

# Quick recap

Day 4

# Nanopore sequencing

- The group collectively prepared a library!
  - 16 positive samples
  - 4 negative controls
  - 20 barcodes (NBD 1-20)
- Flowcell check was good (1414 pores)
  - Compared to 1431 at warranty QC (performed 1/02/2024)
- Run with live basecalling



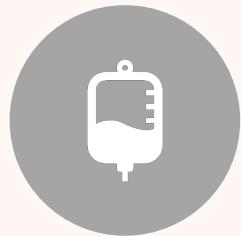
# Problems/changes during prep

- QC check failed with an error because wifi not connected
  - Can make MinKNOW run offline
  - Or only need connection for start of a run
- We didn't pack enough Short Fragment Buffer (SFB)
  - We used a lower volume (125 instead of 250ul)
- Some problems with small volumes
  - Barcode volumes were small and hard to pipette (another aliquot provided)

# Run metrics



LIBRARY ADDED TO  
FLOWCELL:  
200FMOL



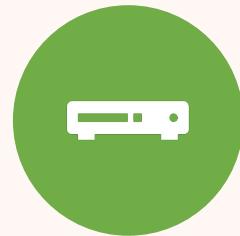
PORE OCCUPANCY  
~60%



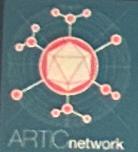
RUN TIME: 2H 37M



READS:  
1.67 MILLION



ESTIMATED BASES:  
971.33MB  
(MEGABASES)



# RAMPART

A set of small, semi-transparent navigation icons typically found in web browsers like Chrome or Firefox.

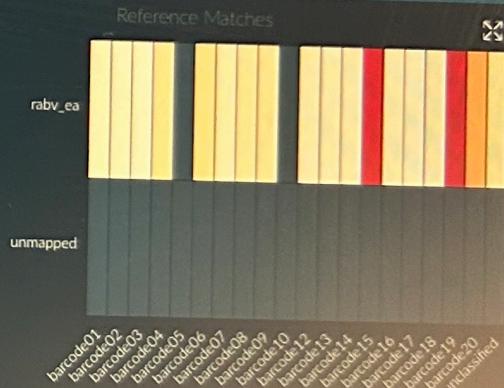
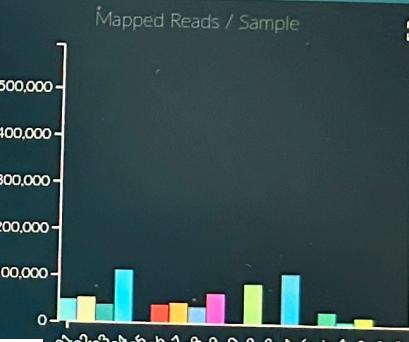
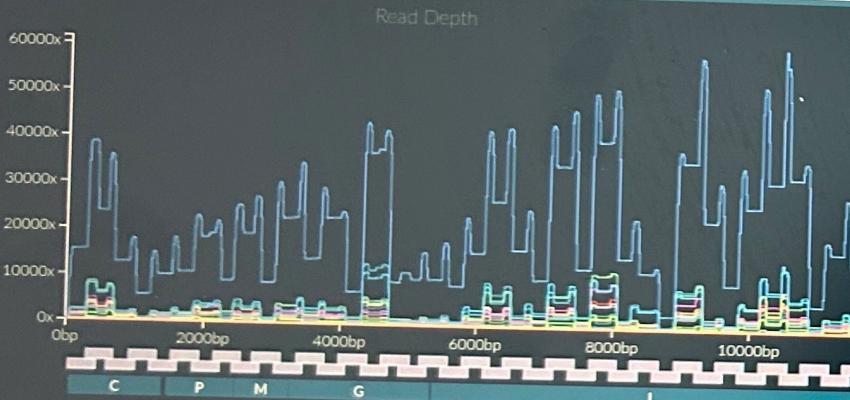
Experiment: Started @ 2024-02-15T07:05:03.284

1256082 reads mapped | 1409945 processed | calculating rate... | Data last received 05m08s ago  
Server messages 07:07:23 new data (t=9164s, 1256082 mapped, 1409945 processed)

