Long-read sequence analysis

Sequencing technologies

Question 4

What is a long read?

- Short read: 50-300 bp, often paired-end (Illumina sequencing)
- Long read: > 1kb, up to 20 Mb:
  - single molecule sequencing or
  - 3rd generation sequencing

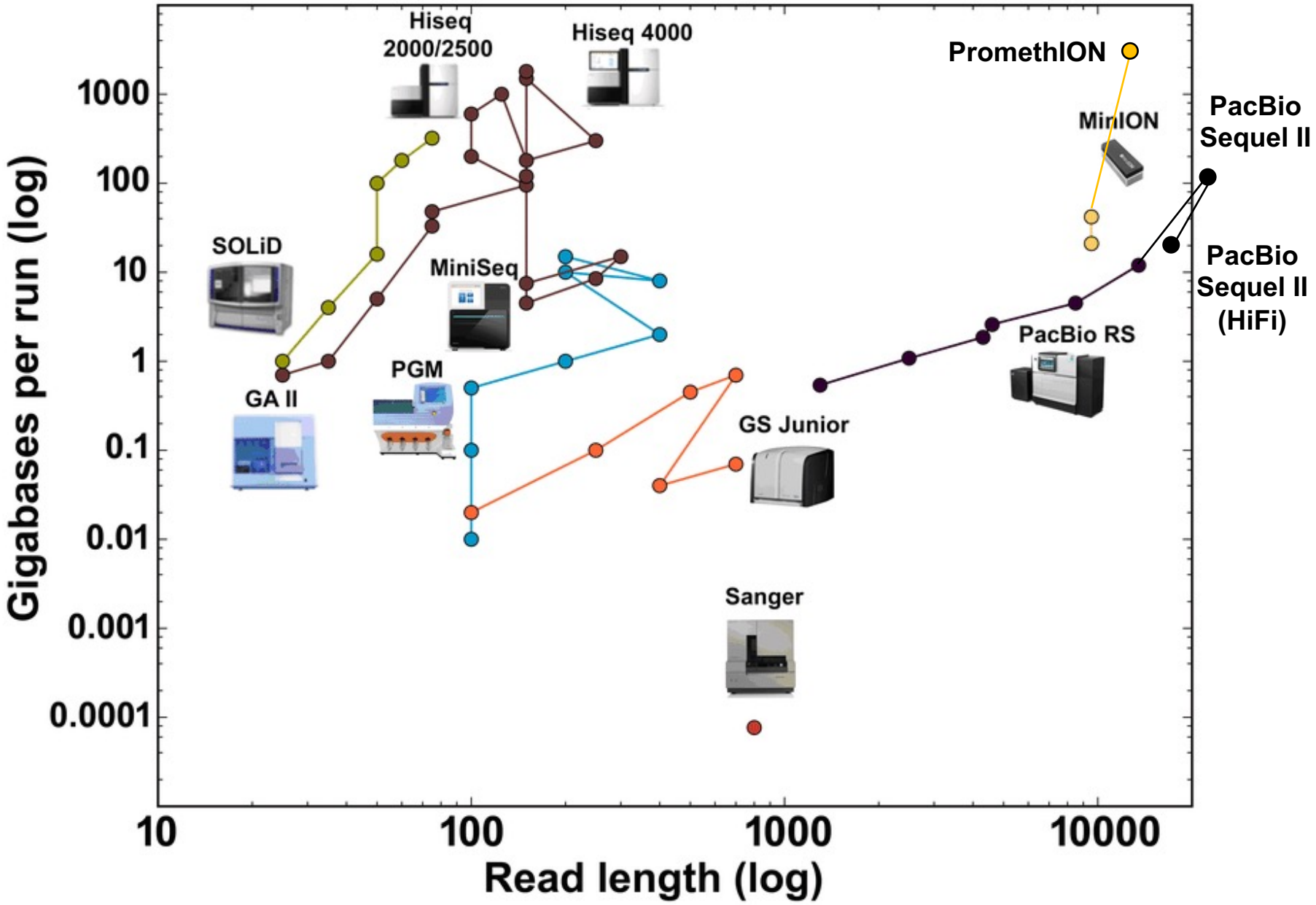


Image from: G. Silva (2016)

