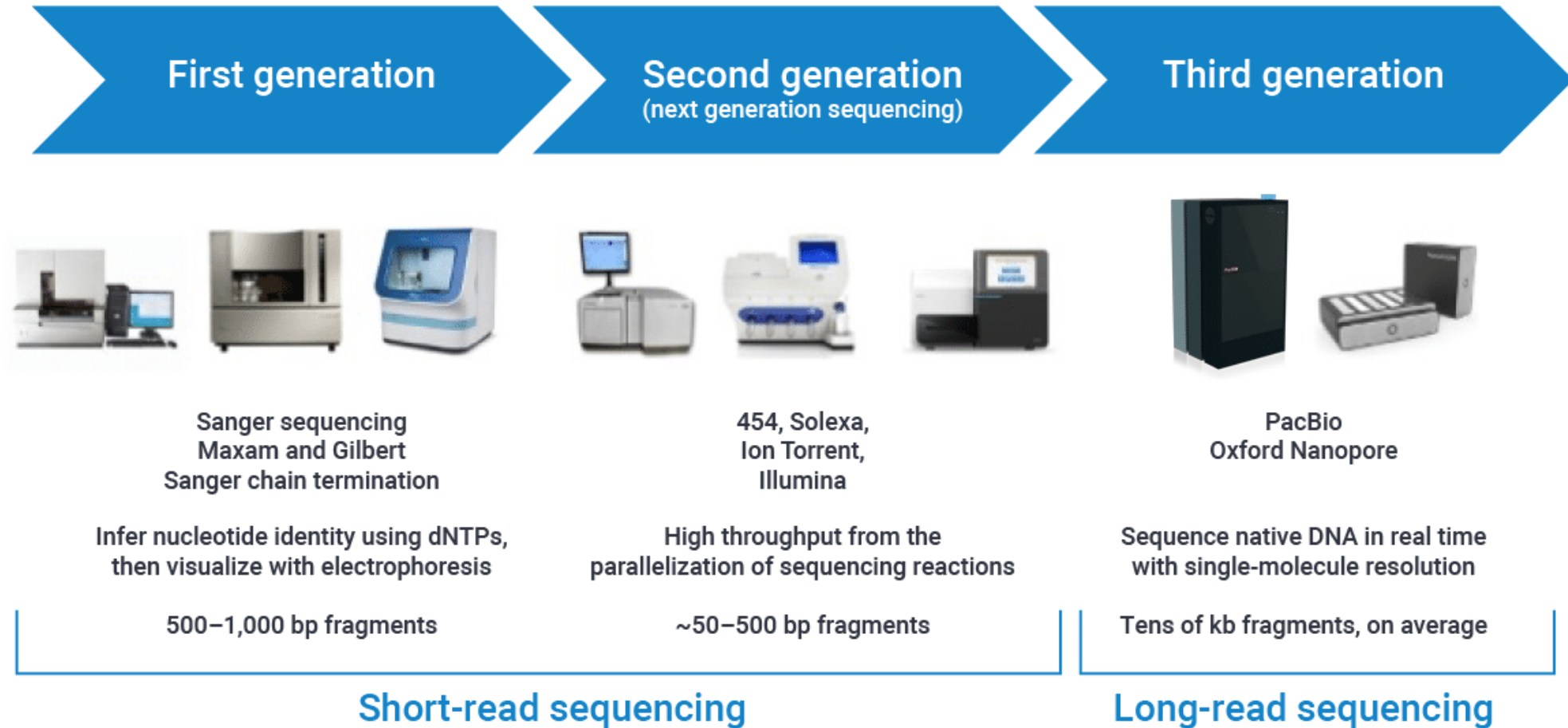


Sequencing

MBDP-105

Modern sequencing techniques



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

Short-read sequencing

Illumina

Illumina library preparation

Size selection

Correct amount of input DNA to avoid under- and overtagmentation

Adapter ligation

