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# Introduction to Oxford Nanopore Technologies

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# Pre-Test 16S Sequencing using ONT

# Oxford Nanopore in a snapshot

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## Technology

- Continuum of devices to suit all throughput needs and work environments:
  - **MinION**: personal DNA sequencing in the lab or in the field
  - **GridION**: compact benchtop system to run and analyse up to 5 MinION/Flongle Flow Cells simultaneously
  - **PromethION**: for high-throughput and ultra-high-throughput applications
  - **Flongle:** the quickest, most accessible and cost-efficient sequencing system for smaller tests and experiments

## Large and rapidly growing customer base

- Sequencers in >120 countries
- Presence in key global institutes

## Global operations


- UK Offices: Oxford (HQ) & Cambridge
- US offices: NYC, Cambridge & San Francisco
- Asia Pacific offices: Shanghai, Beijing, Tokyo, Singapore
- Distributors in Europe, Asia, Africa and Americas

## Applications

- >8600 publications and growing fast
- Broad applications: population sequencing, cancer, infectious disease, plant/agriculture, environmental research, education
- Initial customers scientific researchers, but applied markets now coming online
- Technology being used extensively in the global push to monitor COVID-19 outbreak

## Committed to continual innovation and excellence

- Culture of continuous improvement
- >2500 patents & applications
- More than half generated in-house
- 32 Global Academic Partnerships
- Additional innovation through supplier relationships

# Our Goal

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## Our goal: to enable the analysis of anything, by anyone, anywhere

### Life science research: understanding the biology of any organism

Human genetics | Cancer | Plants | Animals | Microbial organisms

### As a foundation for emerging real-world impact

**Health**
Oncology
Human disease
Infectious disease
Immunology and transplant
Reproductive health

**Agriculture**
Livestock
Crops

**Industry**
Biomanufacturing
Food safety

**Environment**
Biodiversity
Biosecurity

**Biosecurity**
Pathogen surveillance

**Education**
Genomics literacy

**Consumer**

# Nanopore Technology in the Field

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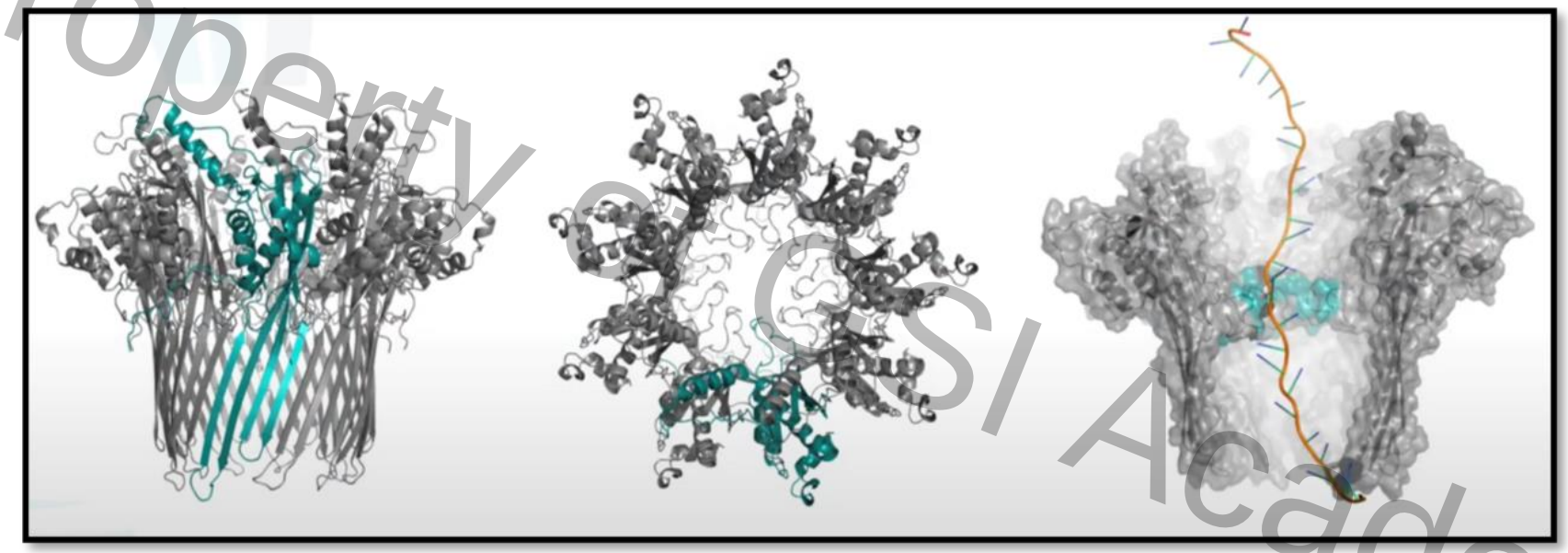
**MinION in the field:** on the International Space Station, by the Indian Ocean, in the Arctic, and under the sea. (NASA/NEEMO)

