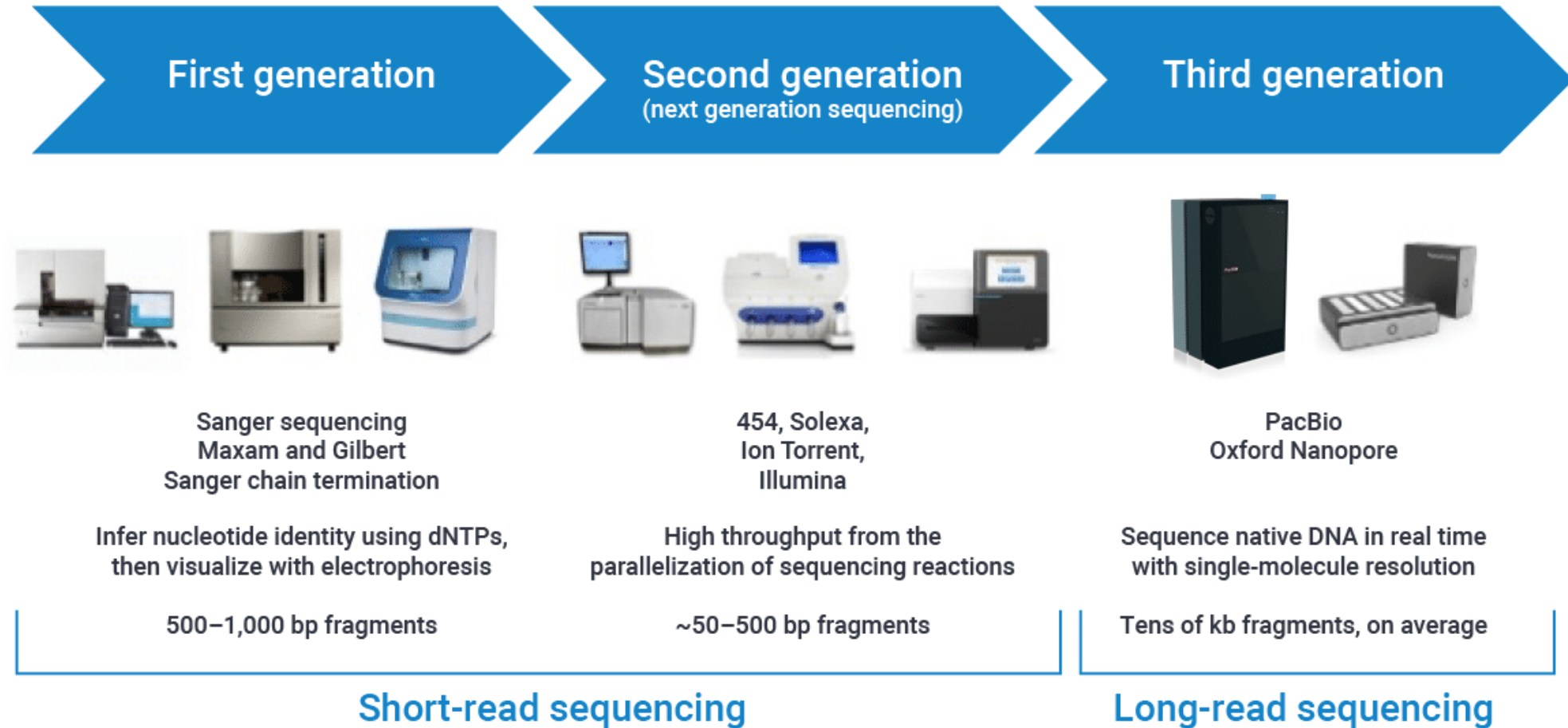


Nanopore sequencing

MMB-114

Modern sequencing techniques



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

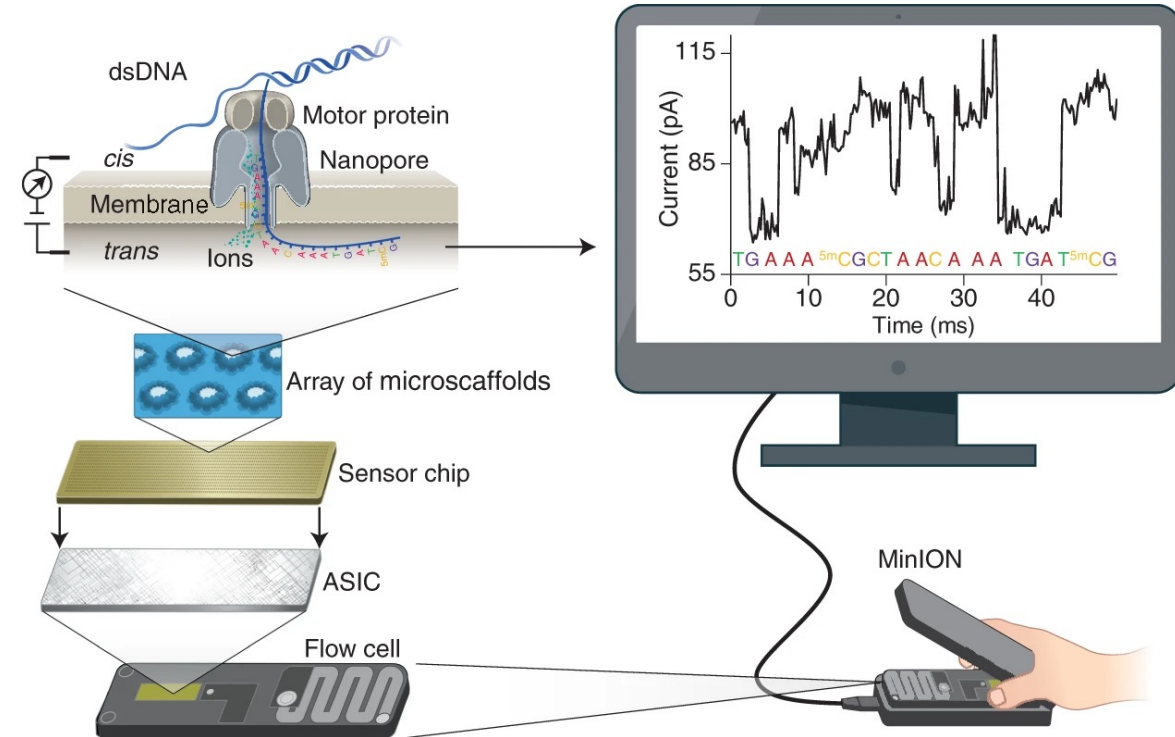
