ROSLIN

THE UNIVERSITY of EDINBURGH
Royal (Dick) School of Veterinary Studies

The Digital One Health – Oxford Nanopore Workshop

Ministry of Water and Environment
REPUBLIC OF UGANDA

CENTRAL PUBLIC HEALTH LABORATORIES
of The Republic of Uganda
MINISTRY OF HEALTH

MAAIF
Ministry of Agriculture
Animal Industry and Fisheries

Digital One Health Laboratory

The Roslin Institute,
University of Edinburgh

digital-one-health.github.io/doh-ont-workshop

Biotechnology and Biological Sciences Research Council

MAKERERE UNIVERSITY

THE ROYAL SOCIETY

The Digital one health Laboratory

What we will cover today

- Introductions (everyone)

- Overview of the workshop
- Overview of the protocols
- Important concepts

- Feedback from participants
- Questions

Workshop website:

Introductions

Trainers

- Vesa Qarkaxhija
- Bryan Wee
- Frank Chilanga
- Adrian Muwonge
- Emmanuel Ssebaggala

Participants

- Julius Sseruyange
- Arinaitwe Eugene
- Tusabe Godwin Wenka
- Nakanjako Gladys Kiggundu
- Kia Praiscillia
- Ankunda Penrose
- Bulyaba Lydia Namutale
- Katumba Godfrey
- Nabatta Esther
- Olum George William
- Franklin Mayanja
- Daniel Eurien

What are we doing ?

Aims:

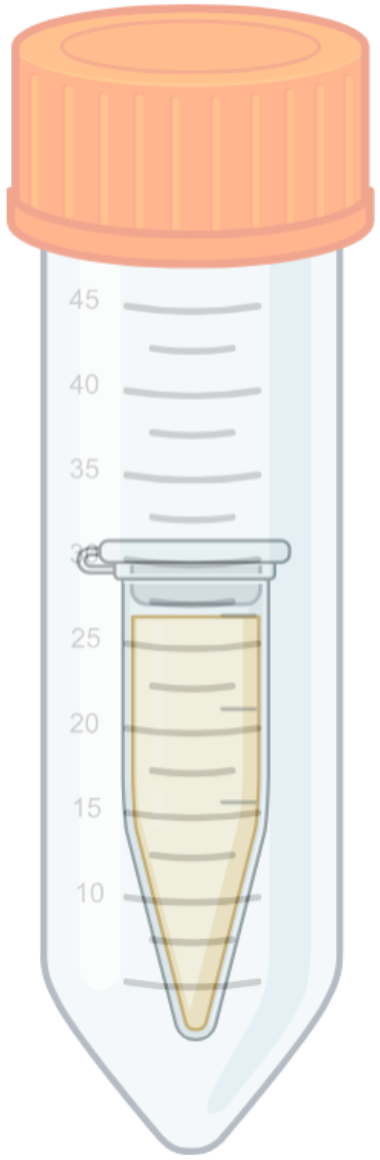
- To familiarise with DNA extraction and purification methods suitable for long read sequencing
- To familiarise with Oxford Nanopore library preparation, loading and sequencing
- Generate first few whole genome sequences for the DOH pilot project

Day 1

Time	Session	Who
09:00	*Registration and morning coffee*	All
09:15	Opening ceremony	All, Dr Susan Nabadda, Dr Adrian Muwonge
09:45	Part 1: DNA Extraction 1 (3 Hours)	11 participants and trainers only
12:45	*Lunch Break*	11 participants and trainers only
13:30	Part 2: DNA Extraction 2 (2 Hours)	11 participants and trainers only
15:30	*Afternoon break*	11 participants and trainers only
15:50	Bioinformatics overview (MinKNOW & EPI2ME)	11 participants and trainers only
16:20	Digital One Health showcase (Bodastage)	11 participants, trainers & Emmanuel Ssebaggala
17:00	END	Everyone

Day 2

Time	Session	Who
09:00	*Arrival and morning coffee*	11 participants and trainers only
09:30	Bioinformatics overview (EPI2ME)	11 participants and trainers only
10:30	Part 3: Preparing a sequencing library (2 hours)	11 participants and trainers only
12:30	Lunch	11 participants and trainers only
13:30	Part 4: Starting a sequencing Run (1.5 hours)	11 participants and trainers only
15:00	*Afternoon tea*	11 participants and trainers only
15:30	Bioinformatics analysis (EPI2ME)	11 participants and trainers only
16:30	END	Everyone

