

# Sample-sequence-interpretation pipeline



A wide-angle photograph of the University of Glasgow's main building complex. The buildings are made of light-colored stone and feature multiple gabled roofs and tall, ornate towers. In the foreground, there are several large, leafy trees with autumn-colored leaves (yellow, orange, and red). The background shows a hilly landscape under a blue sky with scattered white clouds.

**WORLD  
CHANGING  
GLASGOW**

**A WORLD  
TOP 100  
UNIVERSITY**

Biology

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

Published: August 18, 2023 doi: [10.3791/65414](https://doi.org/10.3791/65414)

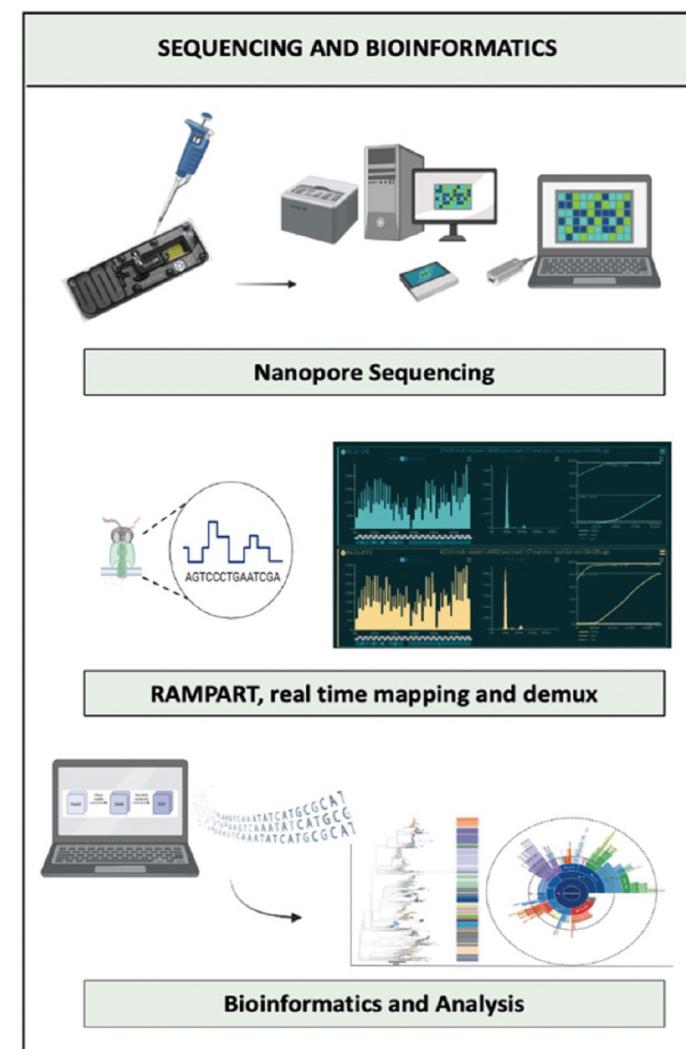
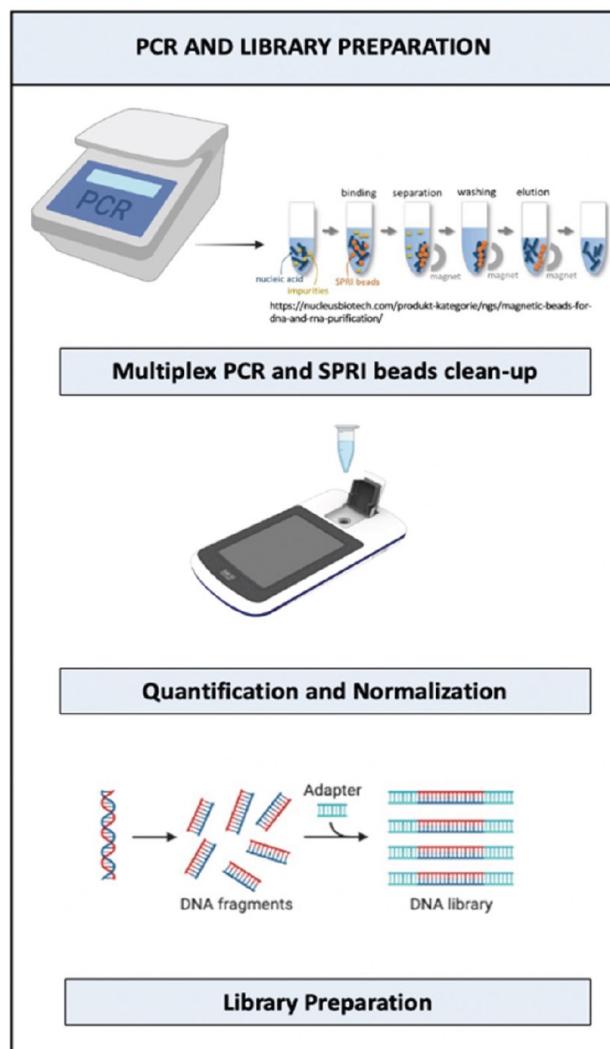
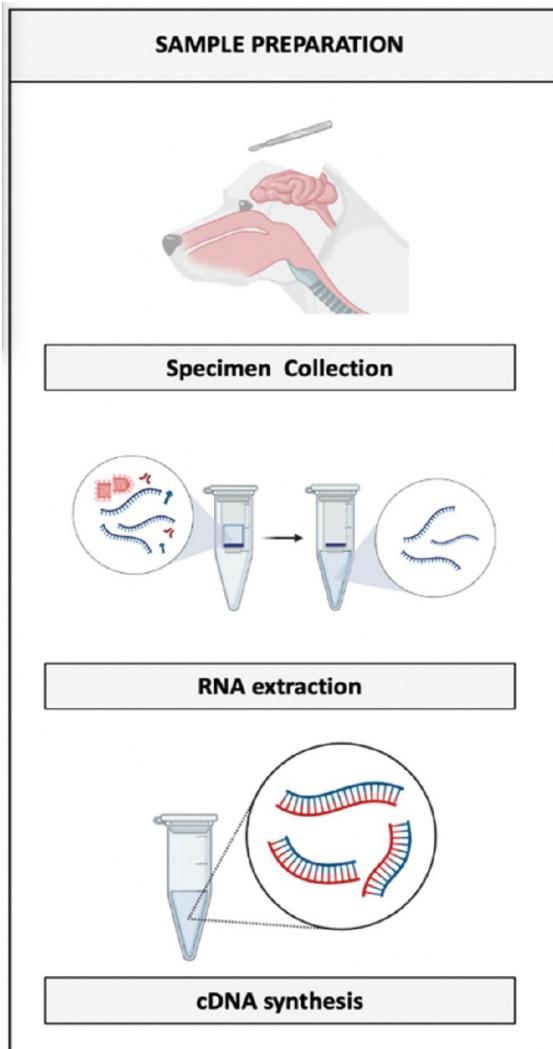
Criselda Bautista<sup>1,2</sup>, Gurdeep Jaswant<sup>1,3,4,5</sup>, Hollie French<sup>1,6</sup>, Kathryn Campbell<sup>1</sup>, Rowan Durrant<sup>1</sup>, Robert Gifford<sup>1,6</sup>, Grace S. N. Kia<sup>7,8</sup>, Brian Ogoti<sup>3,9</sup>, Katie Hampson<sup>1</sup>, Kirstyn Brunker<sup>1,6</sup>



