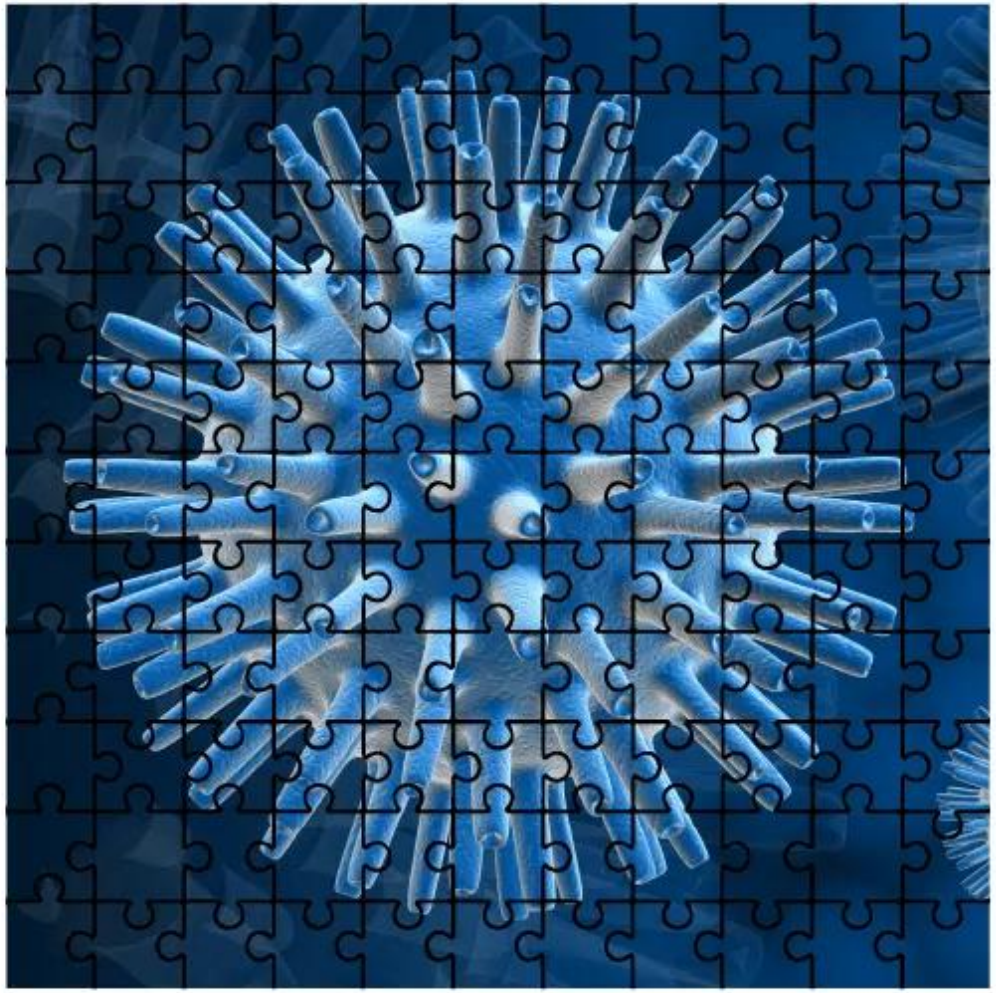


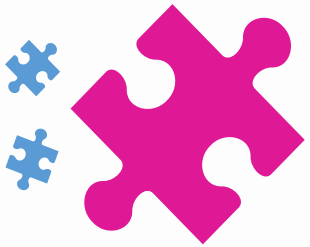


What is long read?

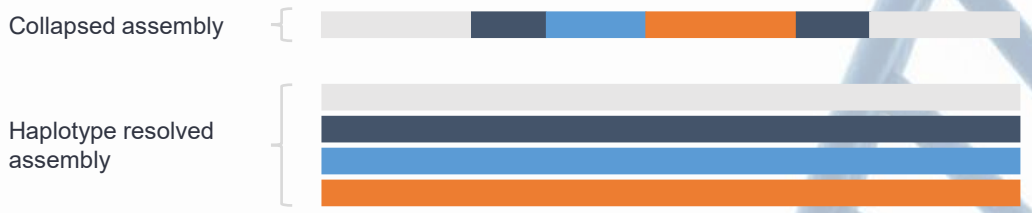
- Sequencing but not short reads
  - 1kb or way longer in general
- Linked reads, long reads, optical genome mapping
- There is no ‘best’ technology
- Main technologies
  - Nanopore
  - Pacbio
  - Linked reads

No ‘one ring’ to rule them all

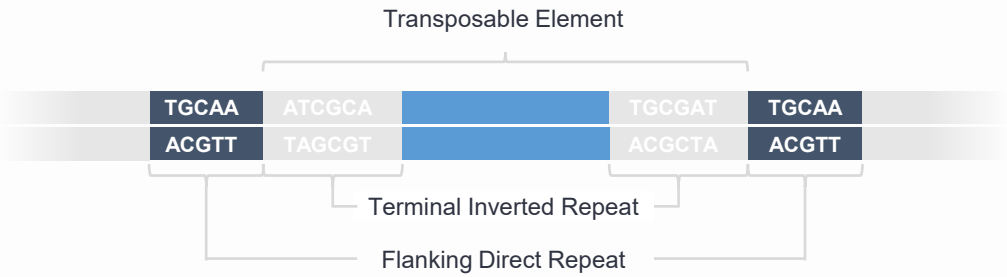
Why long reads?



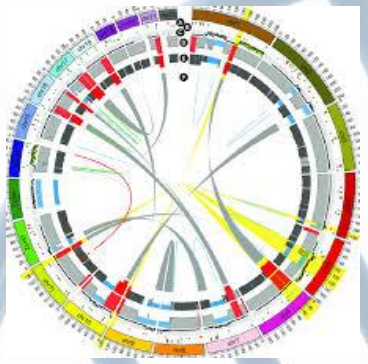
Bigger is better – for assembly or mapping



Longer reads help distinguish different haplotypes



Long reads span repeats and accuracy allows differentiation between copies of long repeats

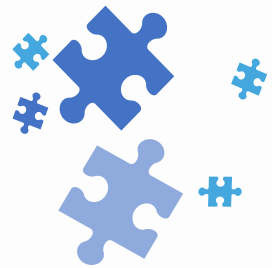


Better handling or aneuploidy, SV's and complex genomes

Long reads— the four Cs to take into account

Assemblies using long reads check all the boxes of a high-quality genome assembly

