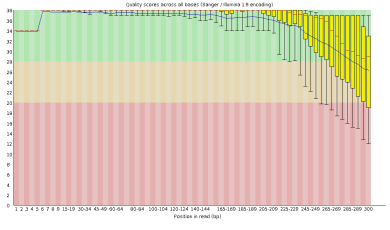
NGS - quality control, alignment, visualisation

Sequencing technologies

Major applications

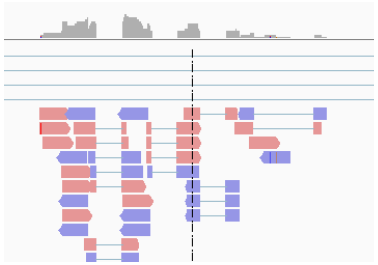
- Transcriptome characterization
  - e.g. RNA-seq
- Epigenome characterization:
  - e.g. ATAC-seq
- DNA-protein interactions:
  - e.g. ChIP-seq
- Whole genome (assembly)
- Variant detection
- Metagenome characterization
- Any others?

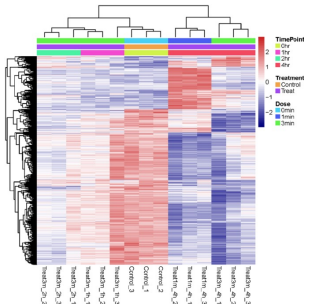
Sequencing



Quality control

Alignment





Down-stream analysis

Visualisation

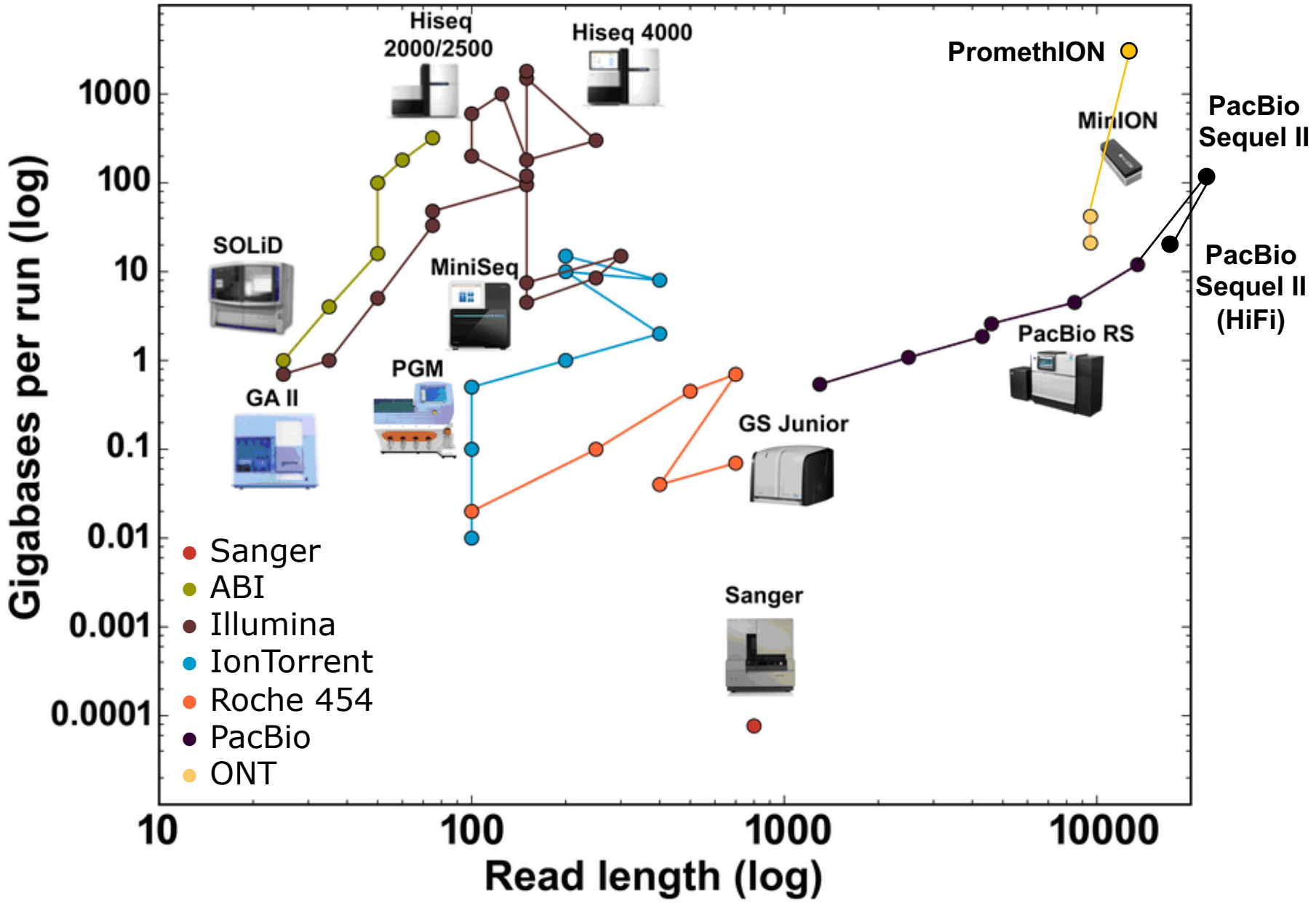


Image from: G. Silva (2016)

This course

- 2nd generation:
  - Illumina
- 3rd generation:
  - Pacific Biosciences
  - Oxford Nanopore Technology

