Third Generation Sequencing: A Zika Virus Case Study

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

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Third Generation Sequencing Platforms

	Second Generation sequencing	PacBio: Single Molecule Real-Time Sequencing SMRT	Oxford Nanopore Technology: MinION
Read length (bp)	300 max (600 paired)	15,000	90,000
Ave yield (Gb)	12	8	12-20
Cost Per Run ($)	2500	850	~800
Hardware Cost ($)	195k	695k	1k
Observed Error Rate	<1%	~12-1%	~12-1%

Nanopore technology

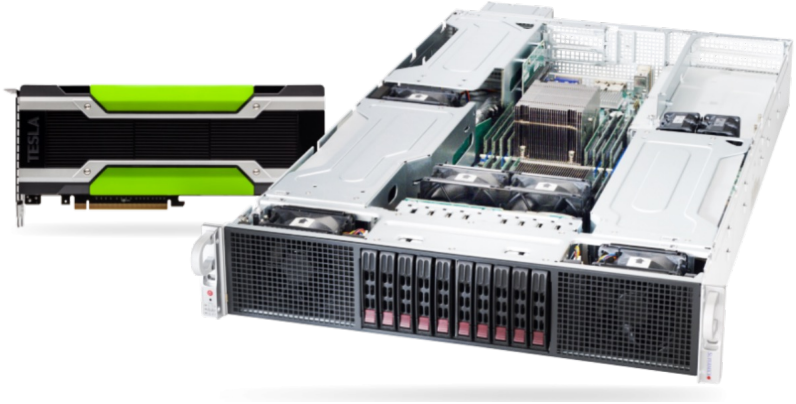
PromethION

GridION

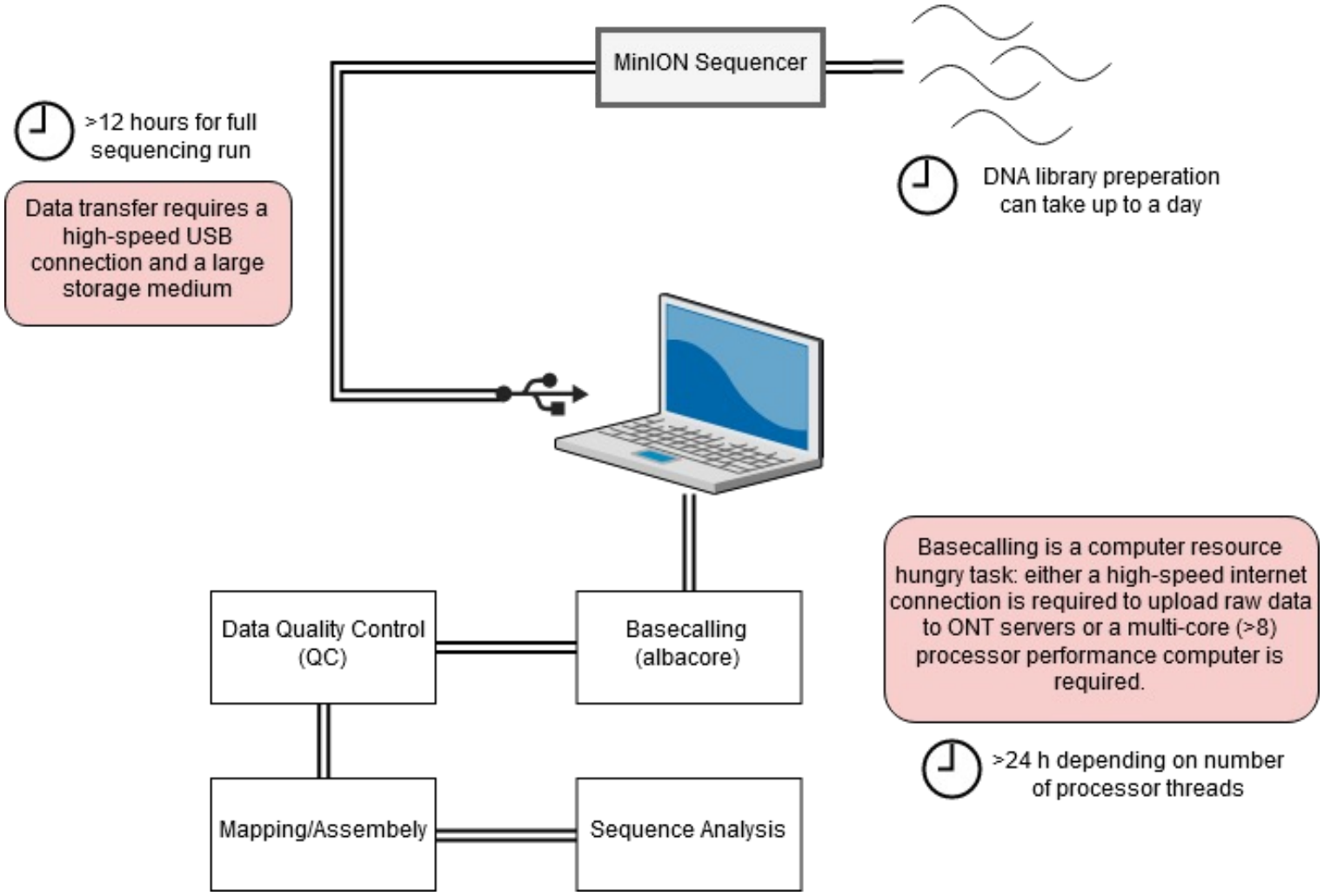
MinION

Flongle

HPC + GPU



Sequencing Pipeline

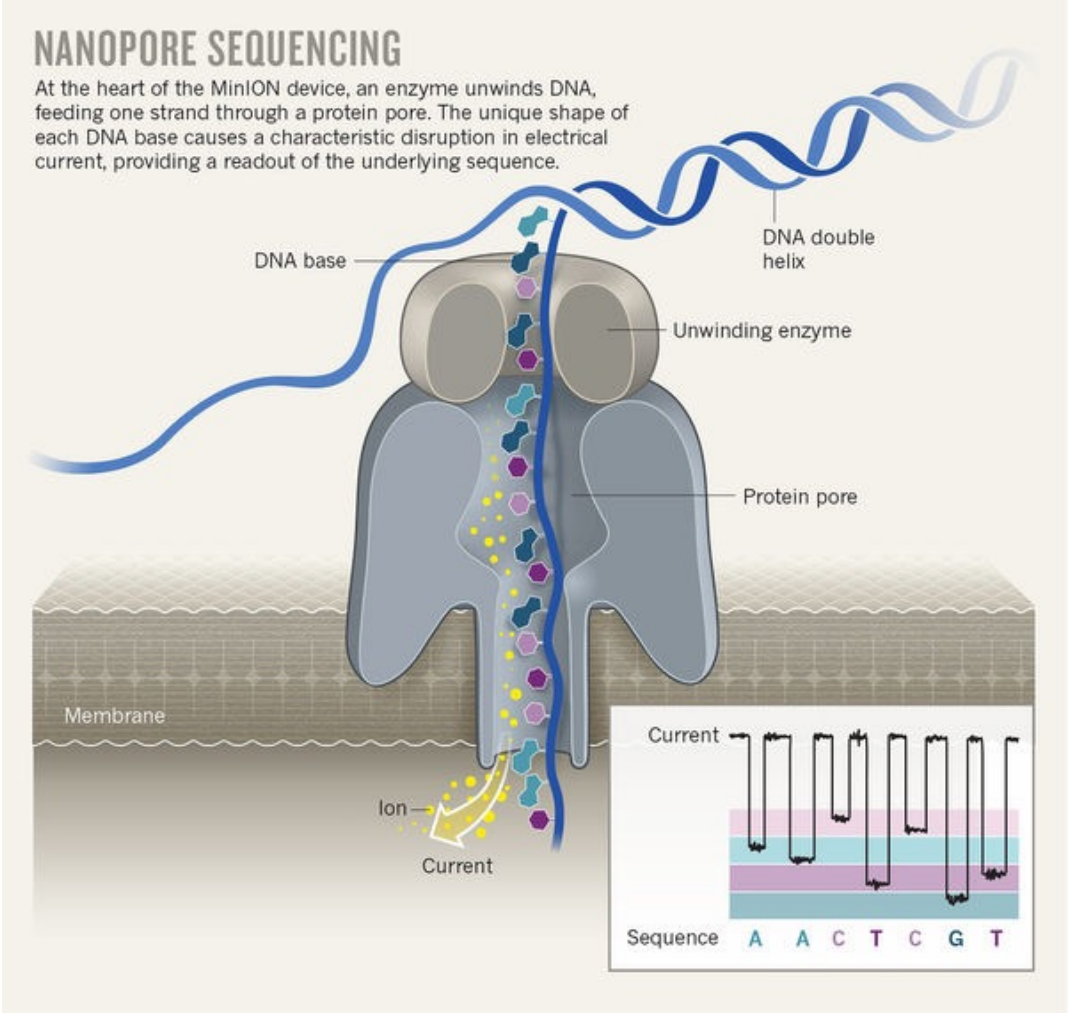


MinION – Nanopore Sequencing Technology

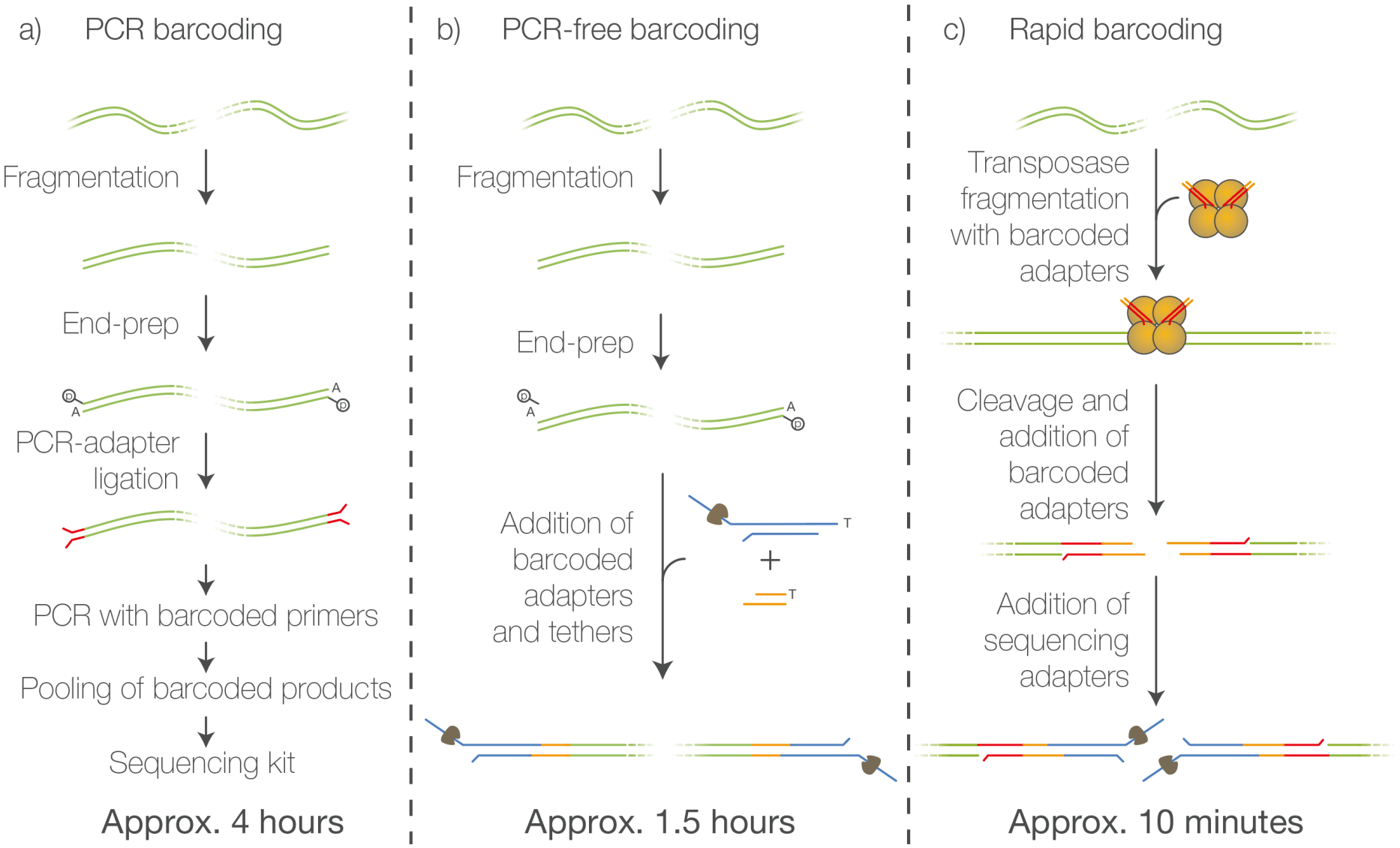
"Portable, real-time sequencing analyses"