[bit.ly/3HNX2tT](https://bit.ly/3HNX2tT)



# 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



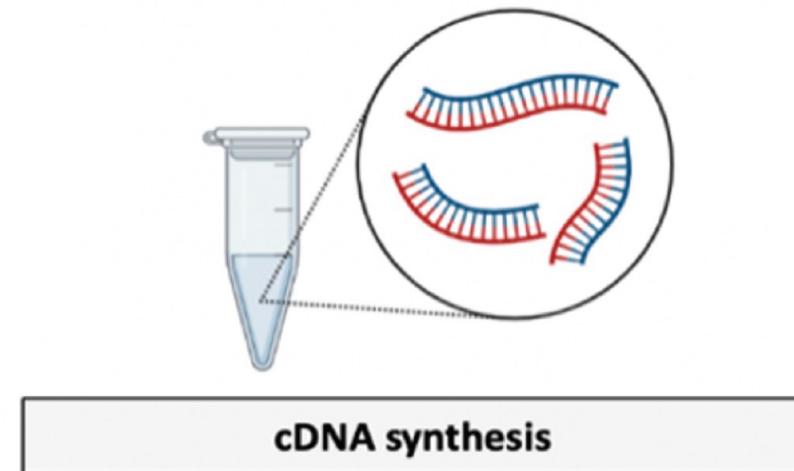
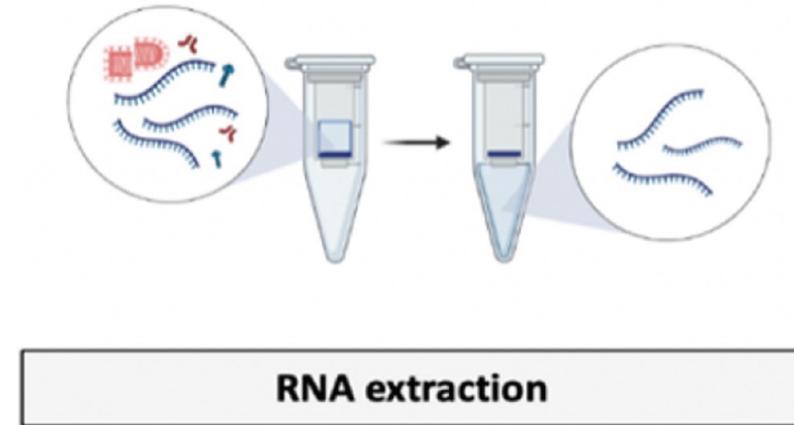
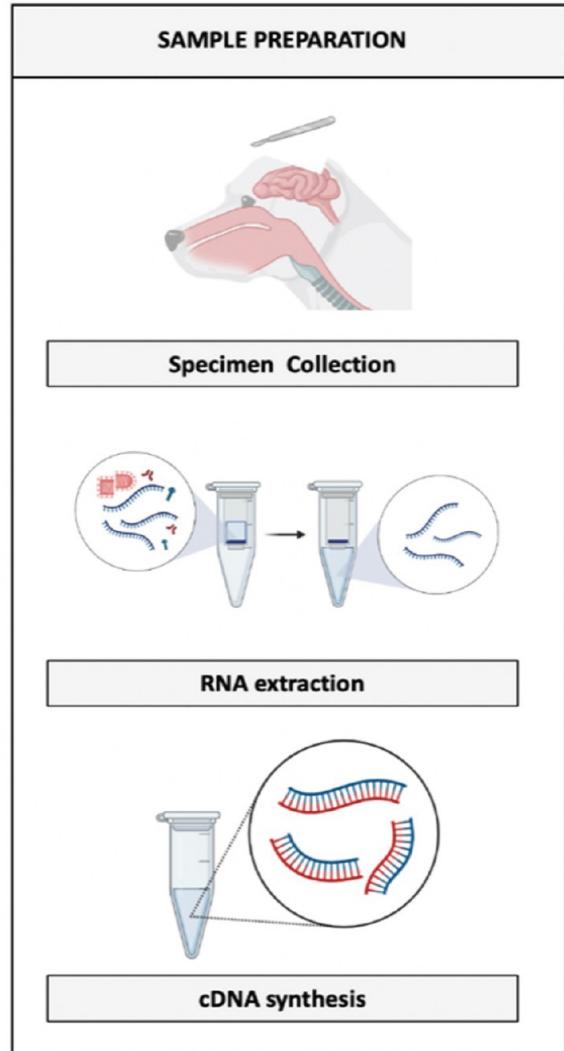
## Sample storage