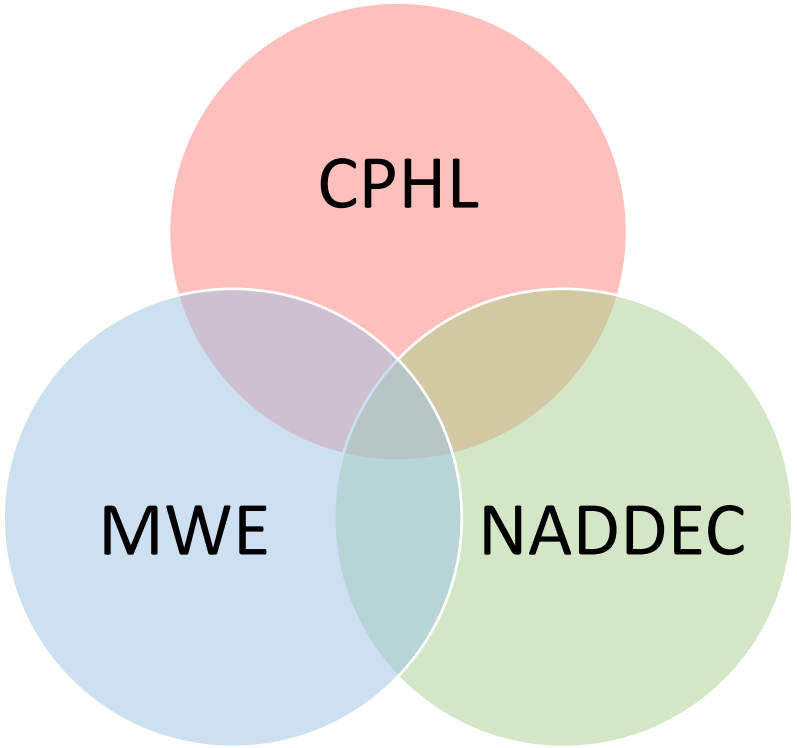


To bring to the workshop

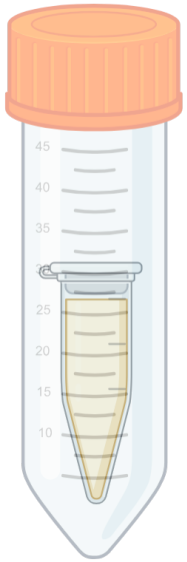
- Each participant to have 2x different samples
- Overnight LB/nutrient broth culture of Bacterial isolate part of pilot project
- 1.5ml Eppendorf
- Best to transport in Eppendorf placed in falcon tube.
- Centrifuge at 5000Gs @ 3mins to pellet
- Bring it to CPHL on Thursday 22 Feb
- OR bring it on the day of the workshop

Any questions?

Samples AMR surveillance



- *E. coli* from each institution's AMR surveillance program
- Please send us a list of isolates with time and location metadata so that we can identify overlapping strains



Lab session overview

Day	Parts	Important steps	Duration
Day 1	**Part 1:** DNA Extraction 1	Cell lysis, DNA extraction	3 hours
	Part 2: DNA Extraction 2	DNA cleanup	2 hours
Day 2	**Part 3:** Preparing a sequencing library	Adding barcodes and adaptors to DNA and another clean up	2 hours
	Part 4: Starting a sequencing Run	Getting the sample onto the flow cell and starting the sequencing run	1.5 hours

What are the protocols used?

Edited versions of:

- Qiagen Manual Purification of High-Molecular-Weight Genomic DNA from Gram-Negative Bacteria (DNA Extraction)
- ProNex® Size-Selective Purification System Technical Manual, TM508 (DNA Purification)
- Oxford Nanopore Technologies (ONT) Rapid sequencing gDNA – Barcoding SQK-RBK114-24

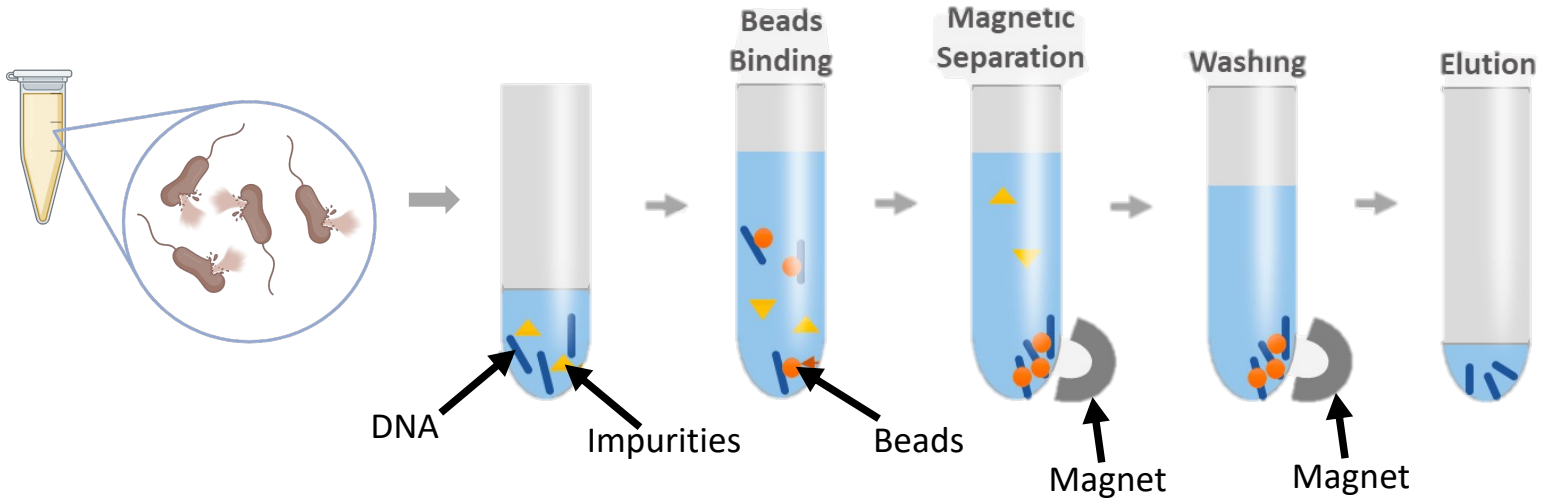
We will also provide flow cell wash, reuse, and storing protocol (Flow Cell Wash Kit EXP-WSH004) but this will not be covered by the workshop.