- Very portable – can take to the field*
- Produces long reads compared to NGS platforms
- Can produce very high depth and coverage – high data yield.

- Still requires sophisticated lab techniques for library preparation
- Has a very high error rate compared to Illumina
- Is still under development – high dependency on community contributions
- Data is very challenging to process and analyse



Library preparation and loading

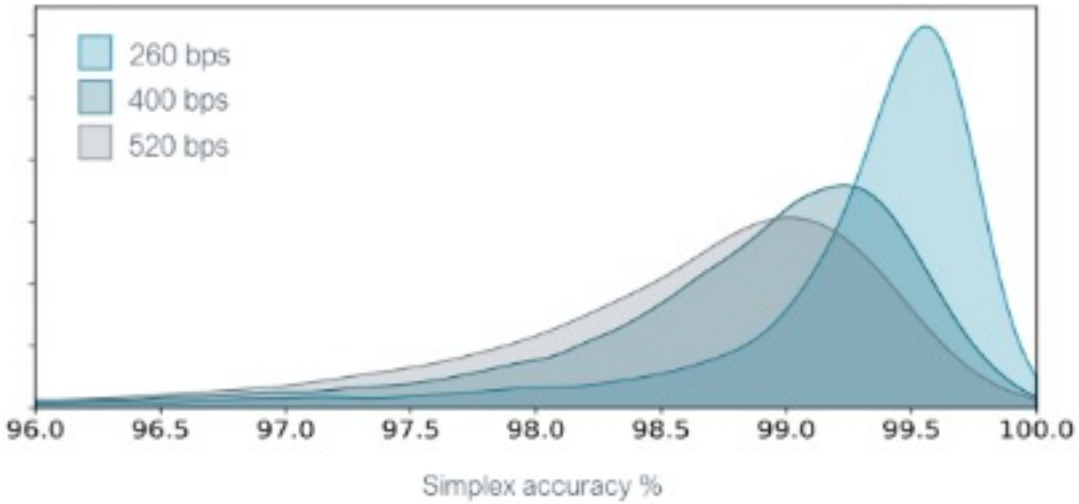


Flow cells and chemistries

- Currently ONT sell R9.4.1 and R10.4.1 flow cells.
- Different chemistries produce varying qualities and yields of read data.
- Kit 9, 10, 12 and 14 chemistries are available.
- Specific kits are used for specialised applications and are not always compatible with each other/ flow cells / barcodes.
- Kits and flow cells require specific basecalling models.

Sequencing errors

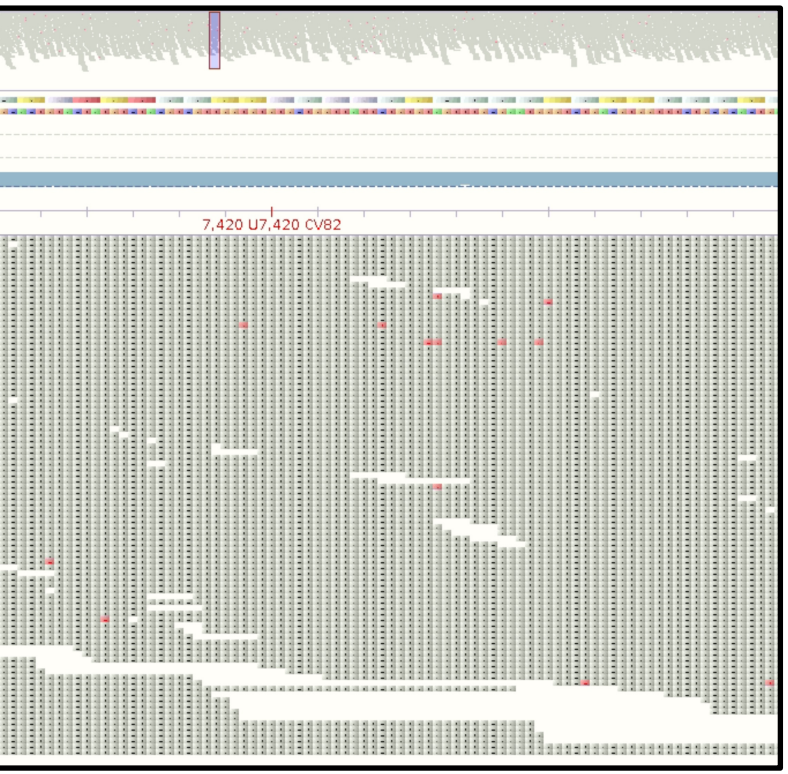
- The major caveat of nanopore sequencing is the high error rate.
- It is a common misconception that nanopore errors are all random. **Some are systematic**.
- ONT have been working on Q20+ sequencing chemistries, which decrease the sequencing error rate significantly.
- Duplex basecalling combines the data from the complementary strand to increase base call confidence.