# Oxford Nanopore Technologies

# The Heart of Nanopore Technology

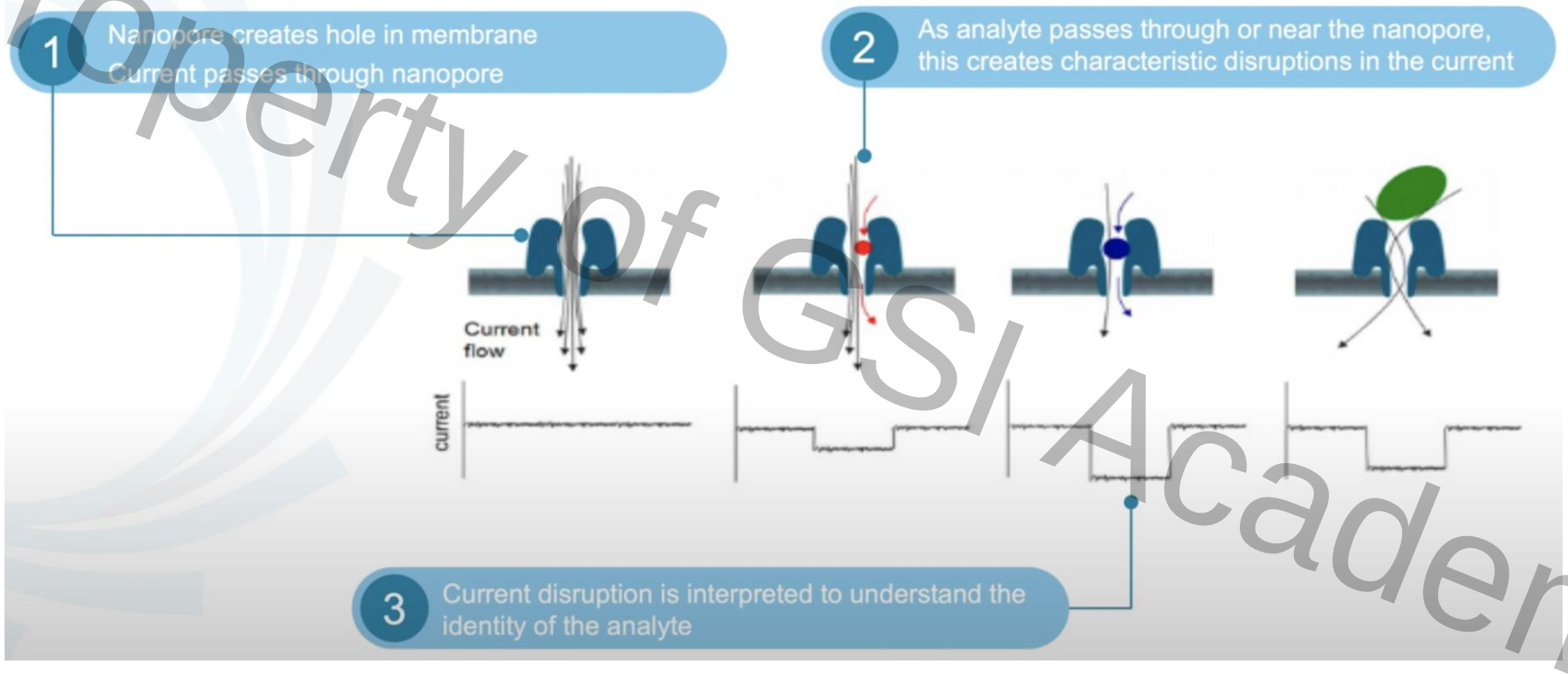




- In nature, protein nanopores embedded to cell membrane and acting as gateways between two systems
- Protein nanopores used in Nanopore Sequencing Technology have been carefully engineered through mutating key residues in the barrel of the pore to make it suitable for sequencing