CONTIGUITY



High-contig N50 for assembly

COMPLETENESS

Mapping in difficult regions

CORRECTNESS

AGTCCGTCAATGT
GCAATAGACAGTC
TACAGTTGGACAT
GCAGATACAGATA

High base accuracy + phased alleles

COMPUTE

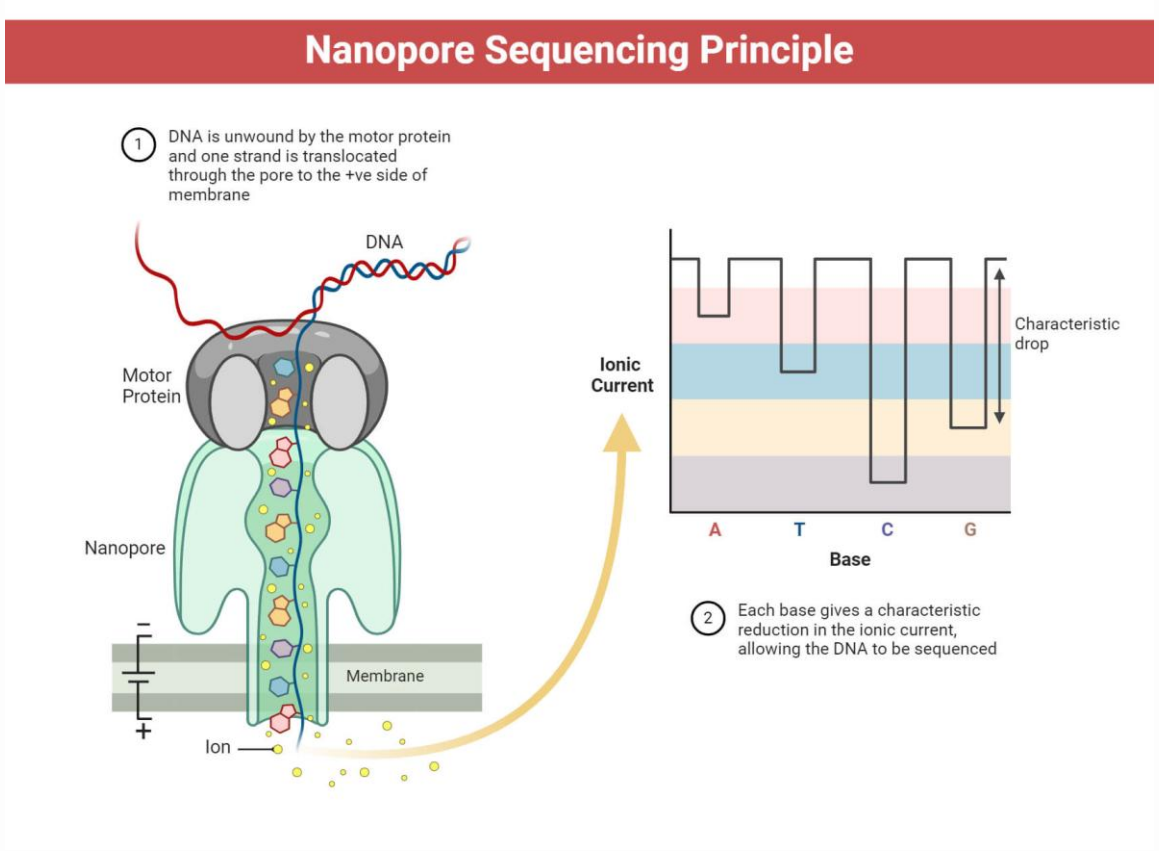
Large data requirement

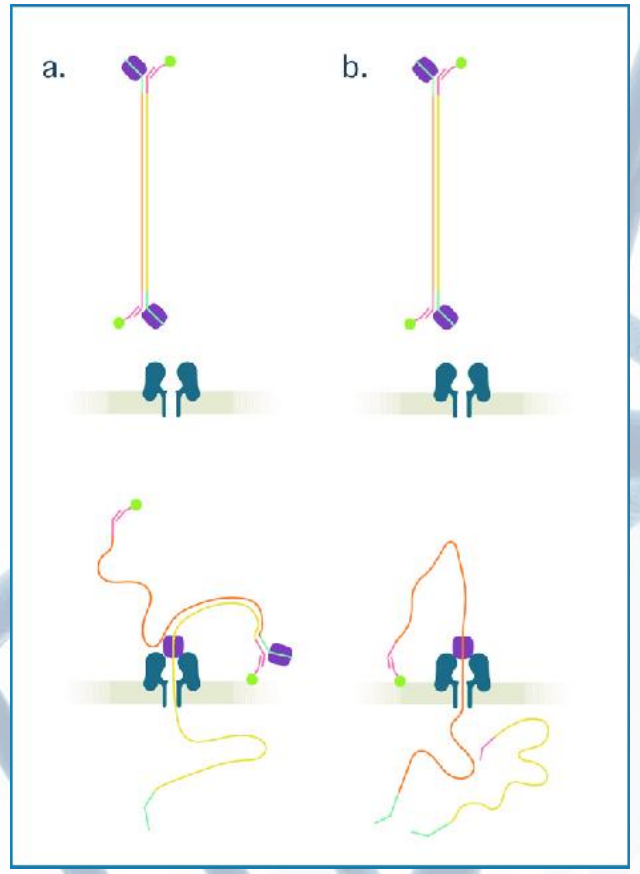
Large compute requirement

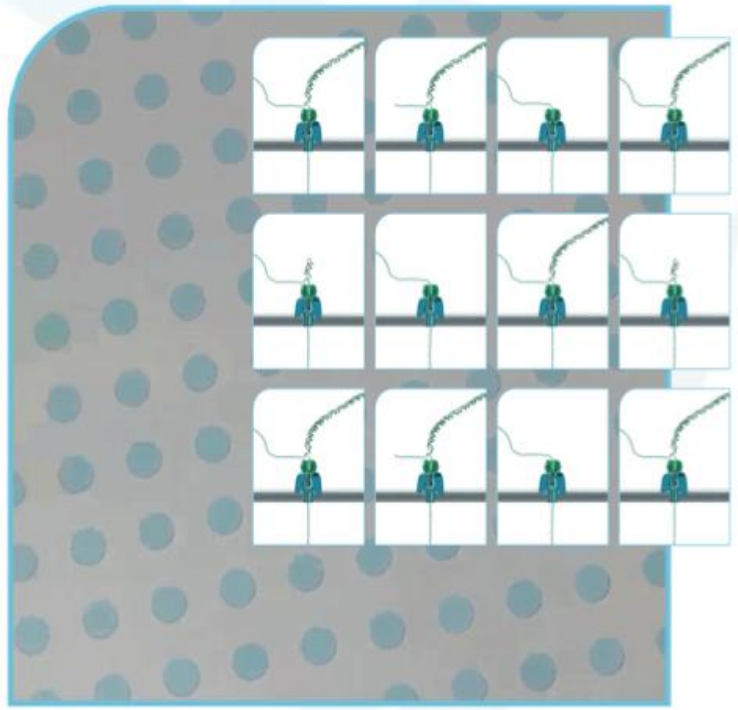
Nanopore sequencing

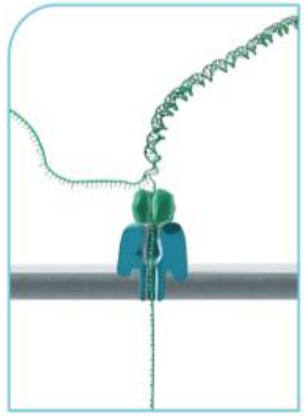
- MinION
- GridION
- PromethION

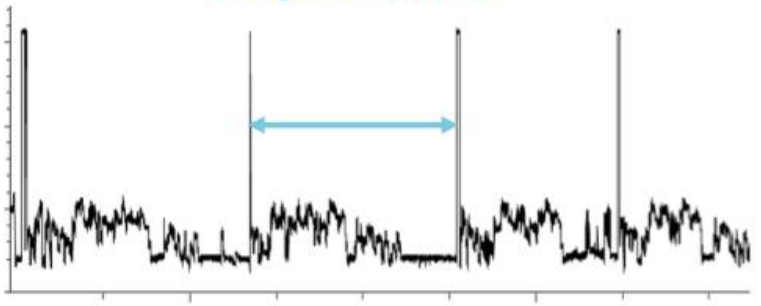
Oxford Nanopore sequencing

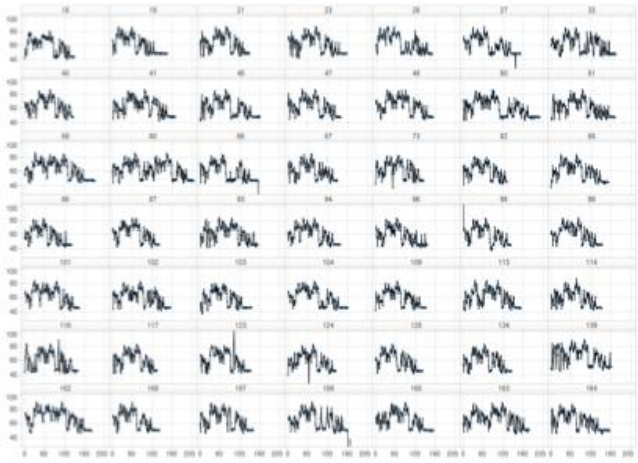








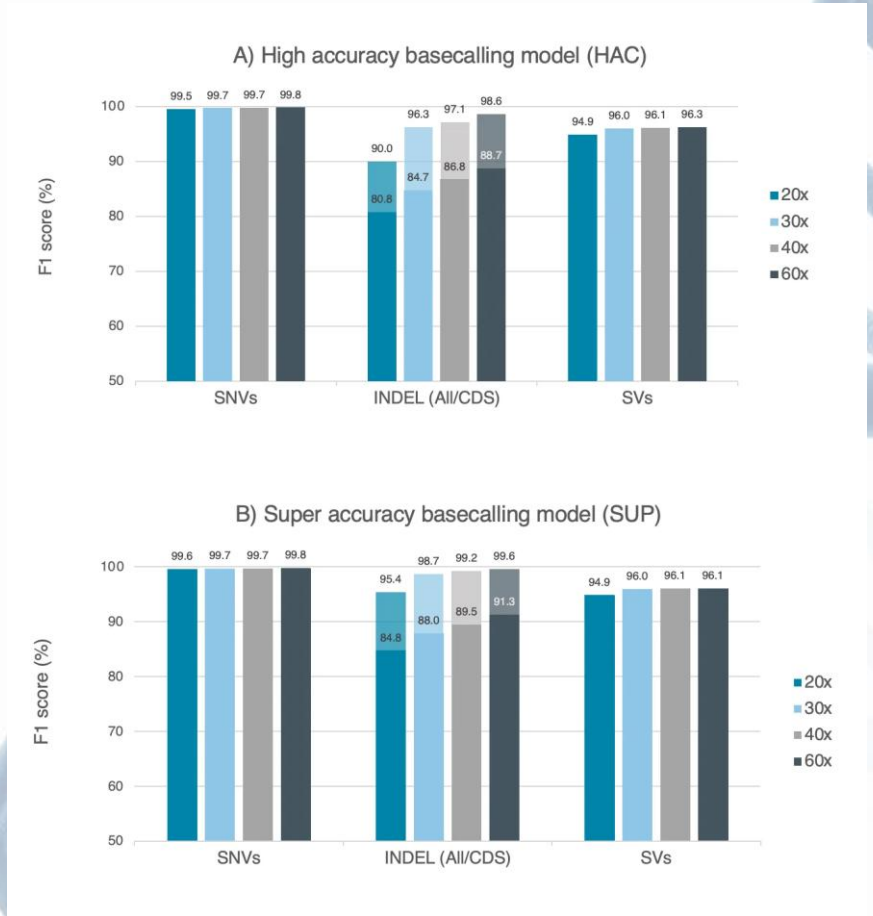




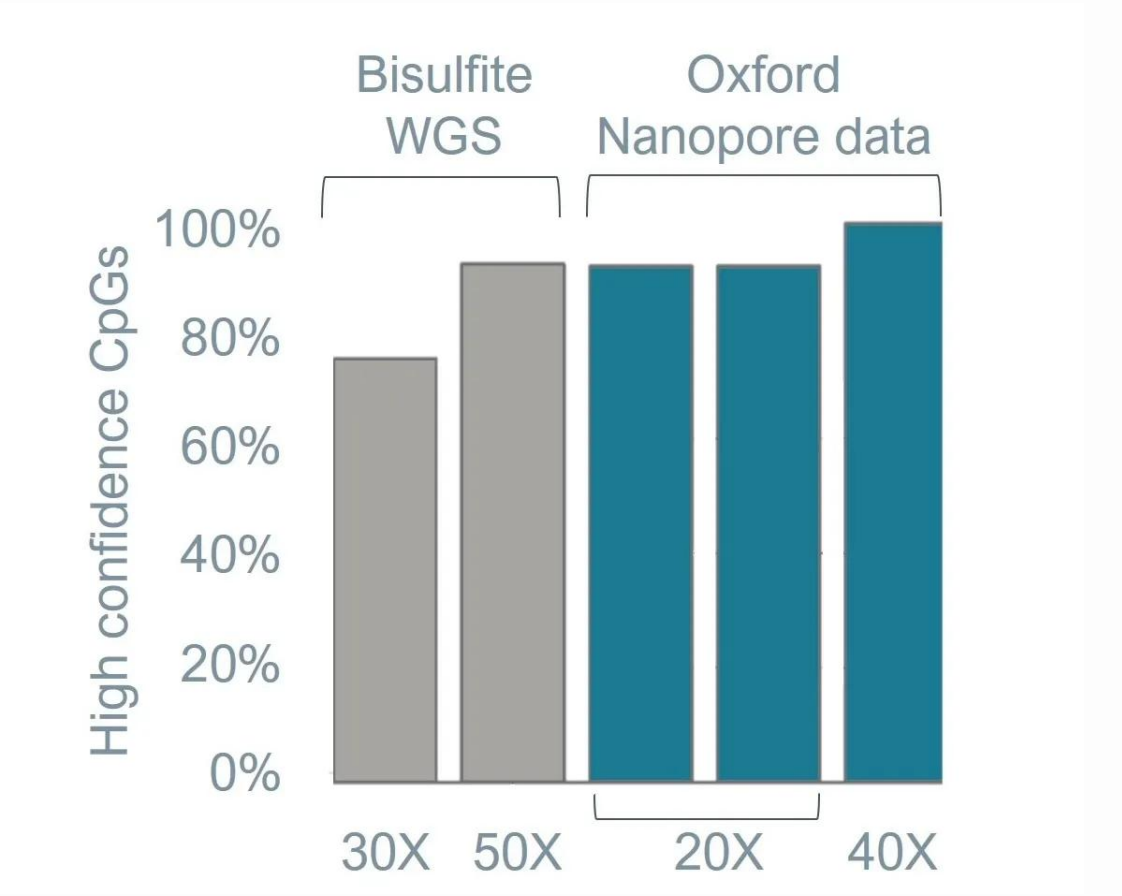
- Data acquired as full length reads – real time
- Data throughput = No. pores x average speed/pore

