# Nanopore sequencing

## MMB-114

# Modern sequencing techniques


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

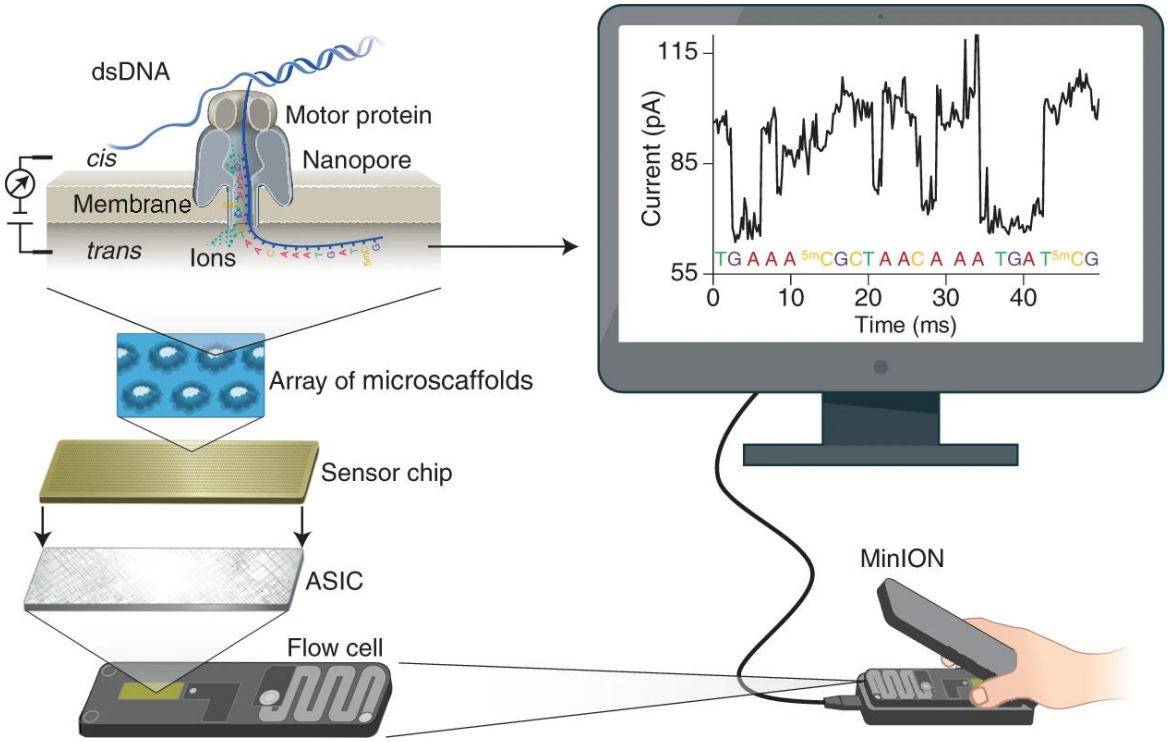
# Oxford Nanopore

- 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

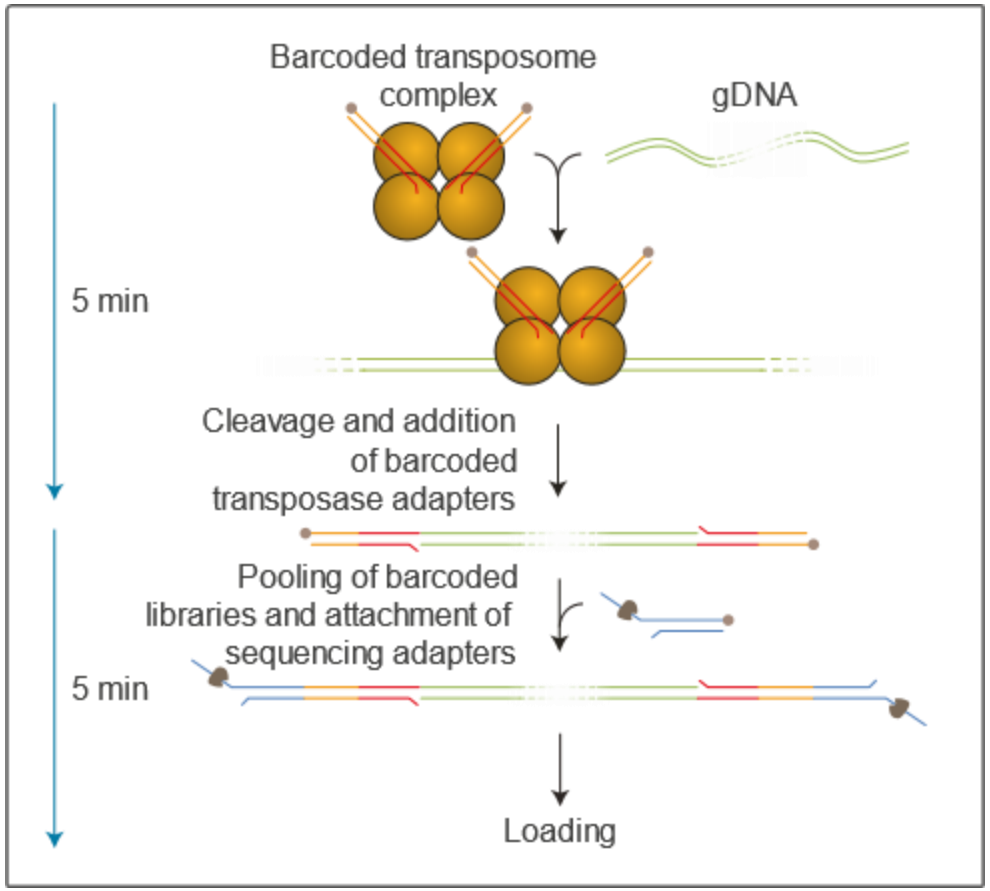
# Multiplexing

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