Equipment

- Thermomixer
- Benchtop centrifuge + Thermal cycler (can be replaced with Bento Lab)
- Fluorometer (Qubit or Quantus)
- Magnetic rack
- Pipettes (P1000, P200, P20, P10) + tips

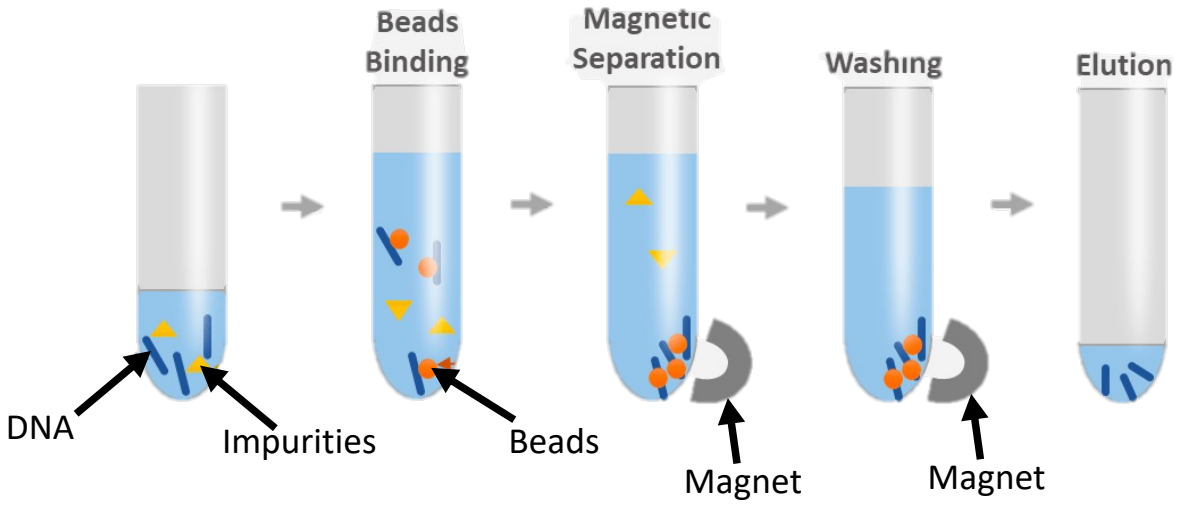
DNA extraction

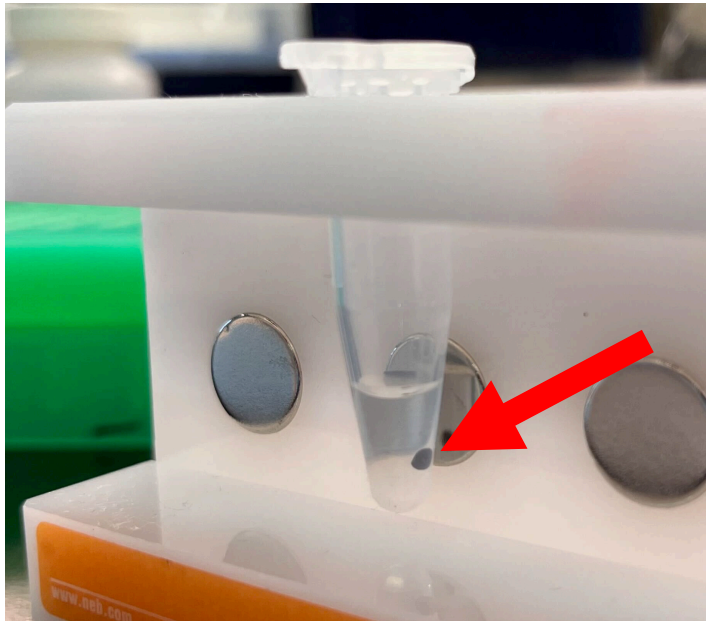
- Bacterial cell isolation and **Lysis**
- Use Magnetic beads to **Bind** DNA
- **Wash** off impurities whilst retaining DNA bound beads on Magnet
- **Elute** DNA off beads



DNA purification

- Use **Size selective** Magnetic beads to **Bind** DNA
- **Wash** off impurities whilst retaining DNA bound beads on Magnet
- **Elute** DNA off beads





Be gentle when pipetting – Why?