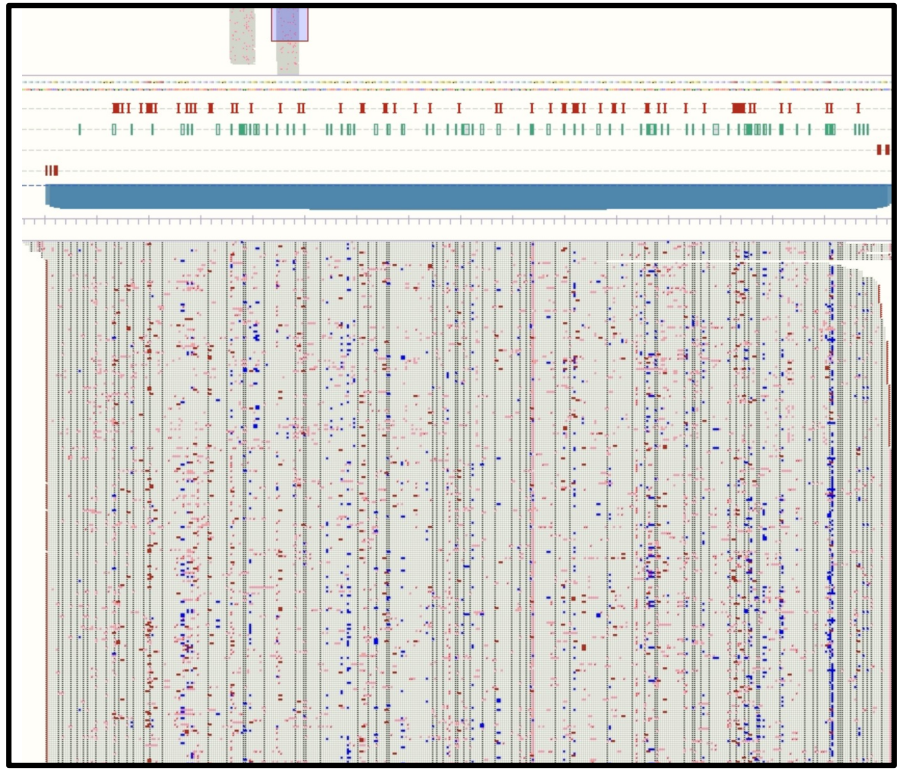
Run condition	Translocation speed	Theoretical max output (PromethION Flow Cell)	Modal accuracy (simplex)
Accuracy	260 bps	185 Gb	99.6% (Q24)
Default	400 bps	285 Gb	99.2% (Q21)
Output	520 bps	370 Gb	~99% (Q20)