# How Nanopore Sequencing Works



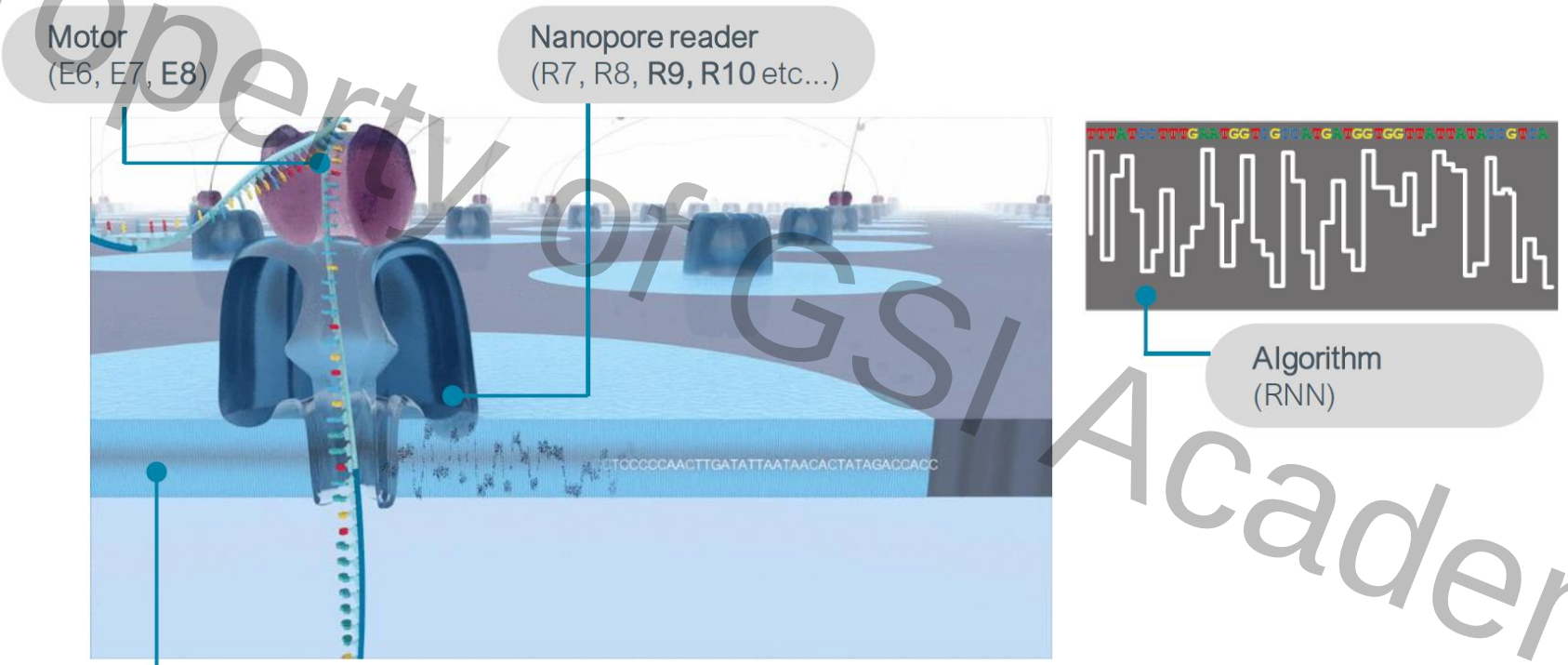


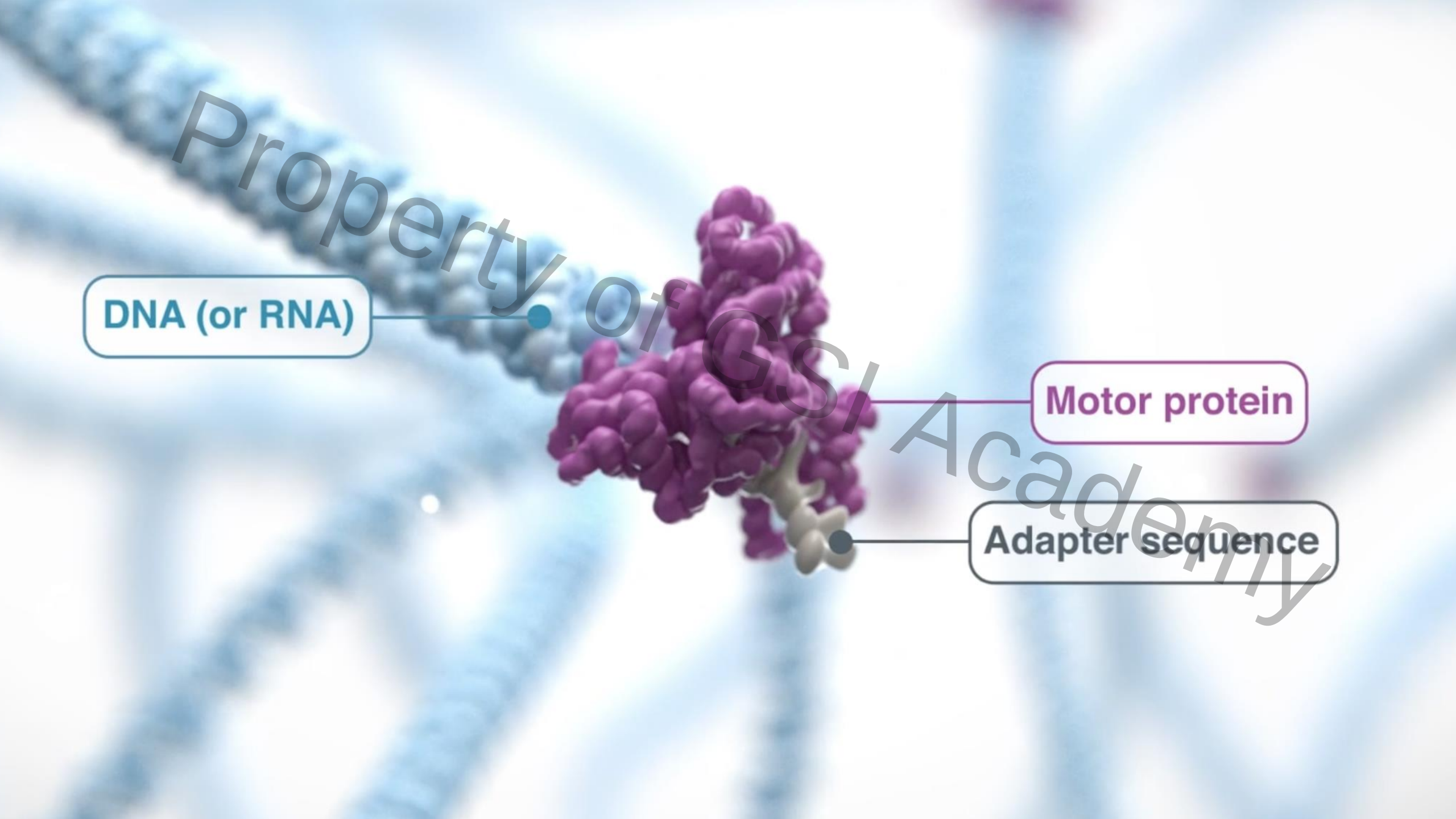

- Nanopore embedded in electrically resistant membrane so that ionic current can pass through the nanopore when a voltage is applied
- This system could be used to distinguish 5 standard RNA and DNA bases and also modified bases in those molecules