From squiggles to sequencing

- Basecaller: transform squiggles into base calls
  - Fast base calling: live action
  - HAC: the usual
  - SUP: the accurate ➔ most diagnostics
  - Duplex: the ‘super’ accurate but wasteful



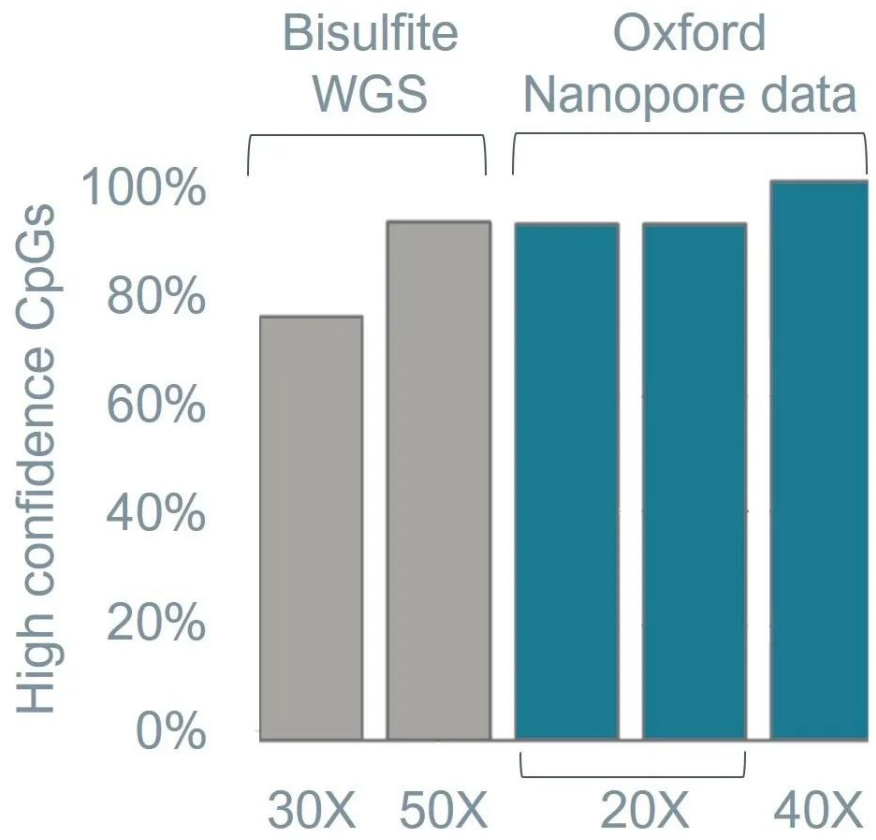
Methylation calling



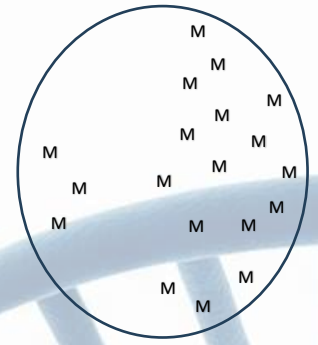
BASE RATE FALLACY

Molecule	Modification	Molecular context	Raw read accuracy (SUP)
DNA	5mC	CpG	99.5%
	5mC	All	99.4%
	5mC/5hmC	CpG	99.2%
	5mC/5hmC	All	98.7%
	6mA	All	99.7%
	4mC/5mC	All	97.6%
RNA	m6A	DRACH	99.7%
	m6A	All	98.7%
	pseU	All	97.6%
	m5C	All	97.9%
	Inosine	All	98.8%
	2'OMe-A	All	99.2%
	2'OMe-C	All	98.7%
	2'OMe-G	All	98.2%
	2'OMe-U	All	96.7%