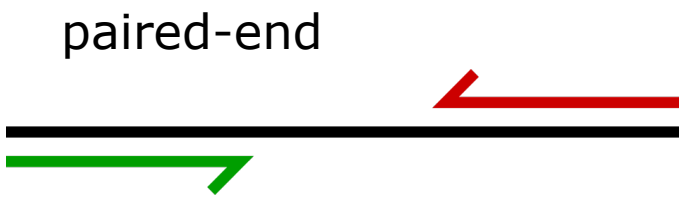
Quiz Question 4

Illumina sequencing

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

Illumina sequencing

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

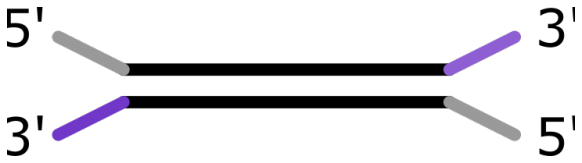


Illumina libray prep

shear DNA

↓

Ligate adapters



↓

Barcode + p5/p7 sites

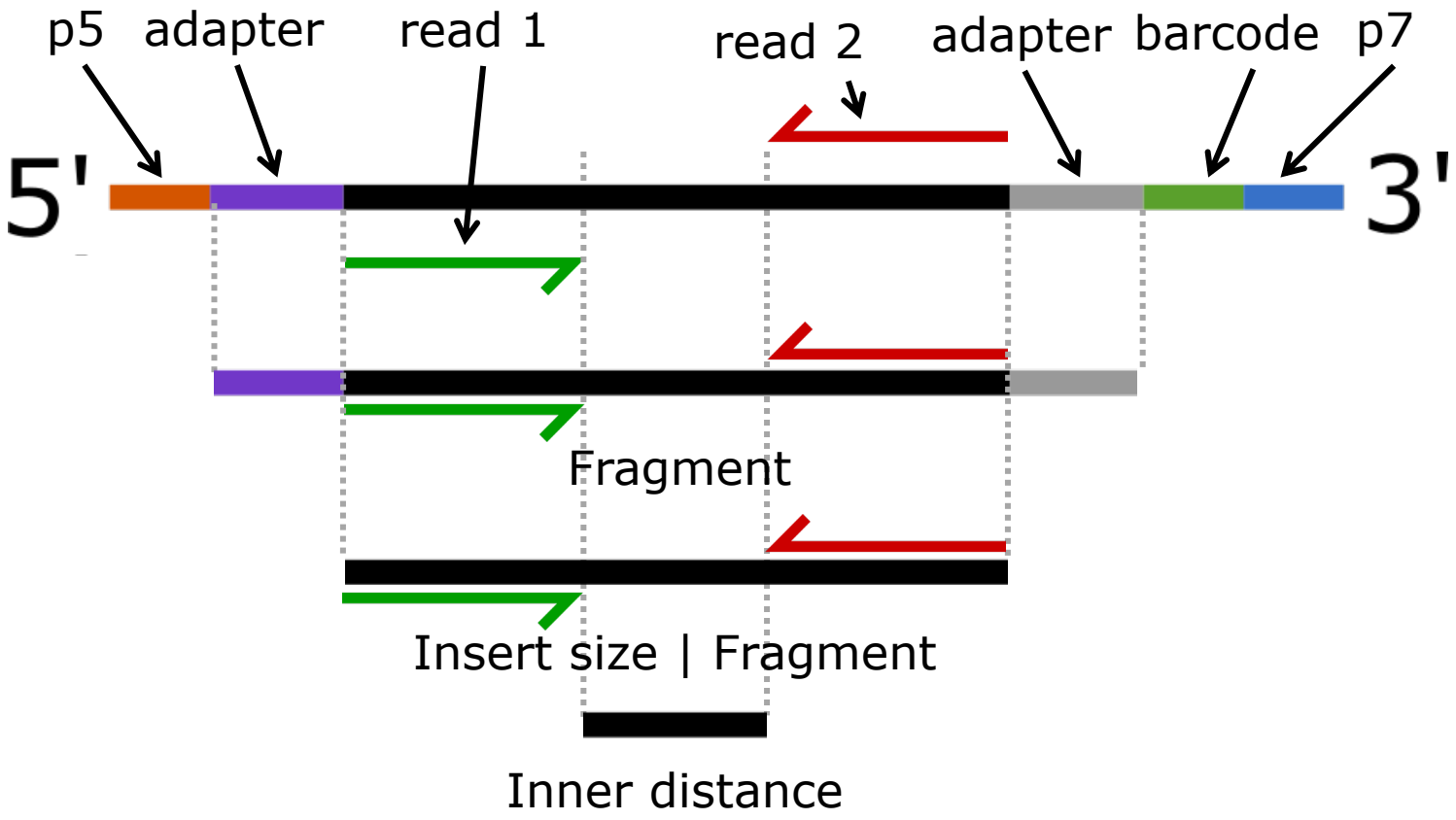