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





# RNA extraction – cDNA synthesis





# Multiplex PCR

**primalscheme**  
primer panels for multiplex PCR

Design a new scheme

Reset defaults

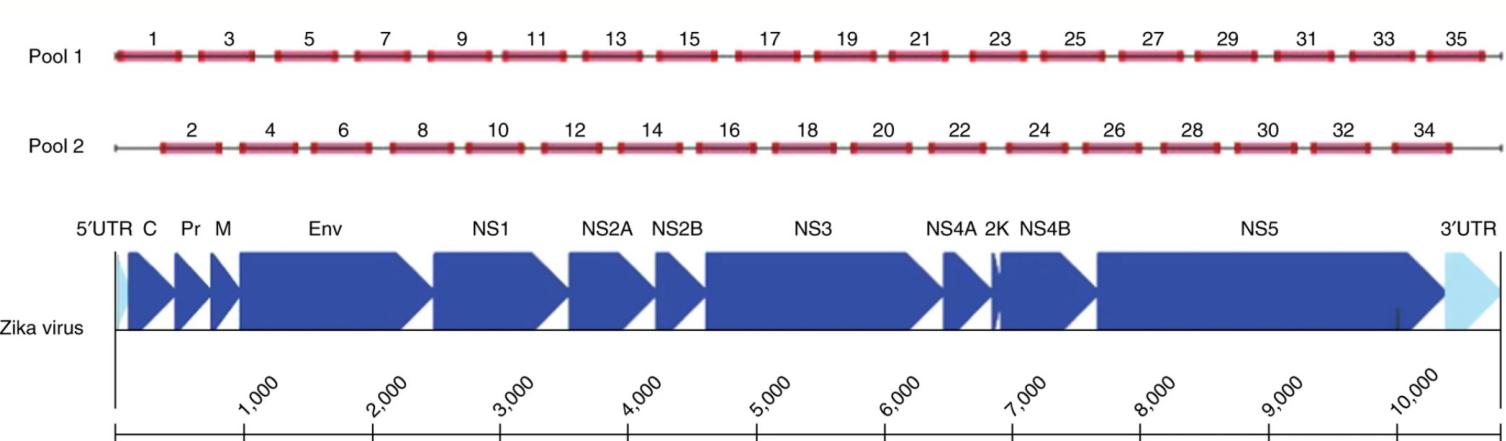
Upload FASTA file... Select a file...

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

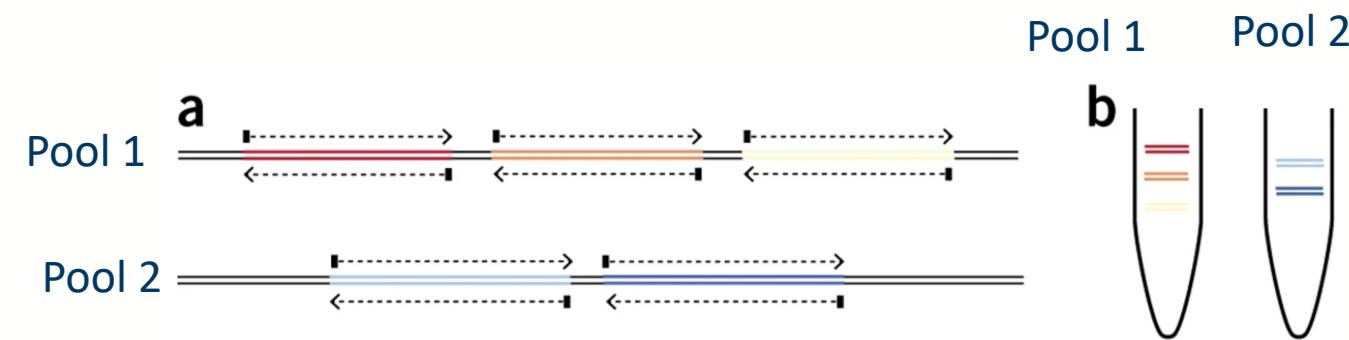
Must be used, other polymerase won't work