Methylation calling

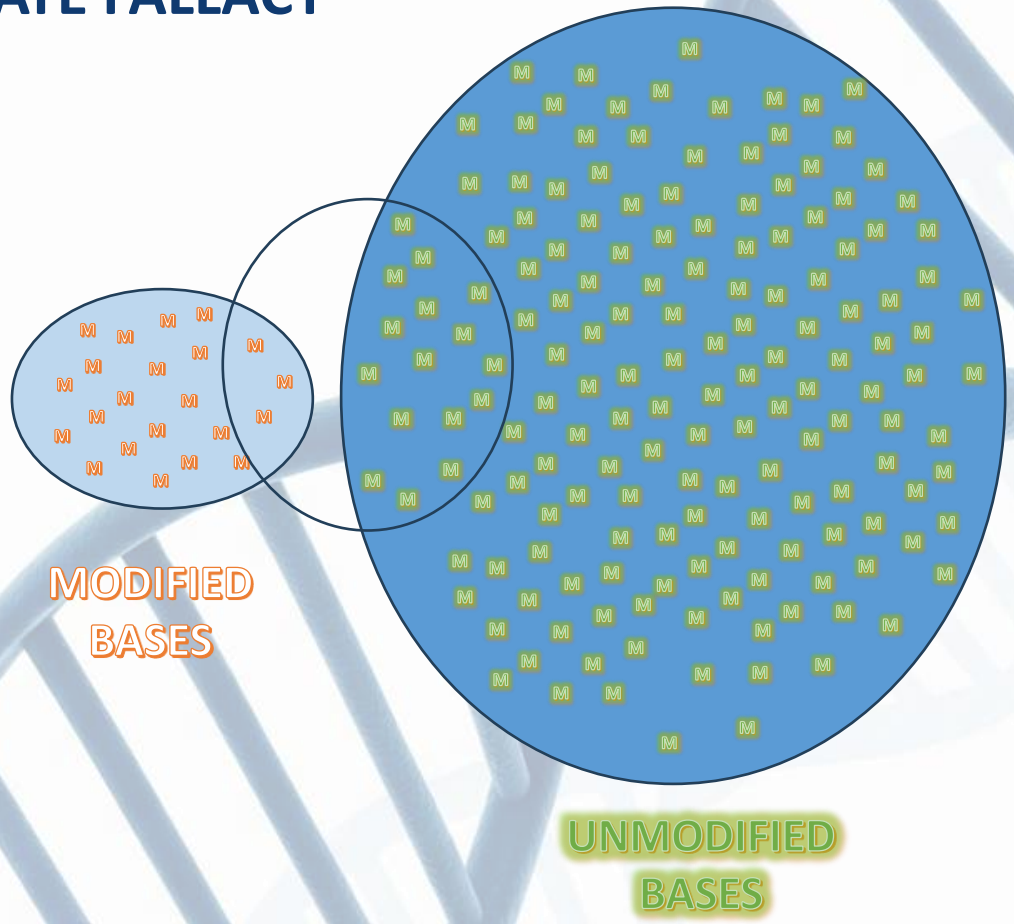


BASE RATE FALLACY



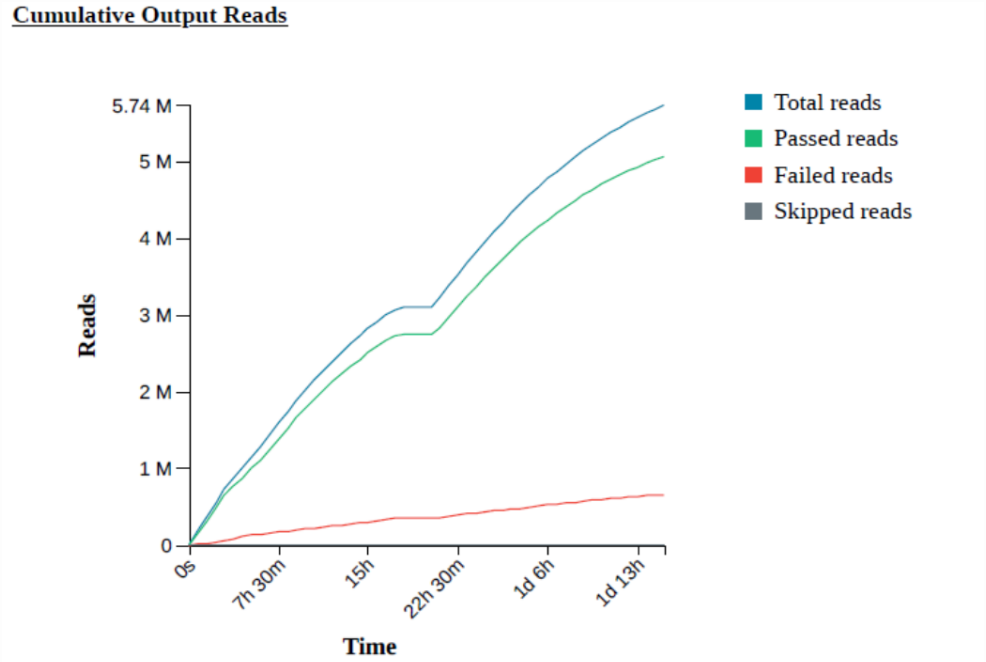
Methylation calling

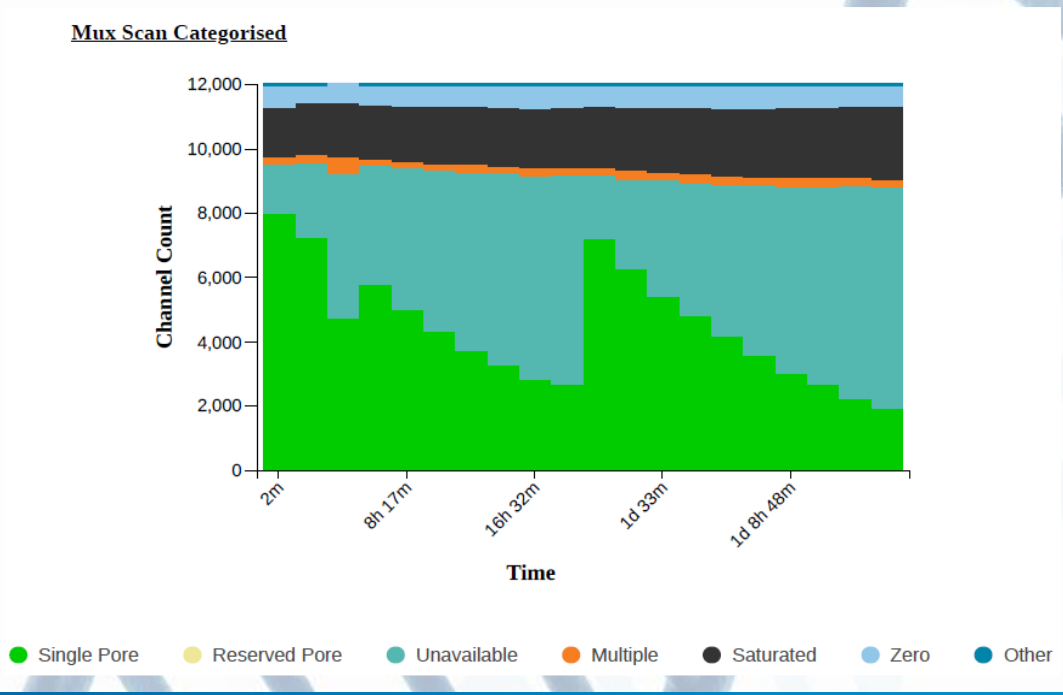
BASE RATE FALLACY



Run QC on Nanopore

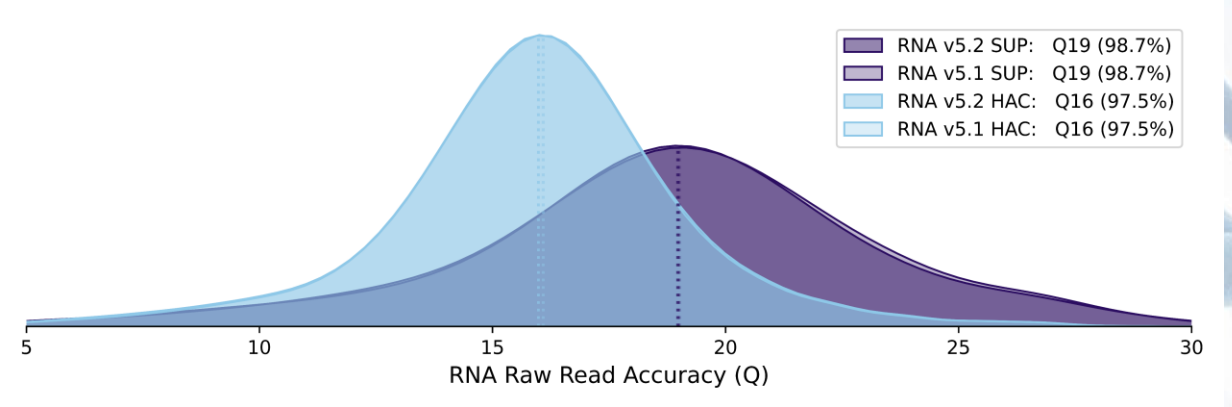
- Every flowcell is different, variation becoming less
- Nuclease wash (and refueling) increases output





Run QC on Nanopore

- Q-score need to be stable
- New flowcell chemisty improves Q-score
- Barcode selection → select high quality door
- SUP basecalling for variant calling necessary



Pacbio Revio

100M
ZMW / run

360 Gb
HiFi yield per run

24 hr
Sequencing time

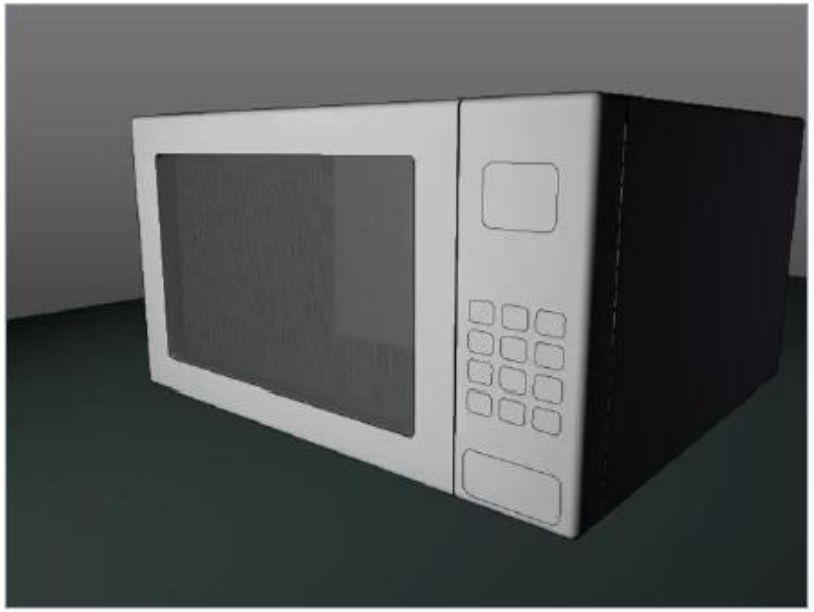
15-18 kb
Read length

5mC
DNA methylation

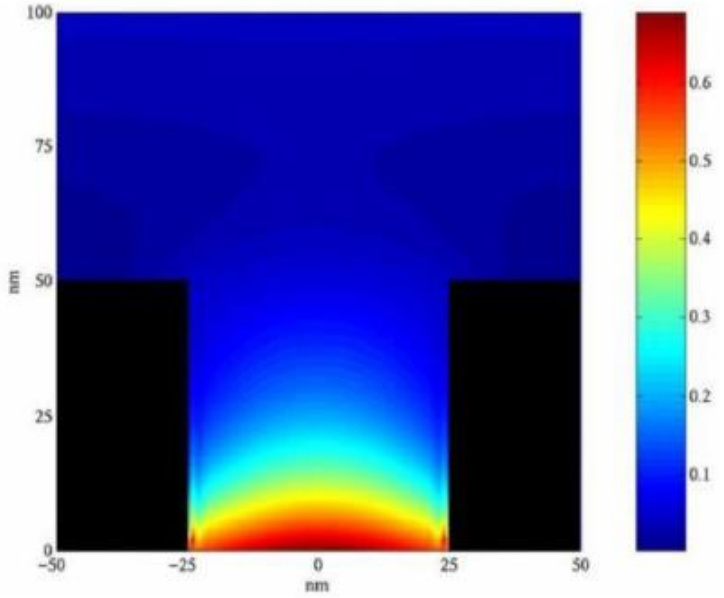
90% ≥Q30
Base quality

Revio™ system

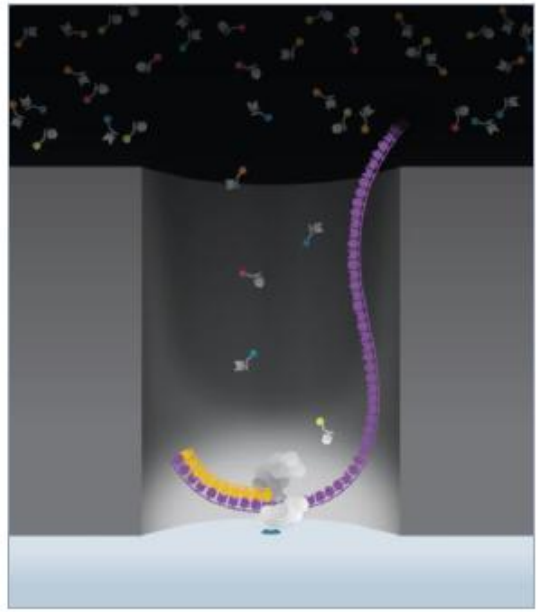
ZERO-MODE WAVEGUIDES (ZMWS) ENABLE HIGHLY SENSITIVE OPTICAL-BASED DETECTION OF SINGLE MOLECULES



