Sequencing

MBDP-105

Modern sequencing techniques

First generation

Second generation (next generation sequencing)

Third generation













454, Solexa, Ion Torrent, Illumina

High throughput from the parallelization of sequencing reactions

~50-500 bp fragments





PacBio Oxford Nanopore

Sequence native DNA in real time with single-molecule resolution

Tens of kb fragments, on average

500-1,000 bp fragments

Sanger sequencing

Maxam and Gilbert

Sanger chain termination

Infer nucleotide identity using dNTPs,

then visualize with electrophoresis

Short-read sequencing

Long-read sequencing

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

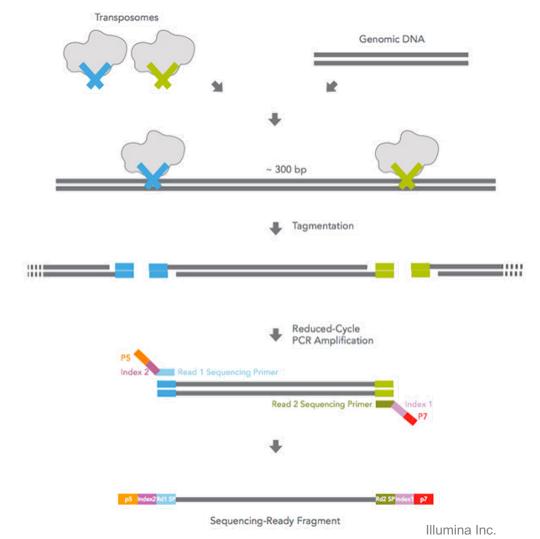
Flow cell adapters

Sequencing primers

Sequencing indexes

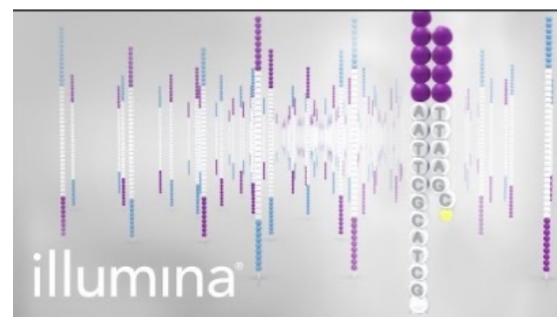
Optional indexes for multiplexing

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

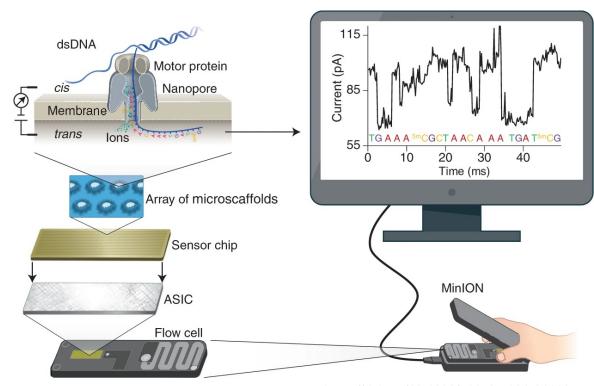
- Different instruments, same technology
- PromethION
 - 1–48 flow cells (specific)
- GridION
 - 1–5 flow cells
- MinION
 - 1 flowcell
- Flongle smaller flow cell



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
- Sample1:

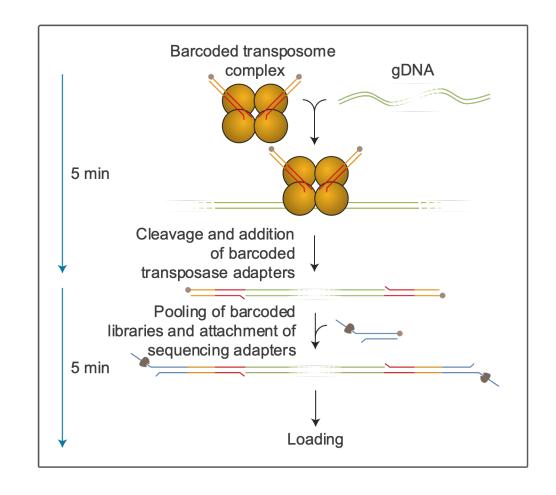
AAGAAAGTTGTCGGTGTCTTTGTG

• Sample 2:

TCGATTCCGTTTGTAGTCGTCTGT

. . .

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



Nanopore sequence quality

