Sequencing

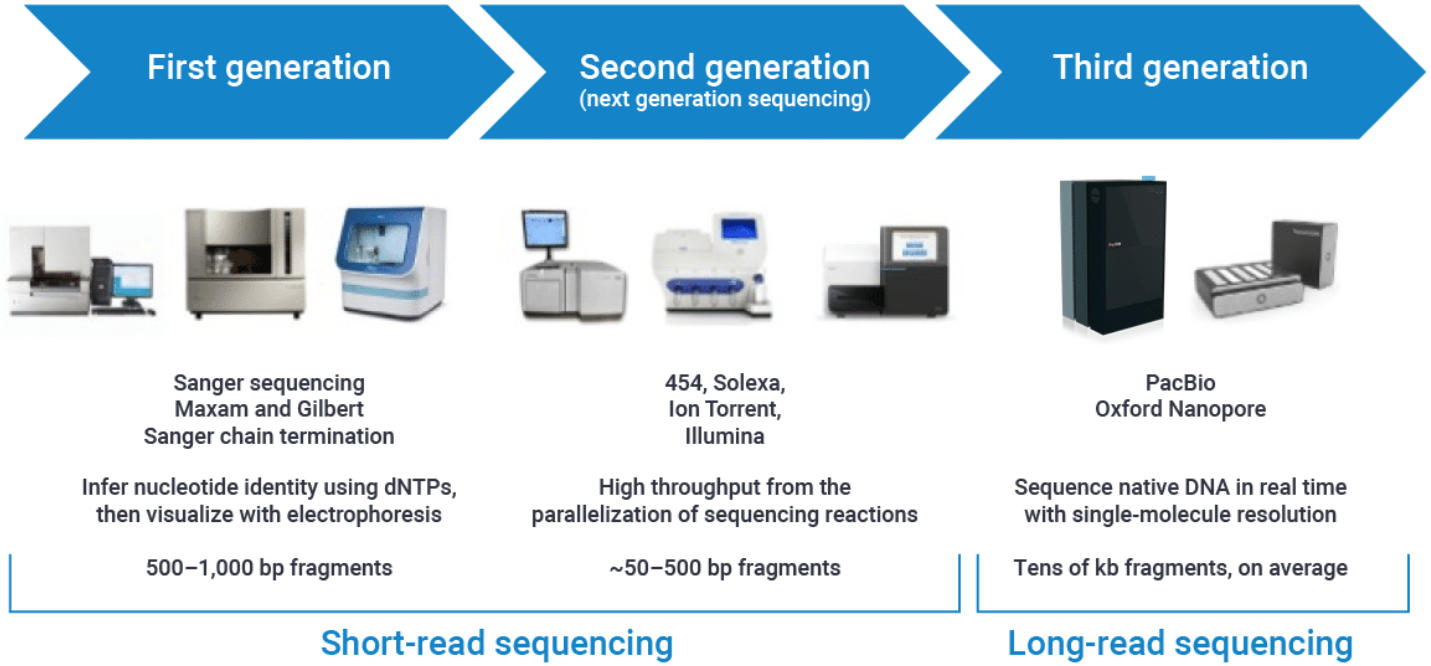
MBDP-105

Modern sequencing techniques

https://www.pacb.com/blog/the-evolution-of-dna-sequencing-tools/

Short group discussion

- What are the pros and cons (1–3) of the different sequencing technologies for whole genome sequencing?