Sequencing errors

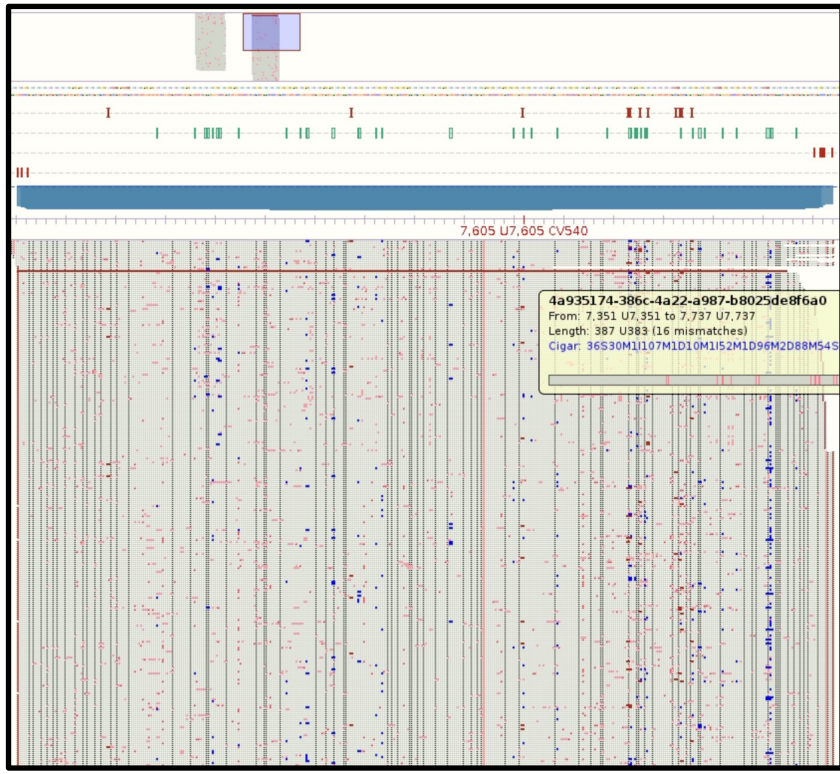
Illumina



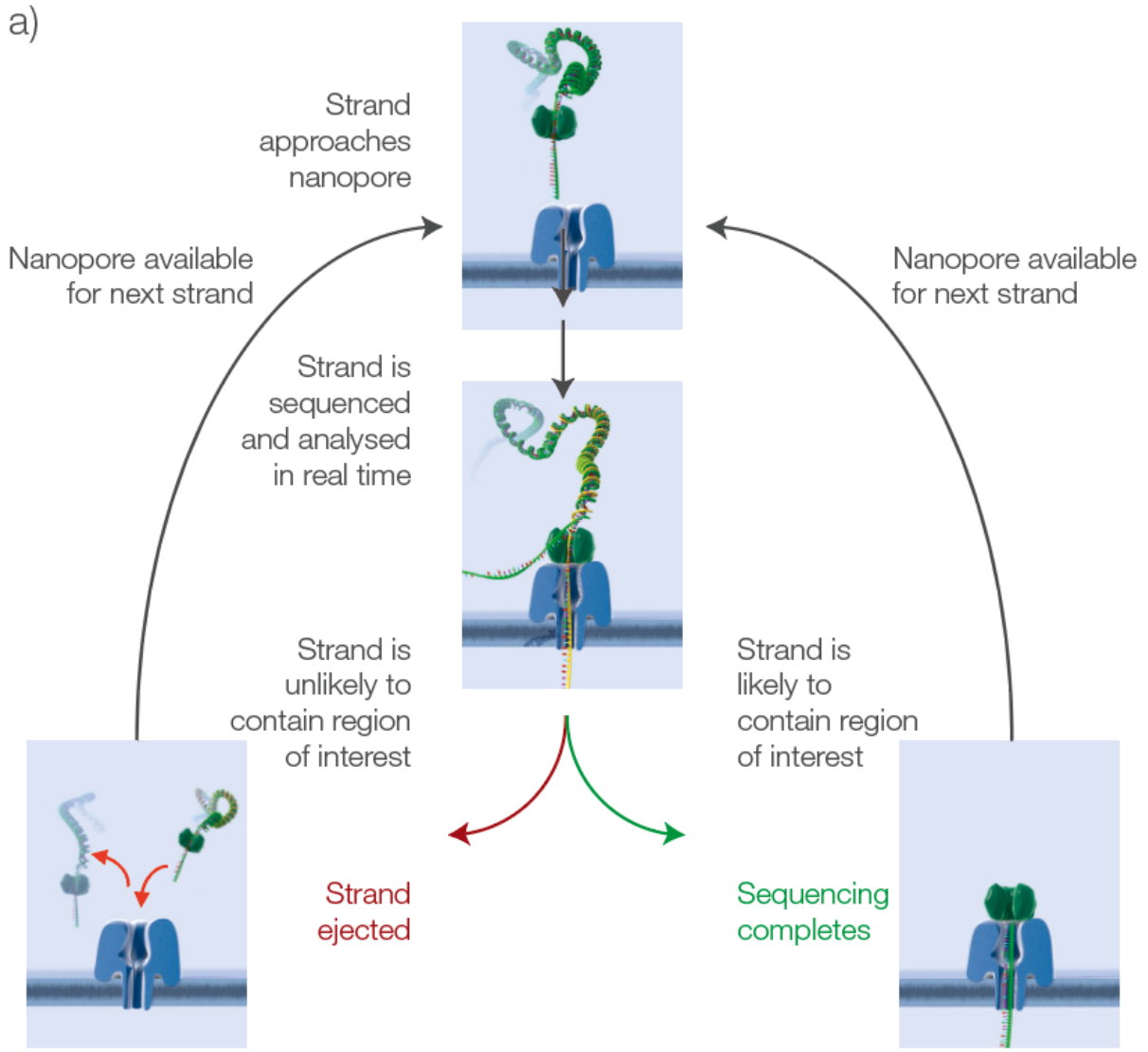
Nanopore: Guppy



Nanopore: Bonito

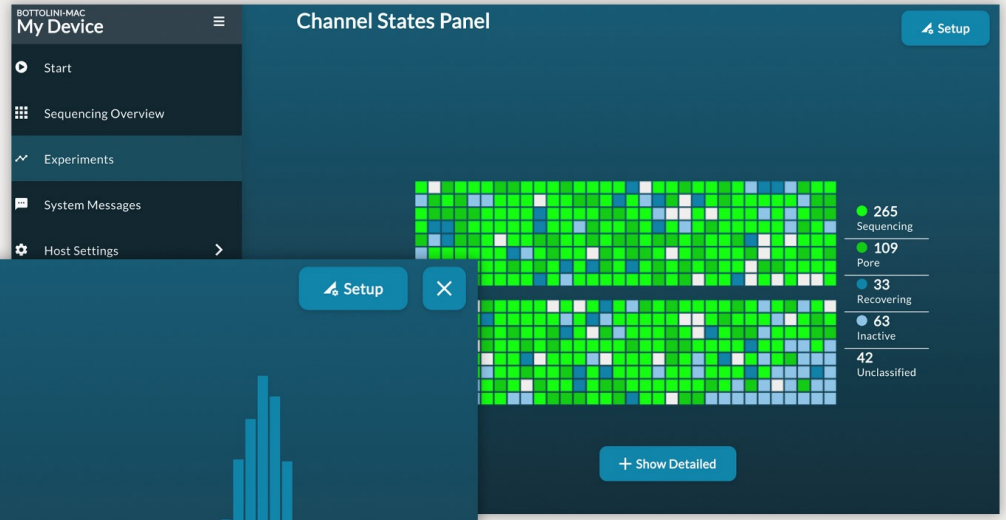


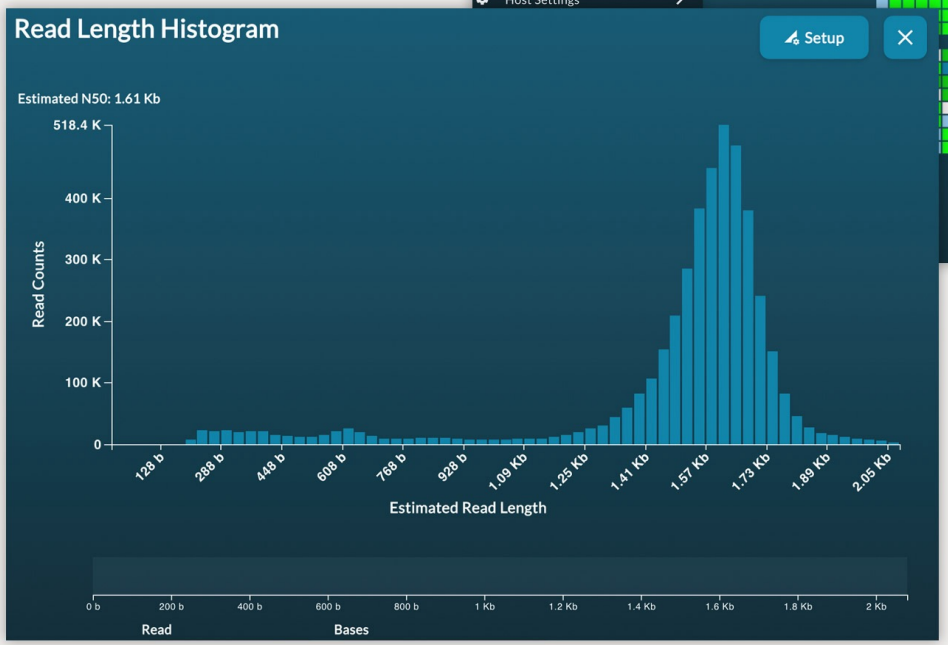
Adaptive sampling



Adaptive sampling allows the automatic selection of an unlimited number of regions of interest during a sequencing run, without the need for additional library-preparation steps

MinION run QC





Analysis Pipeline

Basecalling

Converting the electrical signals generated by a DNA or RNA strand passing through the nanopore **(fast5)** into the corresponding base sequence of the strand (**fastq)**.

