

Sample-sequence-interpretation workflow



Whole Genome Sequencing for Rapid Characterization of Rabies Virus Using Nanopore Technology

Criselda Bautista^{1,2}, Gurdeep Jaswant^{1,3,4,5}, Hollie French^{1,6}, Kathryn Campbell¹, Rowan Durrant¹, Robert Gifford^{1,6}, Grace S. N. Kia^{7,8}, Brian Ogoti^{3,9}, Katie Hampson¹, Kirstyn Brunker^{1,6}



Author Spotlight: A Cost-Effective Genomic Workflow for Advancing Rabies Control in Resource-Limited Settings

02:47 min • 1,271 VIEWS



Sample Preparation, RNA Extraction, PCR Clean Up, and Quantification for Whole Genome Sequencing of Rabies Virus (RABV)

03:08 min • 58 VIEWS



Sequencing, Live and Offline Basecalling, Analysis, and Interpretation of Rabies Virus (RABV) Genome

05:00 min • 58 VIEWS

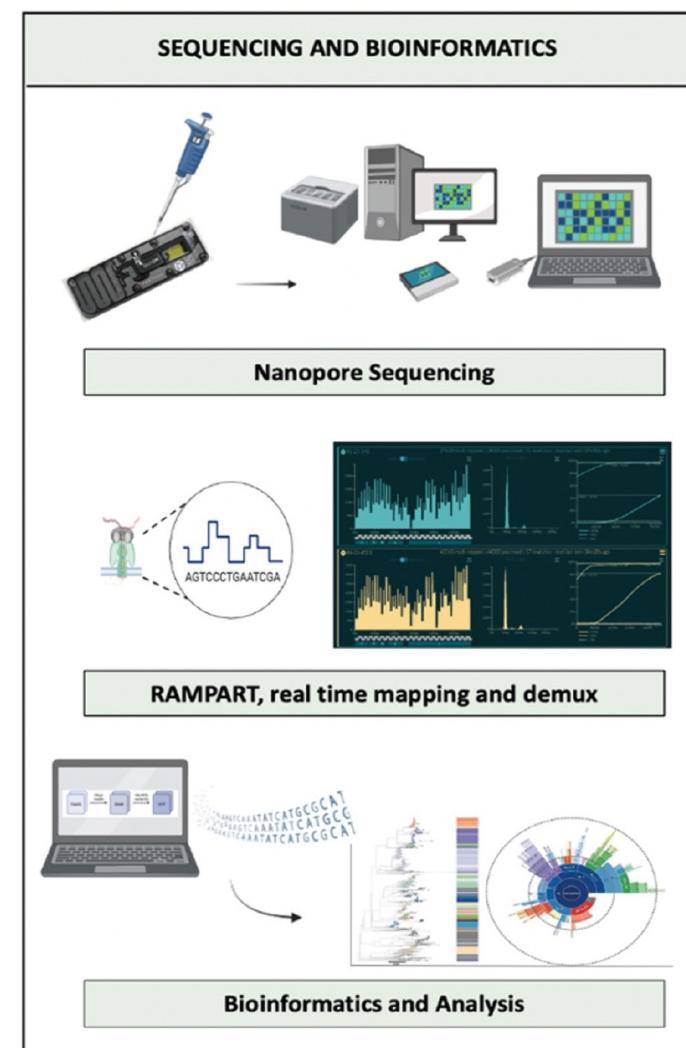
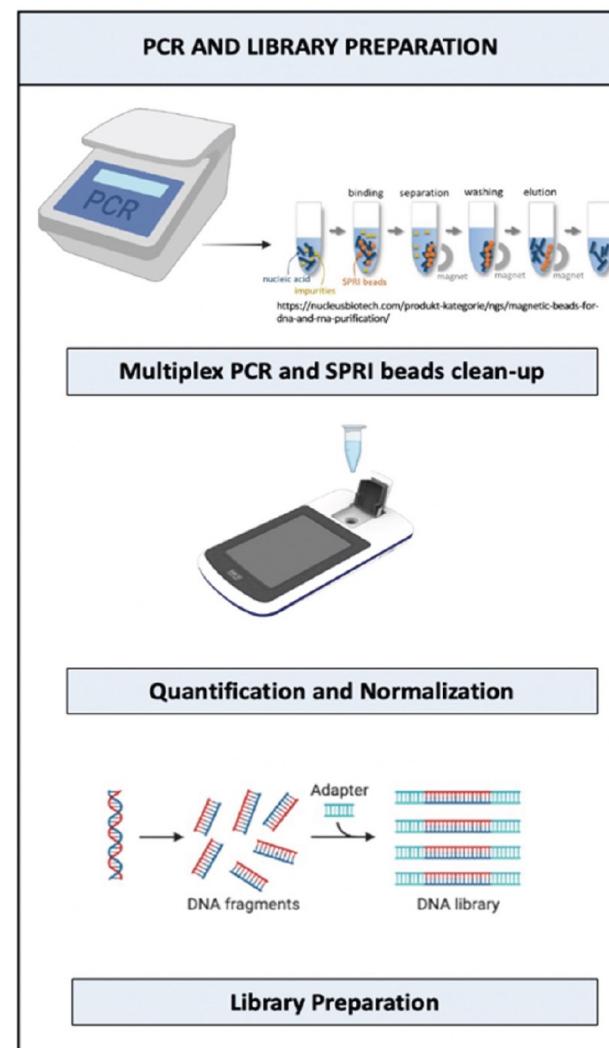
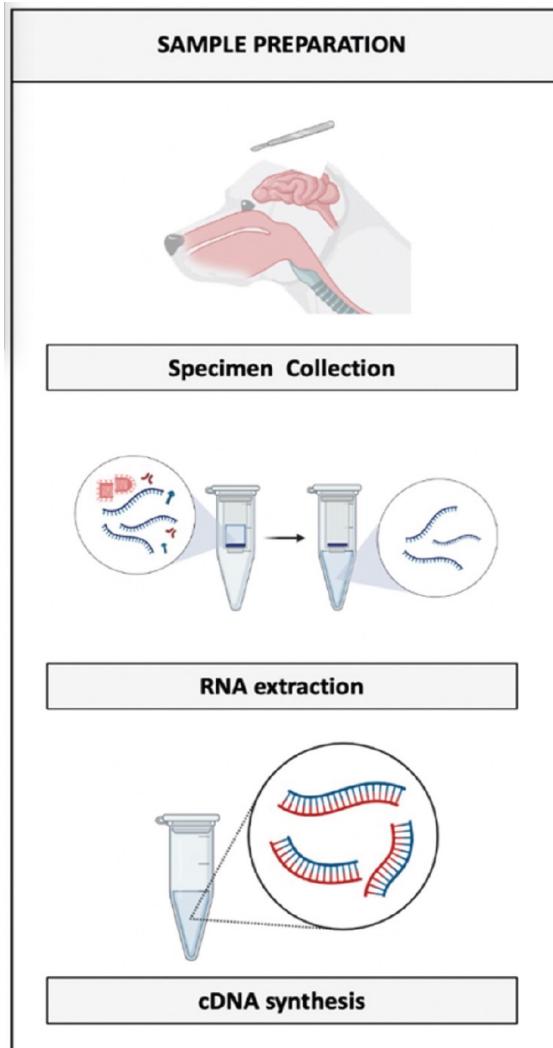