Illumina sequencing

- Sequencing-by-synthesis: 2nd generation sequencing
- Massive throughput: up to $500x10^9$ bases/run
- Most used platform today

illumina®

Illumina sequencing

- 50 – 300 bp
- Paired-end (or single-end)

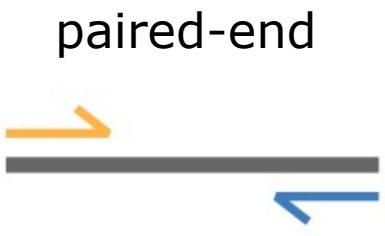
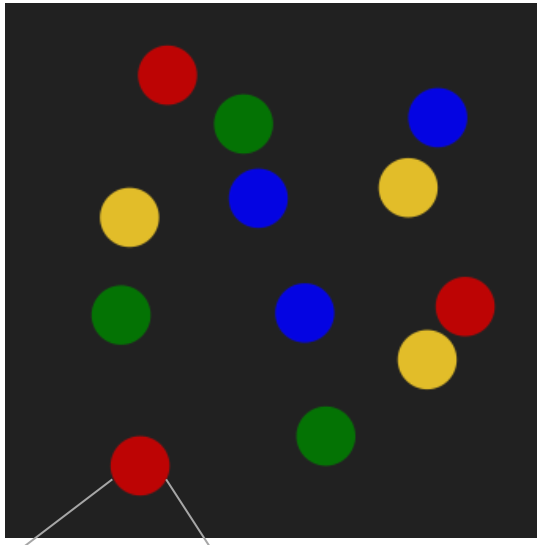


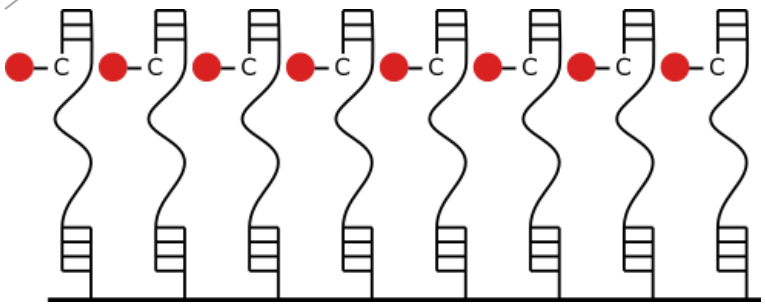
Image from: Illumina (2020)

Question 5

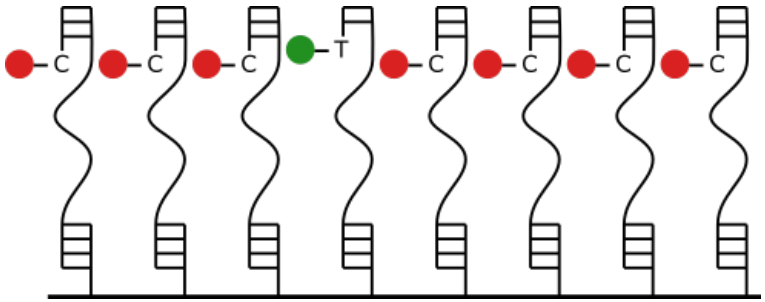
Illumina - limitations

- Maximum read length: 300 bp
- How to reconstruct:
  - Repeats?
  - Isoforms?
  - Structural variation?
  - Haplotypes?
  - Genomes?
- Why not longer read lengths with Illumina?





in phase



out of phase

Long reads (3rd generation)