Guppy:

- Integration with MinKNOW GUI
- Demultiplexing and kit selection built in
- GPU optimised

Bonito

- Experimental with high performance
- Model training

Quality control

The PDF from **MinKNOW** and tools such as **pycoQC** can useful in understanding metrics.

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

Different requirements for mapping or assembly.

Mapping, assembly and variant calling

There are specialised tools for assembling and mapping nanopore reads.

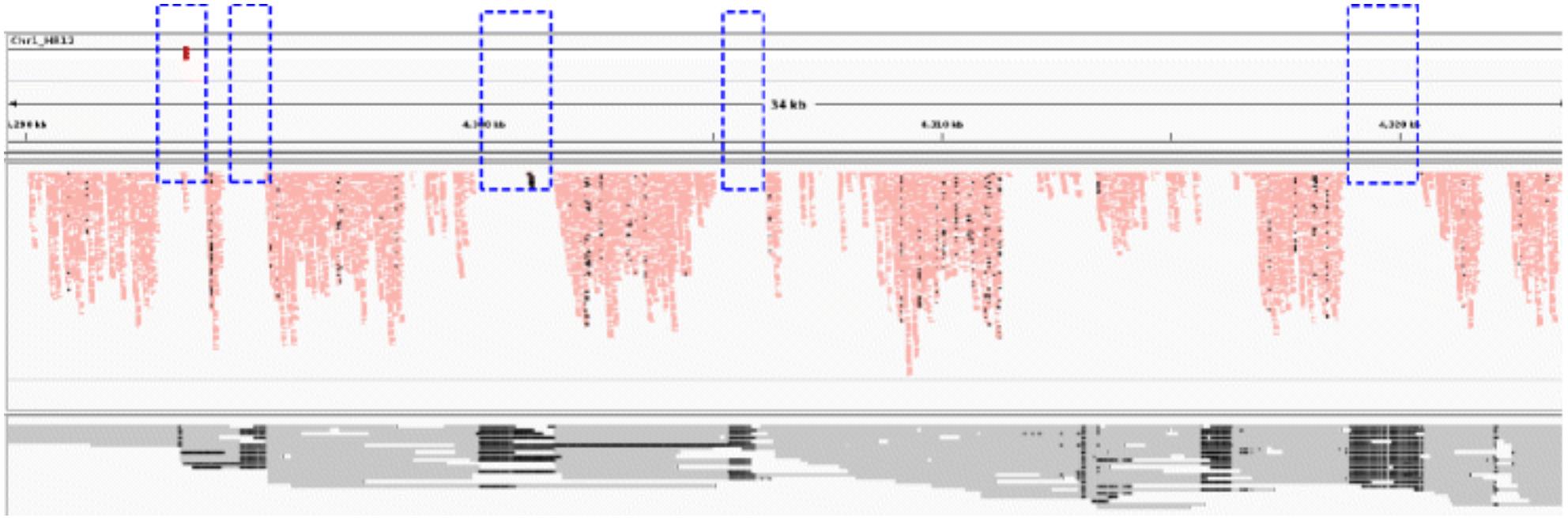
Mapping:

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

Assembly:

- Flye
- Unicycler
- SPAdes
- Canu

Hybrid assembly



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Hybrid assembly helps resolve ambiguities with higher coverage and differing read lengths

SARS-CoV-2 Sequencing

ARTICnetwork
http://artic.network | @NetworkArtic

SARS-CoV-2 sequencing workflow

1 **Specimens collected**

Clinical samples and isolates

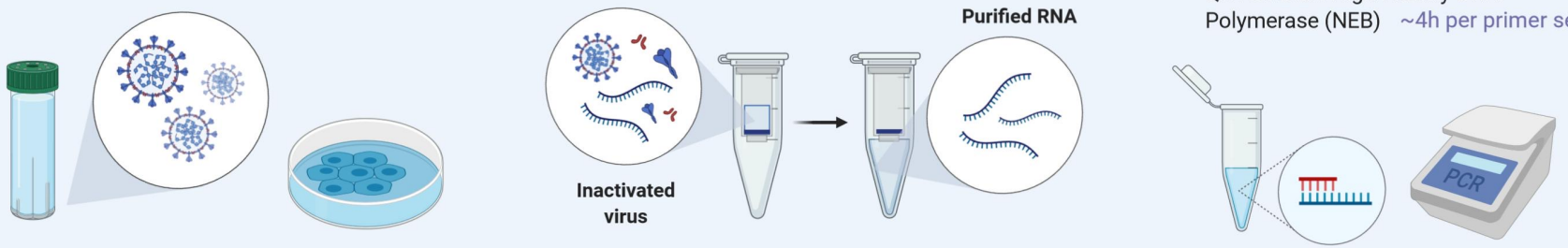
2 **Viral RNA extraction**

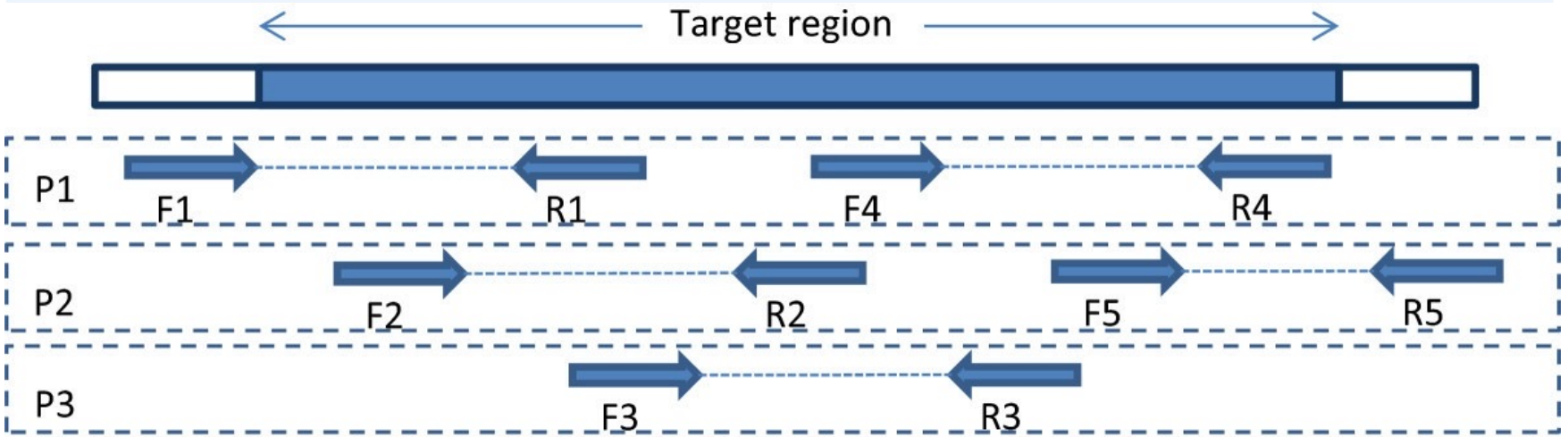
NucleoMag Virus kit (Macherey-Nagel) ~45 min