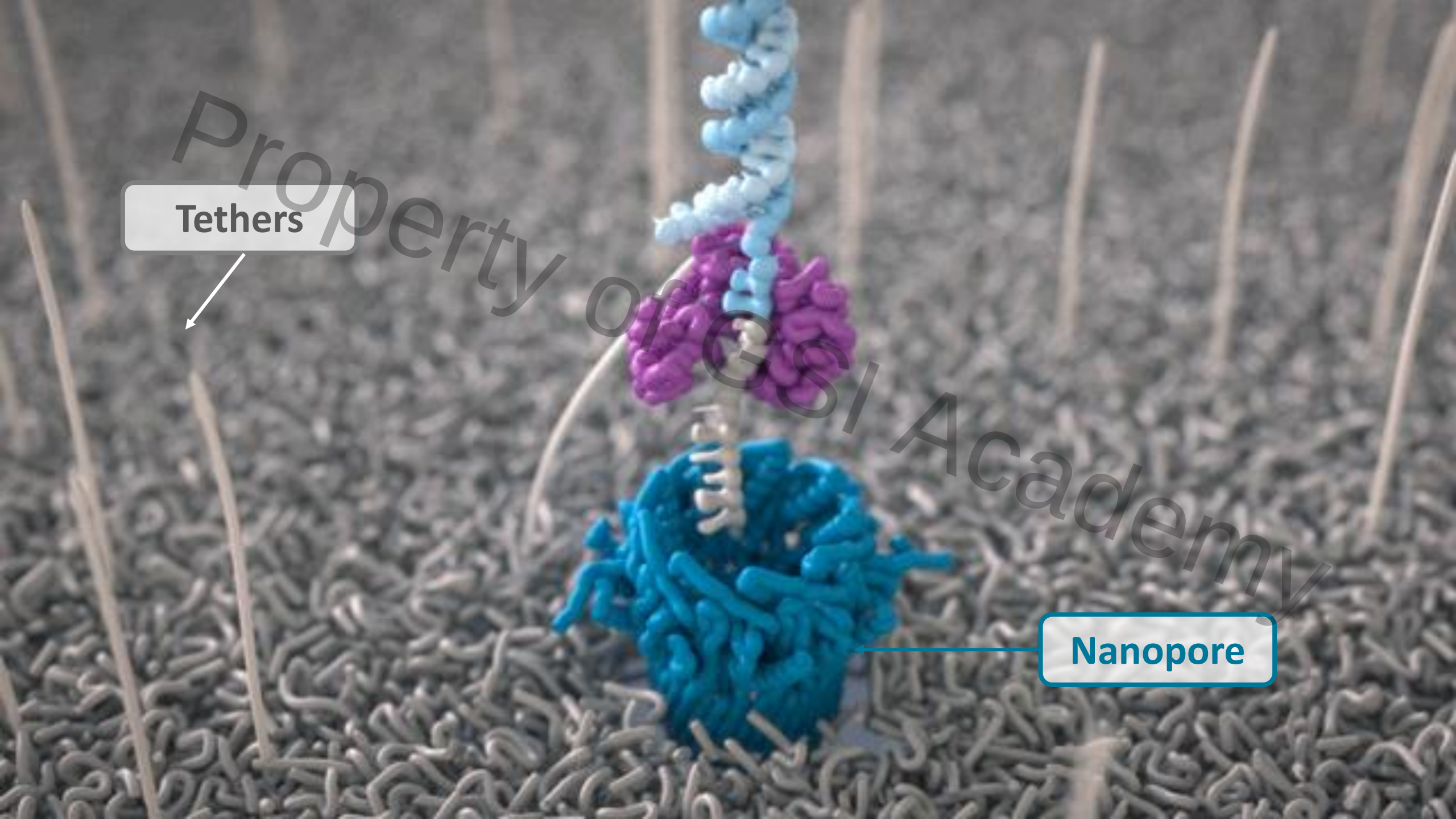
# Nanopore DNA/RNA Sequencing

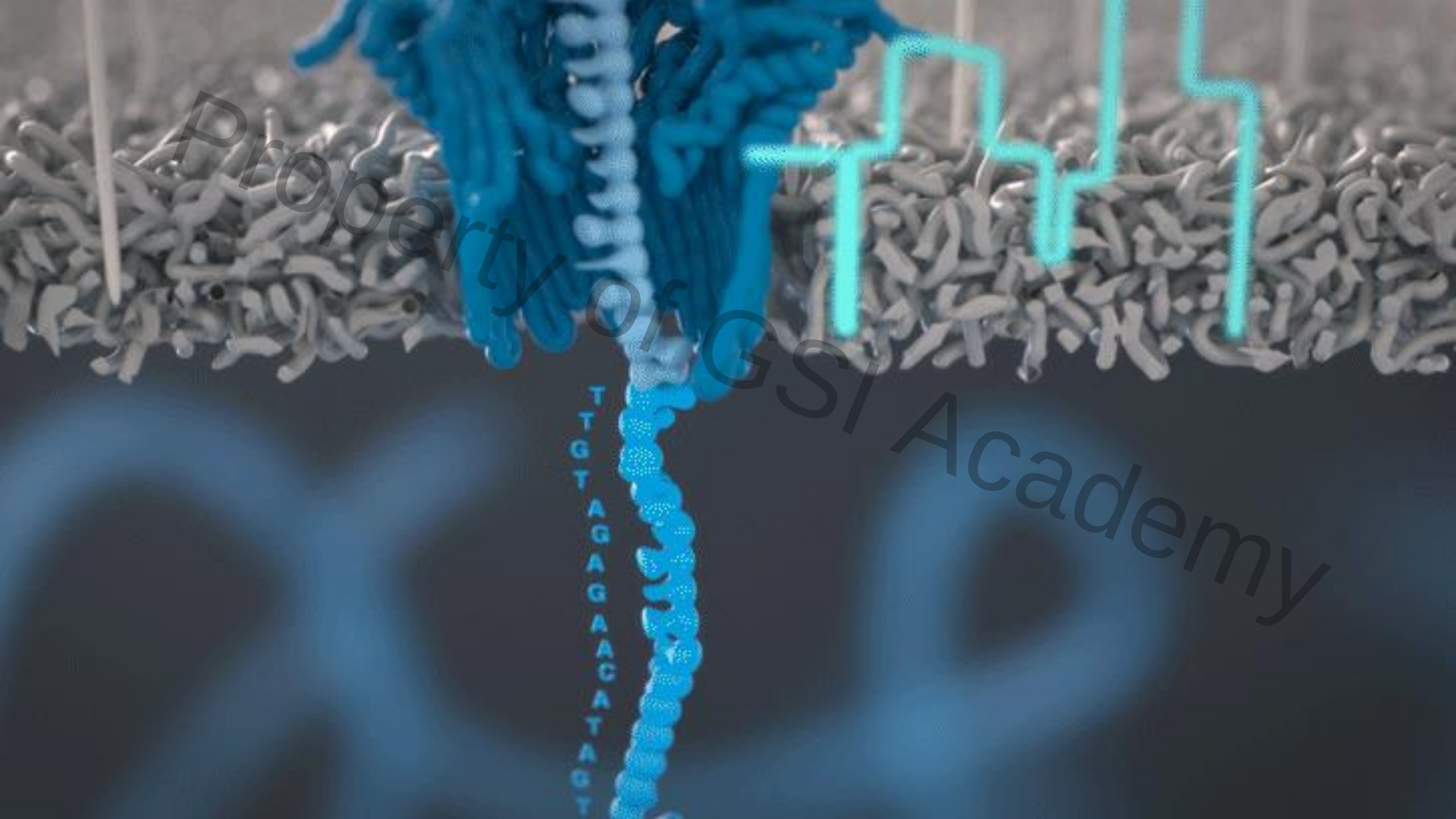
DNA/RNA strand passes through the pore ➔ signal interpreted into sequence data











# Nanopore Sequencing Devices

From the most portable to the most powerful

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| | MinION Mk1B | MinION Mk1C | GridION Mk1 | PromethION P24 | PromethION P48 |
|---|---|---|---|---|---|
| |  |  |  |  | |
| | Portable, highly accessible DNA/RNA sequencing | Portable, fully integrated DNA/RNA sequencing | Self-contained bench-top sequencer with powerful compute | The most powerful benchtop sequencer (P24 or P48) | |
| | TMO: Up to 50 Gb* | TMO: Up to 50 Gb* | TMO: Up to 250 Gb* | TMO: Up to 290 Gb per flow cell* | |
| Flongle | Adapter for MinION/GridION, supports smaller single-use flow cells. TMO: Up to 2.8 Gb |  | | P24 TMO: Up to 7 Tb* | P48 TMO: Up to 14 Tb* |

*TMO: Theoretical Max. Output when system is run for 72 hours (or 16 hours for Flongle) at ~450 bases / second. Outputs may vary according to library type, run conditions etc

# Flexibility for your library preparation

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| | DNA | | | | | | RNA | |
|---|---|---|---|---|---|---|---|---|
| | Ligation-based | Rapid (ligation-free) | Ultra-long | Rapid PCR | 16S | Cas9 | Direct RNA | cDNA PCR |
| Preparation time | 60 mins | 10 mins | 200* mins +1xO/N incubation | 15 mins + PCR | 10 mins + PCR | 110 mins | 105 mins | 210 mins + PCR |
| Input recommendation | 400-1000 ng gDNA or 100-200 fmol | 50-200 ng gDNA | 6M Cells | 1-5 ng gDNA | 10 ng gDNA | 1-10 µg gDNA | 50 ng poly(A)+ RNA | 4ng poly(A)+ or 200 ng total RNA |
| Barcode options | 24 plex 96 plex | 24 plex 96 plex | - | 24plex | 24 plex | 24 plex *coming soon* | *In development* | 24 plex |
| Typical output | ●●● | ●●○ | ●●○ | ●●○ | ●●○ | ●○○ | ●○○ | ●●● |
| Adaptive sampling | ✓ | ✓ | ✓ | ✓ | Not relevant | *In development* | *In development* | *In development* |
| Methylation | ✓ | ✓ | ✓ | - | - | ✓ | ✓ | - |
| Q20+ chemistry Kit V14 upgrade | ✓ | ✓ | ✓ | ✓ | *Coming soon* | *Coming soon* | *New RNA specific chemistry in 2023* | *Coming soon* |
| Highlights | • Base modifications preserved and included<br>• Automatable workflows on various liquid handlers, and XL kits enable production-scale sequencing | | | • Ideal for low input | • Adaptive sampling is a method of software-controlled enrichment and depletion | | • **Base modifications preserved and included** with the Direct RNA Sequencing Kit | |

* Includes DNA extraction

# Features of Oxford Nanopore Technologies



Anything, anyone, anywhere

- No capital cost
- Simple workflow
- Portable and scalable

Real-time and on demand

- Start and stop sequencing as required
- Run single/multiple of flow cells concurrently
- Data available in real time


Information-rich

- High data volume
- High consensus accuracy
- Enable long reads sequencing

# Flow Cell Handling

# What makes up a flow cell?



## TOP SIDE


*Both ports are shown in a closed position


## UNDERSIDE

# Flow Cell Handling and Storage

## Storage



**Shipped in a sealed pouch**

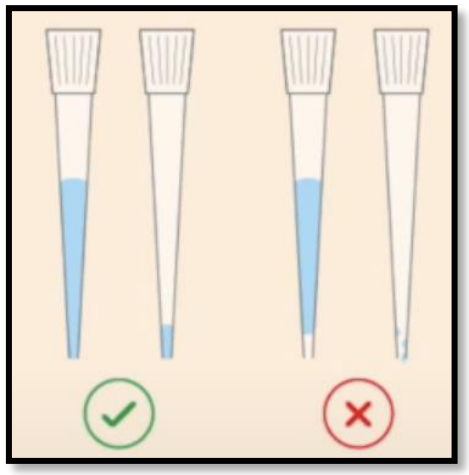
- Flow cells must never be frozen
- 1 month at ambient temperature, unopened
- 3 months at 2-8ºC unopened or opened
  - — Avoid the back of a refrigerator because of the risk of freezing

