

# Third Generation Sequencing: A Zika Virus Case Study

LONDON  
SCHOOL of  
HYGIENE  
& TROPICAL  
MEDICINE



# Third Generation Sequencing Platforms

	NGS: Illumina MiSeq	PacBio: Sequel	Oxford Nanopore Technology: MinION
Read Length (bp)	150	~15,000	~50,000
Read output	~2.5-5,000,000	~500,000	~1,500,000
Cost Per Run (\$)	~1000	~1-2000	~1000
Hardware Cost (\$)	~100,000	~350,000	1000
Observed Error Rate	<0.1% (>Q30)	1-12% (Q10-Q20)	1-12% (Q10-Q20)



# Nanopore technology

PromethION



GridION



MinION

Flongle

HPC + GPU  
(Nvidia > 1080)

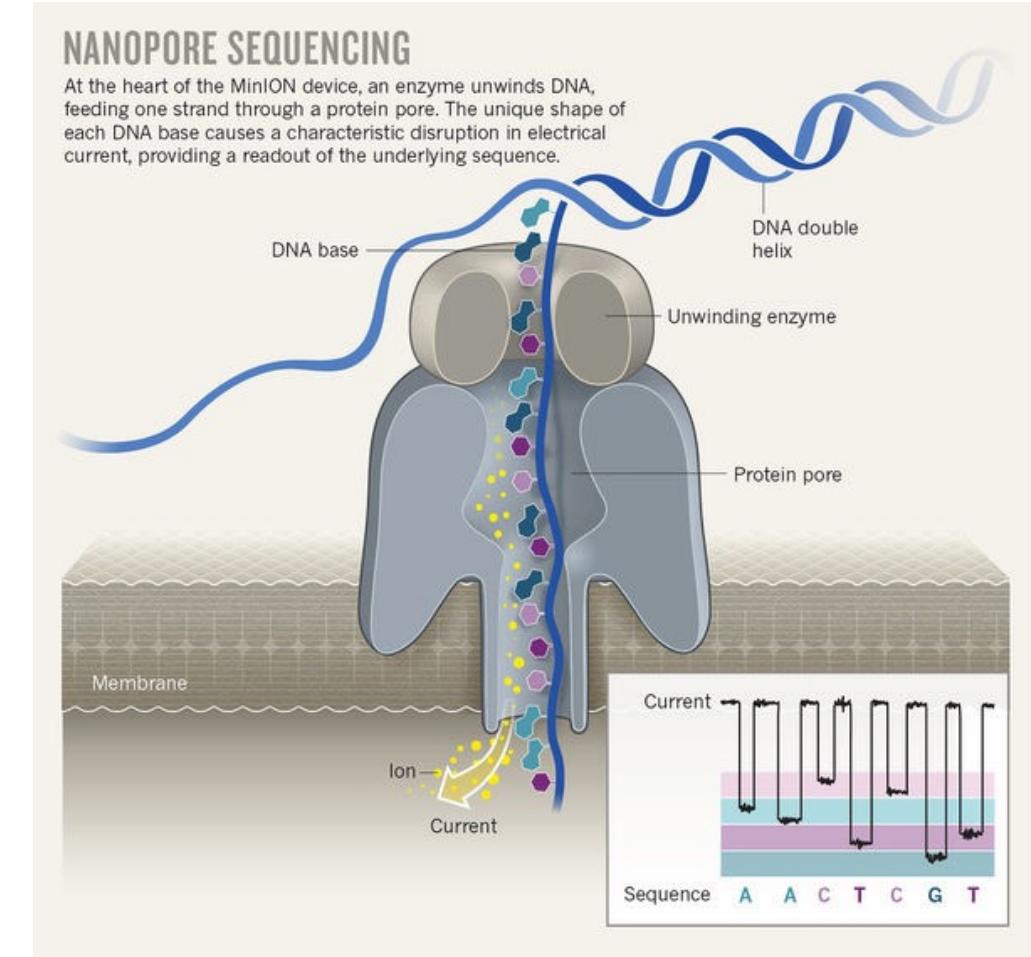


# MinION – Nanopore Sequencing Technology

**“Portable, real-time sequencing analyses”**

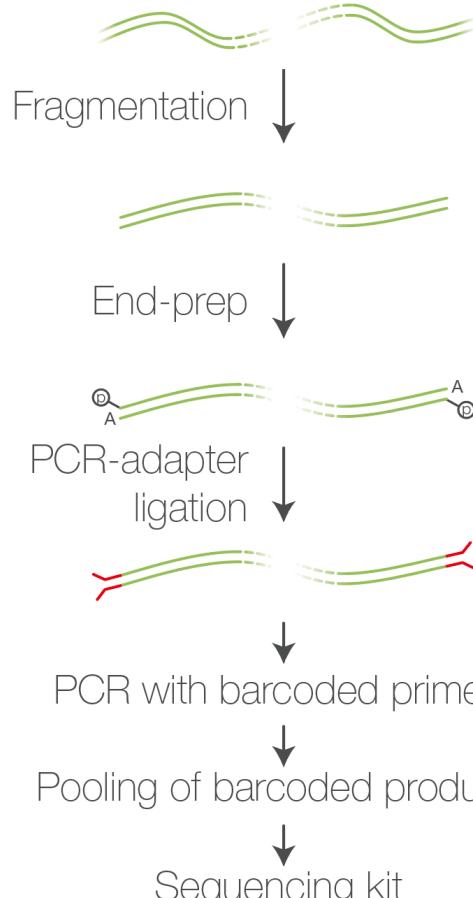
- Portable
- Long reads
- High depth and coverage
- Inexpensive

- Still requires sophisticated lab techniques
- High error rate
- Under development
- Data is challenging to process and analyse