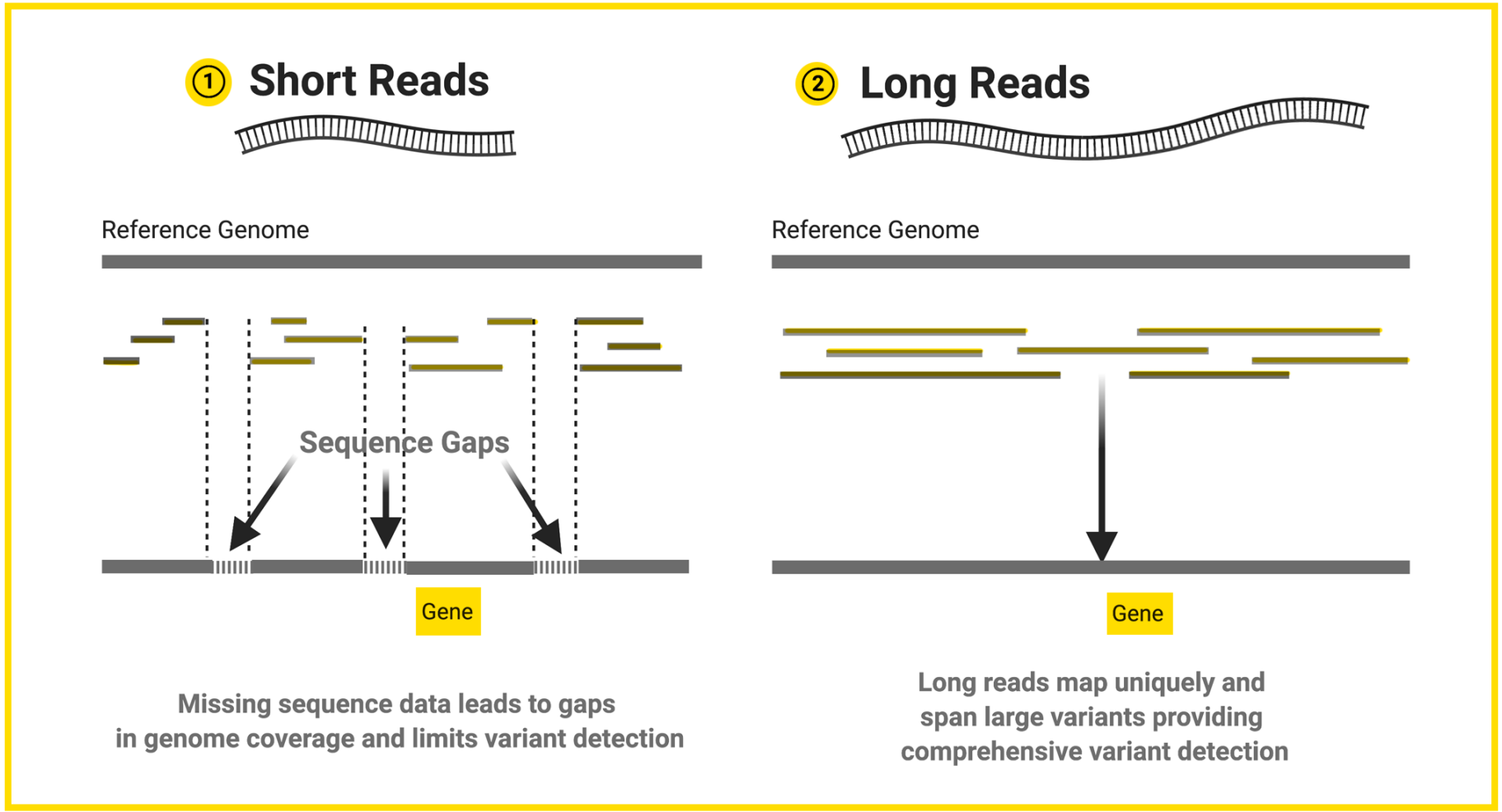
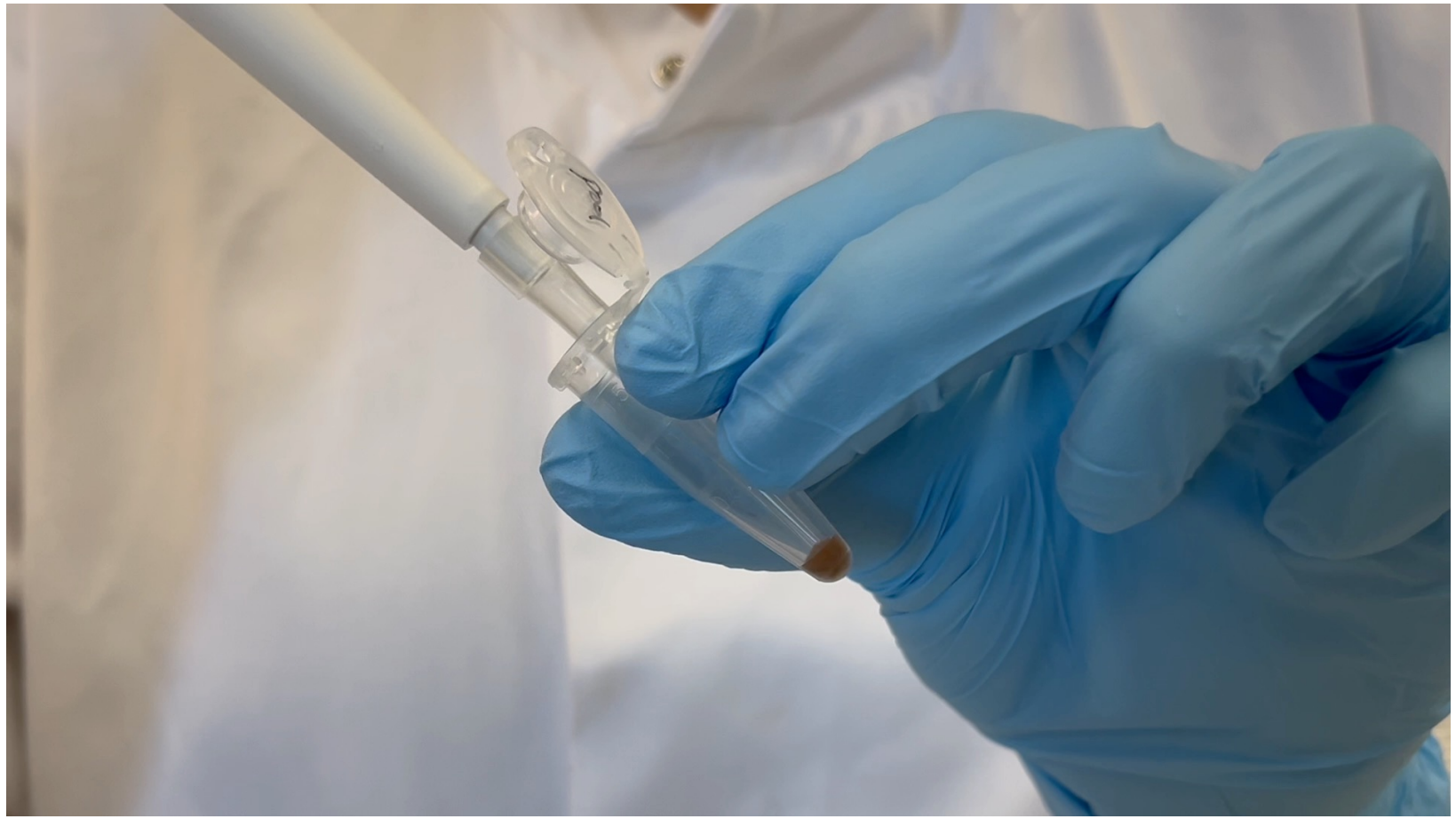