↓

PCR: 8-16 cycles

↓

Sequencing

Some definitions



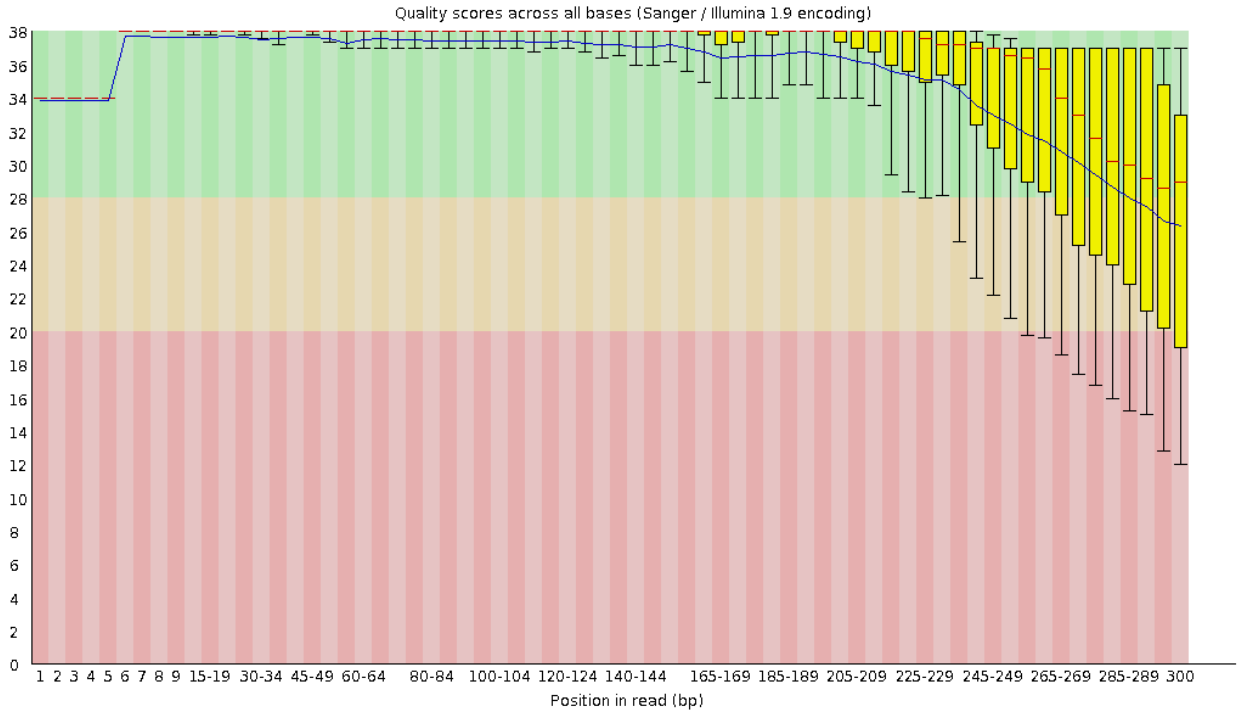
Quiz Question 5

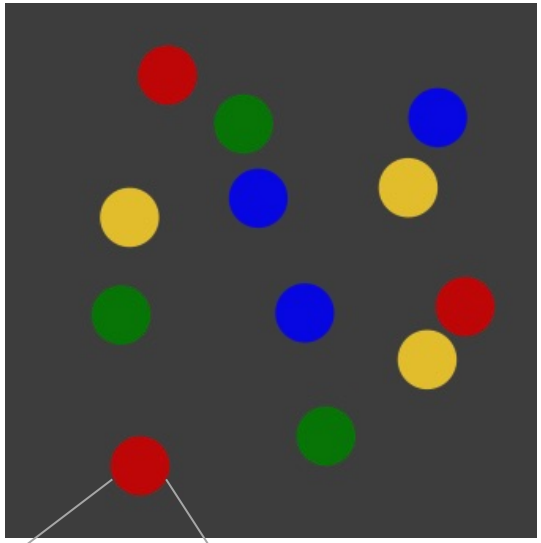
Illumina - limitations

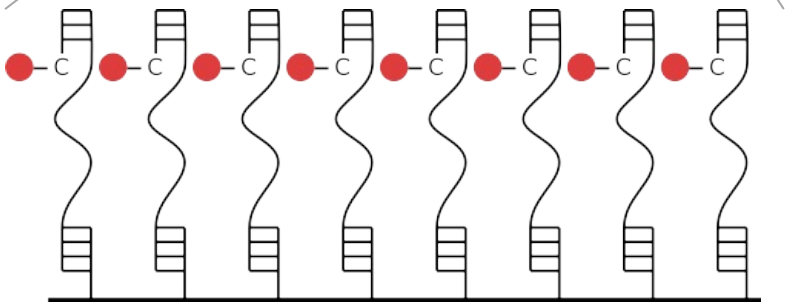
- Maximum read length: 300 bp
- How to reconstruct:
  - Repeats?
  - Isoforms?
  - Structural variation?
  - Haplotypes?
  - Genomes?
- Why not longer read lengths?

Illumina - limitations

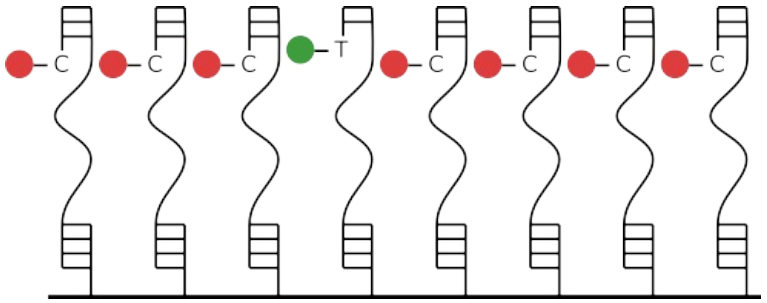
Sequence quality declines towards the end







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

Oxford Nanopore technology

- Based on changes in electrical current
- Well-known for its scalability and portability
- ~95-97% accuracy