- Crux: maximizing signal from a single-molecule base read-out
- Single molecule, so no out-of-phase signal
- Two frequently used platforms:
  - PacBio SMRT sequencing
  - Oxford Nanopore Technology

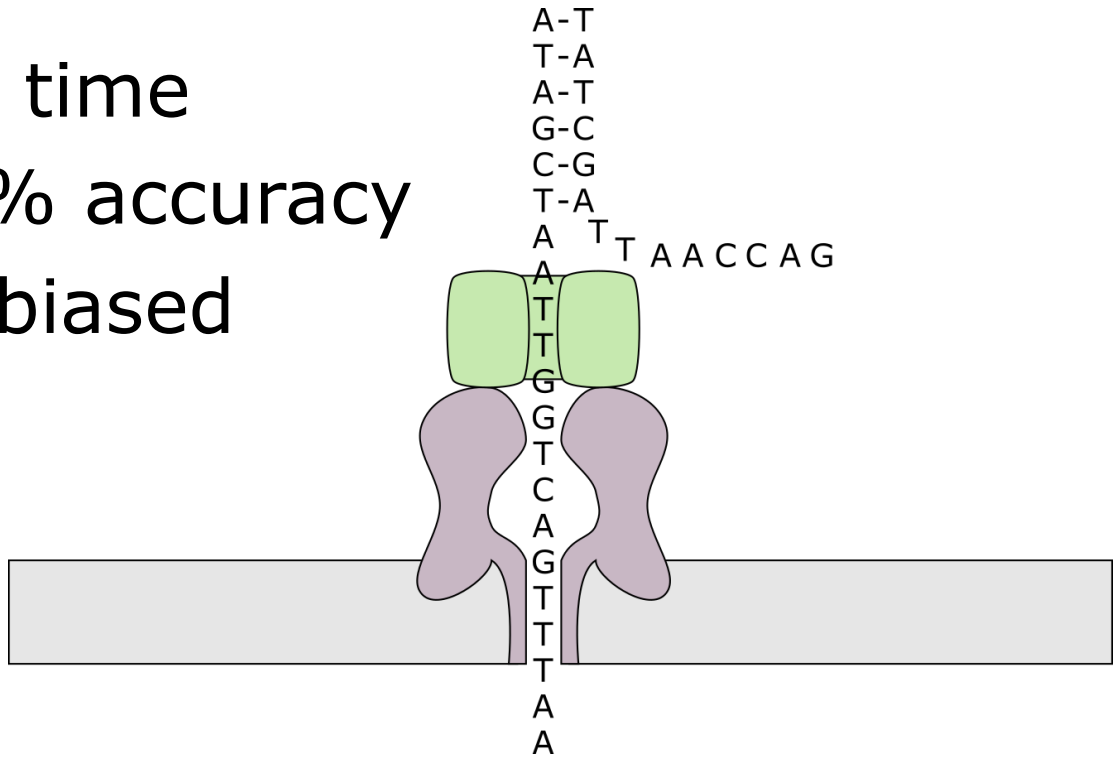
PacBio

Oxford NANOPORE Technologies

Question 6

Oxford Nanopore technology

- Based on changes in electrical current
- Well-known for its scalability and portability
- 4 bp read at a time
- Up to ~95-97% accuracy
- Errors can be biased