Short-read sequencing

Illumina

Illumina library preparation

Size selection

Correct amount of input DNA to avoid under- and overtagmentation

Adapter ligation

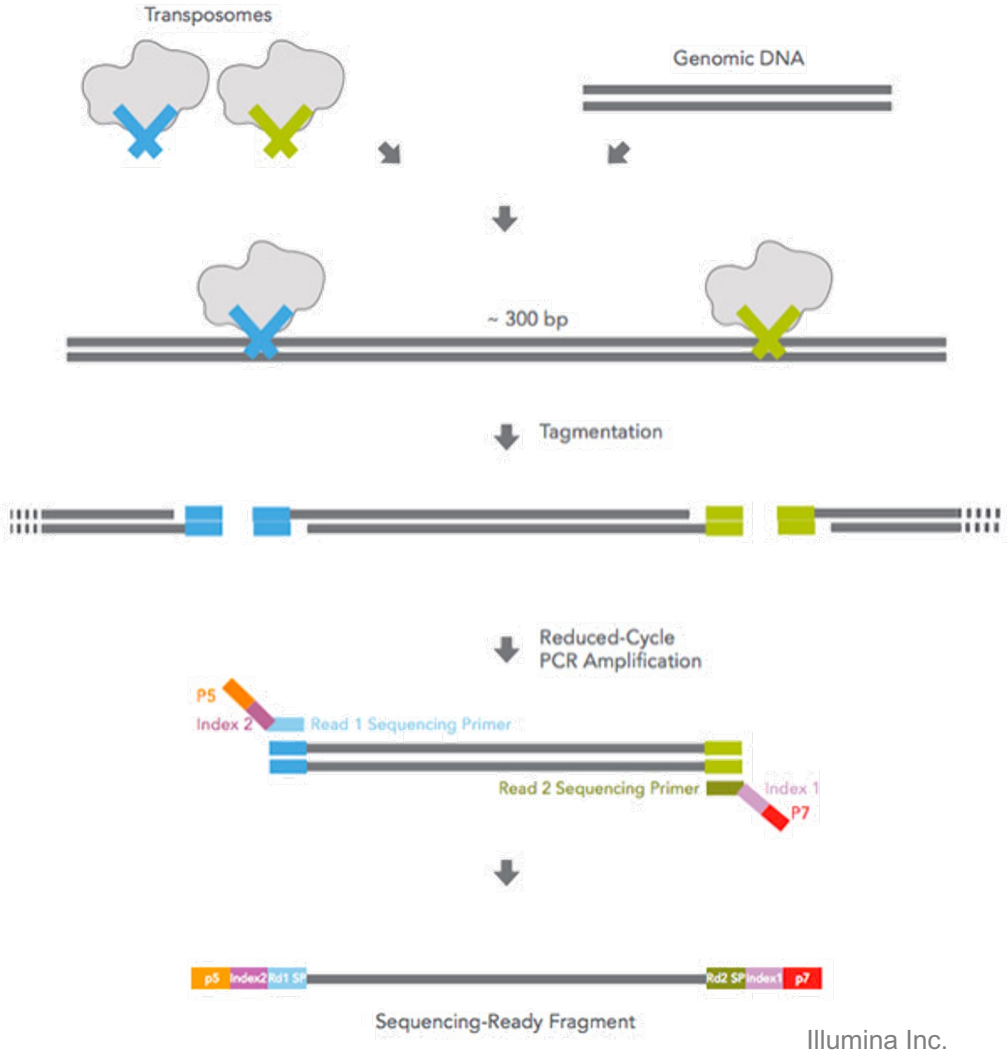
Flow cell adapters

Sequencing primers

Sequencing indexes

Optional indexes for multiplexing

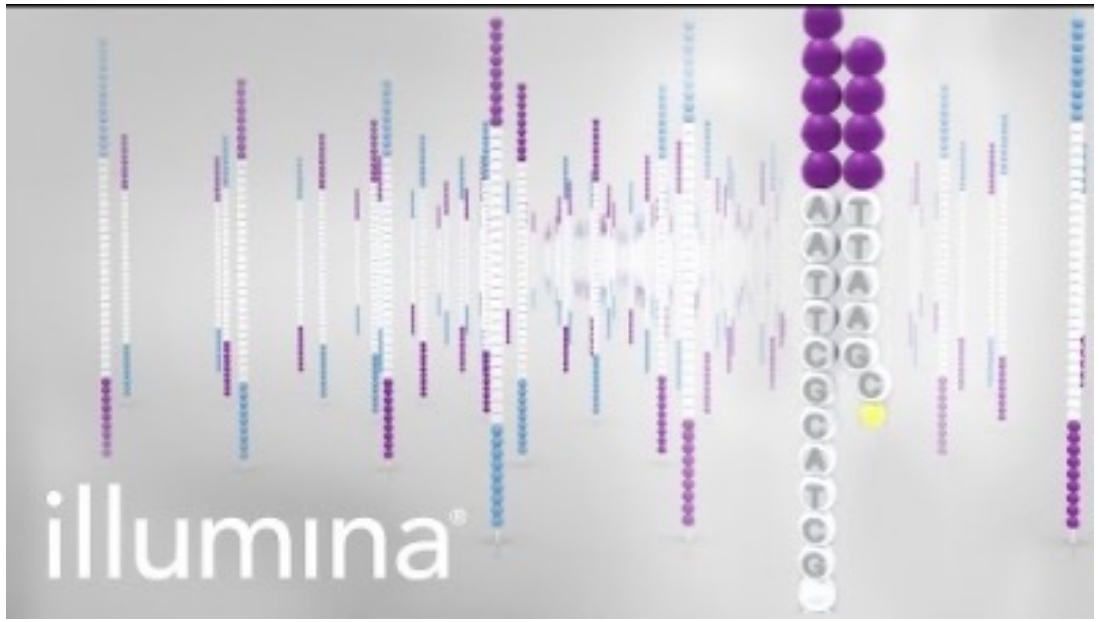
Biases in the first bases have been observed



Illumina Inc.

Illumina sequencing

- Good compromise between size, amount and error rate of reads
