Nanopore sequencing

MMB-114

Modern sequencing techniques

First generation

Second generation (next generation sequencing)

Third generation













454, Solexa, Ion Torrent,

High throughput from the parallelization of sequencing reactions

~50-500 bp fragments

Illumina





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/

Oxford Nanopore

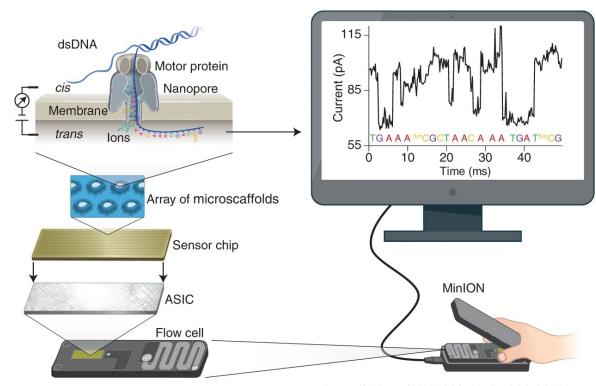
- Different instruments, same technology
- PromethION
 - 1–48 flow cells (specific)
- GridION
 - 1–5 flow cells
- MinION
 - 1 flowcell
- Flongle smaller flow cell



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

Input DNA requirements

- High molecular weight DNA (HMW DNA)
- Single sample ~1 μg DNA
- Barcoding (multiplexing) kits 50 200 ng DNA
- Quality:
 - OD 260/280 of 1.8
 - OD 260/230 of 2.0–2.2

Multiplexing

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

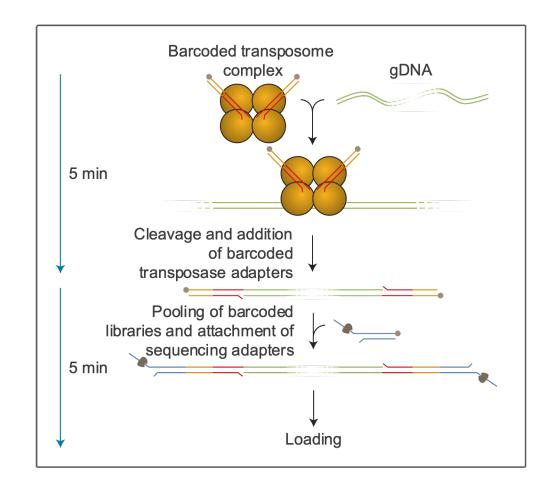
AAGAAAGTTGTCGGTGTCTTTGTG

• Sample 2:

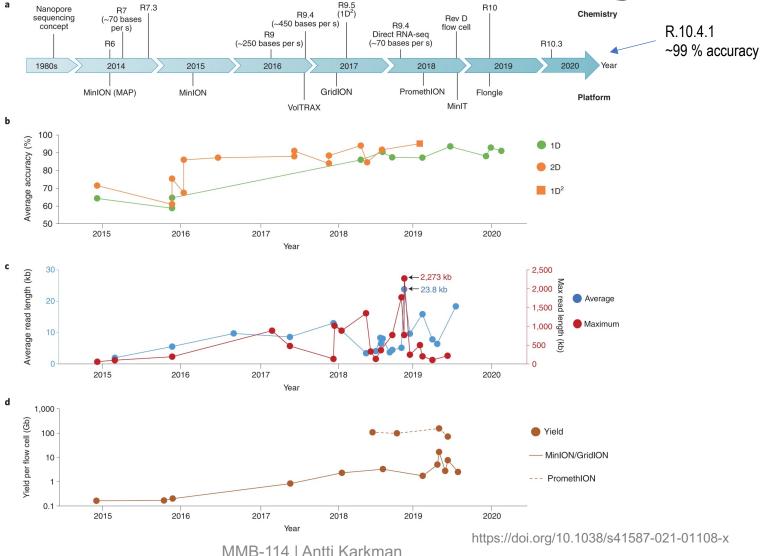
TCGATTCCGTTTGTAGTCGTCTGT

. . .

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



Nanopore sequence quality



Workflow

Monday – DNA purification:

- Purify your DNA using gDNA purification kit
- Measure concentration and quality with Nanodrop

Tuesday – Library preparation and sequencing:

- Barcoding: steps 1-7 on the rapid barcoding kit
- Pooling of the samples --> steps 8-24 in rapid barcoding kit
- Prepping and loading the flow cell
- Sequencing (1-3 days)
- End of the week Basecalling