ONT scalability

1 small flow cell: 1 x 2.8 Gb

Flongle

1 medium flow cell: 1 x 50 Gb

MinION

5 medium flow cells: 5 x 50 Gb

GridION

24-48 big flow cells: 48 x 290 Gb

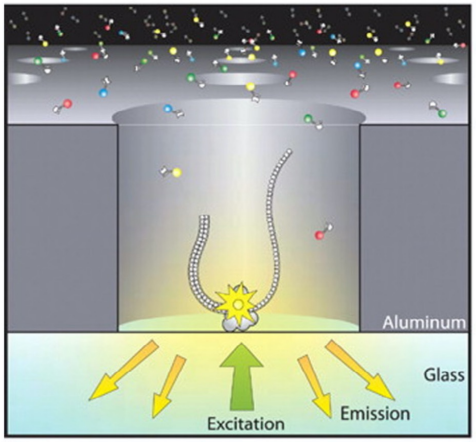
PromethION

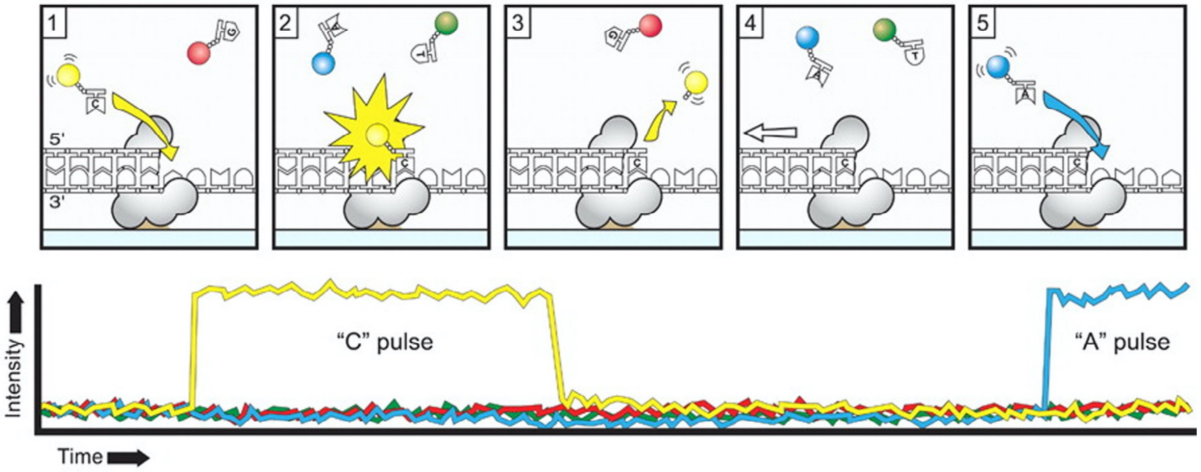
https://nanoporetech.com/products#comparison

ONT library prep

- Standard kit:
  - >1 μg HMW DNA
  - Shearing + size selection is optional
  - Multiplexing requires PCR step

PacBio sequencing





- Polymerase bound to ZMW bottom
- Circular molecules
- Single read out ~90% accuracy
- HiFi: single molecule sequenced multiple times