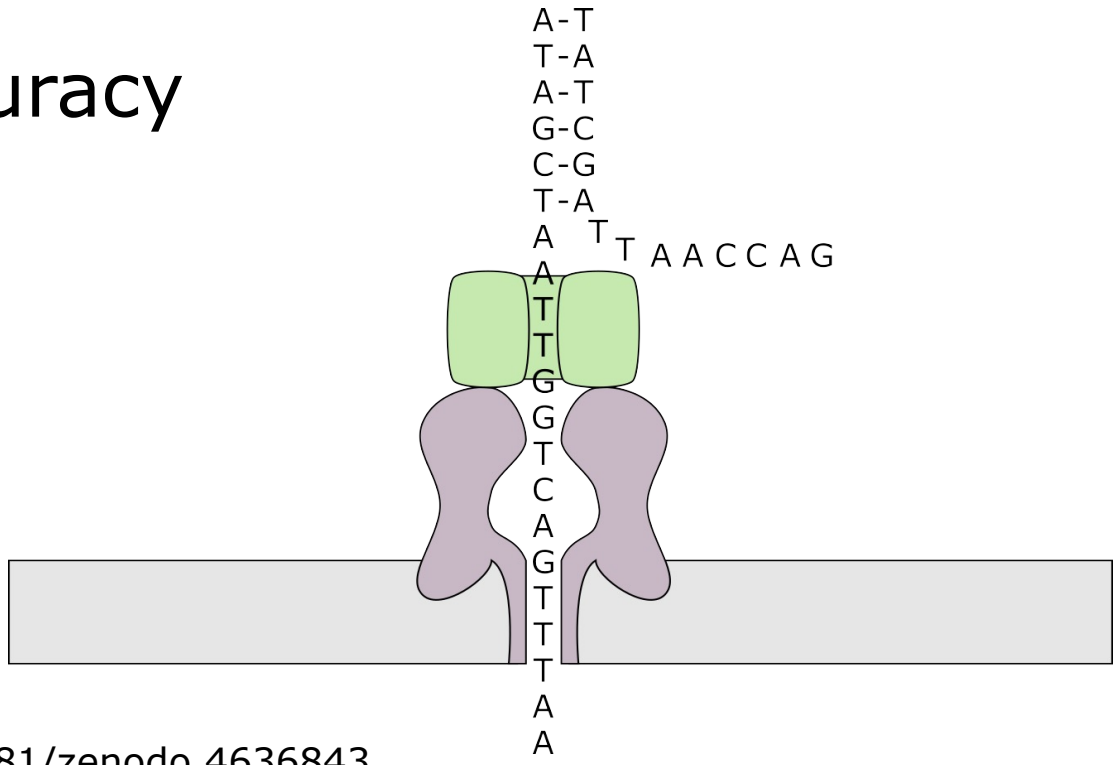
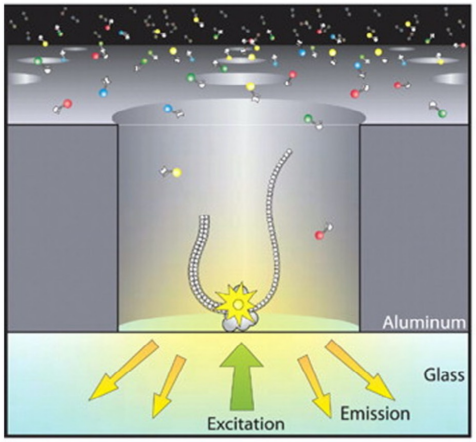
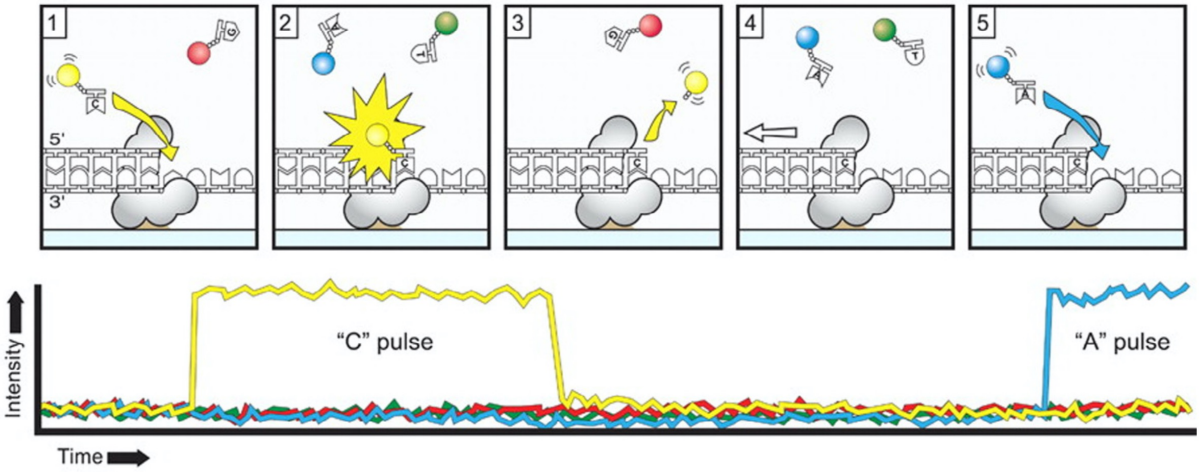