bitly

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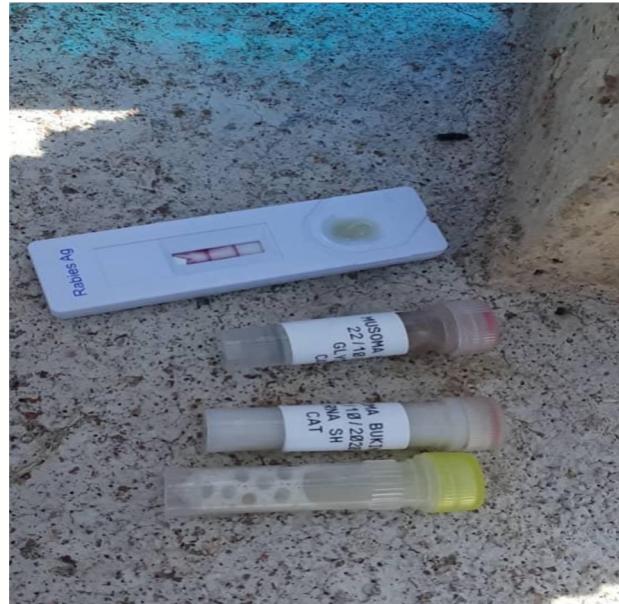


Sample-sequence-interpretation workflow



Sampling – Rabies samples

Sample collection (brain sample)+ rapid test is performed in the field



Biosafety Requirements

1. Pre-Exposure Prophylaxis
2. Proper PPE
3. Proper disposal of carcass

Sample preparation

Sample Quality Check

1. Use RNA stabilization/ preservation buffer
2. Ensure good quality samples
 - a. Properly labelled samples
 - b. Triple packaging system
 - c. Good transport conditions: at least 4°C