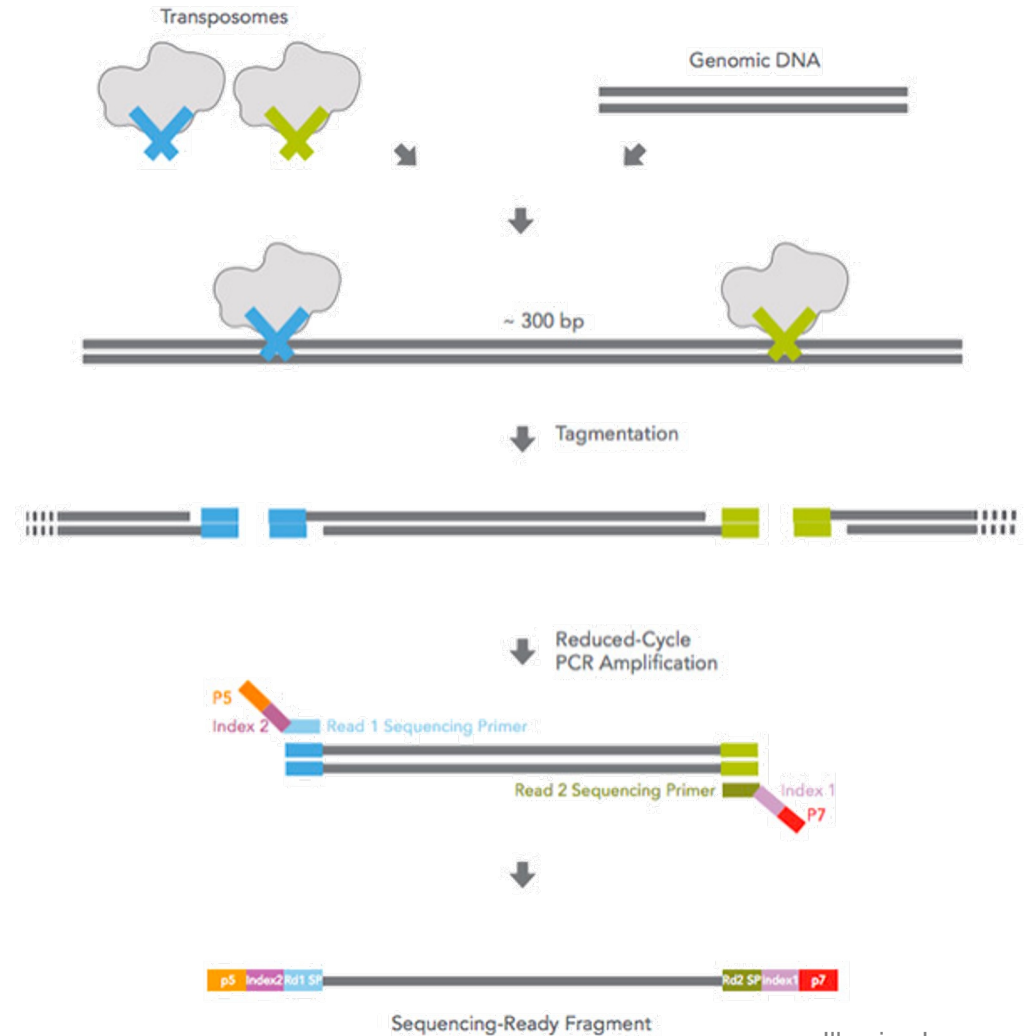
Flow cell adapters

Sequencing primers

Sequencing indexes

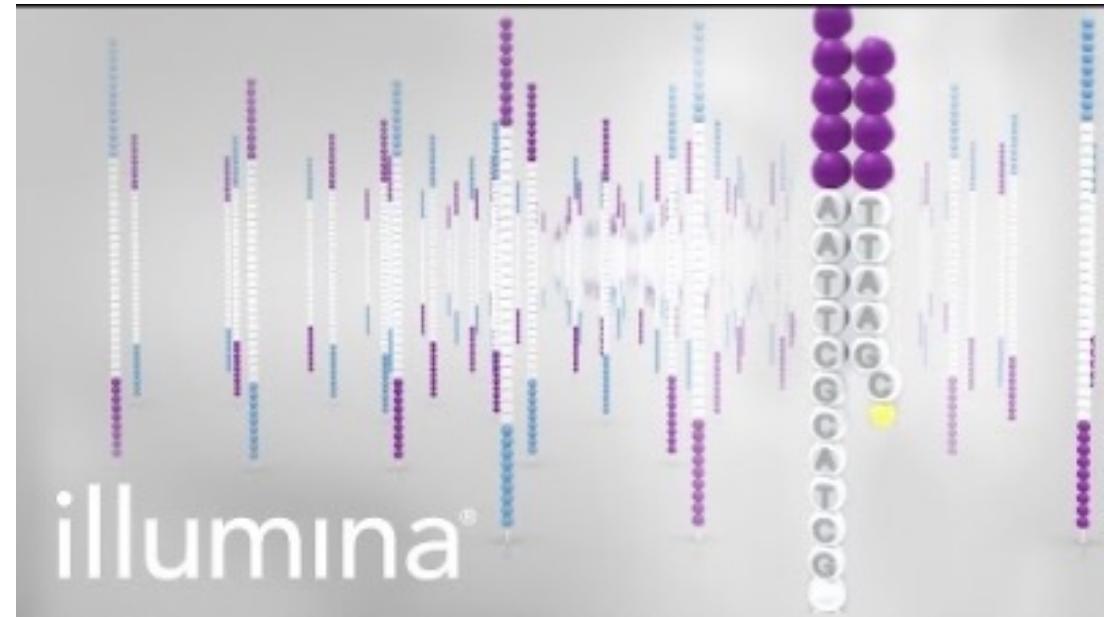
Optional indexes for multiplexing

Biases in the first bases have been observed



Illumina 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

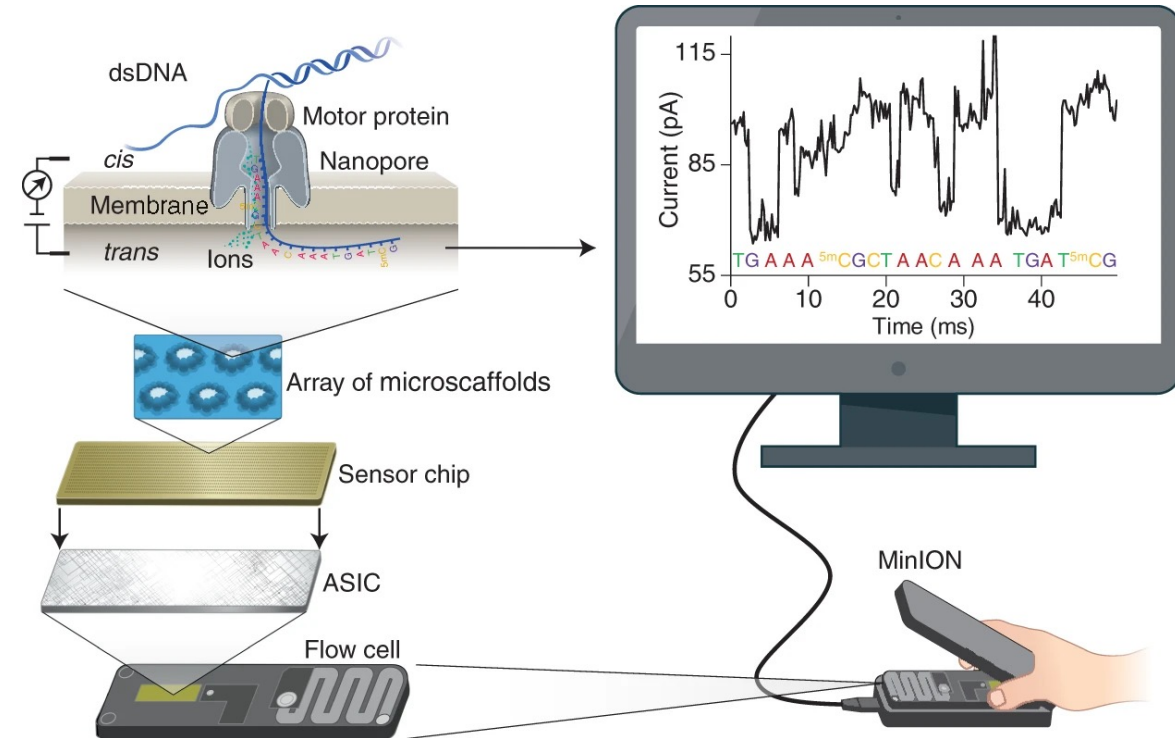