Image from: https://doi.org/10.5281/zenodo.4636843

PacBio sequencing





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

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

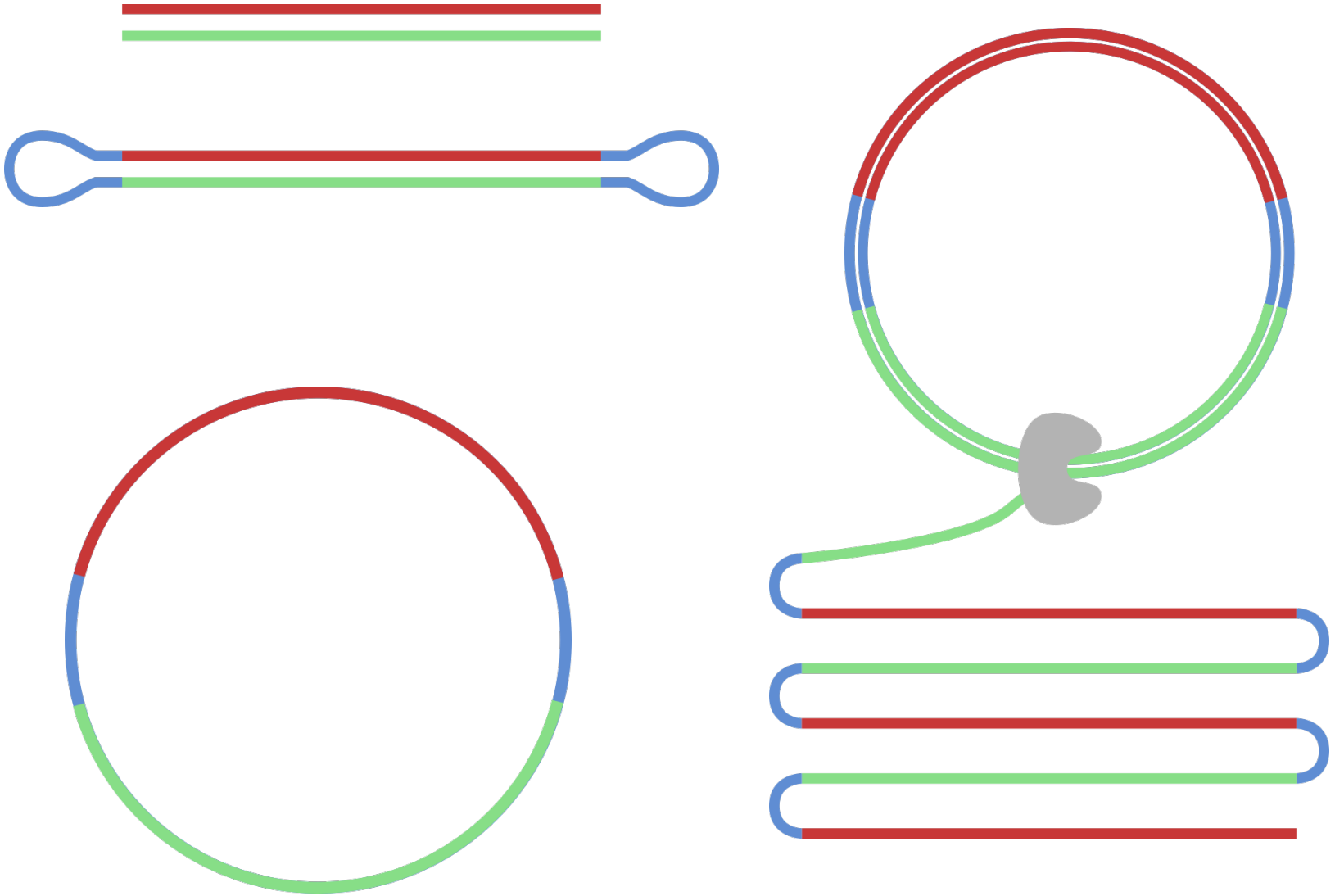


Image from: https://doi.org/10.5281/zenodo.4636860

Quiz Question 6 and 7