Server messages 07:07:23

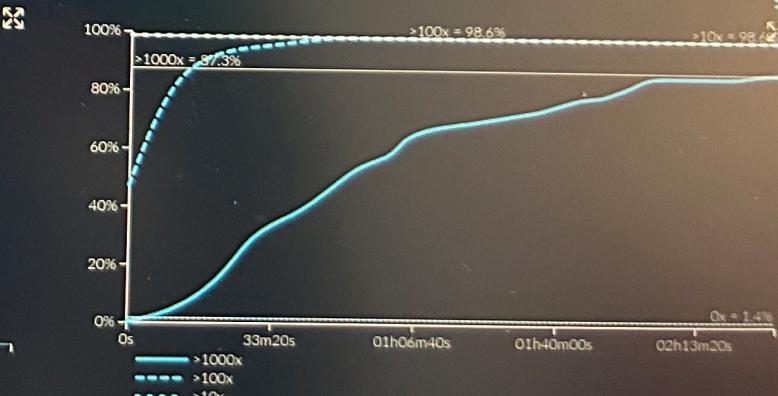
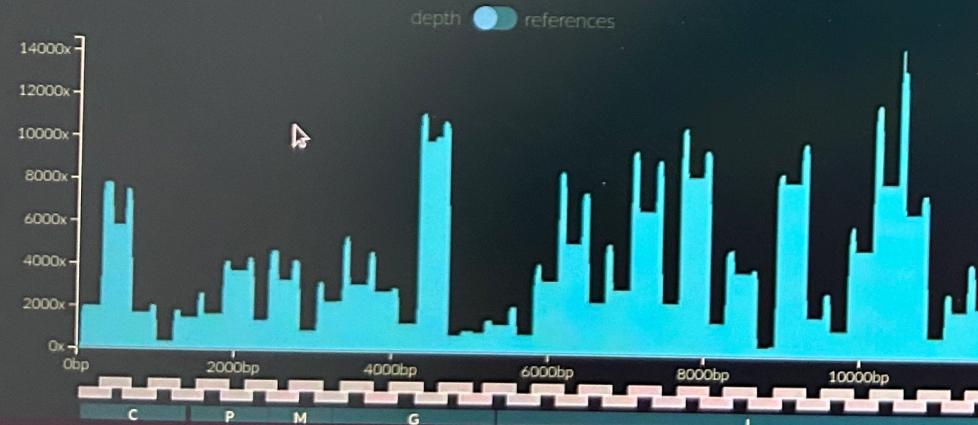
✓ Pipeline: Annotate re; online

### Pipeline: Export reads online



PLATEAU

- barcode01
  - barcode02
  - barcode03
  - barcode04



# What happened after the run?

Performed a flowcell wash

Contains DNase I, which is used to digest any remaining library on a flow cell

Should remove 99.9% of the library

- Some residual DNA may remain & may prefer to use different barcodes on consecutive run (e.g. barcodes 1-24 on 1<sup>st</sup> run, barcodes 25-50 on 2<sup>nd</sup> run)

Can only recover pores lost to the “recovering”/“unavailable” state

Our run: >1000 pores were left active,  
~300 in unavailable state

But I only had 628 pores after washing

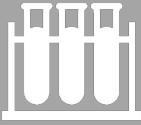
- Found some interesting things on the forum about this...

# Calculating Molarity

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To calculate the molarity of a solution, the number of moles of solute must be divided by the total liters of solution produced.



If the amount of solute is given in grams, we must first calculate the number of moles of solute using the solute's molar mass, then calculate the molarity using the number of moles and total volume.

# DNA/RNA molarity formula

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$$M = w / MW$$

Where:

M: DNA molarity, in mol

w: DNA weight, in g

MW: DNA molecular weight, in g/mol

*If only DNA length is given, the molecular weight is calculated as:*

$$MW = \text{DNA Length (bp)} \times \text{DNA/RNA base weight}$$

The approx. double strand DNA base weight is 660 Dalton, for single strand is 330, for RNA is 340.

Or use this website:

<https://nebiocalculator.neb.com>

# Our calculations

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Groups 1 + 2 = 1.16ng/ul

Groups 3 + 4 = 0.709ng/ul

We used 15ul of each to use 30ul total volume of DNA in adaptor ligation reaction

So...

$$1.16 \times 15 = 17.4 \text{ng}$$

$$0.709 \times 15 = 10.635 \text{ng}$$


$$= 28 \text{ng}$$

## Concentration

# Our molarity

Concentration = 28ng

- $M = w / MW$
- MW = DNA Length (bp)  $\times$  DNA/RNA base weight
- MW =  $400 \times 660 = 264000$

$$\bullet M = w / MW$$

1	=	1e-9	
Nanogram	▼	Gram	▼

- $M = (28 \times 1e-9) / 264000 = 1.06060606061 \times 1e-13 \text{ moles}$
- $\sim 106 \text{ fmol (femtomoles)}$

1	=	1E-15	
fmol [Femtomole]	▼	mol [Mole]	▼

## RAMPART

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*Read Assignment, Mapping, and Phylogenetic Analysis in Real Time.*

RAMPART runs concurrently with MinKNOW and shows you demuxing / mapping results in real time.