# Good Practice of Flow Cell Priming and Loading



## ATTENTION: avoid introducing any air into the flow cell

- During pipetting
- When removing too much buffer
- When drawing air back in through the priming port



### When drawing up solution

- Make sure there's no air plug at the bottom of the tip

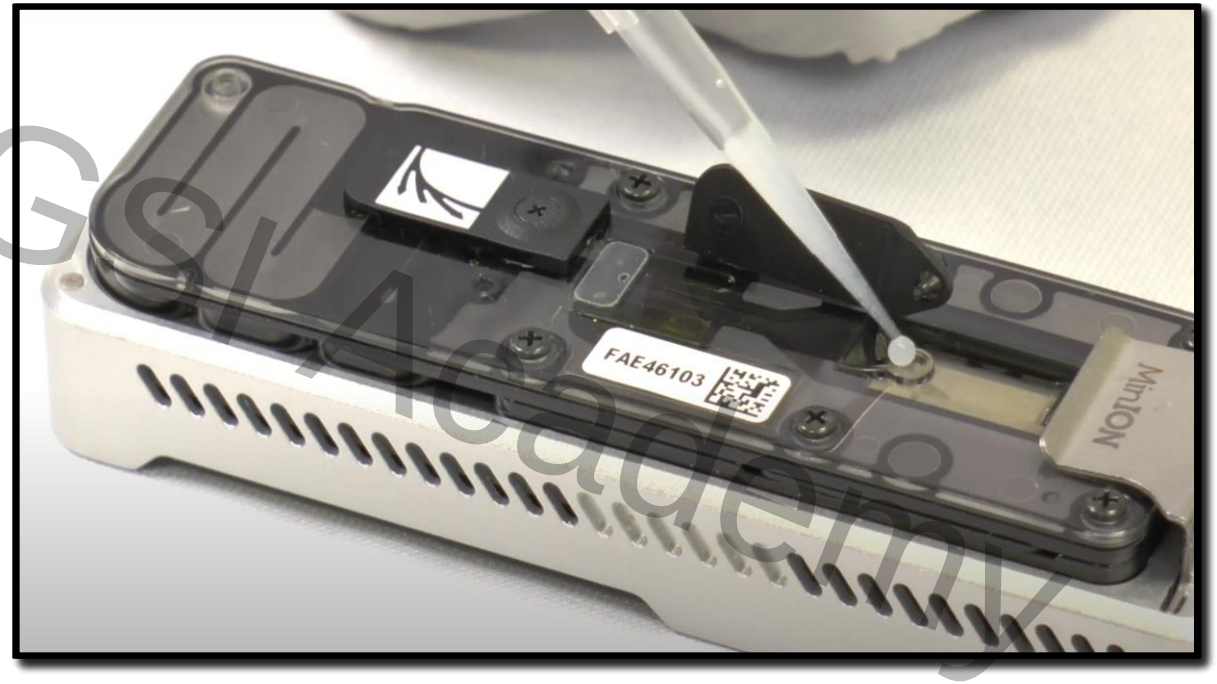
### When ejecting solution

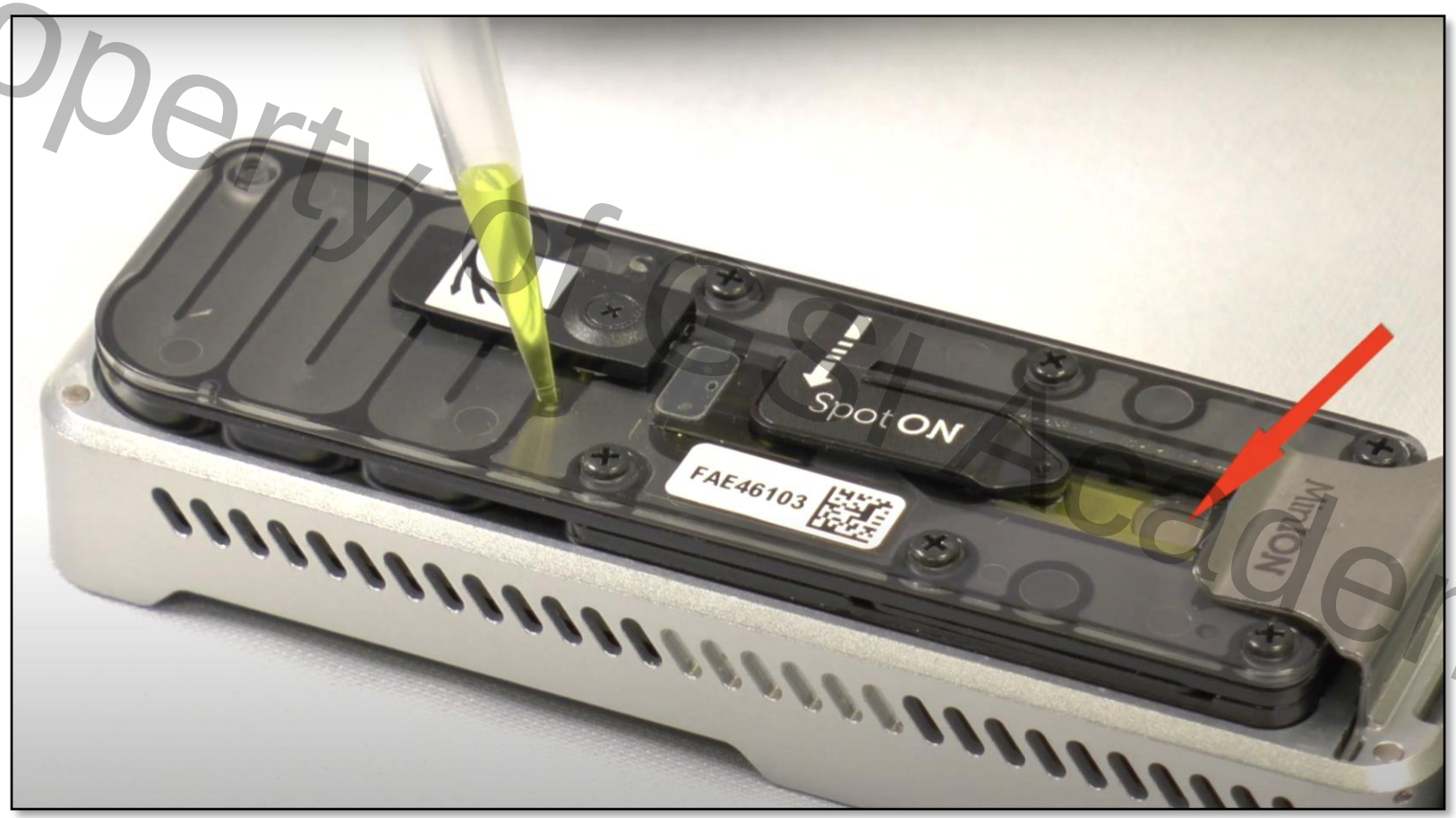
- DO NOT FULLY eject the solution from the tip
- Leave a small volume of solution in the tip end so no air could be introduced into the flow cell

# Priming and Loading Your Flow Cell



- Slide open the priming port to 90°C
- Remove air bubble by drawing back 20-30 µl of buffer
- Load 800 µl priming buffer through priming port
- Incubate 5 minutes
- Open the SpotON port
- Load 200 µl priming buffer through priming port
- Load 75 µl of sequencing mix in a drop wise fashion through the SpotON port






Do you see anything wrong in this image?