Image from: Rhoads A, Au KF. Genomics Proteomics Bioinformatics 2015;13:278–89

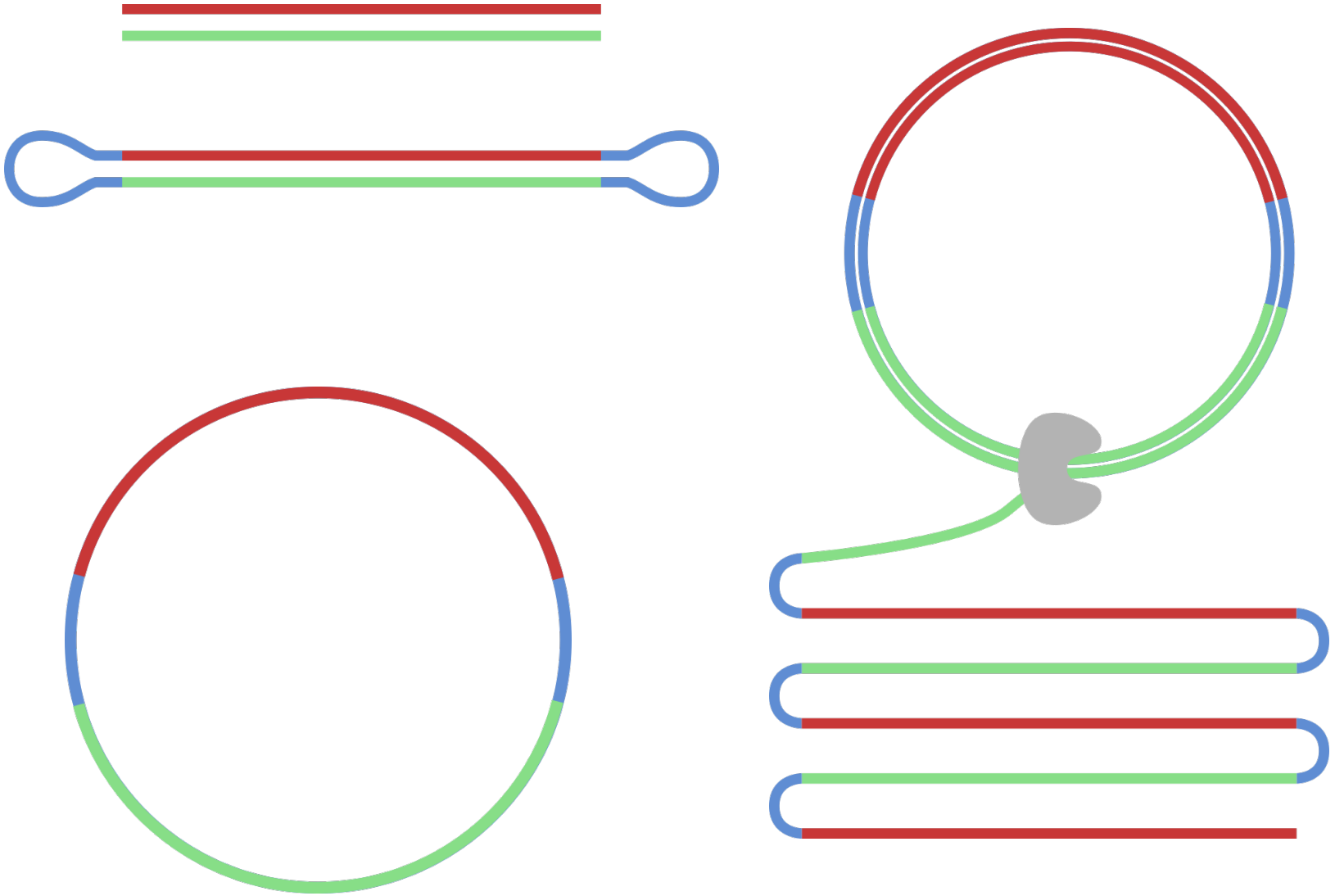
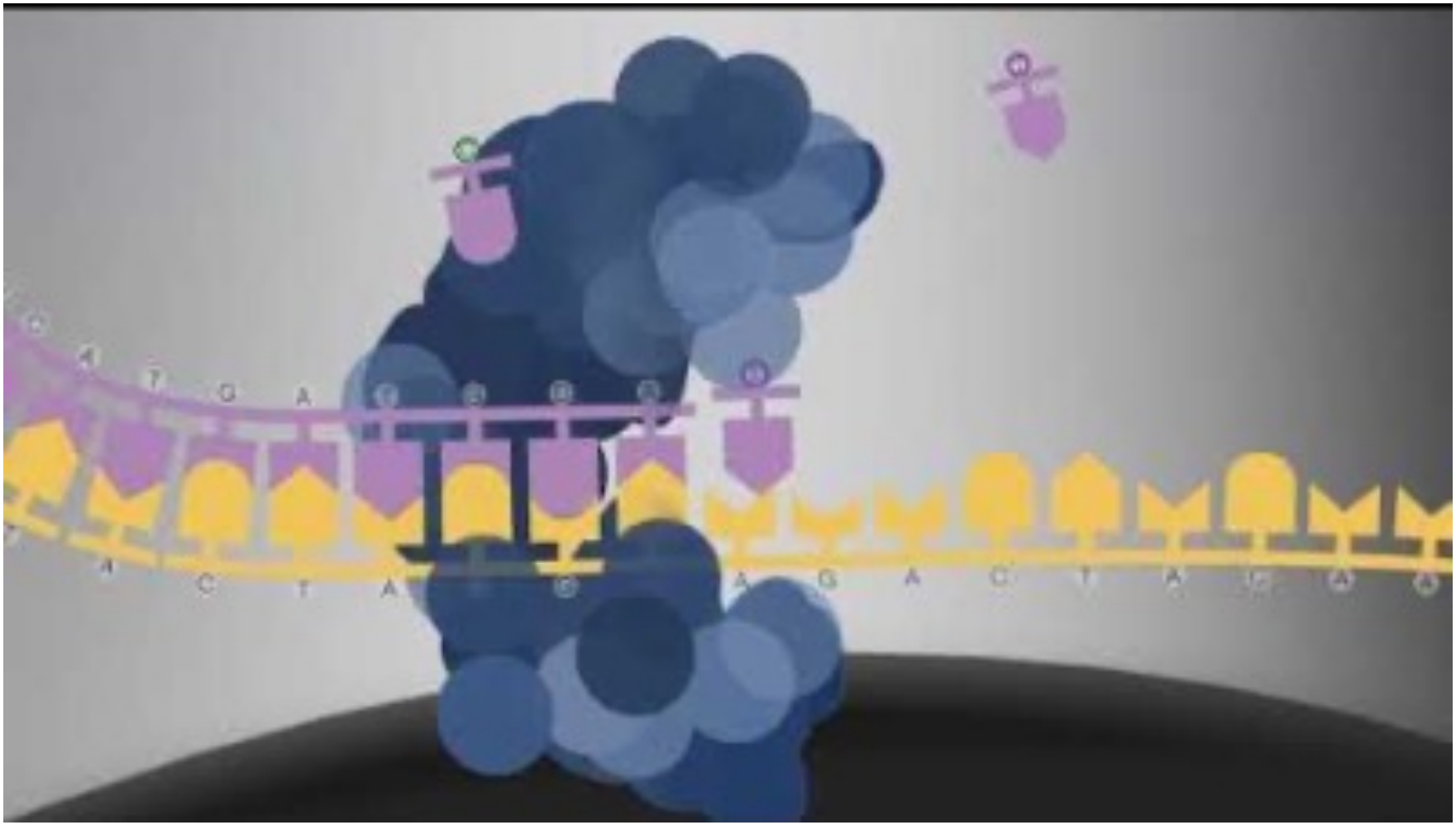