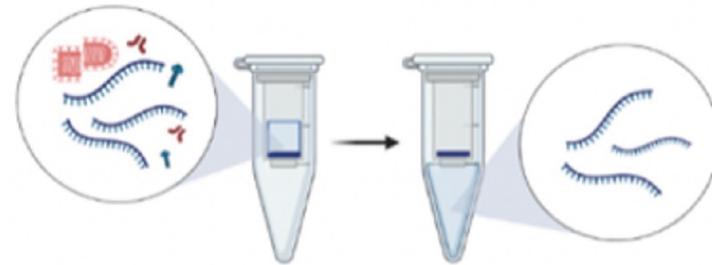
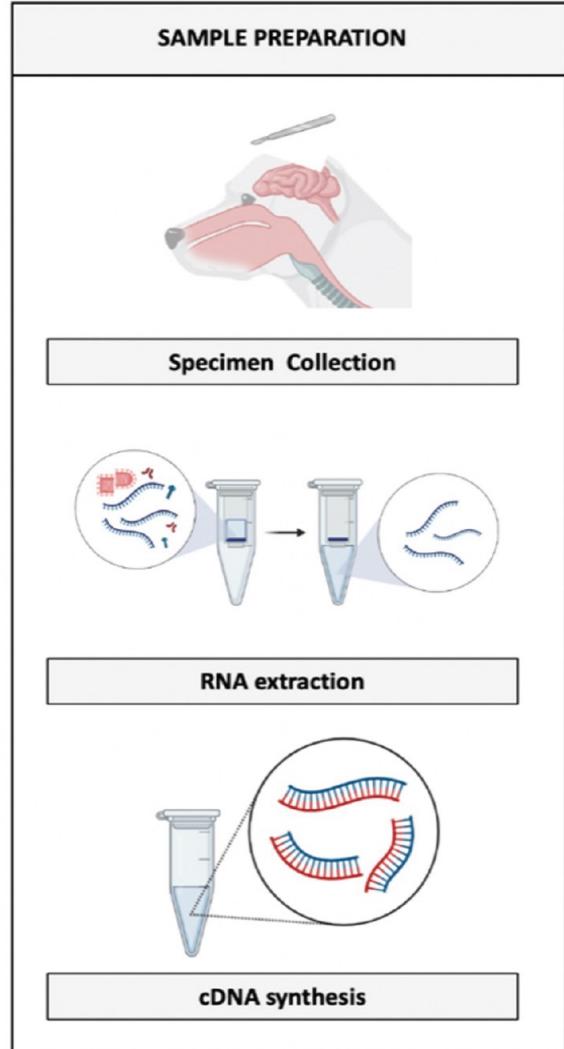
Sample storage

1. Ultra low Freezer -70 / -80
2. Sample in RNA shield - room temp for months

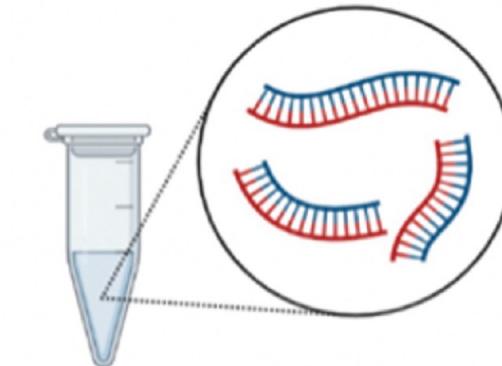




RNA extraction – cDNA synthesis



RNA extraction



cDNA synthesis



Multiplex PCR

primalscheme
primer panels for multiplex PCR

Design a new scheme

Reset defaults

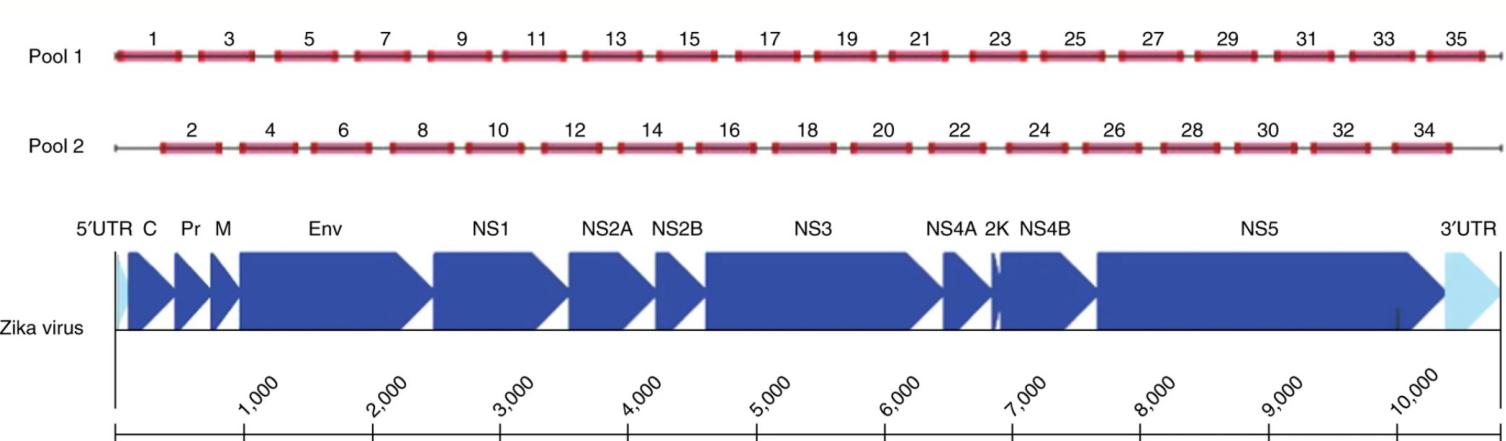
Upload FASTA file... Select a file...

Amplicon size: 400

Options: High-GC mode, Pinned

Scheme name: e.g., nCov-400

Use the standard protocol for these settings.