3 **cDNA synthesis and multiplex PCR**

ProtoScript II First Strand cDNA Synthesis Kit (NEB) ~25 min

Q5 Hot Start High-Fidelity DNA Polymerase (NEB) ~4h per primer set





Real-Time Sequencing = Real-Time Surveillance

- Genome sequencing can provide a high-resolution view of pathogen evolution and is increasingly sought after for outbreak surveillance.
- Sequence data may be used to guide control measures, but only if the results are generated quickly enough to inform interventions.
- In April 2015 the MinION system was transported in standard airline luggage to Guinea and used for real-time genomic surveillance of the ongoing epidemic.
- They presented sequence data and analysis of 142 EBOV samples collected during the period March to October 2015.

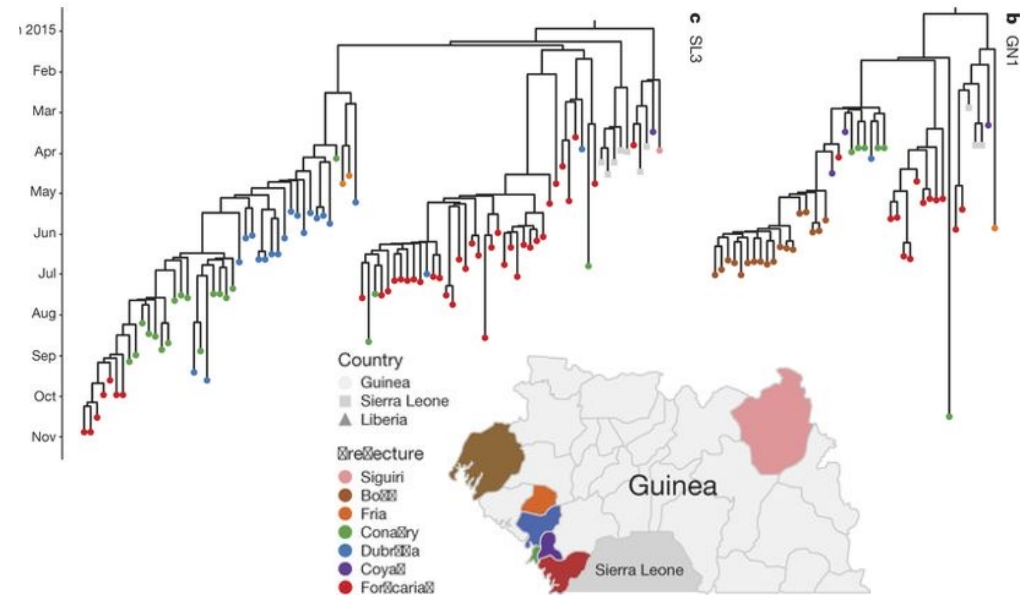
nature
International journal of science

Letter | Published: 03 February 2016

Real-time, portable genome sequencing for Ebola surveillance

Joshua Quick, Nicholas J. Loman [...] Miles W. Carroll

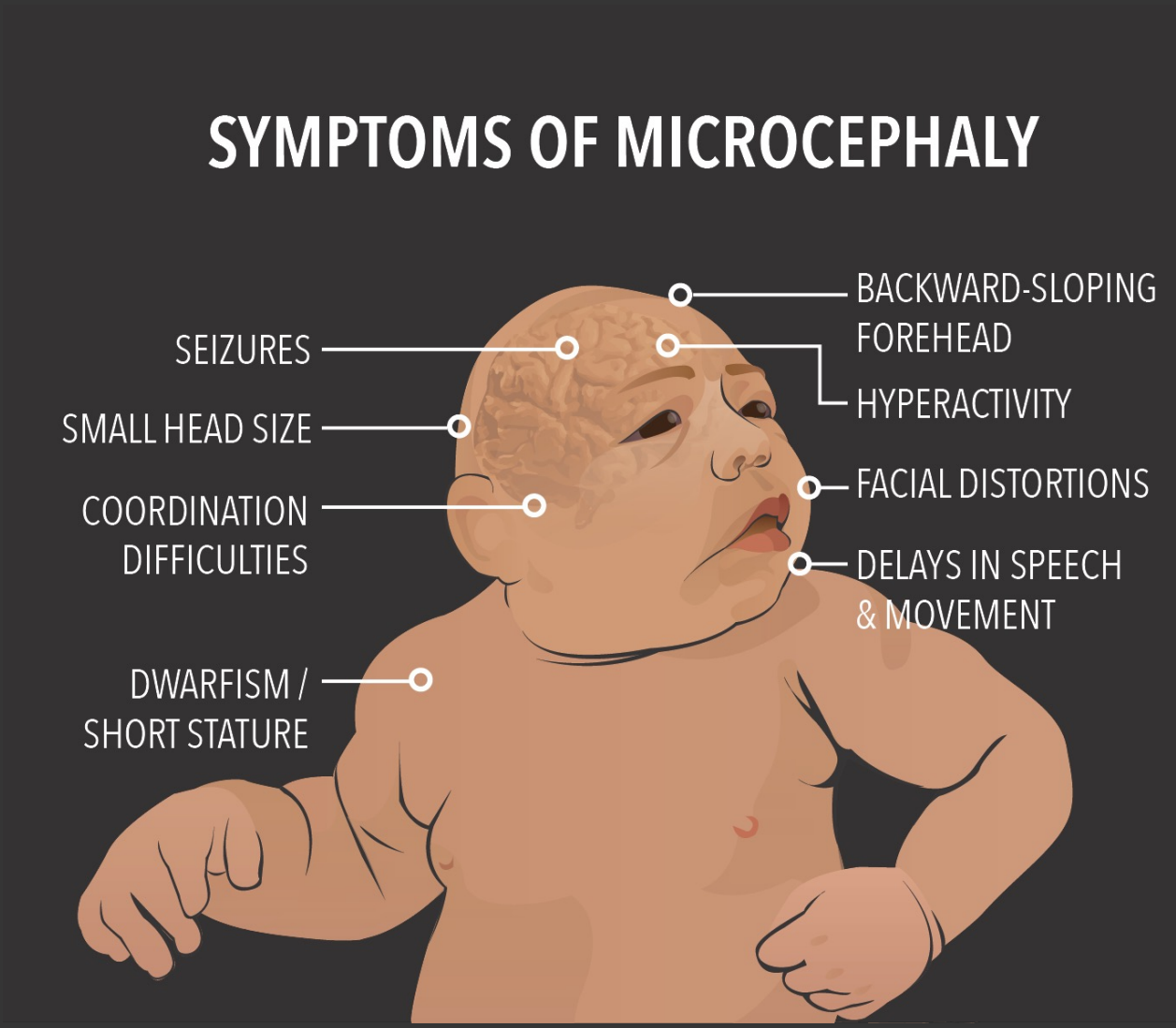
Nature **530**, 228–232 (11 February 2016) | Download Citation