What could possibly happen?

# How to Start Sequencing

# MinKNOW

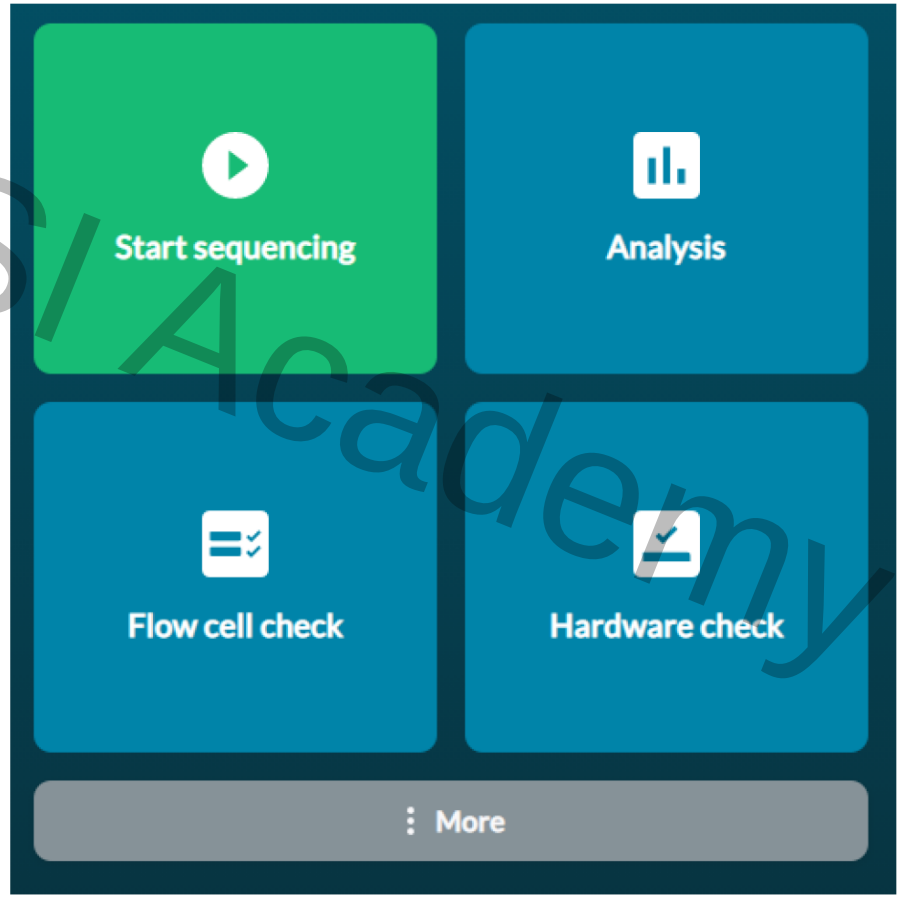
## Primary Interface for Sequencing Experiments



Device control → Data collection → Live basecalling → Analysis

### Features

- **Hardware Check**: confirms correct software and device communication
- **Flow Cell Check**: assesses membrane integrity, count number of pores (warranty for a minimum of 800)
- **Start Sequencing**: setup and start a sequencing run
- **Analysis**: Post-run basecalling, de-multiplexing, alignment