Has a higher Tm than other polymerases

- Mastermix contains all PCR components

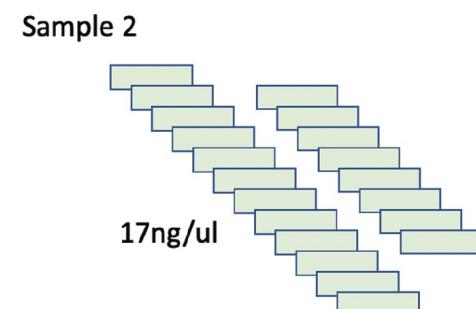
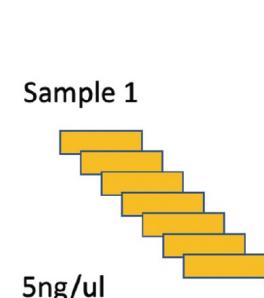
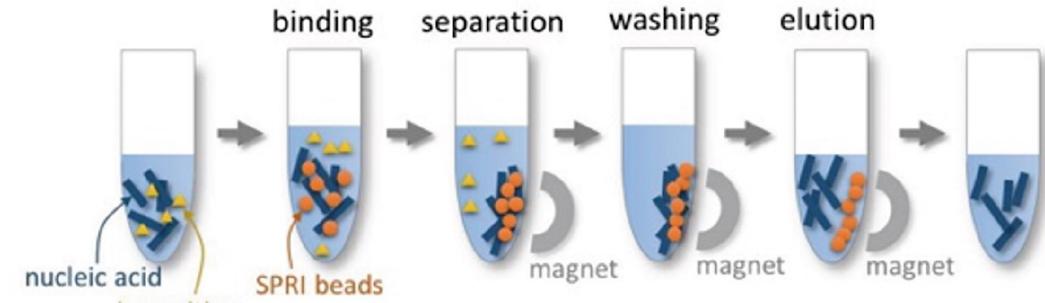
Just combine with cDNA and water

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

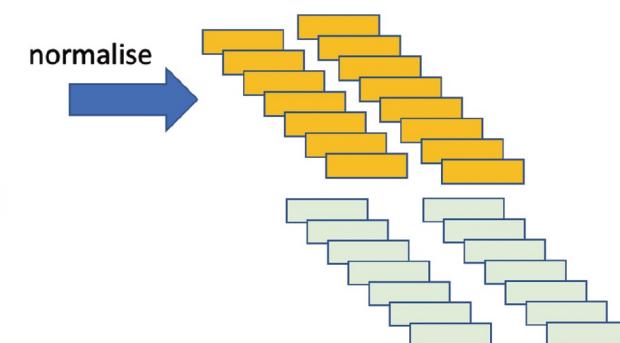


# SPRI Beads Clean-up, Quantification & Normalisation

- *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

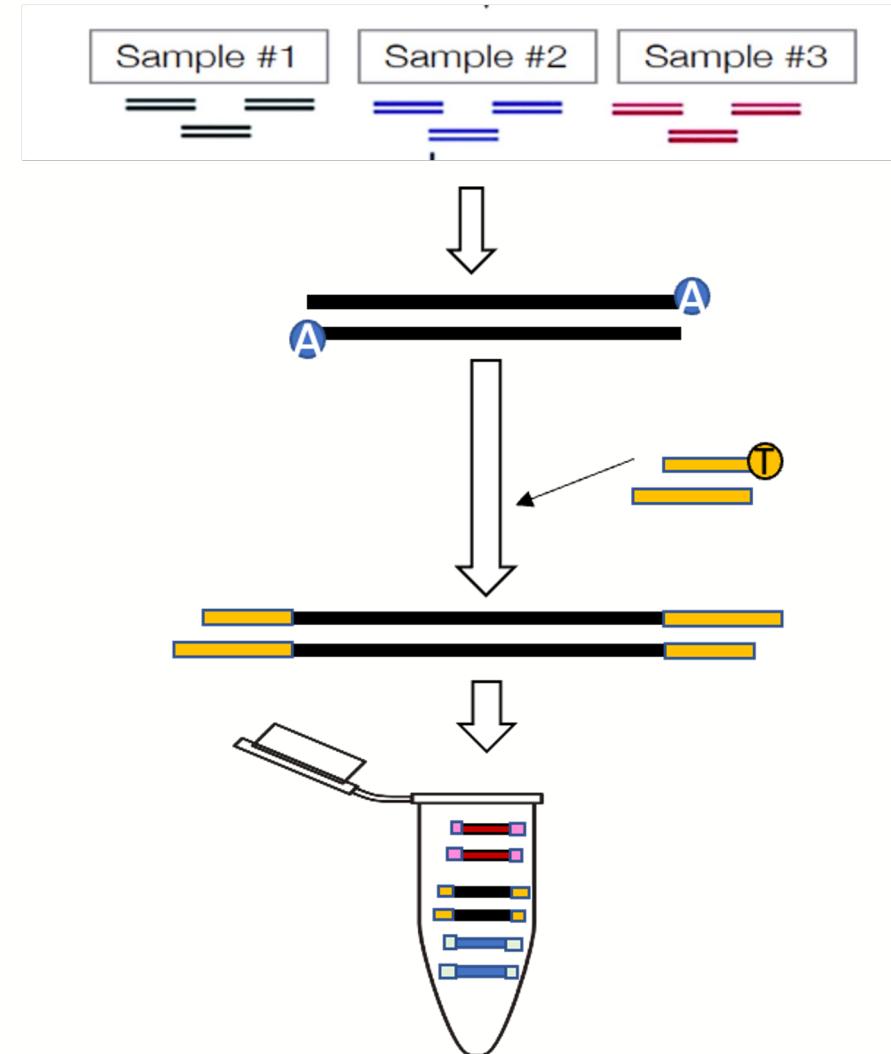
- In order to multiplex samples on one run, we need to BARCODE