<https://artic.network/rampart>

# Estimating required coverage

$$C = LN / G$$

C stands for coverage

G is the haploid genome length

L is the read length

N is the number of reads

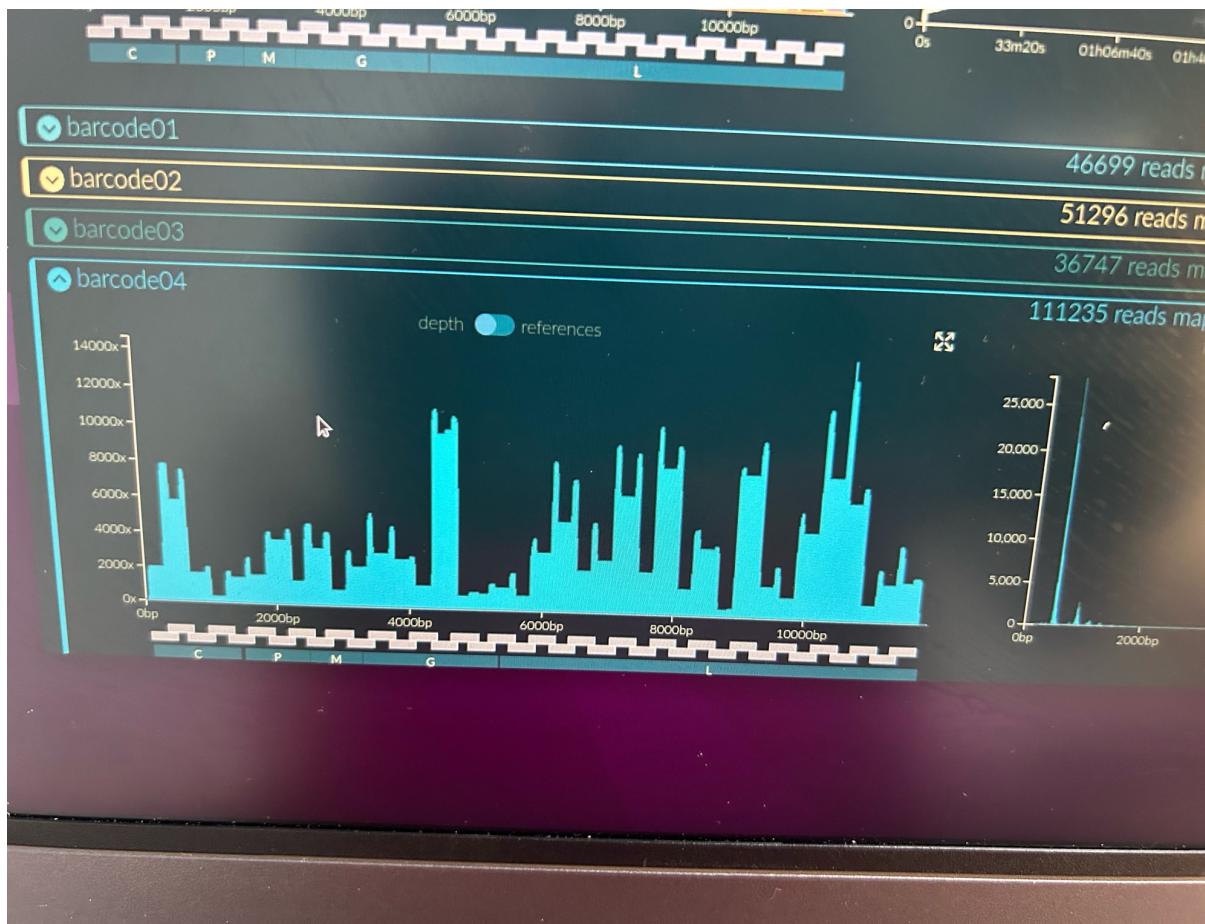
So if I want to get 100X coverage of rabies genome