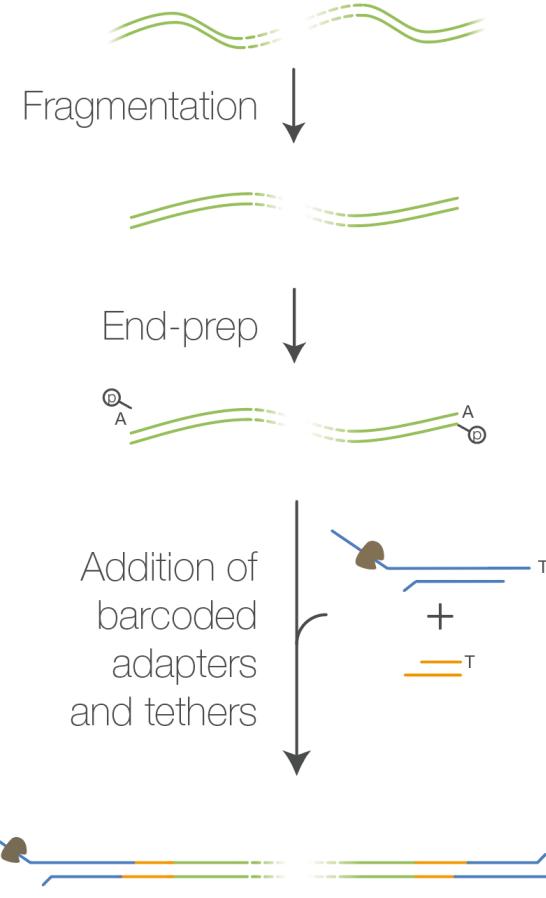


# Library preparation and loading

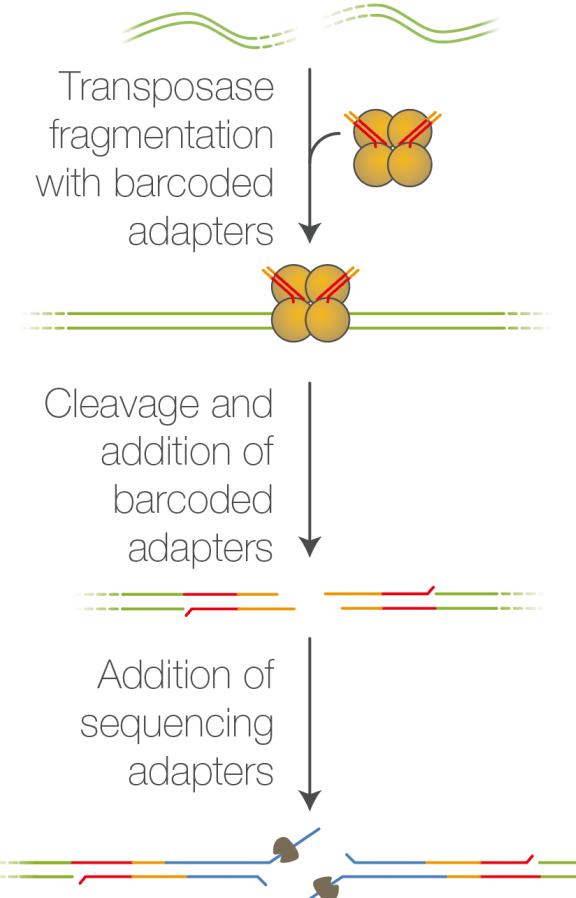
a) PCR barcoding



b) PCR-free barcoding



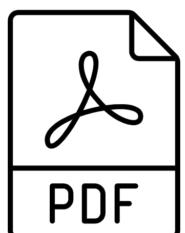
c) Rapid barcoding



# MinION run QC



Remember to save  