## 3. Pool together barcoded DNA

## 4. Clean-up with SPRI beads

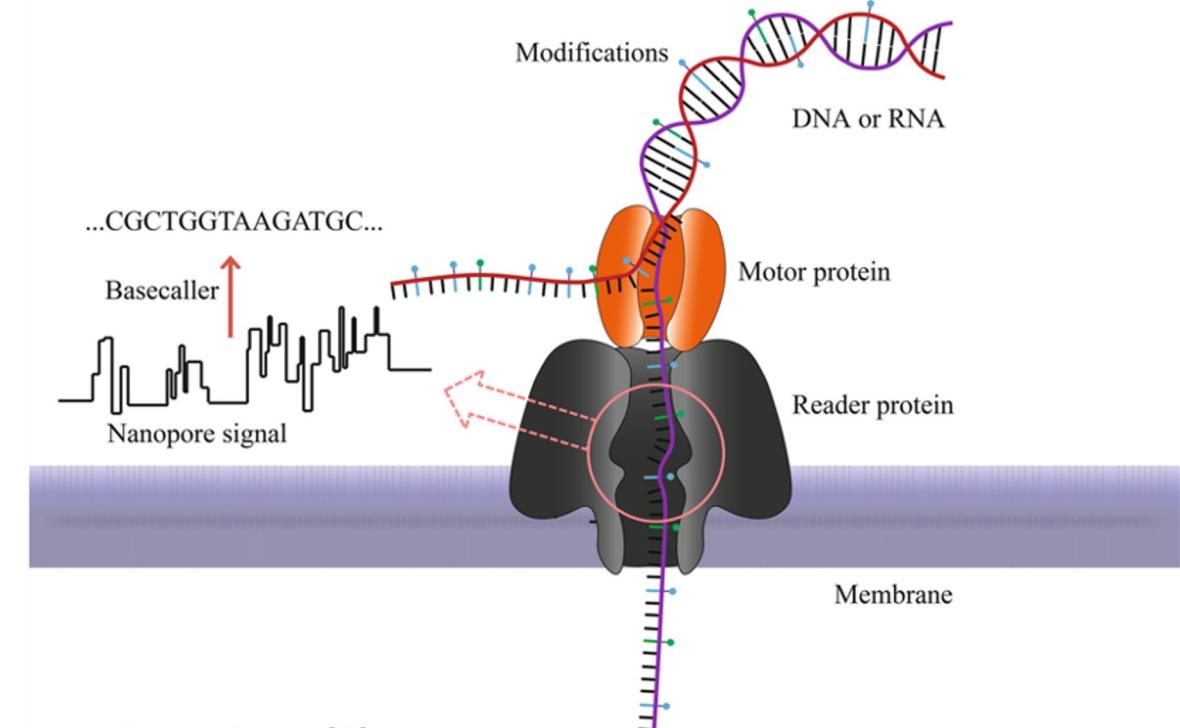
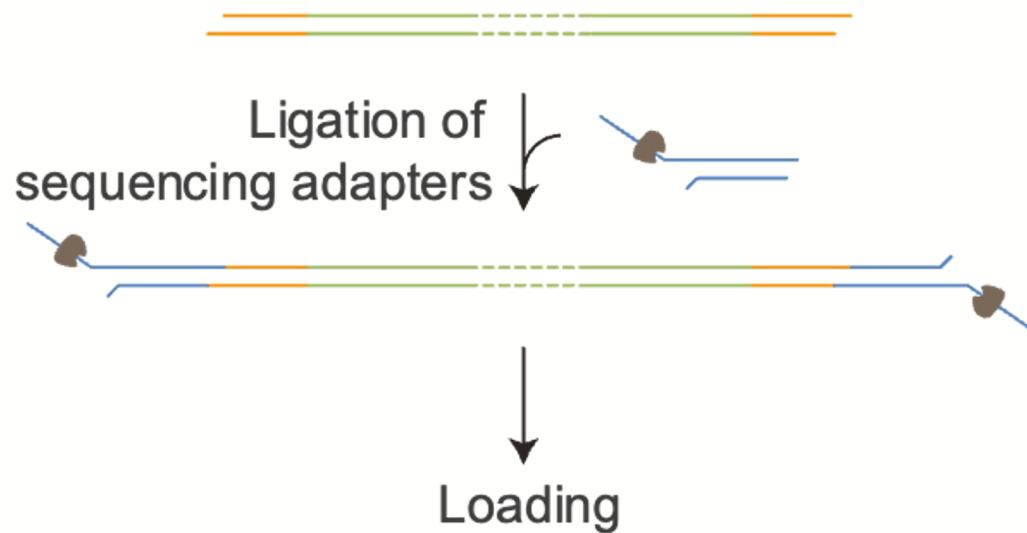
## 5. Quantify with Quantus Fluorometer

## 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



# Sequencing: Minknow and RAMPART interpretation - to be shown during the actual run

1. Positions    2. Kit    3. Run options    4. Analysis    5. Output    6. Review

## Output

Data saved as: Sequencing/

Output location: /data/

Output format: FAST5 (checked), FASTQ (checked), BAM (unchecked)

Filtering: Enabled (green switch)

Qscore: 8 | Readlength: Unfiltered | Read splitting: Enabled

Advanced options

**FAST5 options**

Compression: VBZ

Reads per file: 4000

Save

**Filtering options**

Pass / fail filtering: Setting a minimum and maximum read length will determine the cut-off of reads for the pass and fail folders.