- References: From Genbank, East Africa/Peru/Philippines/Nigeria/Malawi rabies virus WGS
- Short fragments (~400bp)
- Initially developed for Zika sequencing (high Ct clinical samples)
- Generates tiling amplicons for near complete genome coverage

Multiplex PCR

- Q5® Hot Start High-Fidelity DNA Polymerase

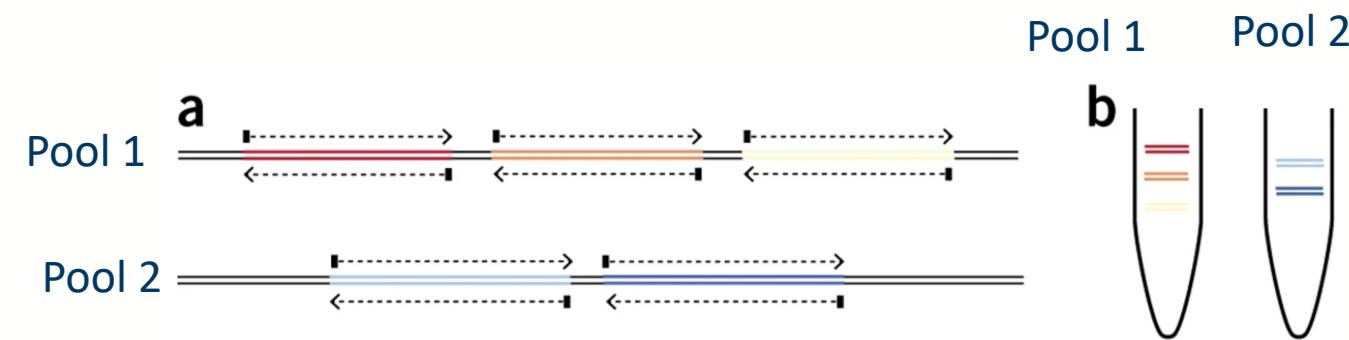
Must be used, other polymerase won't work

Has a higher Tm than other polymerases

- Mastermix contains all PCR components

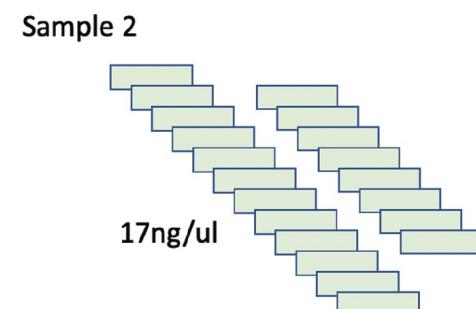
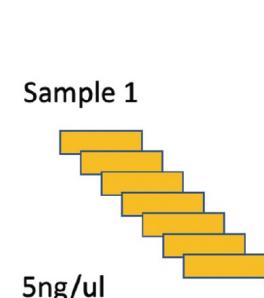
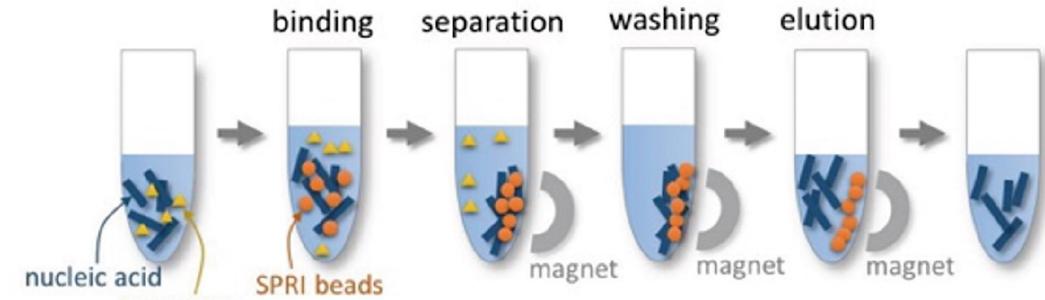
Just combine with cDNA and water

*Require 2 reactions per sample:
Pool A and Pool B*

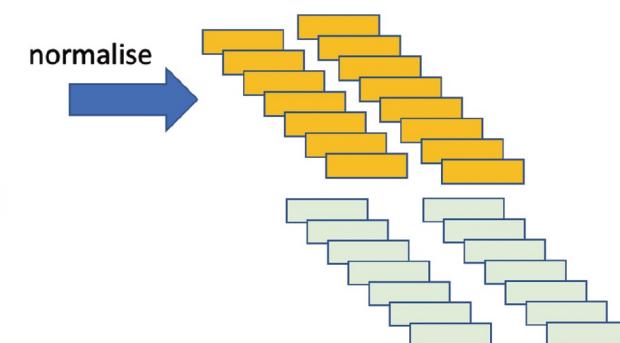


SPRI Beads Clean-up, Quantification & Normalisation

- *SPRI – Solid Phase Reversible Immobilis*
- *Quantification using fluorometer*
- *Variation between samples*
- *Normalisation to even the sequencing coverage across samples*