A. Each ZMW is illuminated from below and works *via* the same principle as a metallic microwave oven door screen



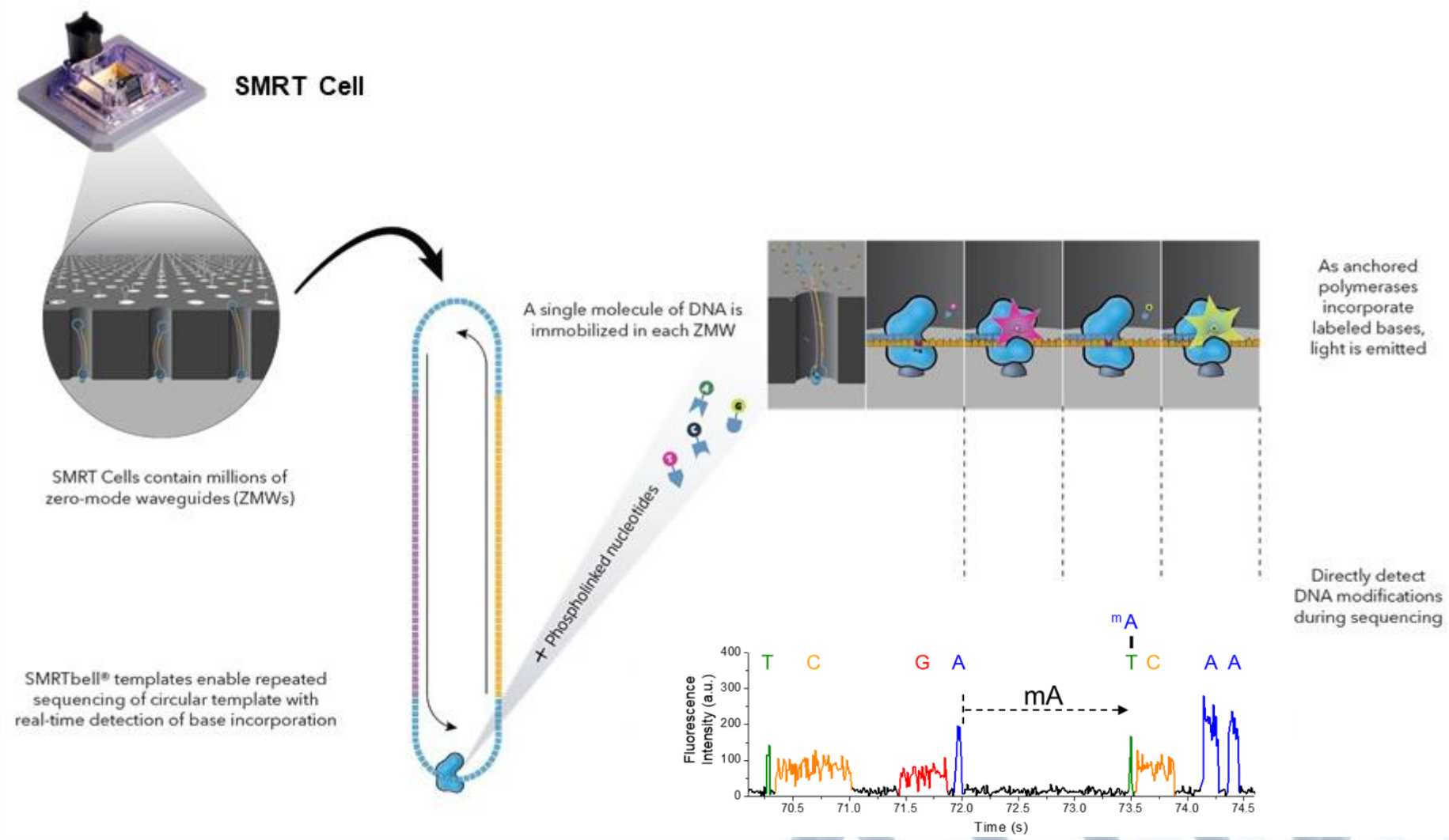
B. Because the illumination wavelength is much greater than the ZMW diameter, light penetrates only nanometers into the ZMW, providing a vanishingly small illumination volume



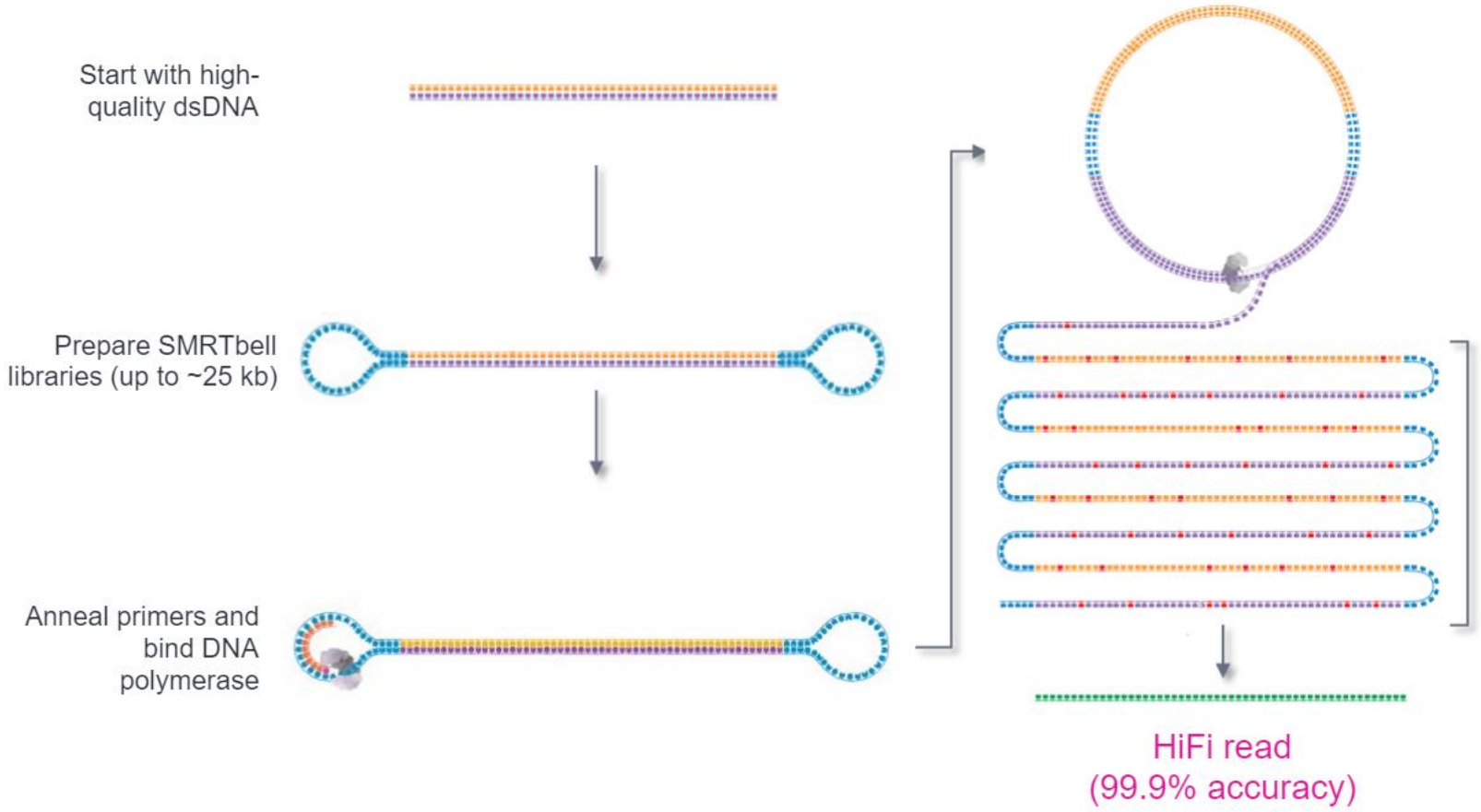
C. A DNA polymerase-template complex is immobilized onto the bottom surface of the ZMW

The Illuminated volume is small enough to enable real-time observation of DNA synthesis at the single-molecule level

SINGLE MOLECULE, REAL-TIME (SMRT) SEQUENCING



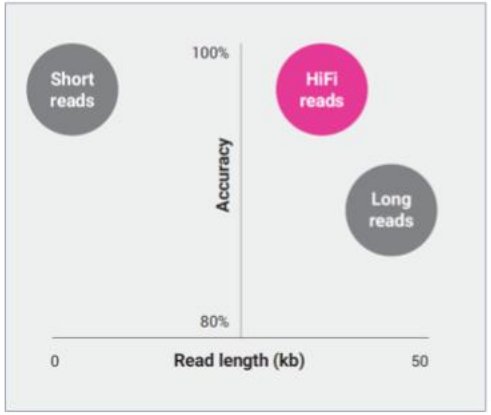
Circular consensus sequencing generates HiFi reads



Circularized DNA is sequenced in repeated passes

Polymerase reads are trimmed of adapters to yield subreads

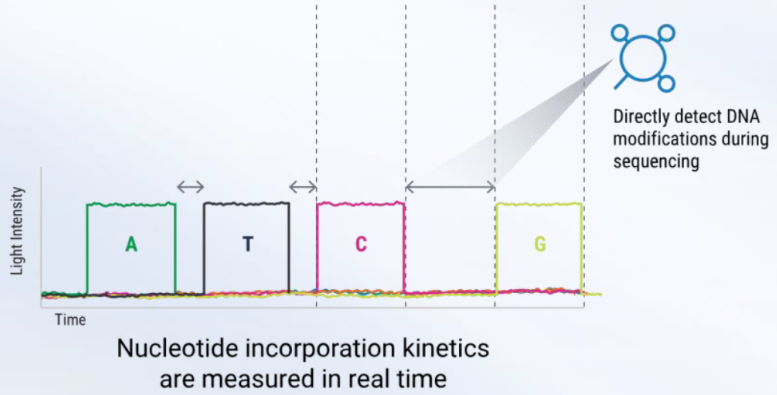
Consensus and methylation status are called from subreads



Benefits of HiFi reads

- Long read lengths up to 25 kb
- High read accuracy ≥99.9%
- Easy library preparation
- Low coverage requirements
- Small file sizes to minimize compute time
- A single technology solution for a range of applications

Multiomic capabilities

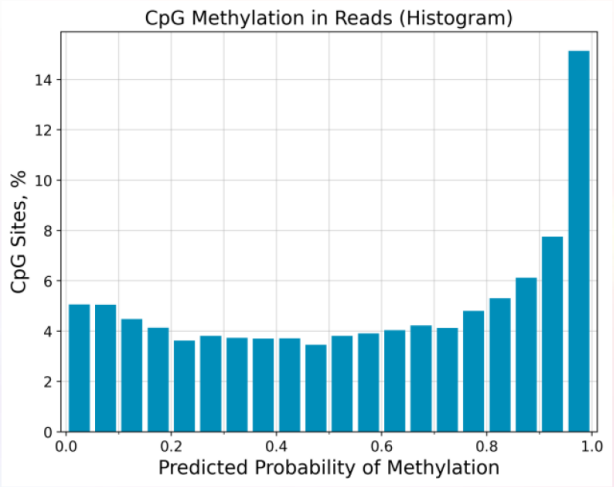


Nucleotide incorporation kinetics are measured in real time

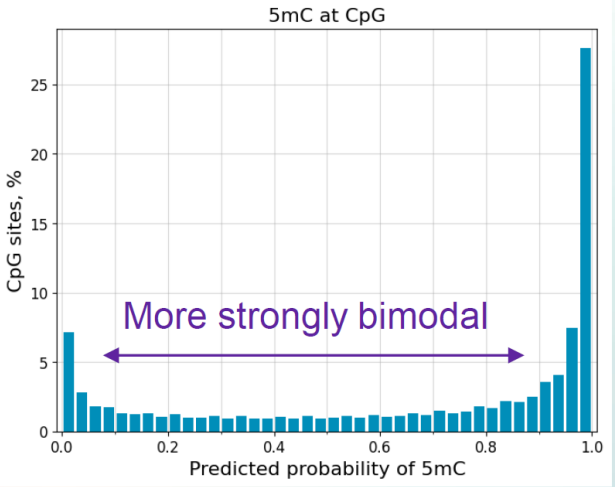
- ✓ Improved accuracy (+10%) and increased confidence of 5mCpG calling
- ✓ On-instrument 6mA caller for multiomic Fiber-seq chromatin assay

5mC at CpG sites

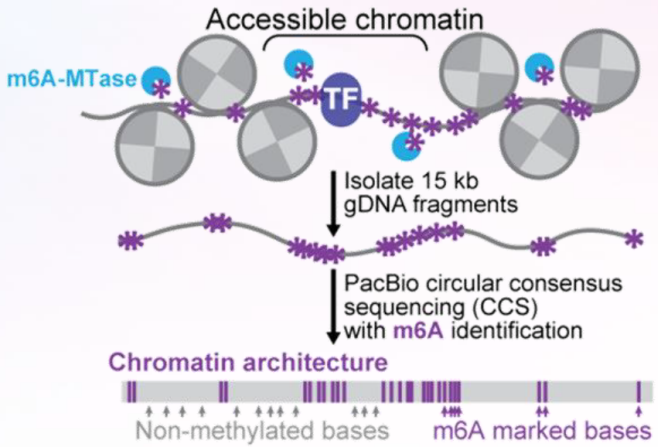
Previous



SPRQ



6mA for Fiber-seq chromatin assay



Fiber-seq chromatin assay is being adopted in HPRC, SMaHT, and other groups.