Qscore: 8

Min readlength (kb): 8

Max readlength (kb): Max readlength

Save

**FASTQ options**

Compression: Gzip

Reads per file: 4000

Save

**Read splitting**

Enable read splitting: Gzip compression

Override read splitting min score

Save

1. Positions    2. Kit    3. Run options    4. Analysis    5. Output    6. Review

## Sequencing

Selected positions

Kit

Selected kit: SQK-LSK109

Expansion packs: EXP-NBD104

Run options

Run length: 72 hours

Minimum read length: 200 bp

Adaptive sampling: Off

Advanced run options

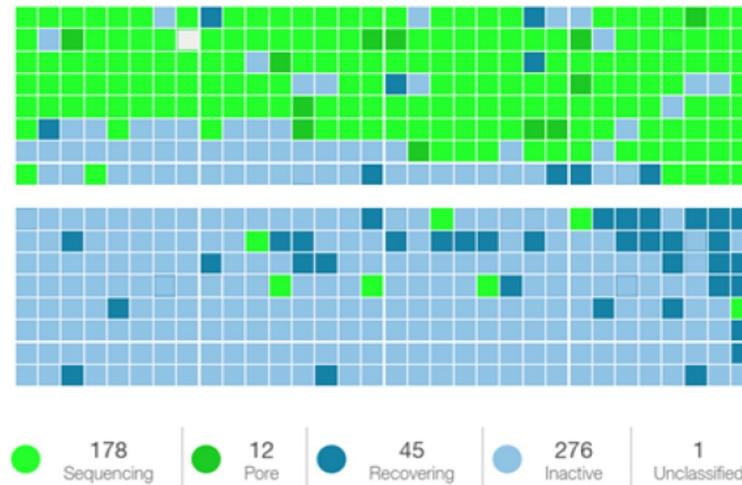
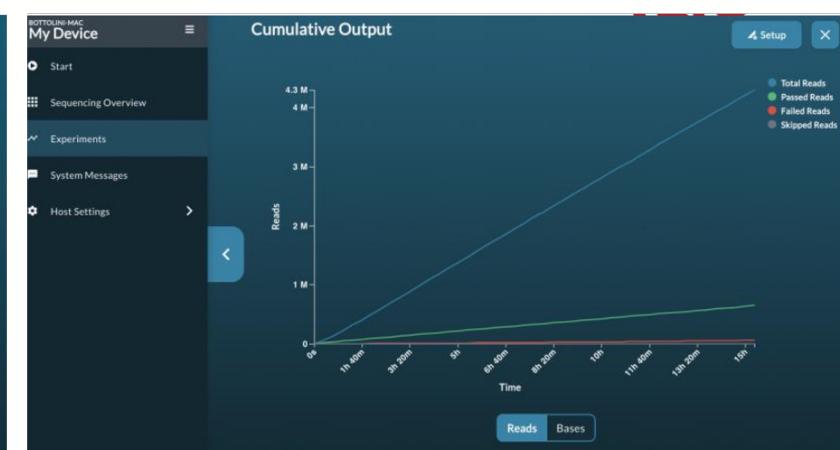
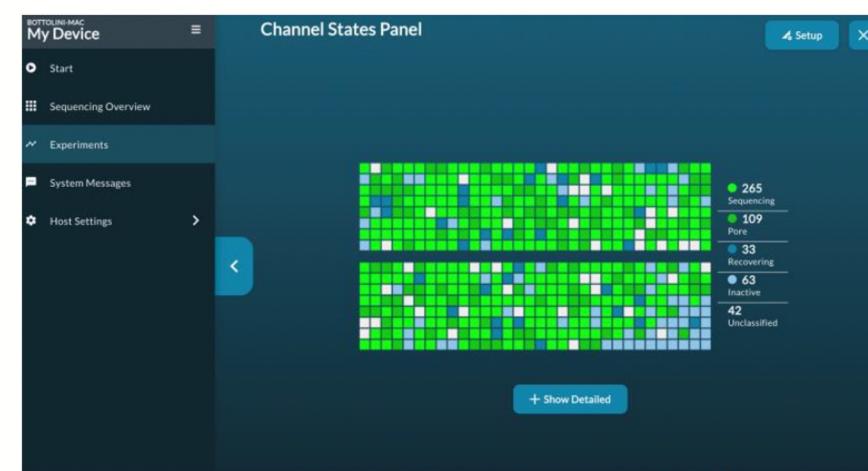
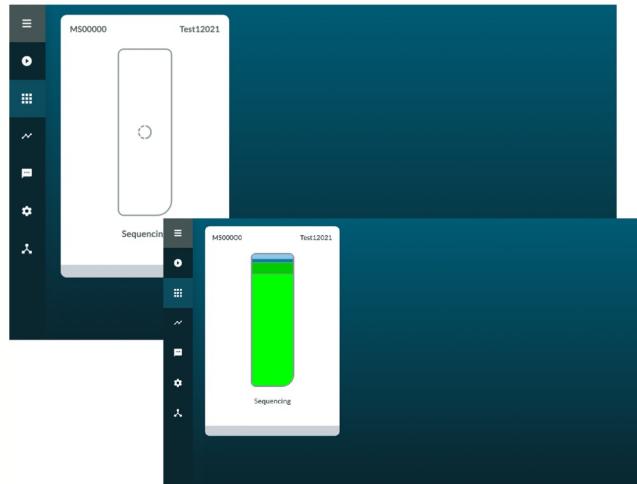
Back to output