normalise



Library preparation

1. End repair/dA tailing

Add a 3'-dA tail to dsDNA

2. Barcoding and barcode ligation

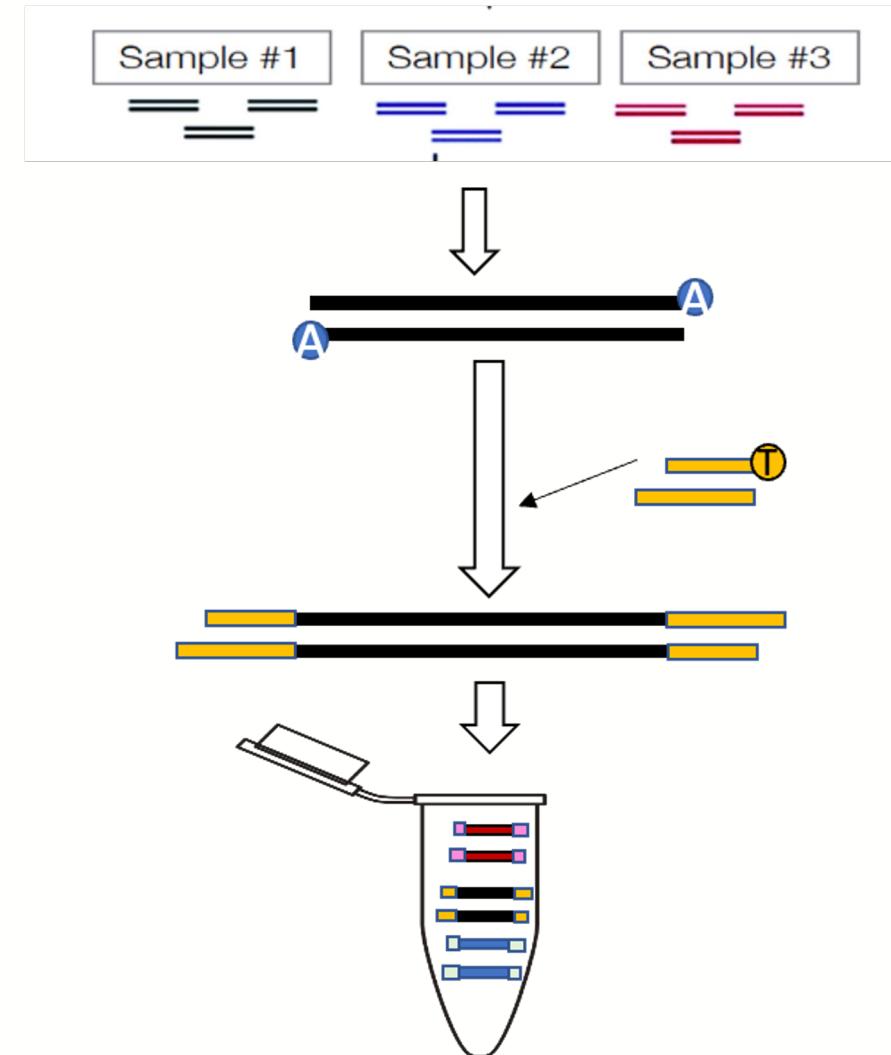
- In order to multiplex samples on one run, we need to BARCODE