Oxford Nanopore

- 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



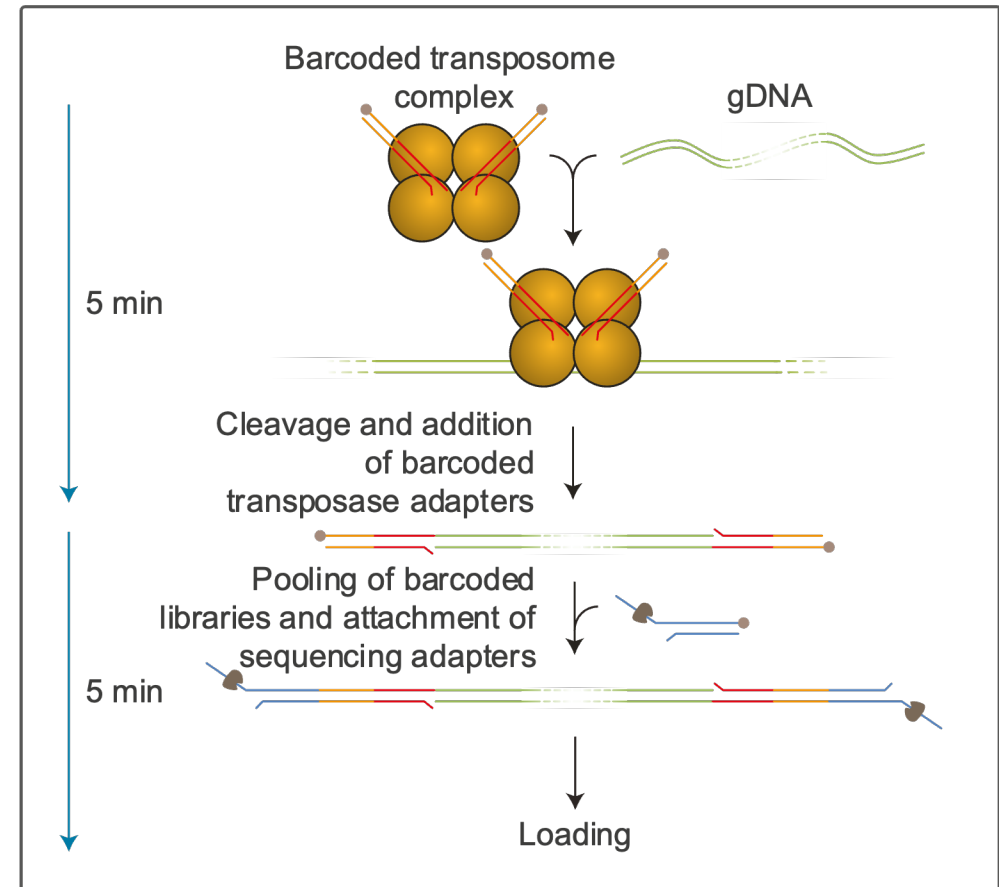
<https://doi.org/10.1038/s41587-021-01108-x>