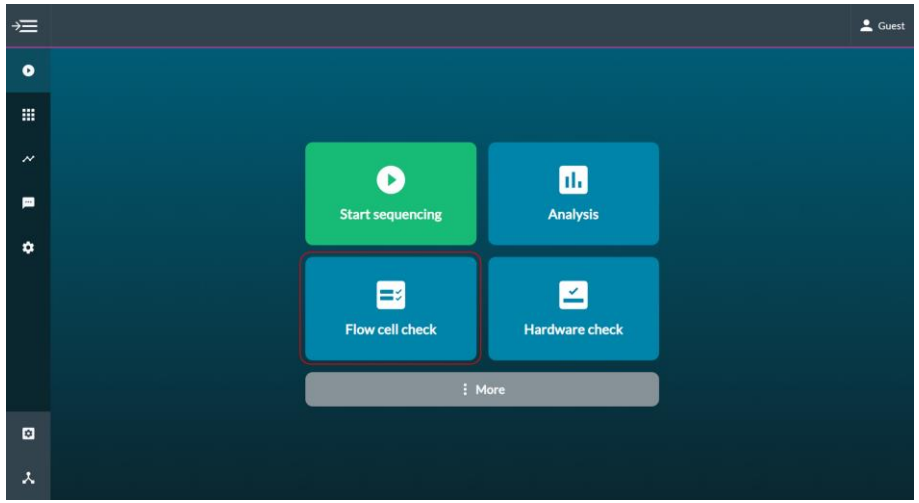


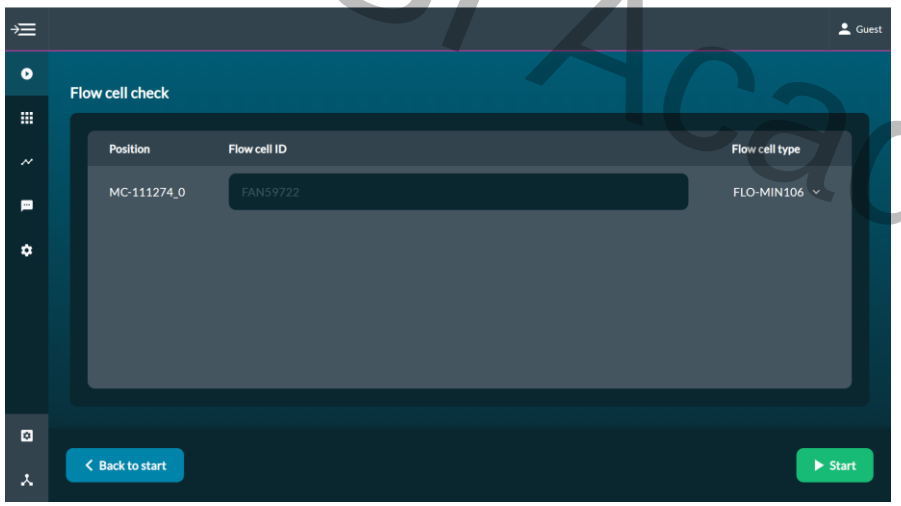
# Flow Cell Check

Before starting the sequencing run

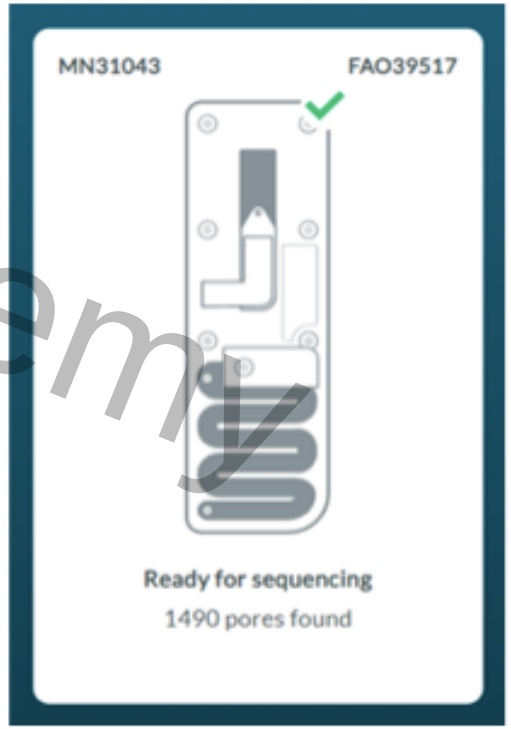


- For MinION, if available pores fall below warranty (800), report the flow cell within 2 days
- Keep flow cells unopened




Green check icon means flow check pass

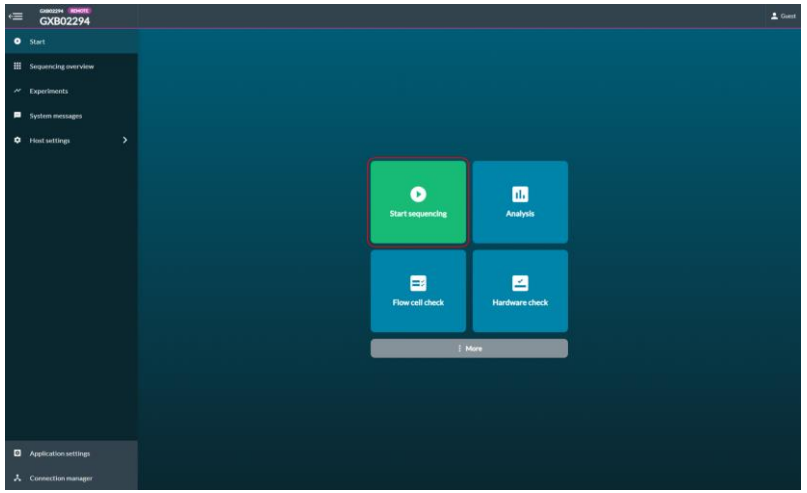


# Start Sequencing

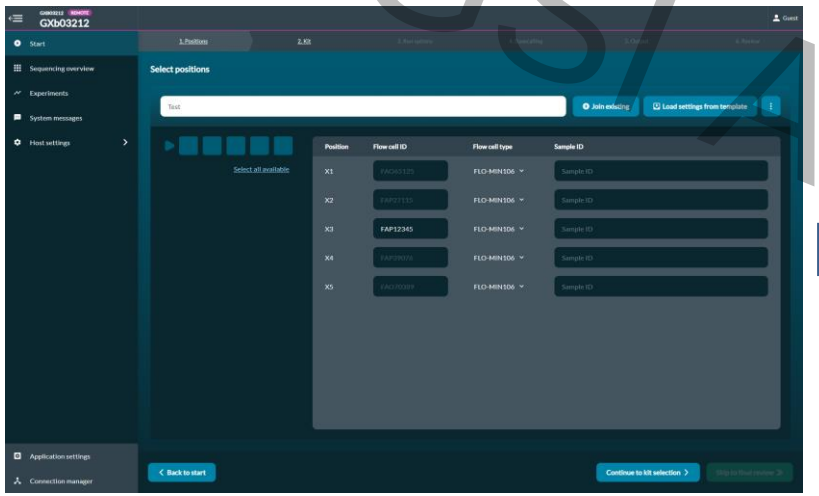


## Select kit and barcode

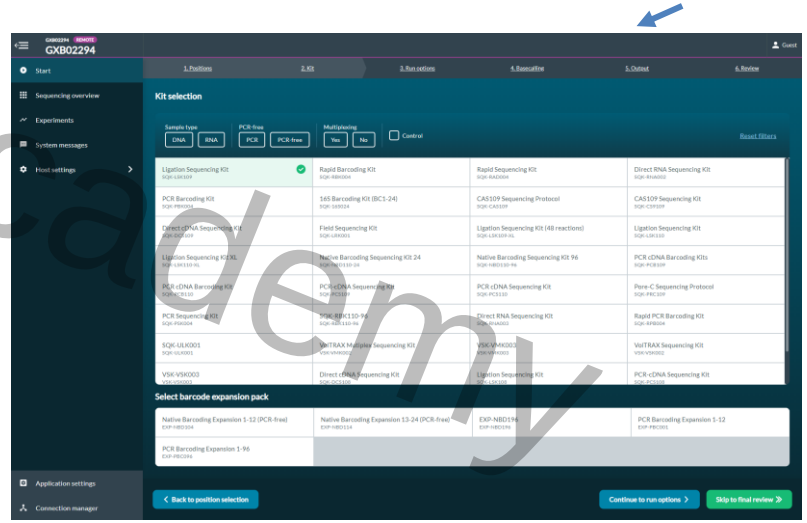
- Two or more barcoding expansion packs can be selected. If a selected kit is not compatible with any barcode expansion pack, barcode options will not appear




Select 'Start sequencing' option




Type in the experiment name, sample ID and choose flow cell type from the drop down menu. Then select 'continue to kit selection'