3. Pool together barcoded DNA

4. Clean-up with SPRI beads

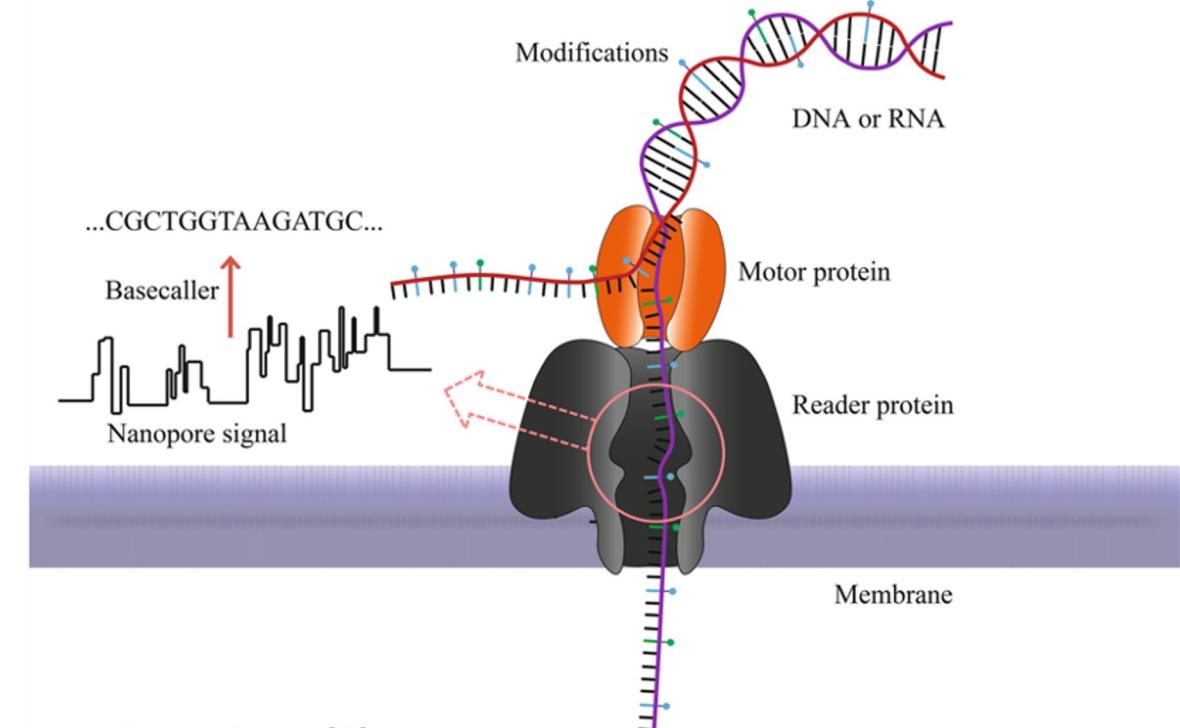
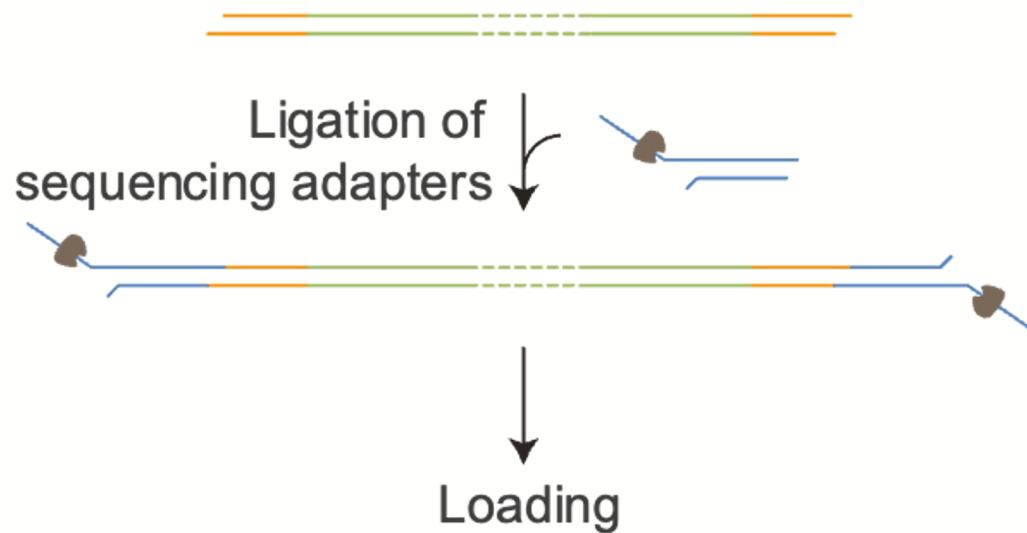
5. Quantify with Quantus Fluorometer

6. Adaptor Ligation



Adaptor Ligation

- Must attach a **nanopore-specific adaptor** to the DNA in order for it to sequence



- Final clean-up to get rid of loose adaptor contaminating library



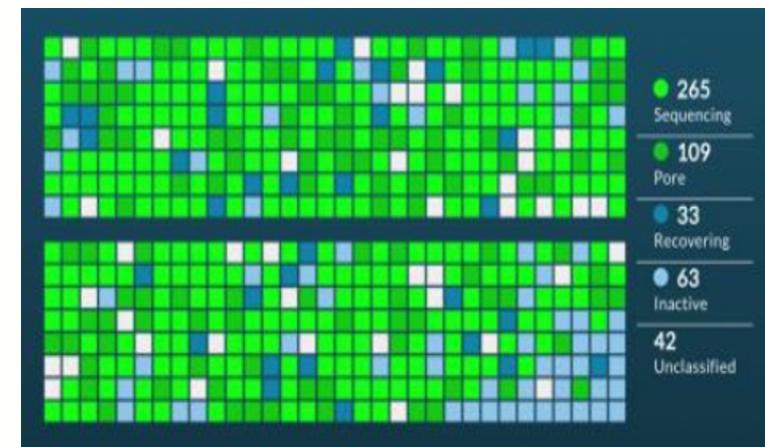
University
of Glasgow

Sequencing: MinNOW



Min**KNOW**

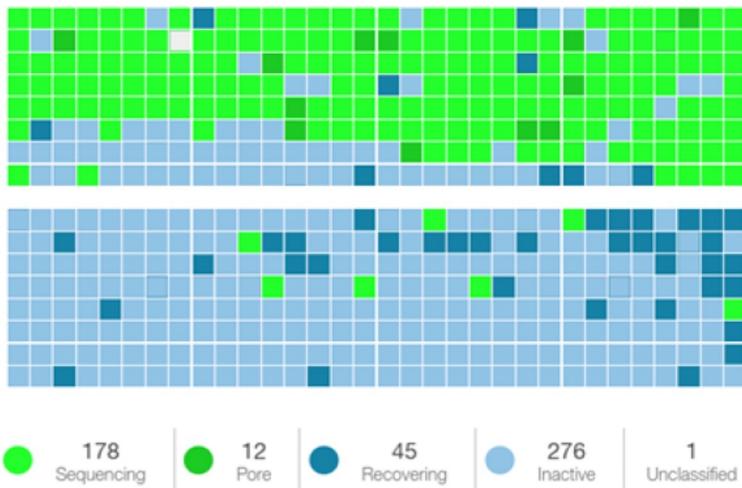
The screenshot shows the MinNOW software interface for sequencing setup. The top navigation bar includes tabs for 1. Positions, 2. Kit, 3. Run options, 4. Analysis, 5. Output, and 6. Review. The 2. Kit tab is active, displaying the selected kit (SQK-LSK109) and expansion packs (EXP-NBD104). Below this, the 3. Run options section shows settings for run length (72 hours), minimum read length (200 bp), and adaptive sampling (Off). A link to 'Advanced run options' is also present. At the bottom are buttons for 'Back to output', 'Save settings as template', and 'Start'.



