Nanopore sequencing

MMB-114

Modern sequencing techniques

First generation

Second generation (next generation sequencing)

Third generation

















Sanger sequencing Maxam and Gilbert Sanger chain termination

Infer nucleotide identity using dNTPs, then visualize with electrophoresis

500-1,000 bp fragments

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

Short-read sequencing

Long-read sequencing

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

Oxford Nanopore

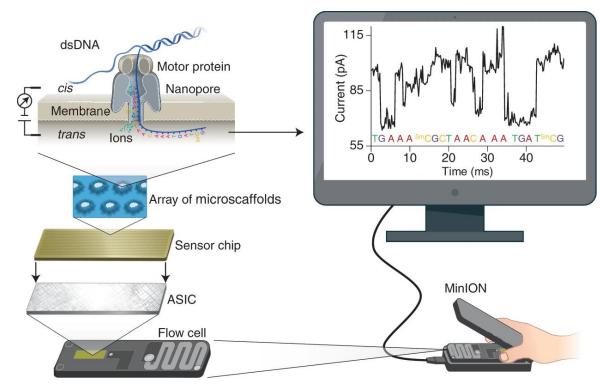
- Different instruments, same technology
- PromethION
 - 1–48 flow cells (specific)
- GridION
 - 1–5 flow cells
- MinION
 - 1 flowcell
- Flongle smaller flow cell



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:

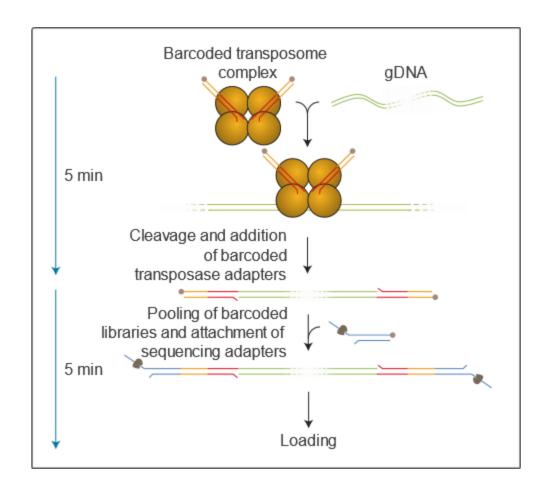
AAGAAAGTTGTCGGTGTCTTTGTG

Sample 2:

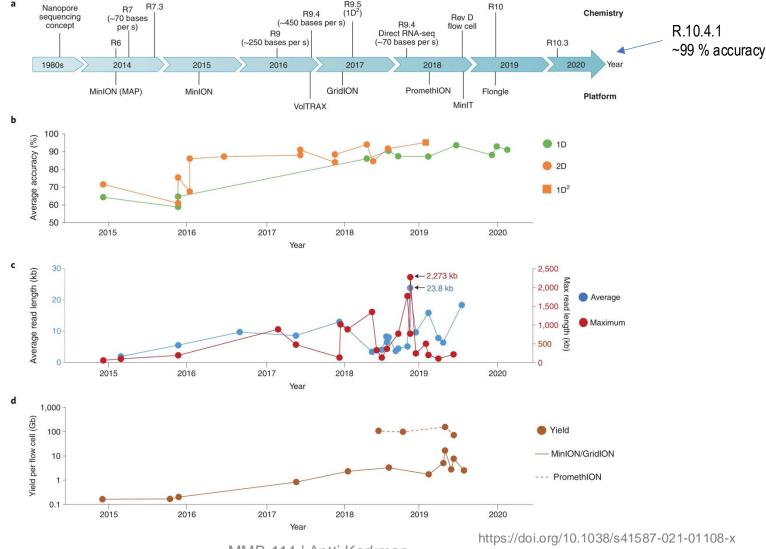
TCGATTCCGTTTGTAGTCGTCTGT

- - -

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



Nanopore sequence quality



Nanopore lab workflow

Barcoding of own DNA **Library Preparation steps:** 1–7 *Everyone*



Group1

Library preparation of ½ samples Library preparation steps: 8–19



Library preparation of ½ samples

Library preparation steps: 20–24



Group2

Library preparation of ½ samples Library preparation steps: 8–19



Library preparation of ½ samples

Library preparation steps: 20–24

Group3

Prepare 80 % ethanol (Consumables)



Watch the video on flow cell prepping

Prime the flow cell

Priming and loading.. steps: 1-6



Load the flow cell

Priming and loading.. steps: 7–12

6 μl to step 8 in loading