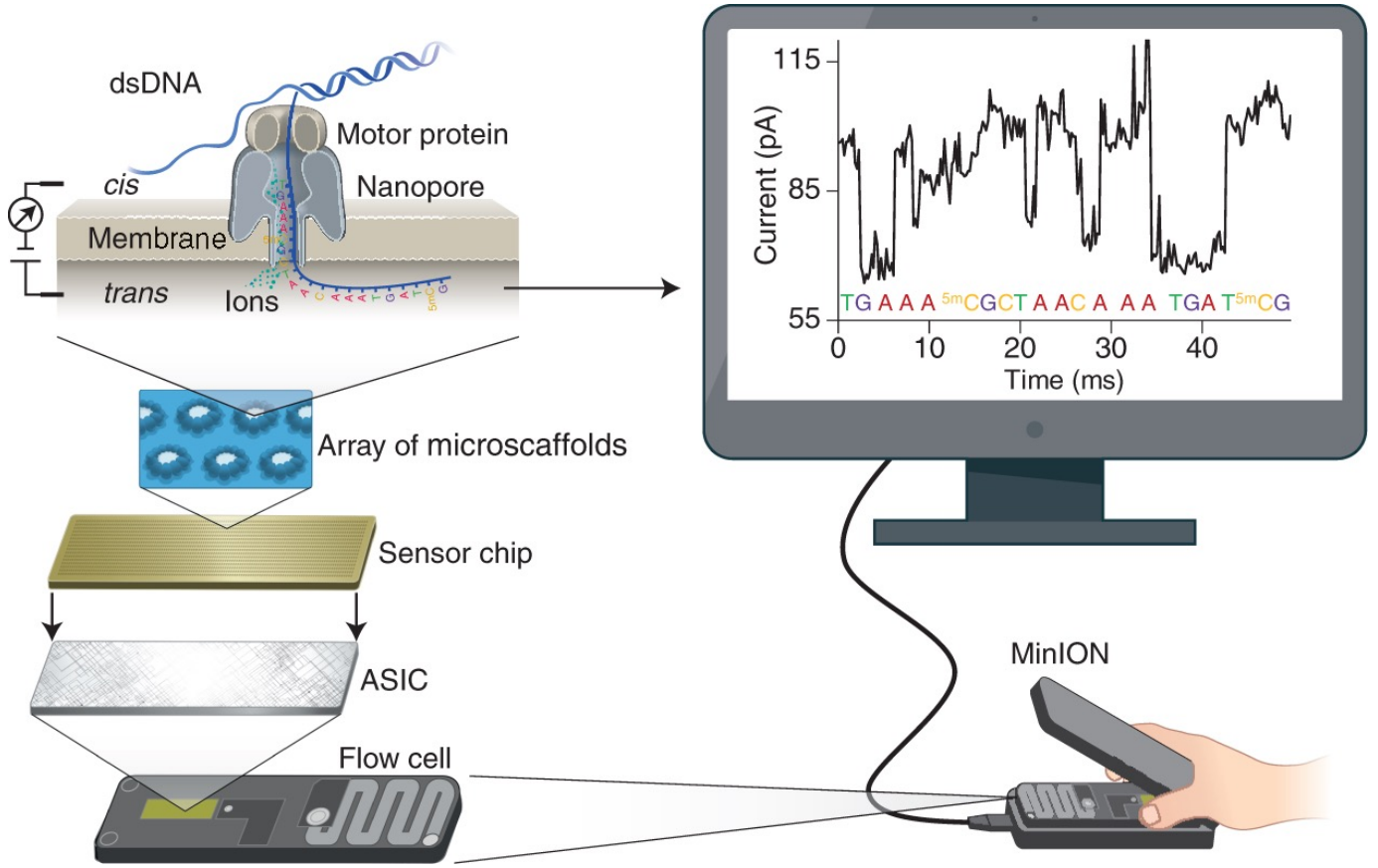