Set-up & monitor
sequencing run in
real-time



Example



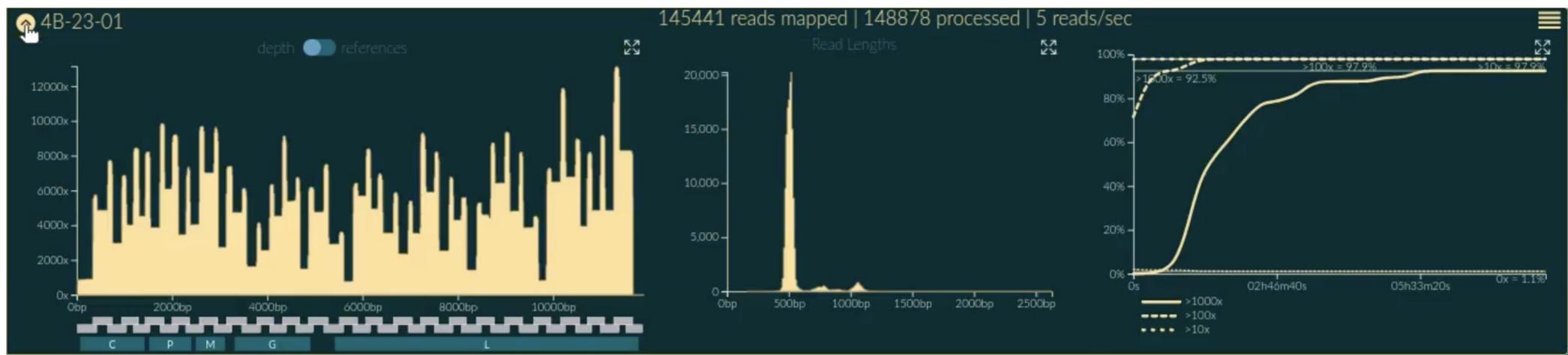
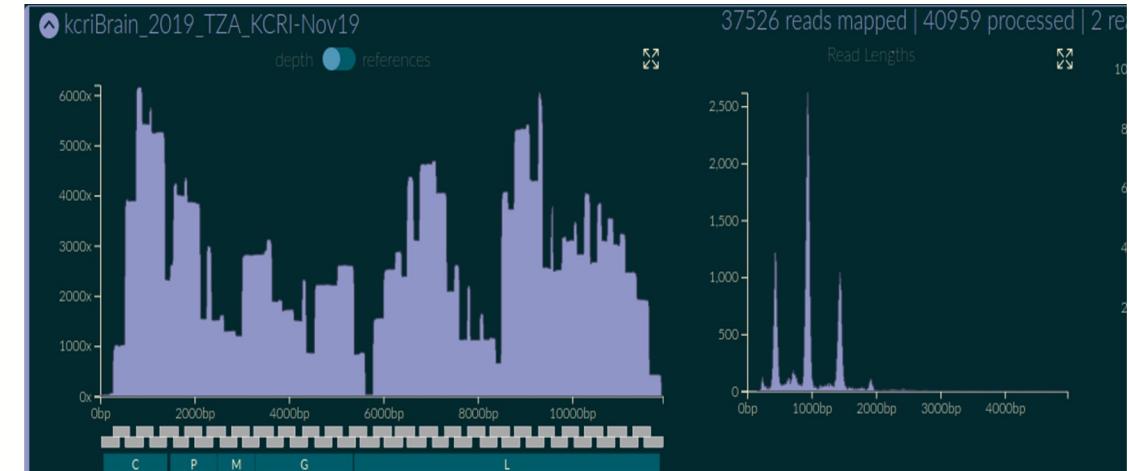
High number of inactive channels

Clear spatial pattern

Indicates an air bubble has been introduced onto the sensor array



Sequencing: RAMPART interpretation



Consensus generation

- **Artic-rabv pipeline**
- **Demultiplexing**
- **Read Filtering**
- **Medaka consensus pipeline**