Save settings as template

Start



# Sequencing: Minknow and RAMPART interpretation - to be shown during the actual run



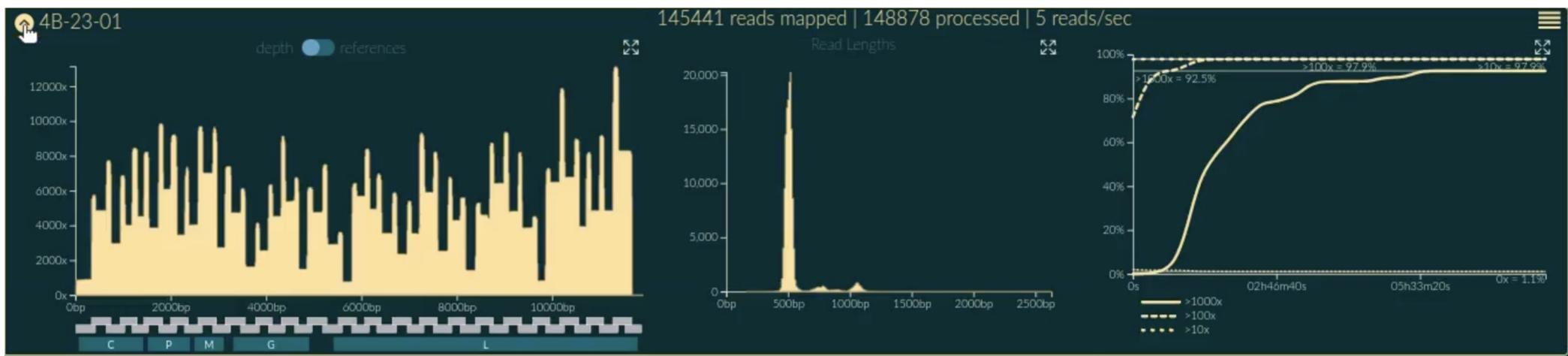
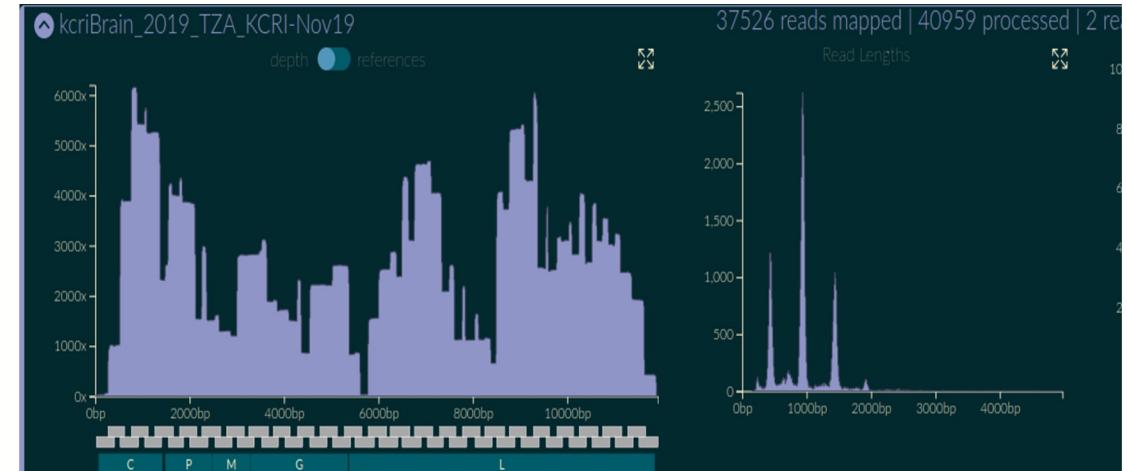
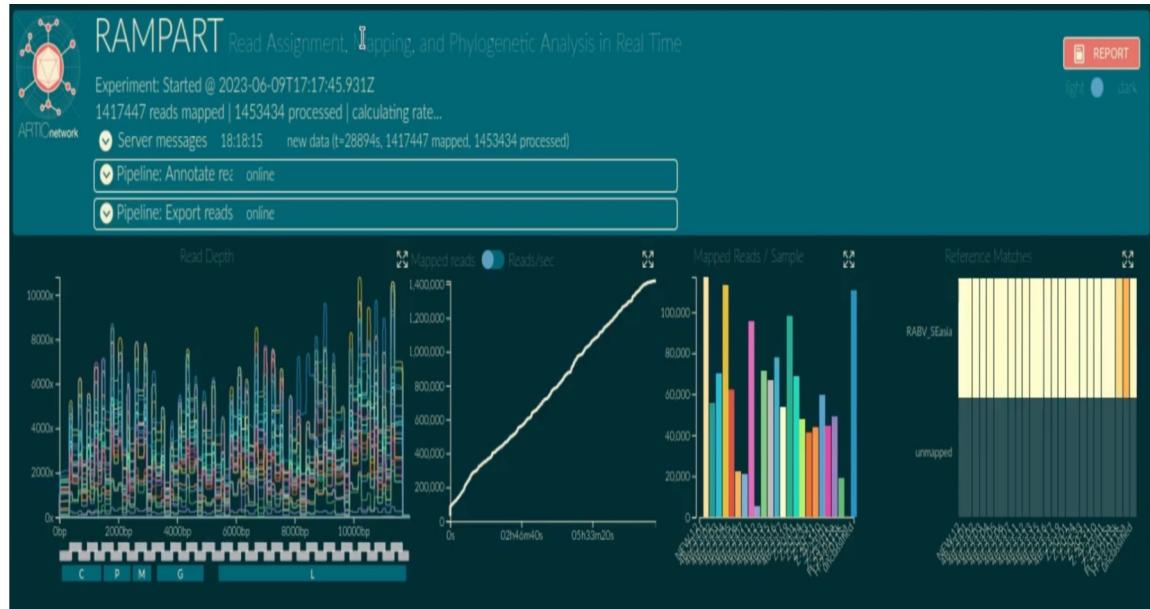
High number of inactive channels