Image By Sarah Sharman, PhD, Science Writer.



Nanopore sequencing

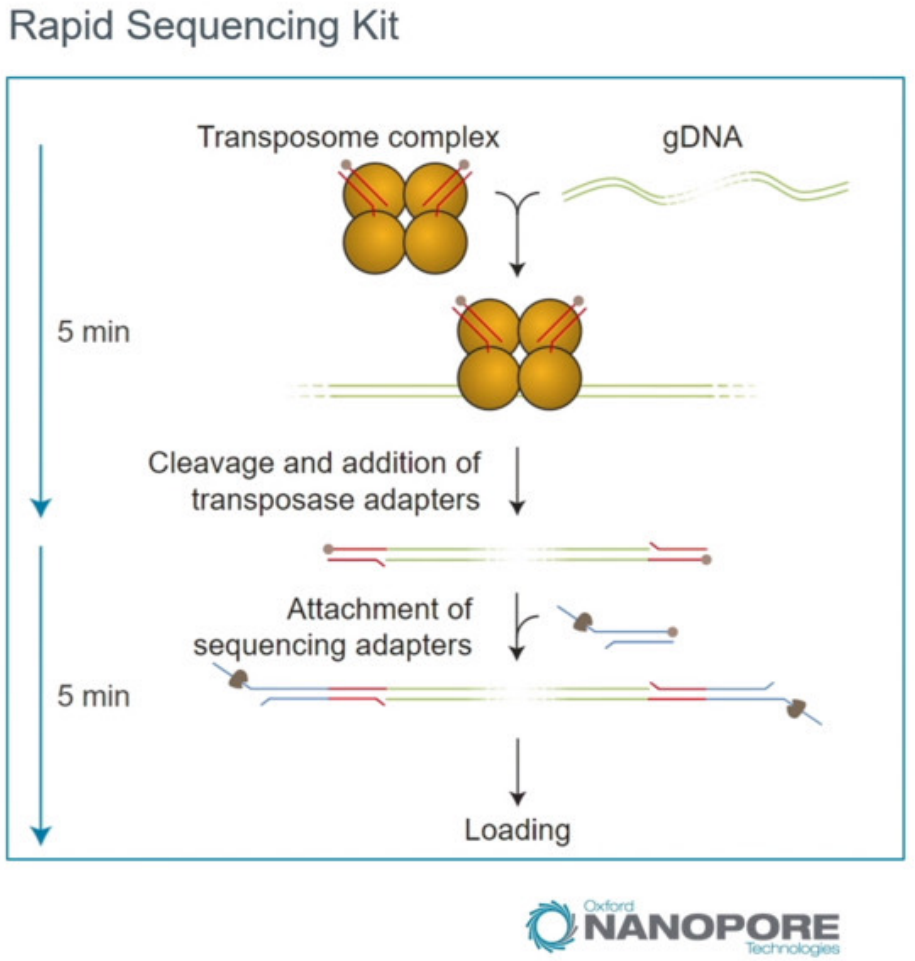
- DNA strands are passed through a protein nanopore
- The electric current changes and these changes are monitored
- The resulting signal is decoded to provide the specific DNA or RNA sequence