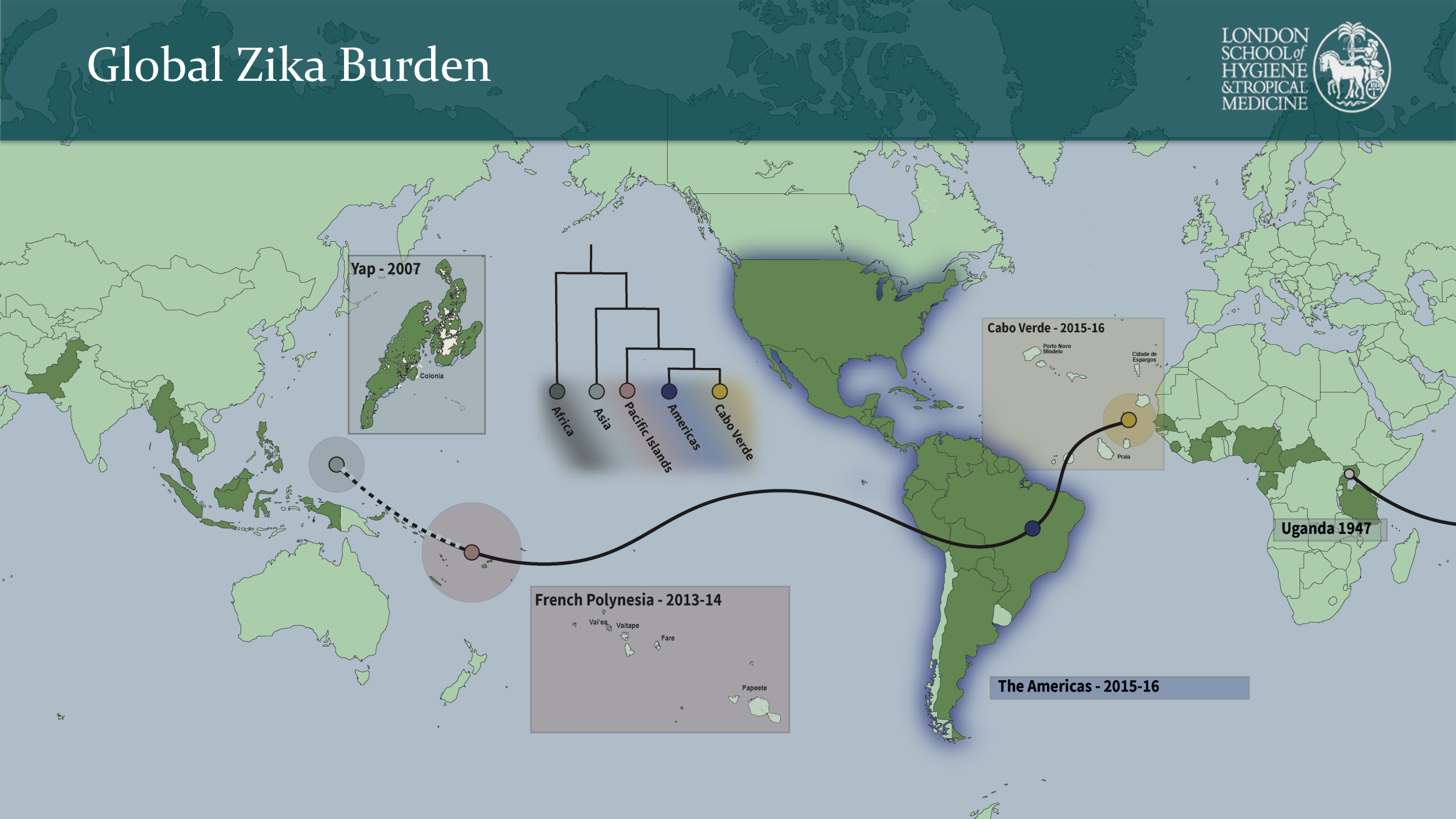
https://nextstrain.org/ebola?dmax=2014-09-26&dmin=2014-08-10

Zika Virus Morbidity

- Zika virus disease is caused by an RNA virus, a member of the *Flavivirus* genus.
- Other flavivirus include Dengue, yellow fever and West Nile Virus.
- The 2015-16 Zika outbreak in South America triggered the most recent WHO PHEIC, following association with microcephaly.
- The primary vector (transmitting agent) is the *Aedes Aegypti* mosquito.



Global Zika Burden



Nanopore Sequencing Data Activity