Summary

Pause point

Pause point

	Process	Timing	Equipment	Reagents
Day 1	CDNA SYNTHESIS	1hr	Thermocycler <i>Optional:</i> centrifuge	LunaScript RT SuperMix Kit
	MULTIPLEX PCR	4-5hr	Thermocycler <i>Optional:</i> centrifuge	SARS-CoV-2 ARTIC primers Q5® Hot Start HF Polymerase 2x mastermix
	(<i>Optional:</i> GEL, PCR CLEAN UP, QUANTIFICATION & NORMALISATION)	(4hrs)		
	POOL AND DILUTE AMPLICONS + QUANTIFY	1hr	Quantus Fluorometer	Quantus DNA reagents
Day 2	ONE POT: END-REPAIR/DA-TAILING AND BARCODE LIGATION	1.5hr	Thermocycler (or heating block), magnetic rack	NEBNext® UltraII™ End-prep kit Blunt/TA mastermix SPRI beads Nanopore Native Barcoding Expansion Kit (1-24)
	ADAPTER LIGATION	1hr	Thermocycler (or heating block), magnetic rack	NEBNext Quick Ligation Module SPRI beads Nanopore library reagents
	PRIME FLOWCELL & SEQUENCING	20min (prime) Sequence until...(max 48h)	MinION Mk1c <i>Optional:</i> laptop	Nanopore library reagents Flowcell (R9.4.1)



Summary

JoVE Journal > Biology

Summary Abstract Introduction Protocol Results Discussion Disclosures Acknowledgements Materials References

Automatic Translation

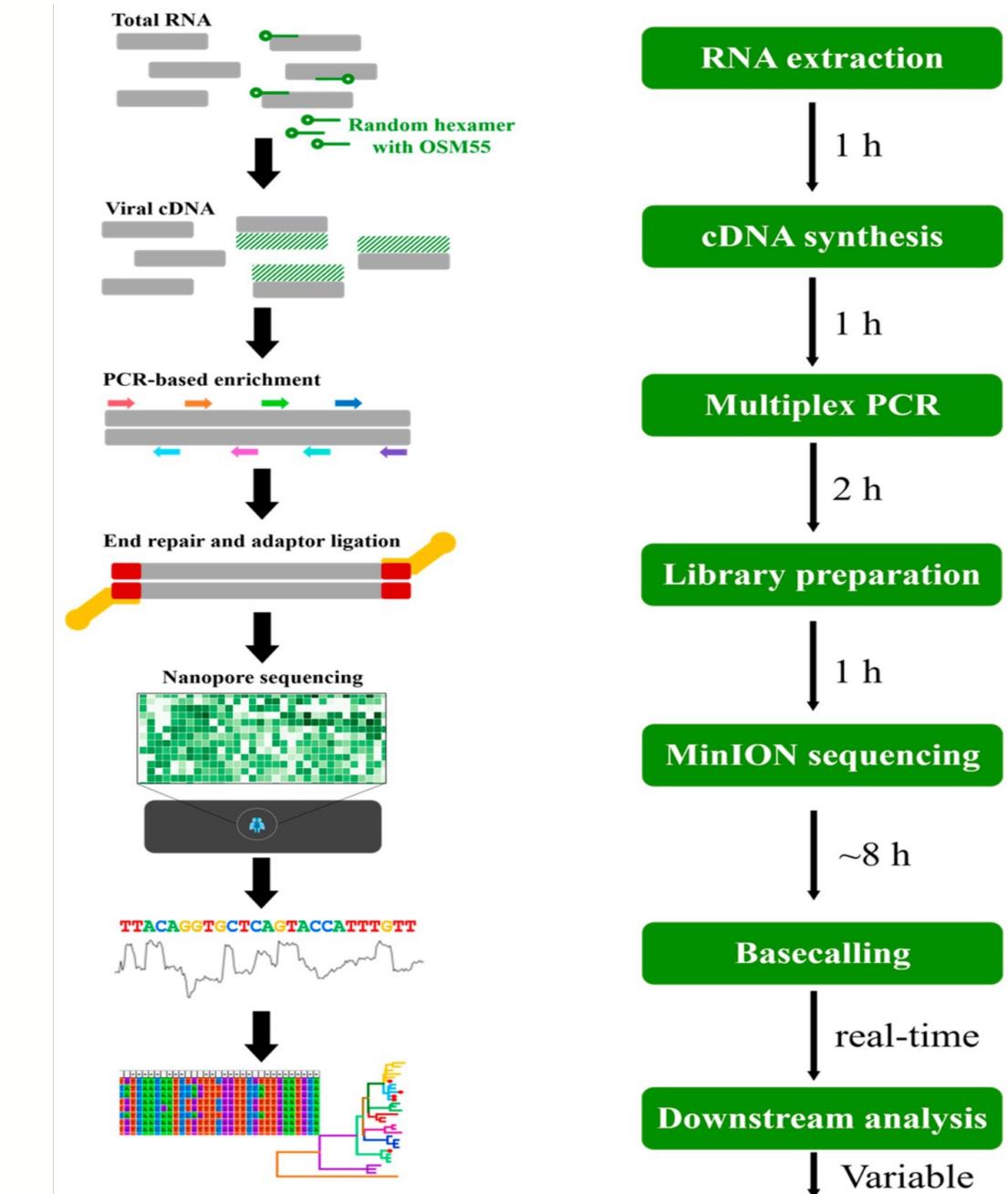
Biology

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Bautista et. al., 2023 [JoVE]



Questions?



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