your PDF report in  
MinKNOW



# Sequencing errors

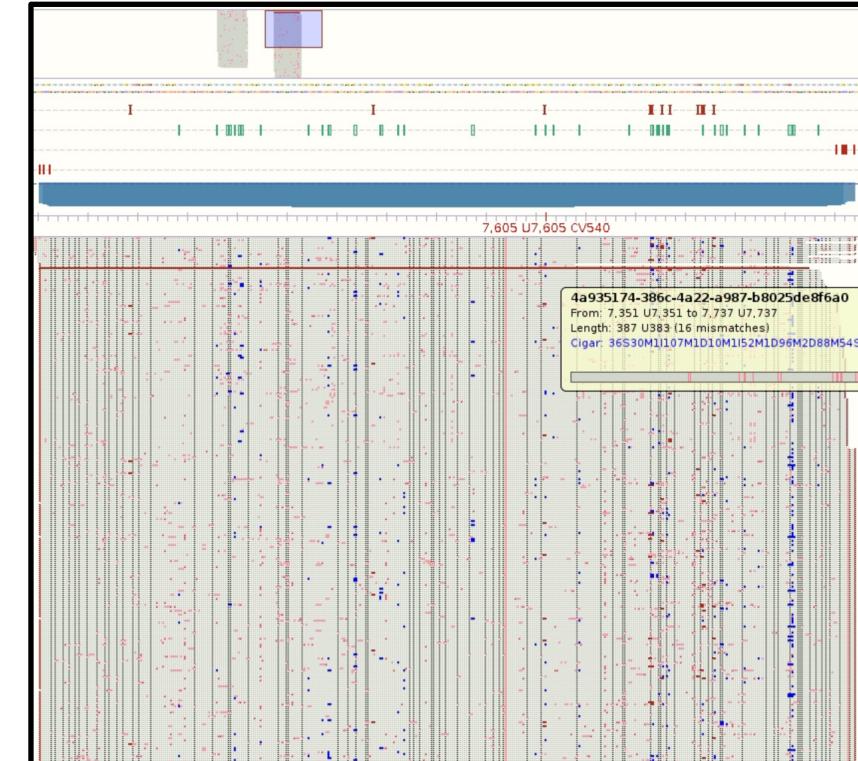
Nanopore sequencing has a high error rate.

- Most nanopore errors are all random but **some are systematic**.
- Accuracy is improving with up to Q20+