Oxford Nanopore

- 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

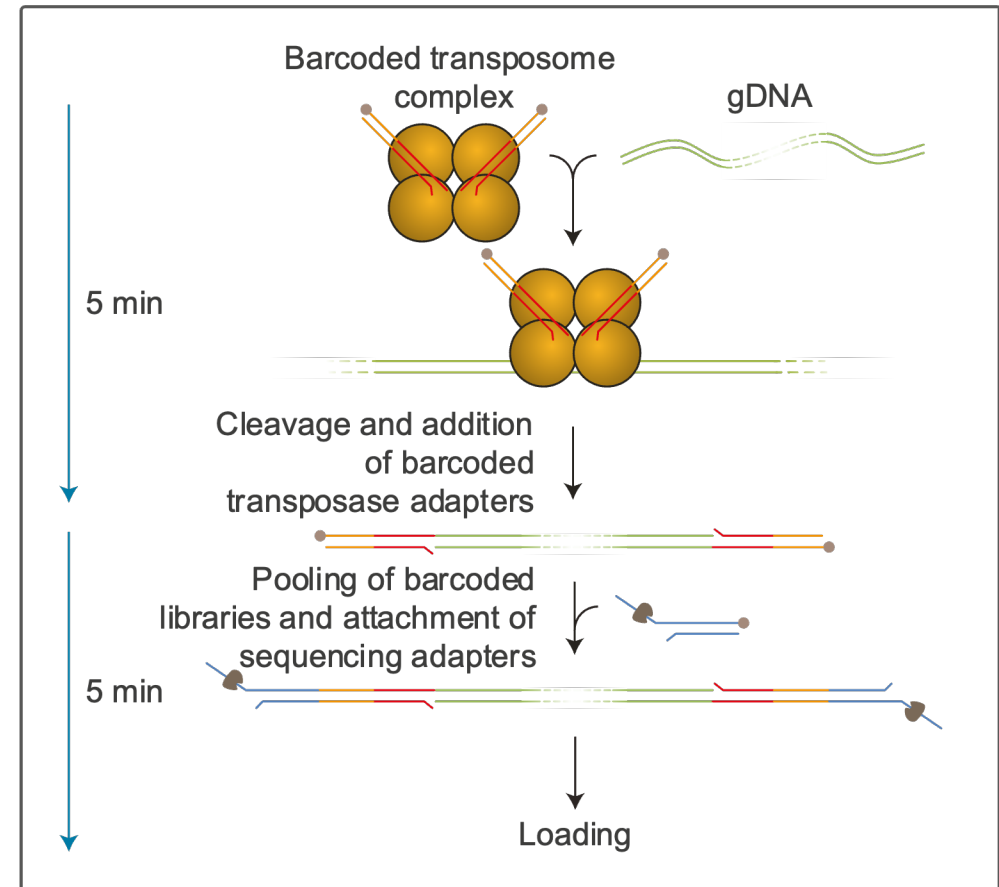
- Flowcell has 512 channels
- Each channels has 4 nanopores
- ~ 450 bases s^{-1}
- Can read DNA and RNA
- Applications:
 - Amplicon sequencing
 - Whole-genome sequencing (WGS)
 - Metagenomics
 - Transcriptomics



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

Multiplexing

- Each sample will get unique barcode (24 nt)
- 24/96 barcodes
- Sample 1:
AAGAAAGTTGTCGGTGTCTTTGTG
- Sample 2:
TCGATTCCGTTTGTAGTCGTCTGT
- ...
- Demultiplexing:
 - Reads will be divided based on the barcode sequence



Nanopore sequence quality