Image from: https://doi.org/10.5281/zenodo.4636860



PacBio Sequel II

- Up to 8M CLR reads/SMRT cell
- 4M HiFi reads/SMRT cell
- start with >5 μg HMW DNA
- Requires shearing + size selection
- Multiplexing requires PCR

	ONT	**PacBio**
Read accuracy	~90-95%	~90% (>99% HiFi)
Read length	up to 2 Mb	up to 30-40 kb (HiFi) up to 200 kb (CLR)
RNA base modifications	Yes (m6A)[1]	No
DNA base modifications	Yes (m5C, m6A)[2]	Yes (m5C, m6A, hm5C)[3]
Throughput (BIF)	~500M reads/run[4]	~4M HiFi reads/run ~8M CLR reads/run

1. Liu, H., et al (2019). Accurate detection of m6A RNA modifications in native RNA sequences. *Nature Communications*, *10*(1), 1–9
2. Liu, Q., et al (2019). Detection of DNA base modifications by deep recurrent neural network on Oxford Nanopore sequencing data. *Nature Communications*, *10*(1).
3. Flusberg, B. A., et al (2010). Direct detection of DNA methylation during single-molecule, real-time sequencing. *Nature Methods*, *7*(6), 461–465
4. 48 flow cells on a PromethIon

Question 7&8