Clear spatial pattern

Indicates an air bubble has been introduced onto the sensor array



# Sequencing: Minknow and RAMPART interpretation - to be shown during the actual run



# 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

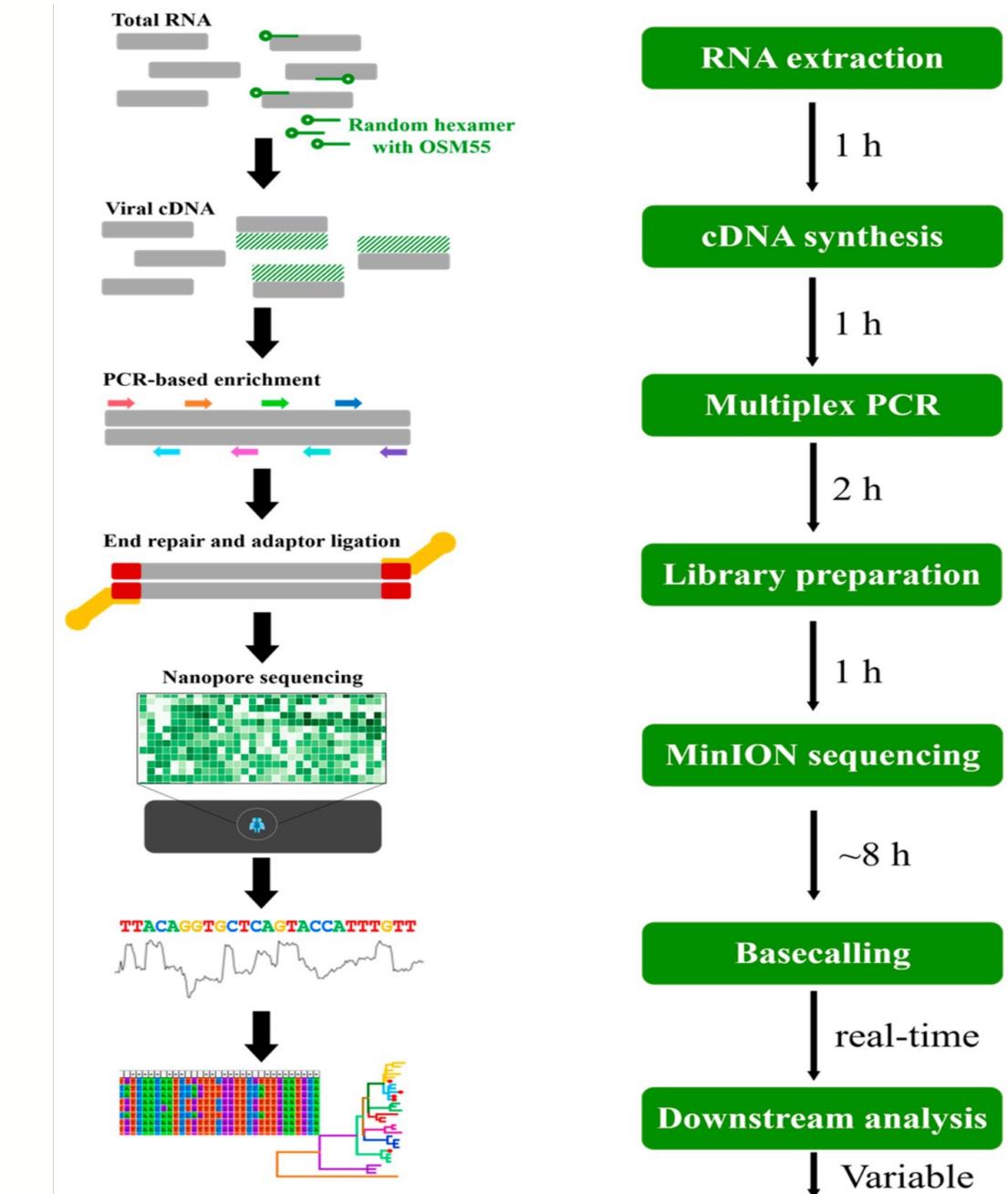
## Biology

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

Published: August 18, 2023 doi: [10.3791/65414](https://doi.org/10.3791/65414)

Criselda Bautista<sup>1,2</sup>, Gurdeep Jaswant<sup>1,3,4,5</sup>, Hollie French<sup>1,6</sup>, Kathryn Campbell<sup>1</sup>, Rowan Durrant<sup>1</sup>, Robert Gifford<sup>1,6</sup>, Grace S. N. Kia<sup>7,8</sup>, Brian Ogoti<sup>3,9</sup>, Katie Hampson<sup>1</sup>, Kirstyn Brunker<sup>1,6</sup>

Bautista et. al., 2023 [JoVE]



# Questions?



[bit.ly/3HNX2tT](https://bit.ly/3HNX2tT)

#UofGWorldChangers  
   @UofGlasgow