- The longer the reads the better.
- Current long-read technologies are becoming more relevant due to lower price and better sequence quality



https://www.youtube.com/watch?v=fCd6B5HRaZ8

Long-read sequencing

Nanopore

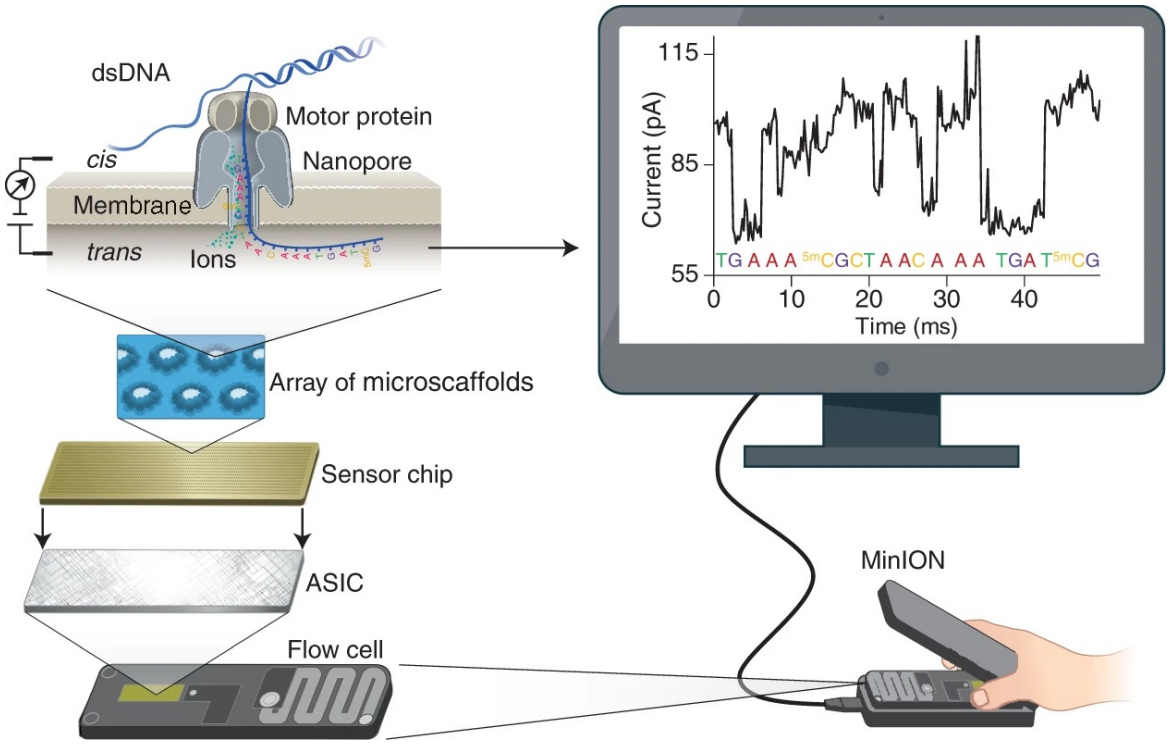
Oxford Nanopore

- Different instruments, same technology
- PromethION
  - 1–48 flow cells (specific)
- GridION
  - 1–5 flow cells
- MinION
  - 1 flowcell
- Flongle – smaller flow cell