The Revio caller simplifies the workflow and unlocks other applications for customers to explore.

The main differences

Competition updates

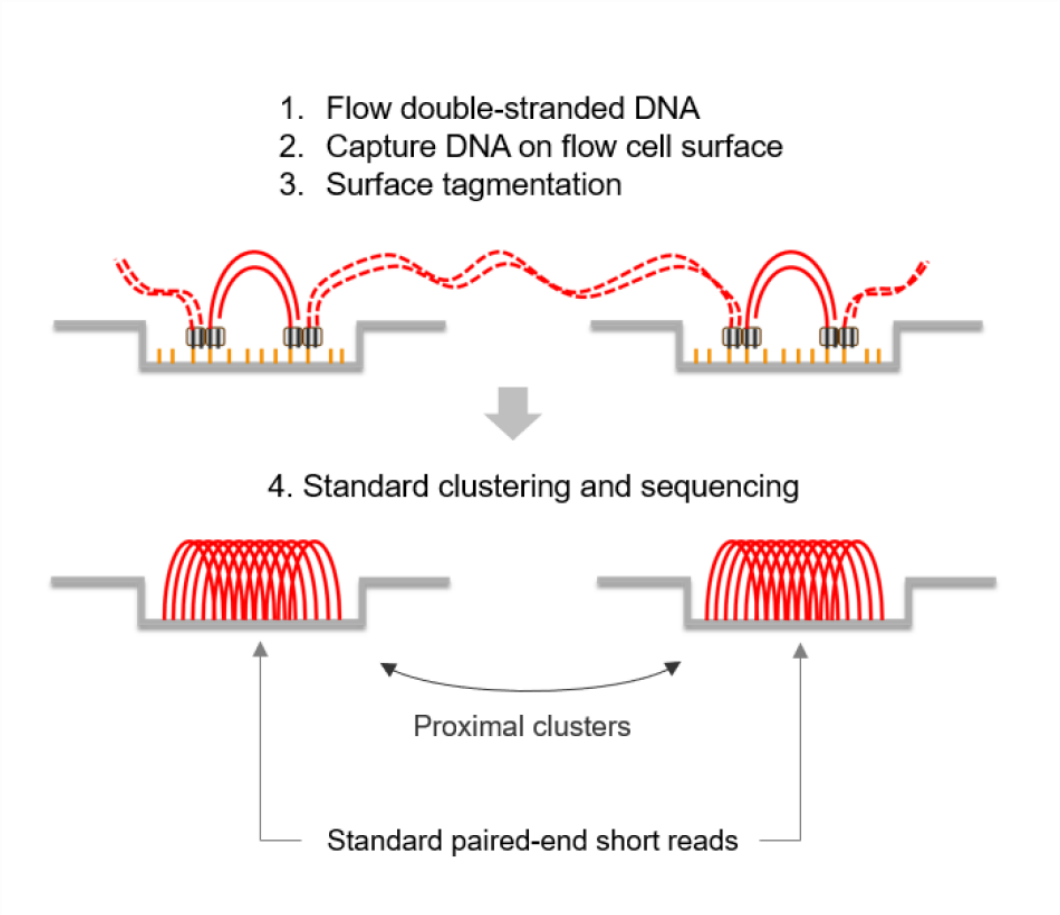
NANOPORE

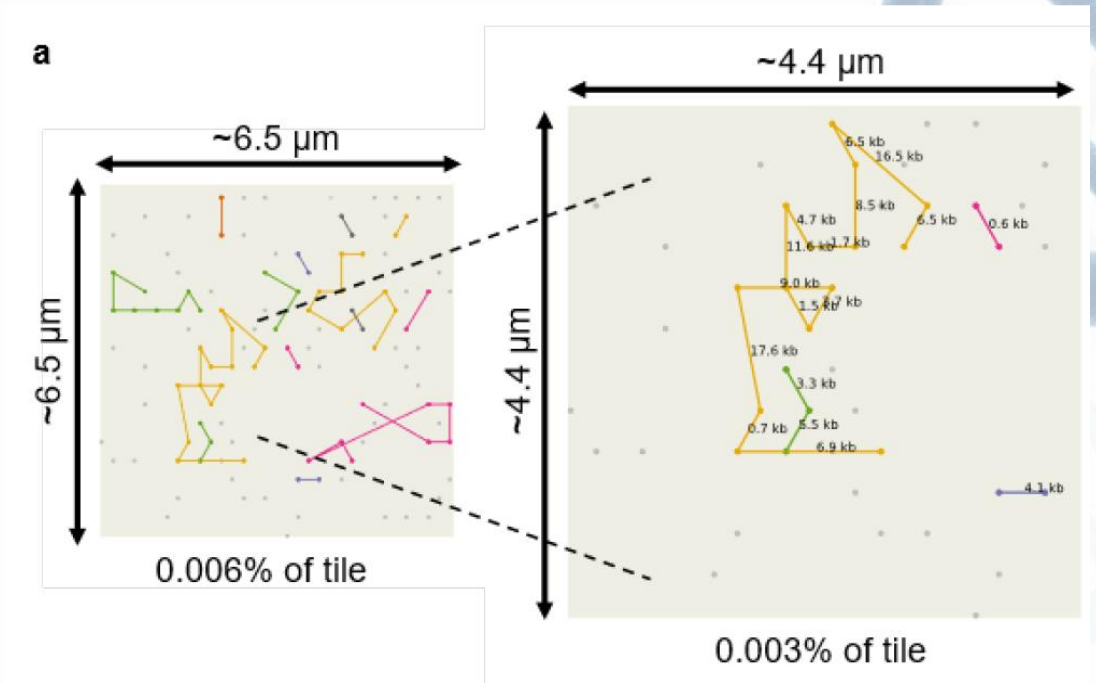
- No-wash flowcells and new chemistry
  - R10.4.1 flowcell
  - Only early 2027
  - ≤15kb fragments need no wash
  - 200Gb output/flowcell
  - Same price

Pacbio

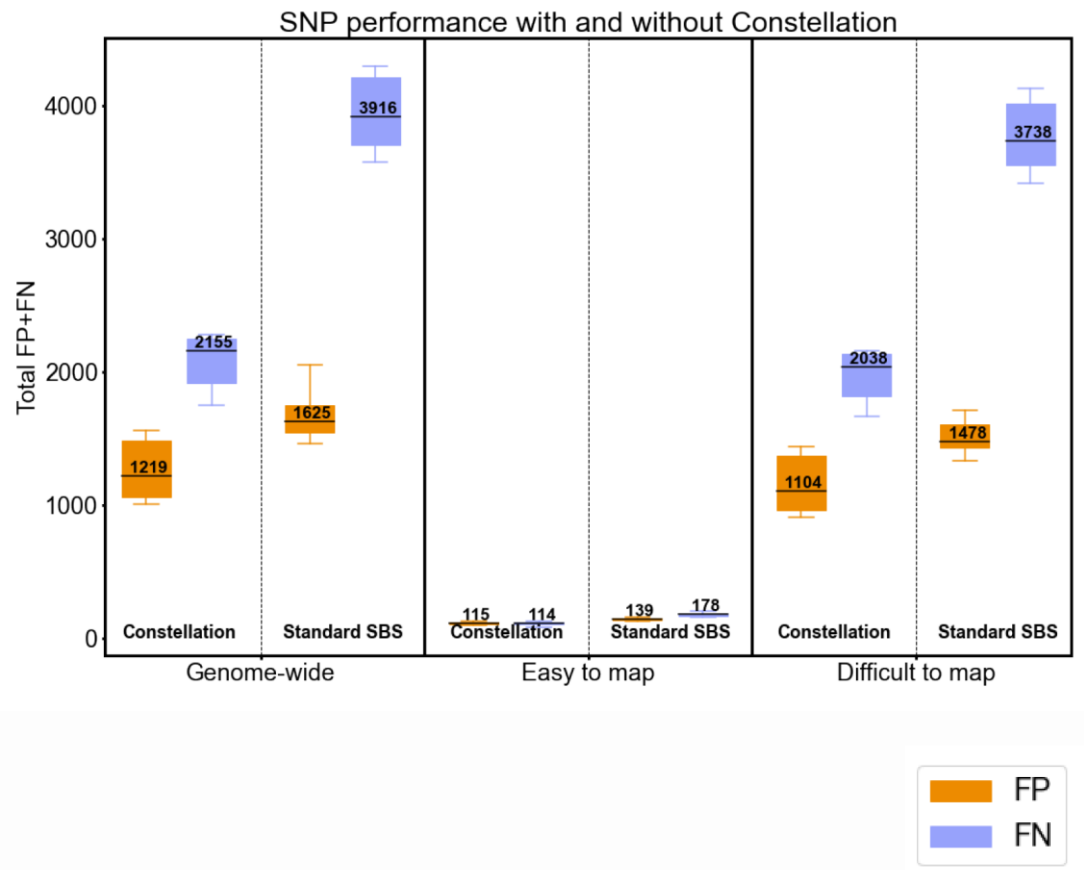
- SPRQ-NX chemistry
  - Multi-use flowcells
  - Accuracy update
  - Output >120Gb/flowcell
  - Same price