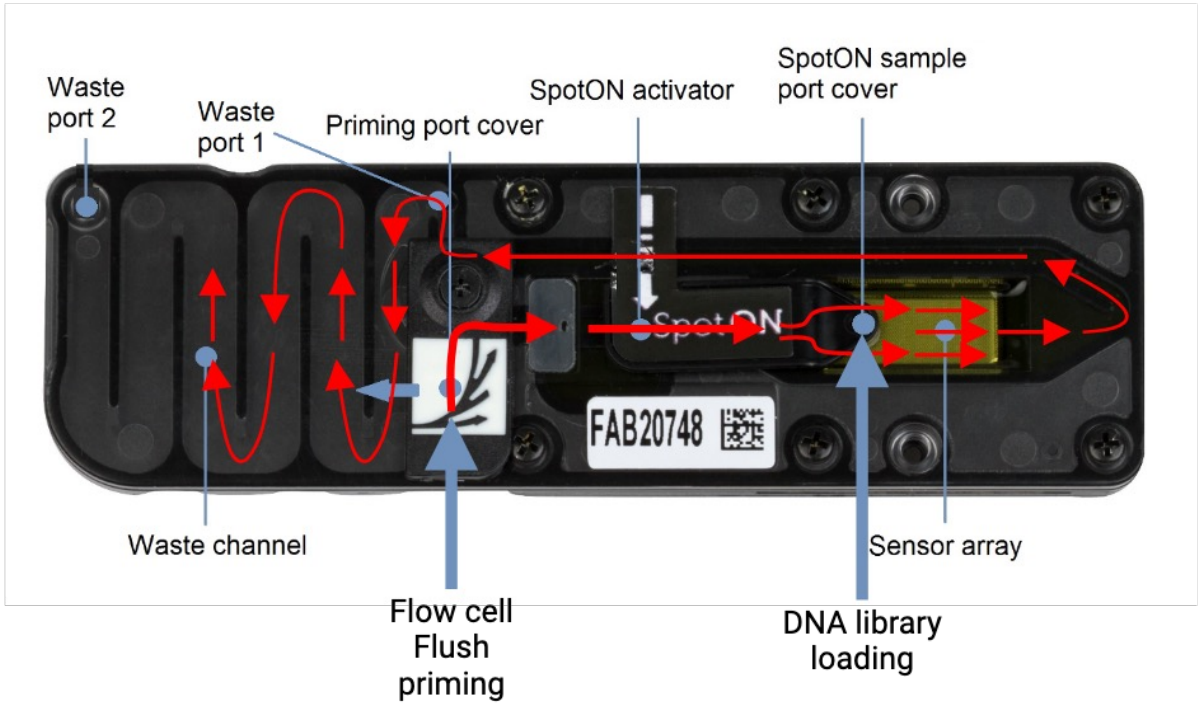
Wang, Y., Zhao, Y., Bollas, A. *et al.* Nanopore sequencing technology, bioinformatics and applications. *Nat Biotechnol* **39**, 1348–1365 (2021). https://doi.org/10.1038/s41587-021-01108-x

Library and Flow cell preparation

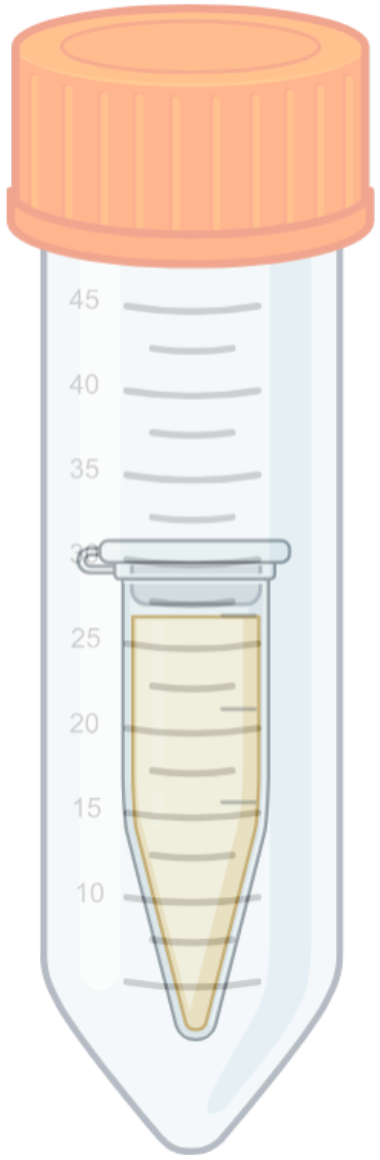
- The kit uses a transposase to cut genomic DNA and attach barcodes to cleaved ends
- Barcoded samples are pooled then cleaned using beads before adding the Rapid Sequencing Adapters to the tagged ends