Flowcell - MinION/GridION

- Flowcell has 512 channels
- Each channels has 4 nanopores
- ~450 bases s^{-1}
- Can read DNA and RNA

- Applications:
  - Amplicon sequencing
  - Whole-genome sequencing (WGS)
  - Metagenomics
  - Transcriptomics



https://doi.org/10.1038/s41587-021-01108-x

Multiplexing

- Each sample will get unique barcode (24 nt)
- 24/96 barcodes
- Sample1:

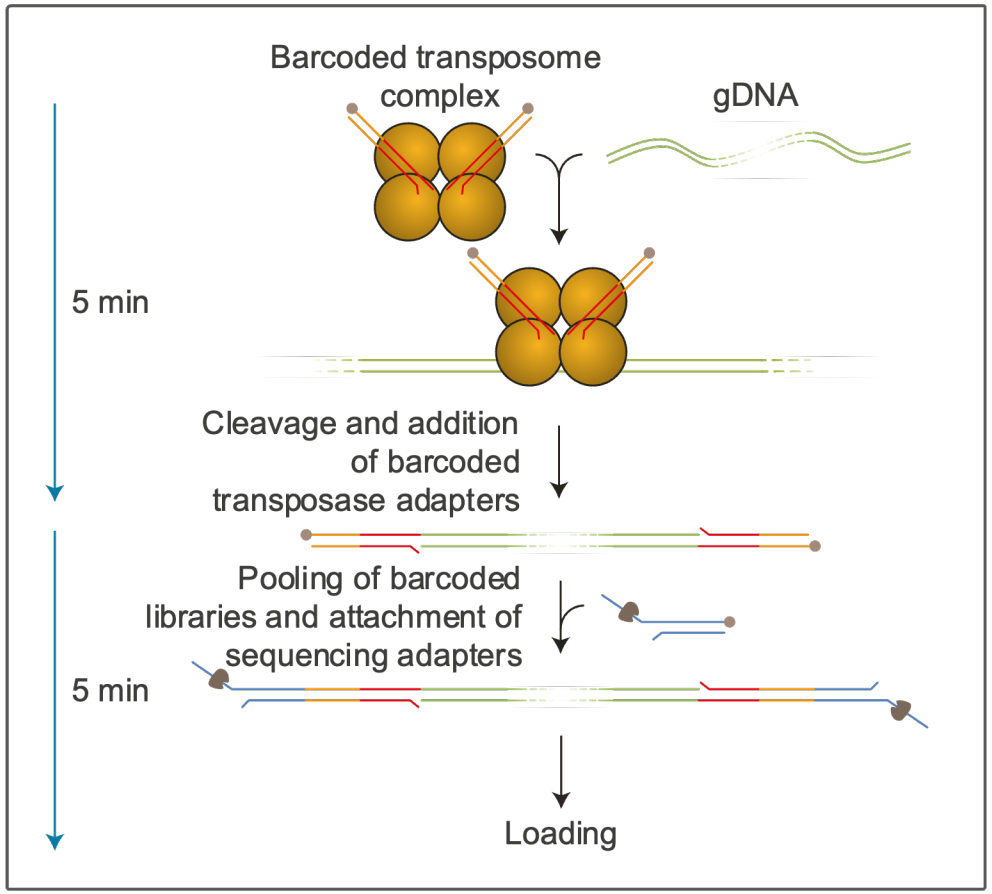
  `AAGAAAGTTGTCGGTGTCTTTGTG`

- Sample 2:

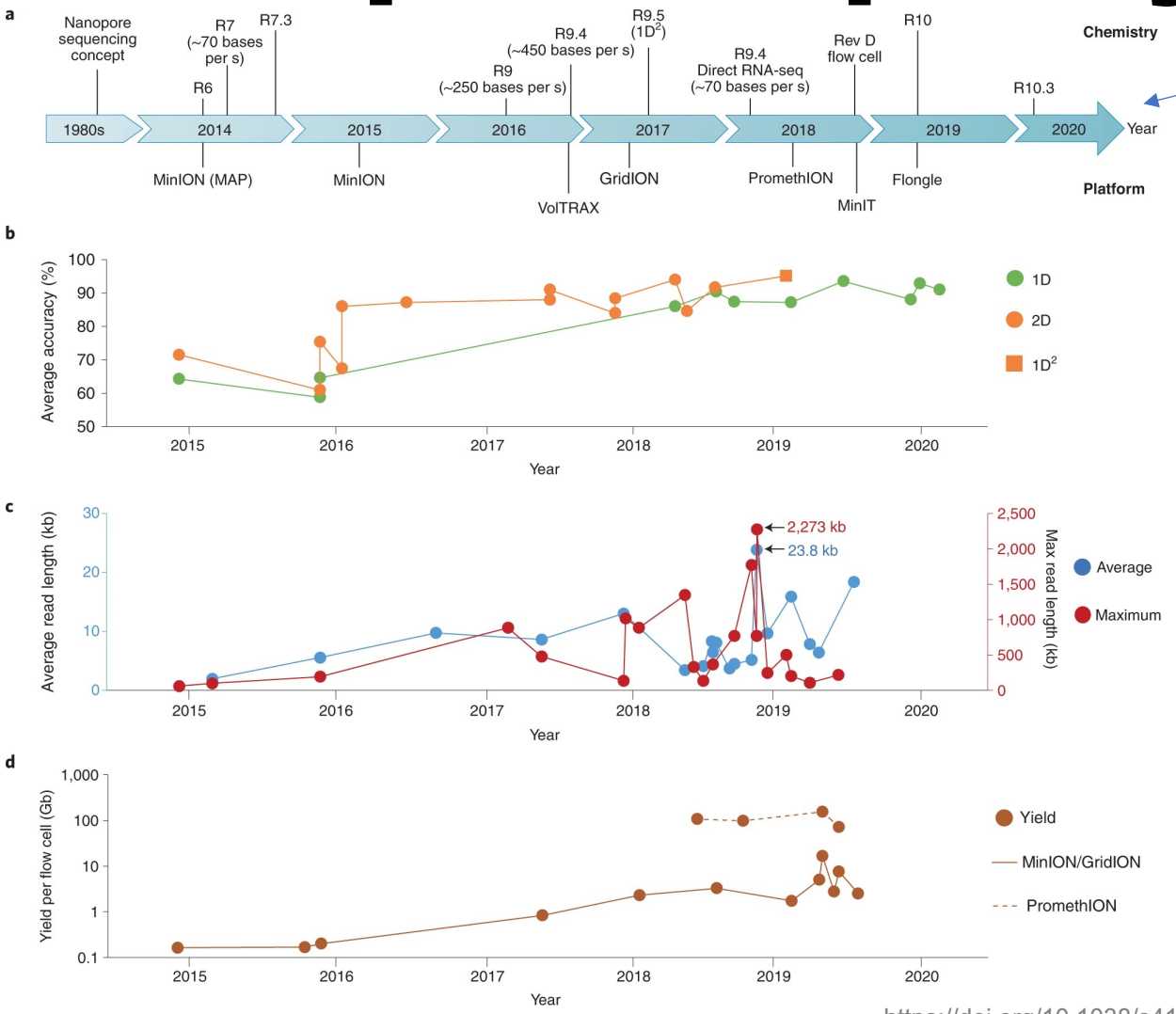
  `TCGATTCCGTTTGTAGTCGTCTGT`

  …

- Demultiplexing:
  - Reads will be divided based on the barcode sequence



Nanopore sequence quality



R.10.4.1
~99 % accuracy

https://doi.org/10.1038/s41587-021-01108-x