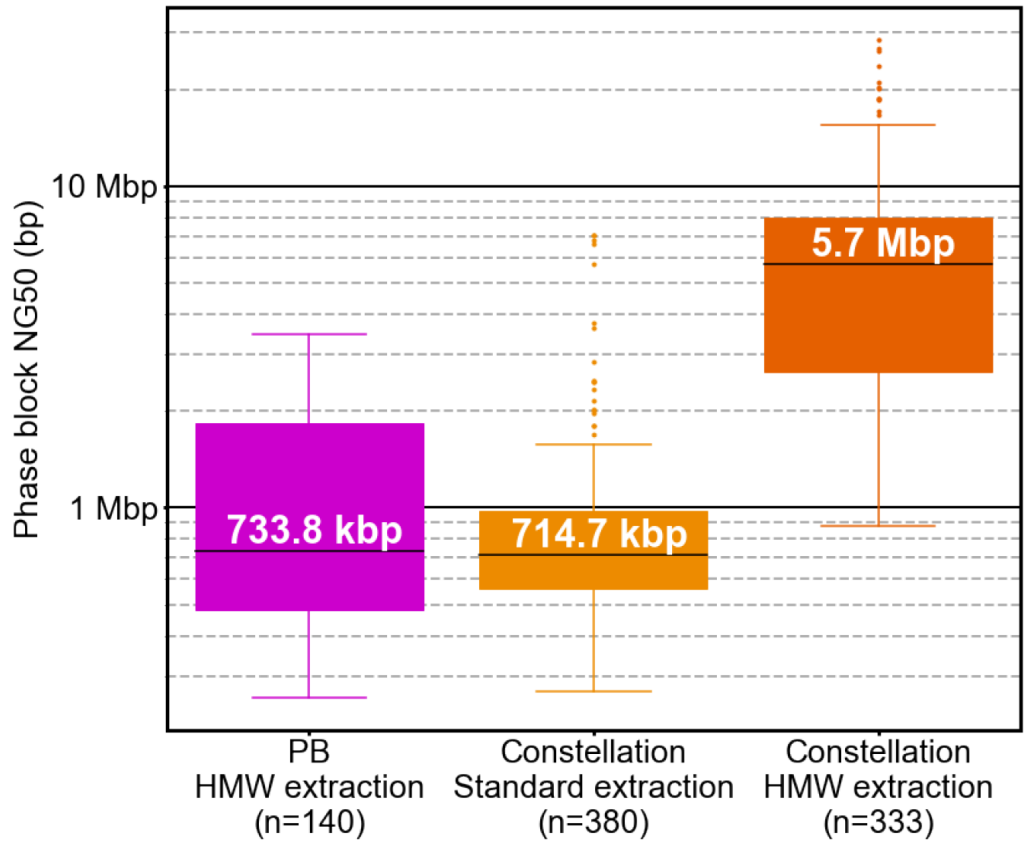
Linked reads: Illumina constellation





Constellation performance





Applications

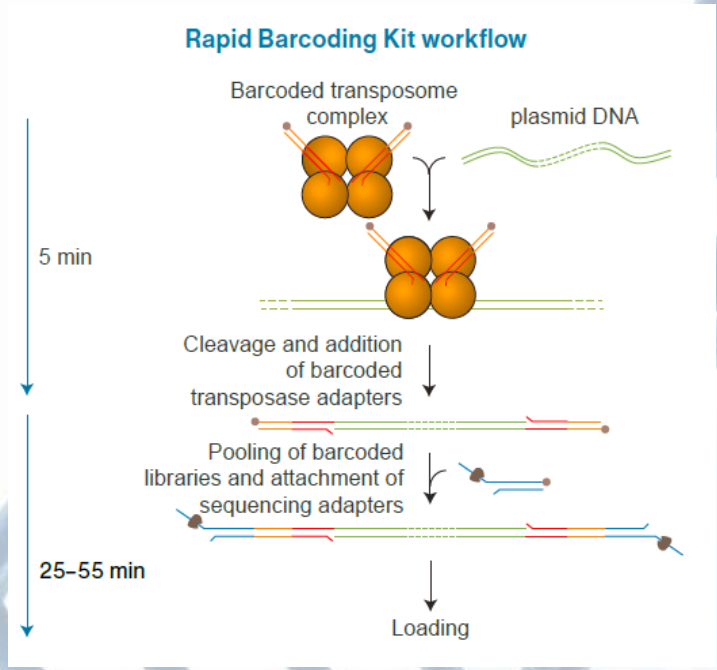
lrWGS: Nanopore or Pacbio?

- Look at the genome and sample to decide what technology to go for
- Input amount
- Symbionts?
- Repetitive regions
- Reference genome
- Genome size
- Ploidy and degree of heterozygosity

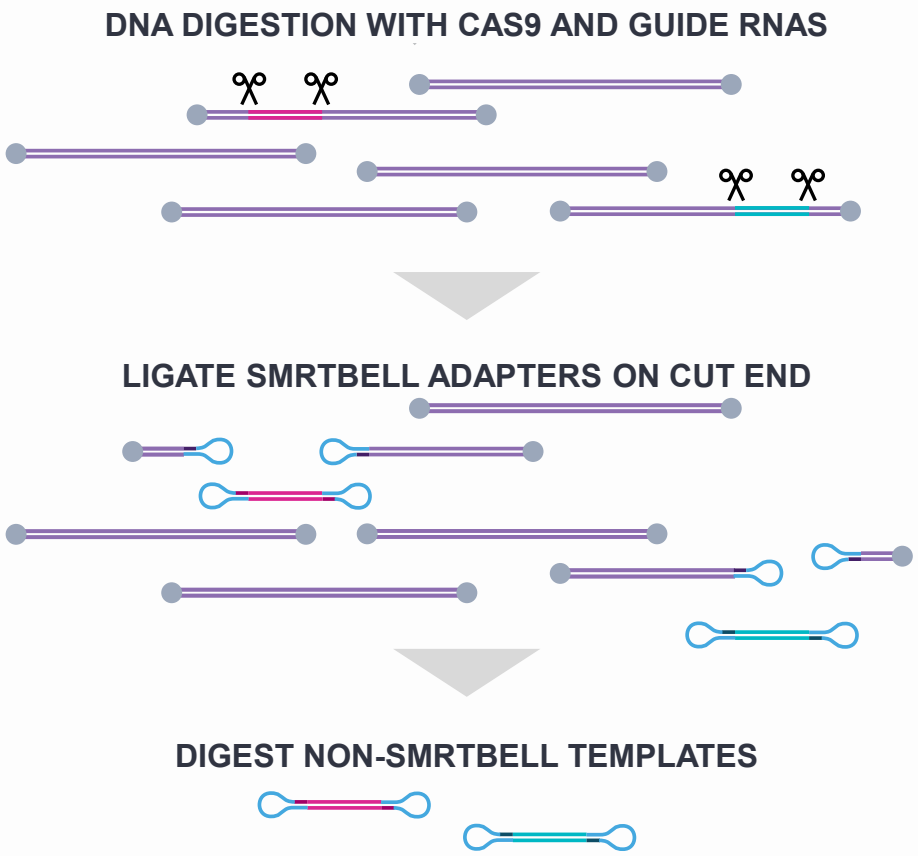
- For human
  - Constitutional: little difference
    - Slight edge for Pacbio on indels
    - Slight edge for Nanopore on SV's in difficult regions
  - Somatic or mosaicism: Pacbio
  - Methylation calling: little difference

Nanopore full plasmid sequencing

- Multiplex up to 96 plasmids
- Full plasmid sequence: no assumptions
- [EPI2Me analysis pipeline]



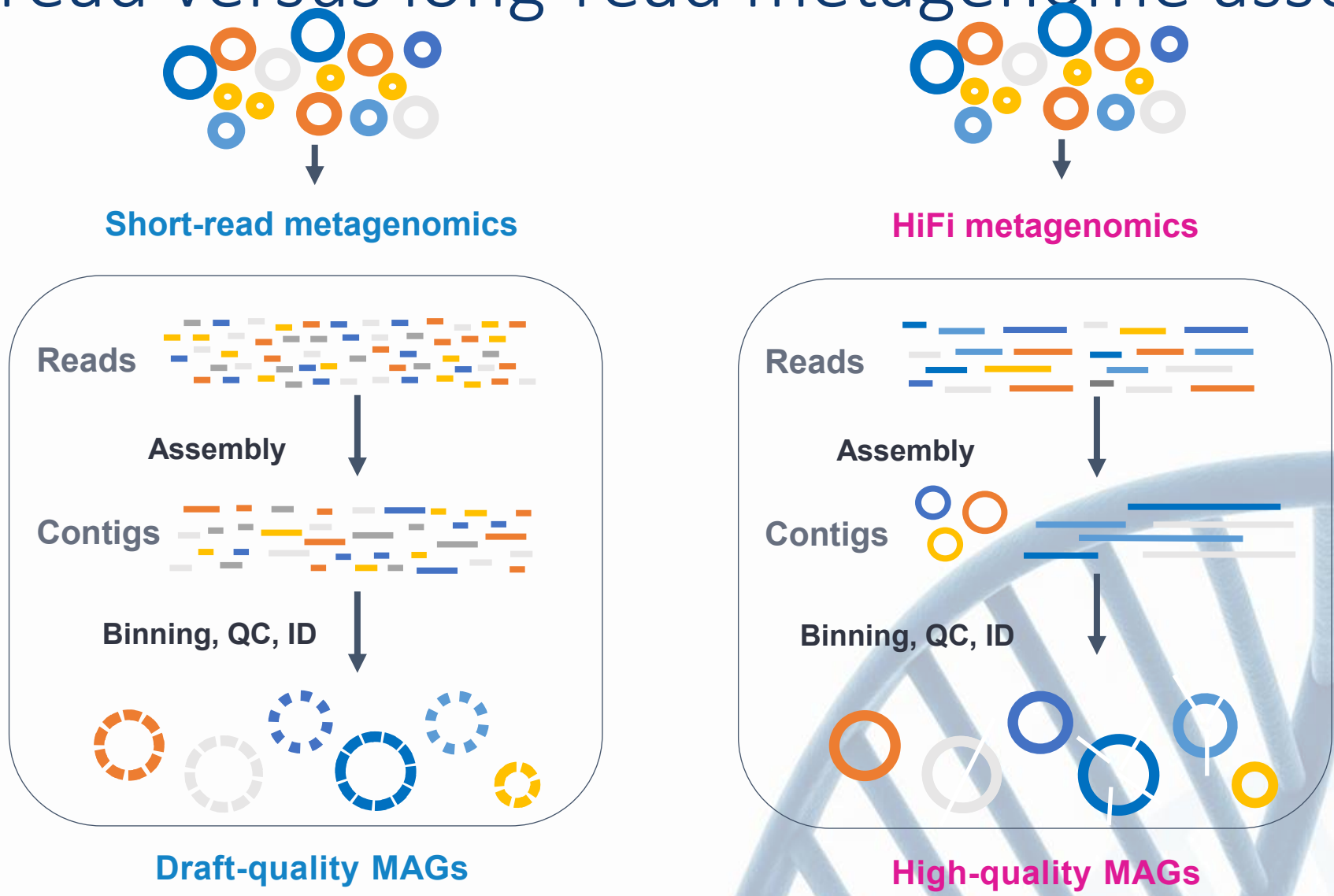
PureTarget enrichment with CRISPR-Cas9 is more accurate than PCR or hybrid capture