Illumina

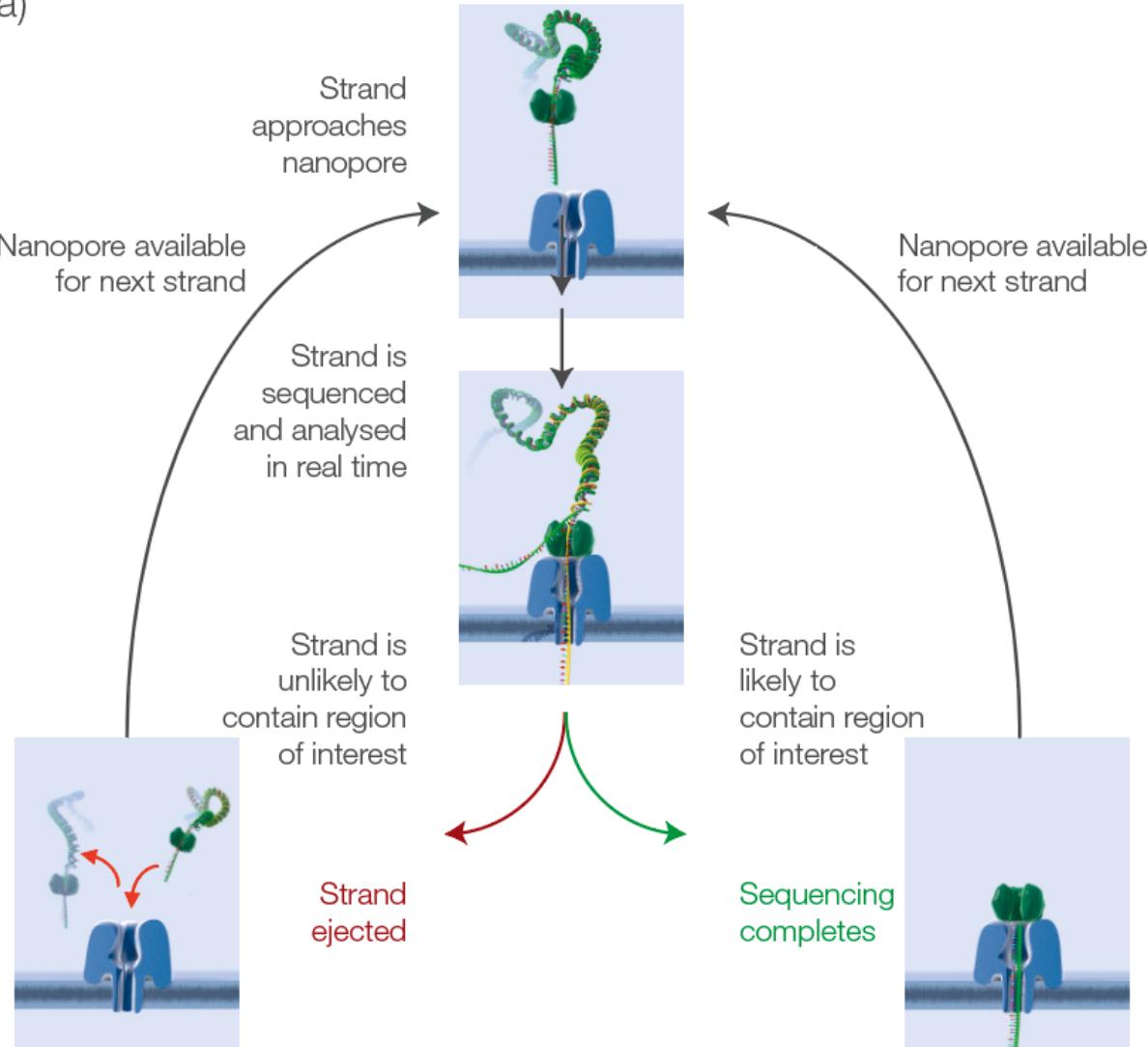


Nanopore



# Adaptive sampling

a)



- Input a fasta sequence
- Real time aligning
- Reject non-target DNA to sequence
- Selective enrichment

# Analysis Pipeline

## Basecalling

Convert the electrical signal (squiggle) into nucleotide bases

**fast5 -> fastq**

### **Packages**

- Guppy
- Bonito
- Dorado

## Quality control

MinNOW software that shows QC in real time

- Demultiplex and adapter trimming (**Guppy, Porechop**)
- Removal of contaminant reads (**Kraken, centrifuge**)
- Quality filtering (**Guppy, Filtlong**)

## Mapping, assembly and variant calling

Special tools

Mapping:

- Minimap2 (-ax map-ont)
  - BWA (-x ont2d)

Assembly:

- Spades

Variant Calling:

- Freebayes

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# Nanopore Sequencing Practical