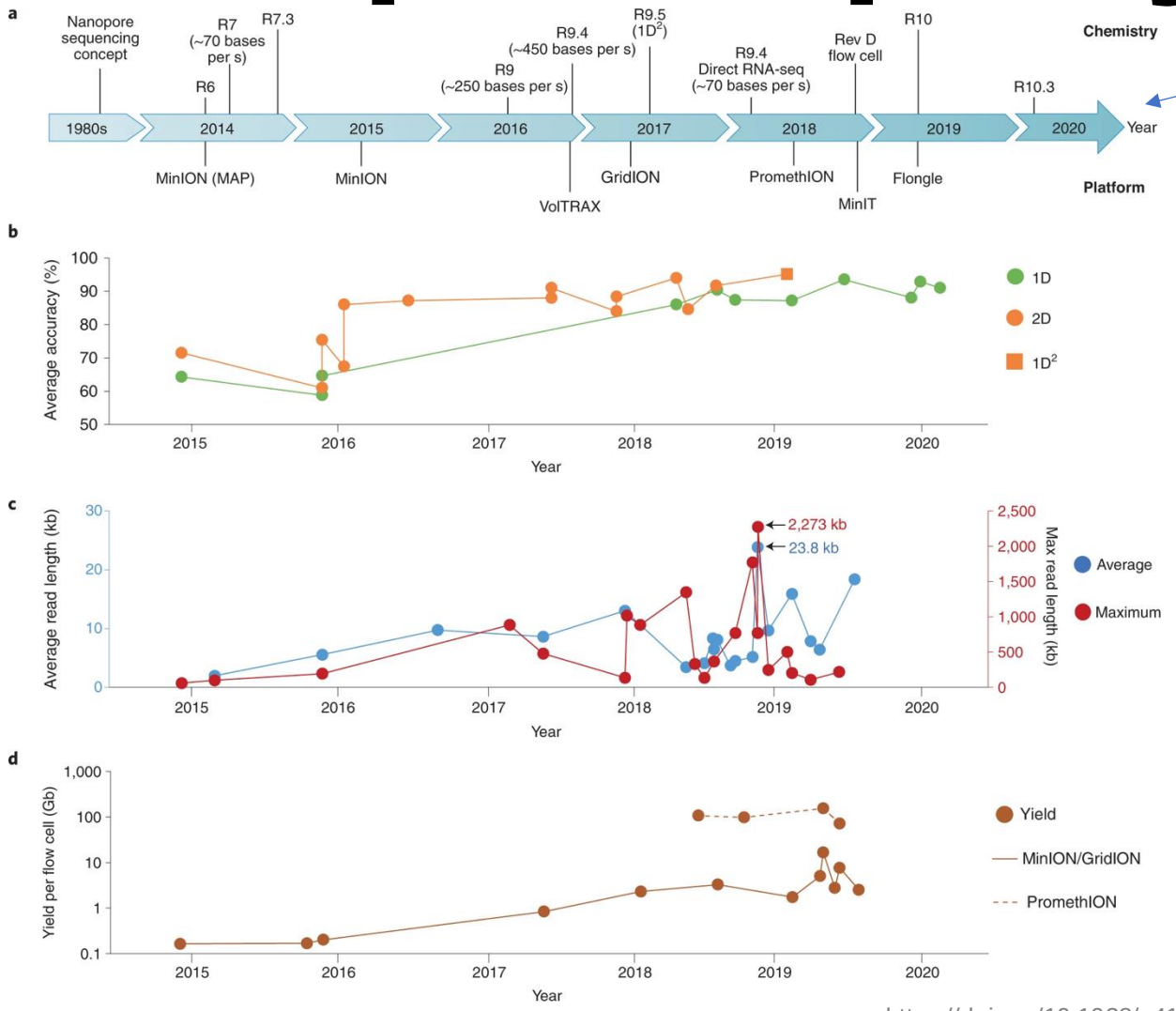
  `AAGAAAGTTGTCGGTGTCTTTGTG`
- Sample 2:

  `TCGATTCCGTTTGTAGTCGTCTGT`

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



# Nanopore sequence quality




R.10.4.1
~99 % accuracy

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

# 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*