	PureTarget (no-amp)	**PCR amplicons**	**Long-read hybrid capture** (Twist)
Uses PCR	No	Yes	Yes
Retain methylation	Yes	No	No
GC dropout	No	Yes	Yes
Size bias	Low	High	High
Replication errors	No	Yes	Yes

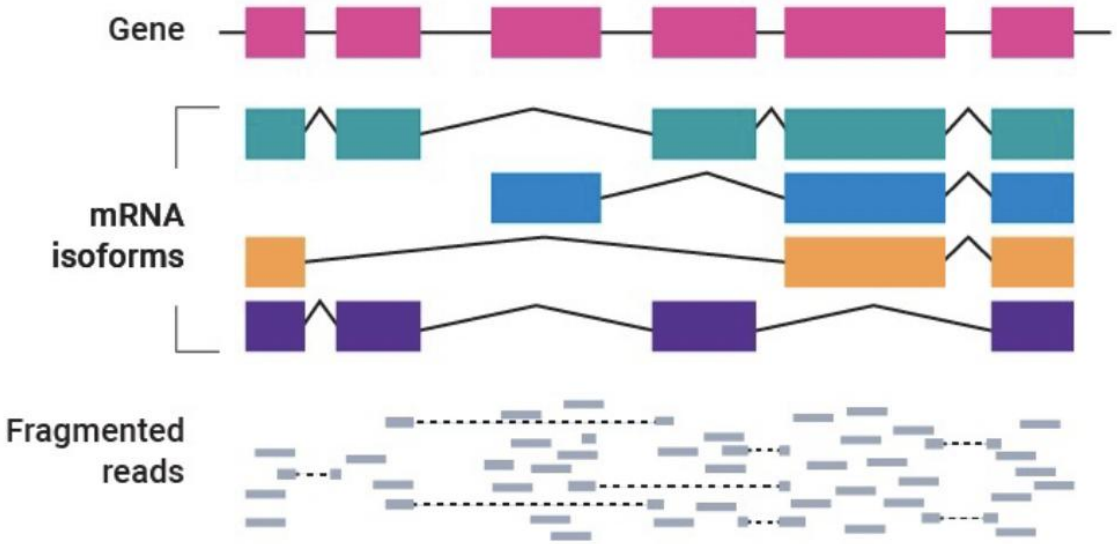
Tsai et al., (2022). *J Mol Diagn.* 24(11):1143-1154. https://doi.org/10.1016/j.jmoldx.2022.08.003.

Short-read versus long-read metagenome assembly



Iso-Seq method delivers full-length transcripts

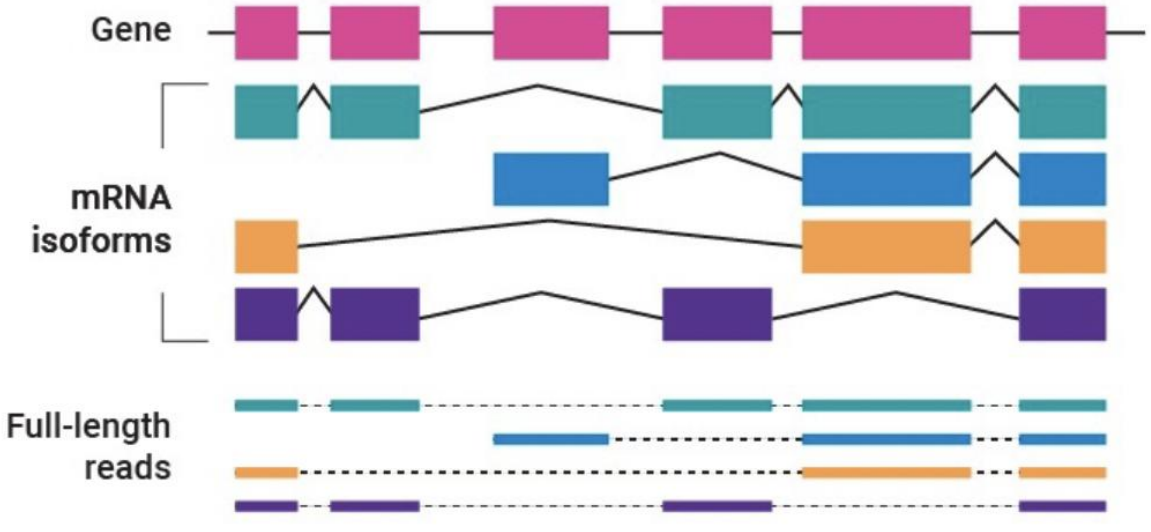
Short read sequencing



Short-read sequencing can only assemble ~20 to 40% of human transcriptomes

PARTIAL view of isoforms

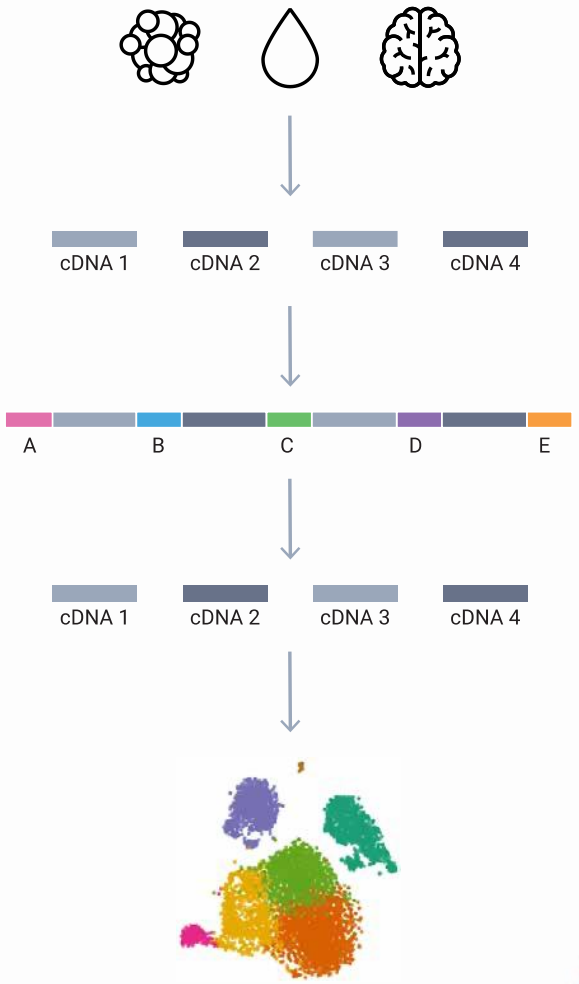
Long read sequencing



PacBio's long-read sequencing offers superior isoform discovery power

COMPLETE view of isoforms

Increasing throughput with concatenation: Kinnex kits



Single-cell
cDNA generation

MAS-Seq
library preparation

PacBio sequencing

Read segmentation +
single-cell isoform analysis

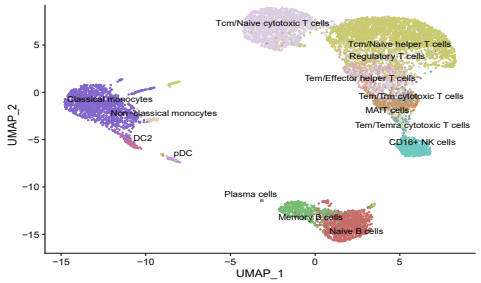
3 varieties of Kinnex kits

Kinnex single-cell RNA kit

Upgrade to *MAS-Seq for 10x Single Cell 3'* kit

Support 10x 3' and 5'; up to 4-plex

40M reads (Sequel II and IIe systems), 80M reads (Revio system)

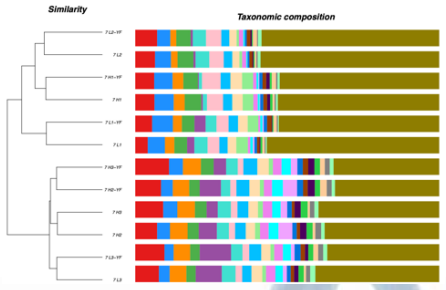


Kinnex 16S rRNA kit

Full-length 16S rRNA for species identification

Up to 1,536-plex

25M reads (Sequel II and IIe systems), 60M (Revio system)

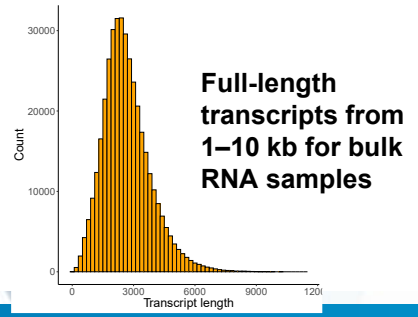


Kinnex full-length RNA kit

Full-length RNA sequencing

Up to 48-plex

15M reads (Sequel II and IIe systems), 40M reads (Revio system)



A long thank you!

Any long questions?