Oxford NANOPORE Technologies



Flow cell fluid direction



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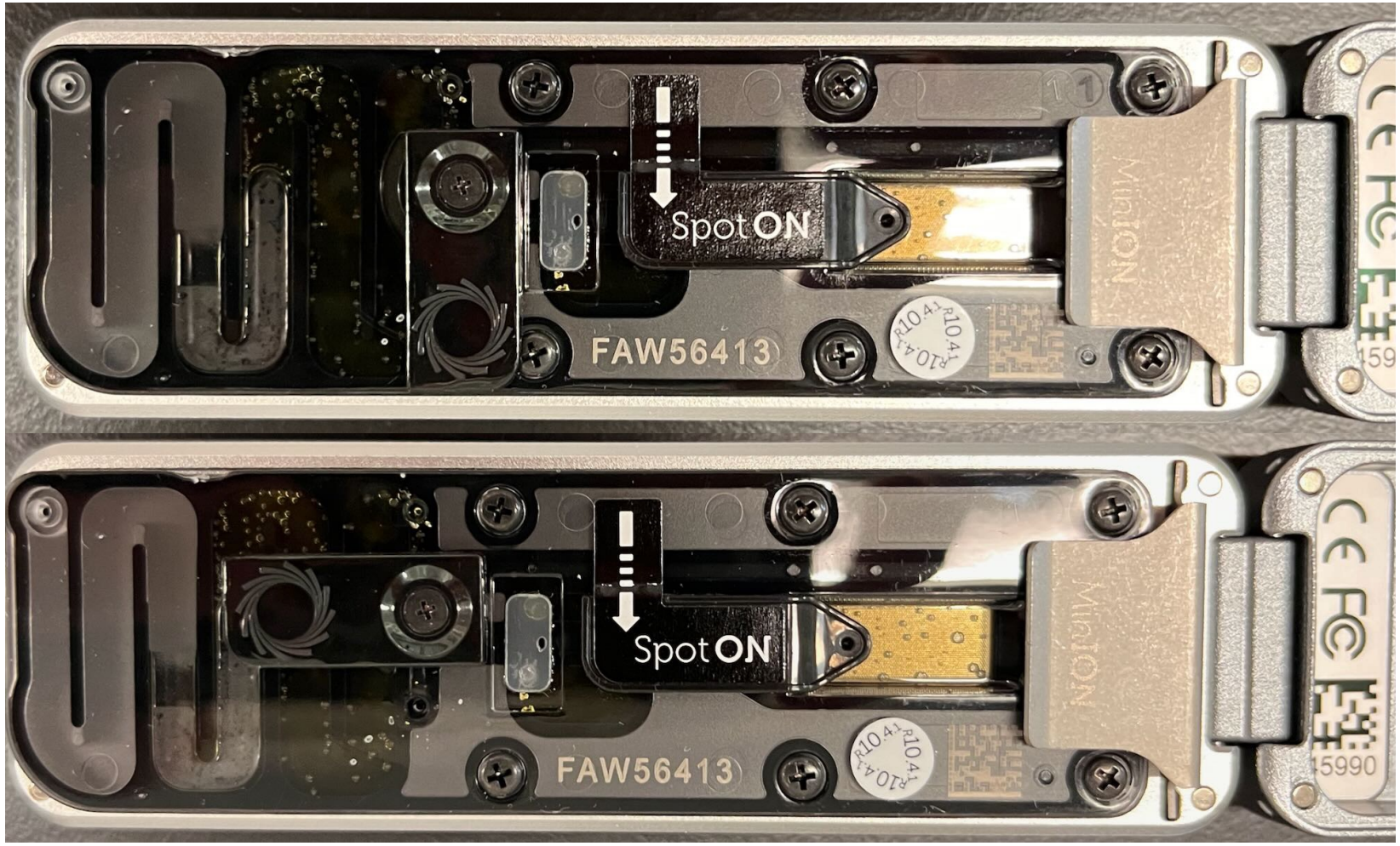

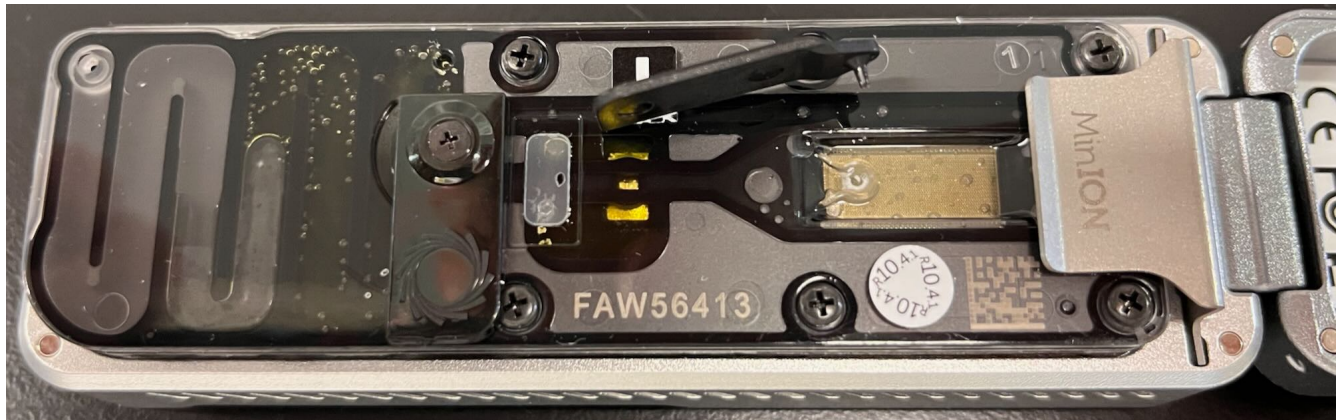

Extra photos of FC showing all ports covered, priming port open (1), spot on port open (2)

Keep at end for reference

Bioinformatics workflow