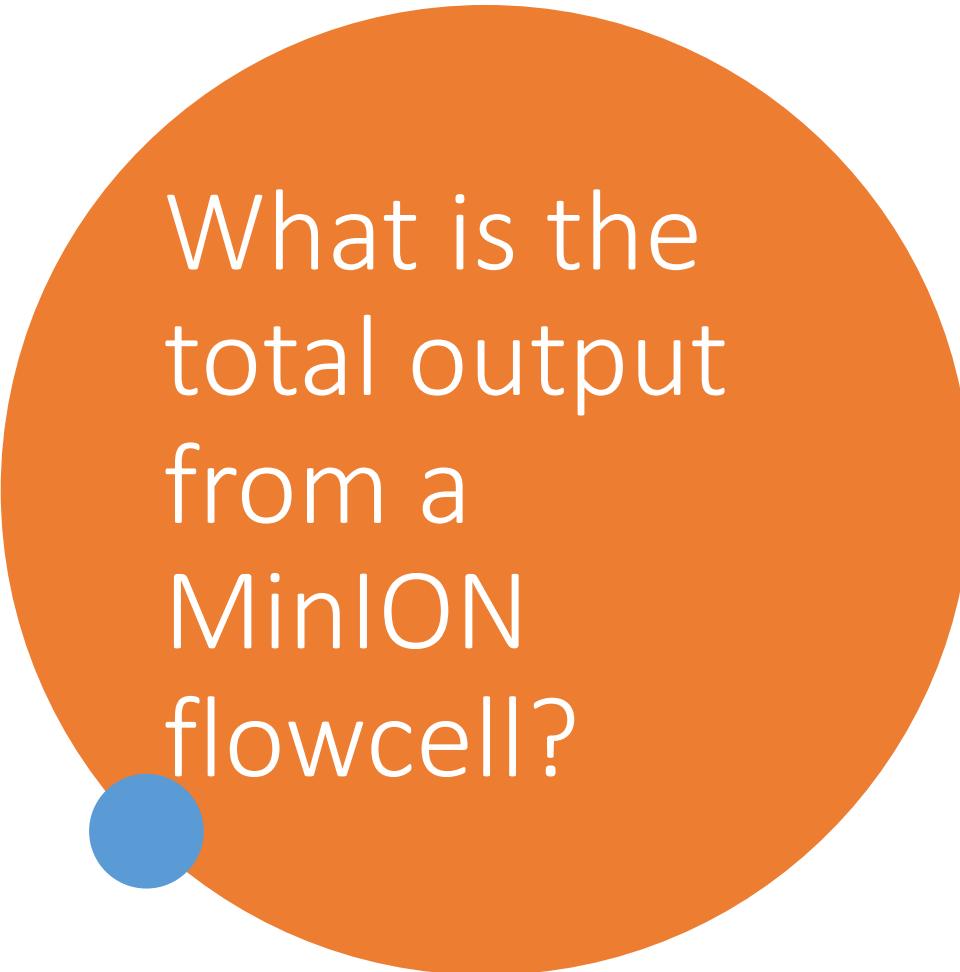
$$100 = 400 \times N / 12000$$

N=3000 reads

However....

# Variation between and within samples





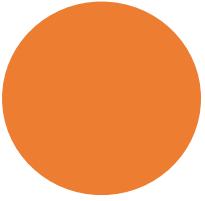
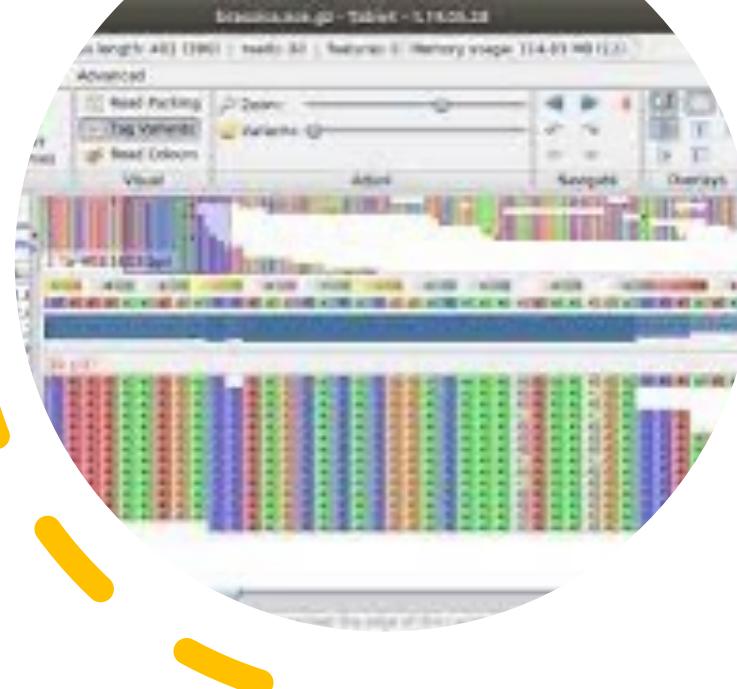
What is the  
total output  
from a  
MinION  
flowcell?

- 50 GB is a theoretical maximum yield
  - ***Highly unlikely to reach***
- Forums suggest about 5-15 GB per run



# Lab-on-an-ssd (*RAGE-on-an-ssd*)

- Think of it as a **computer on a usb harddrive**
- **Plug and play**
- An Ubuntu operating system (Linux)
- Pre-installed software for sequencing and associated bioinformatics



MinKNOW