Input DNA requirements

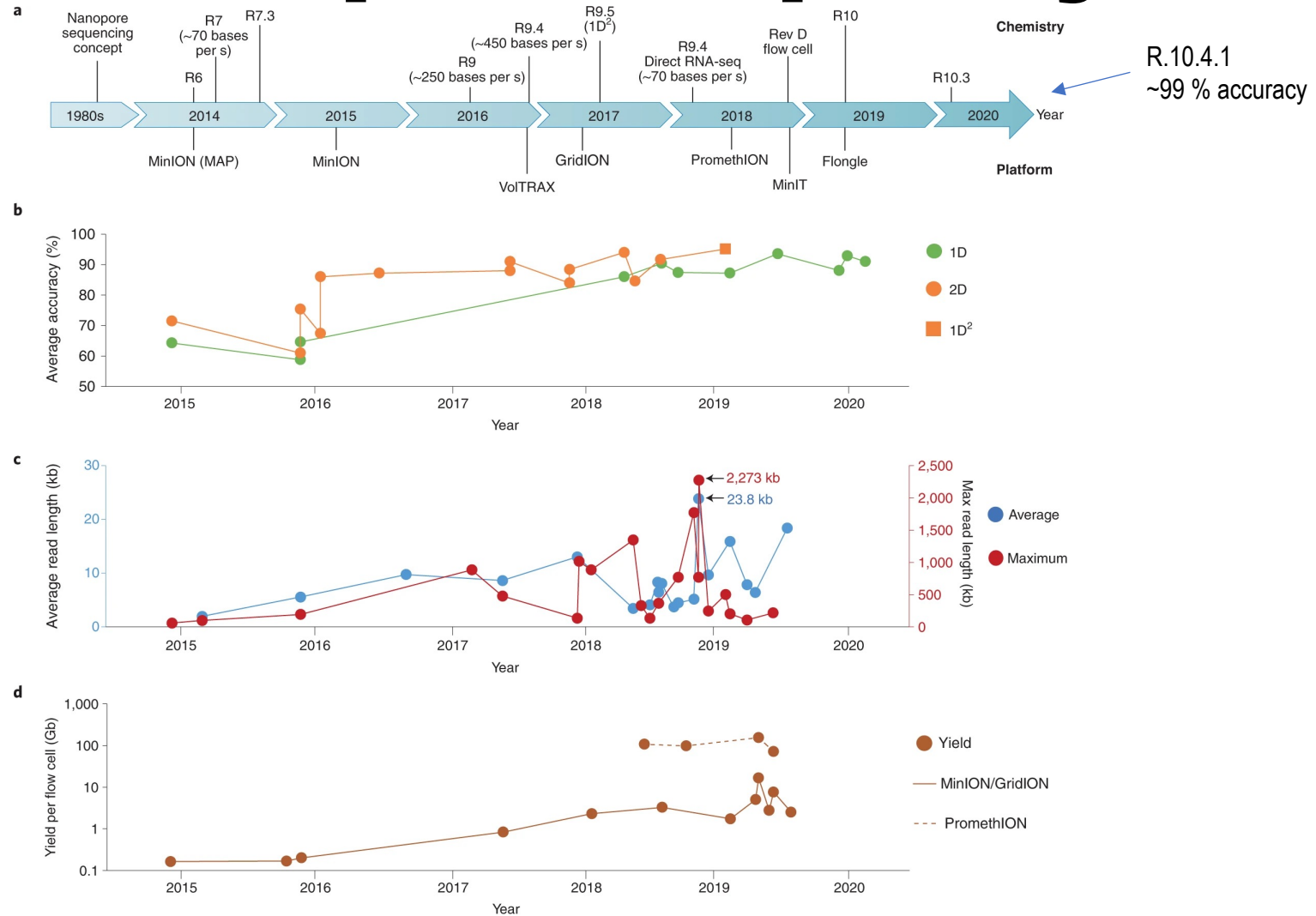
- High molecular weight DNA (HMW DNA)
- Single sample ~1 µg DNA
- Barcoding (multiplexing) kits 50 – 200 ng DNA
- Quality:
 - OD 260/280 of 1.8
 - OD 260/230 of 2.0–2.2

Multiplexing

- Each sample will get unique barcode (24 nt)
- 24/96 barcodes
- Sample 1:
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**