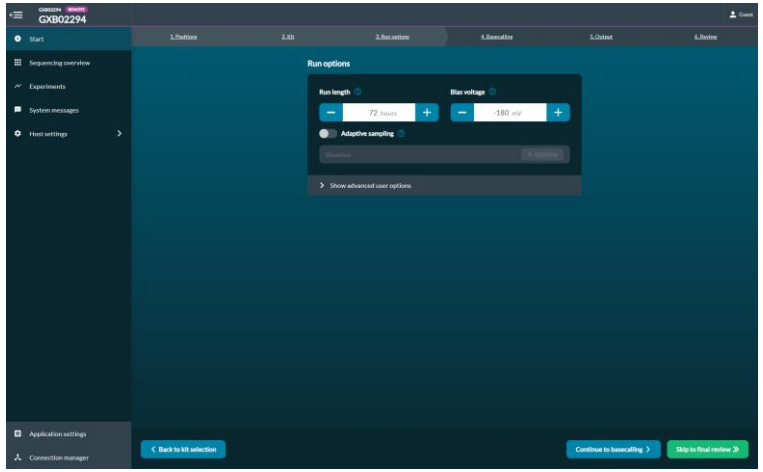

Choose the kit and barcode being used. Then continue to run options

# Start Sequencing

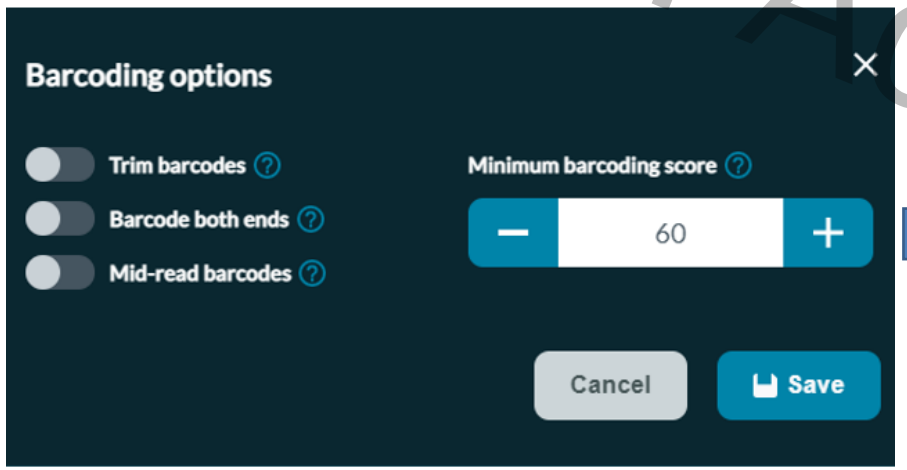


## Customize the run

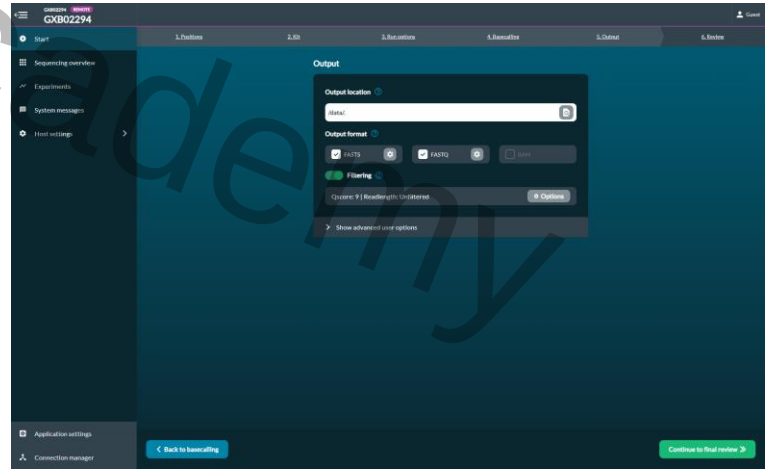
- In Run Options, run length and bias voltage can be altered and adaptive sampling enabled.
- Use the trim barcode options to remove barcodes from demultiplexed reads. Please note, some primer sequences may also be trimmed together with the barcodes.
- Barcoding score can be increased if the user wants to be more confident in barcode detection classification.




Default run time and starting voltage is 72 hours and -180 mV. Then 'continue to basecalling'




Choose basecalling, barcoding and alignment options and use option button to customize a run



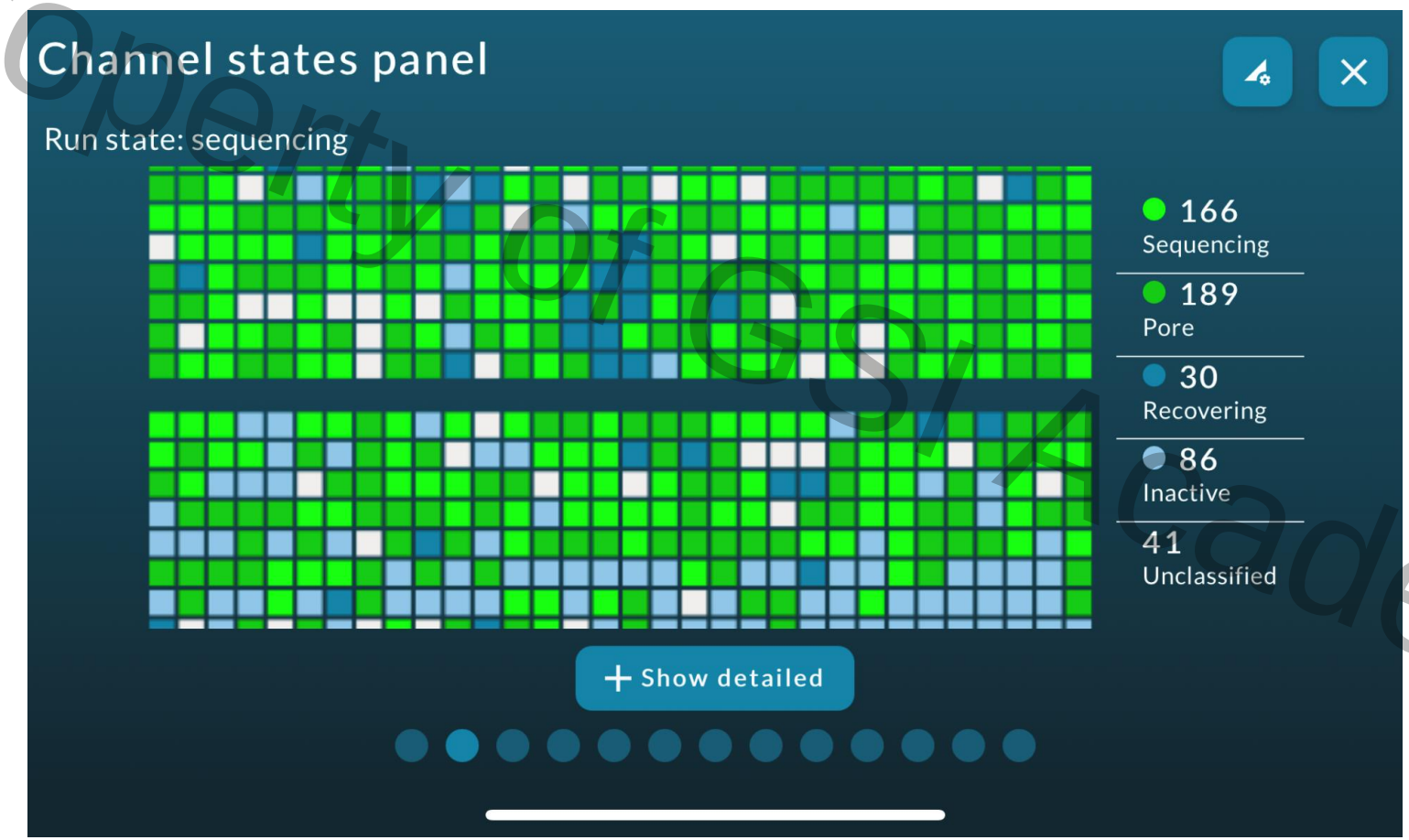

Continue to final review

# MinKNOW User Interface

# Monitoring Your Sequencing Run

## Channel states panel






Pores doing the sequencing

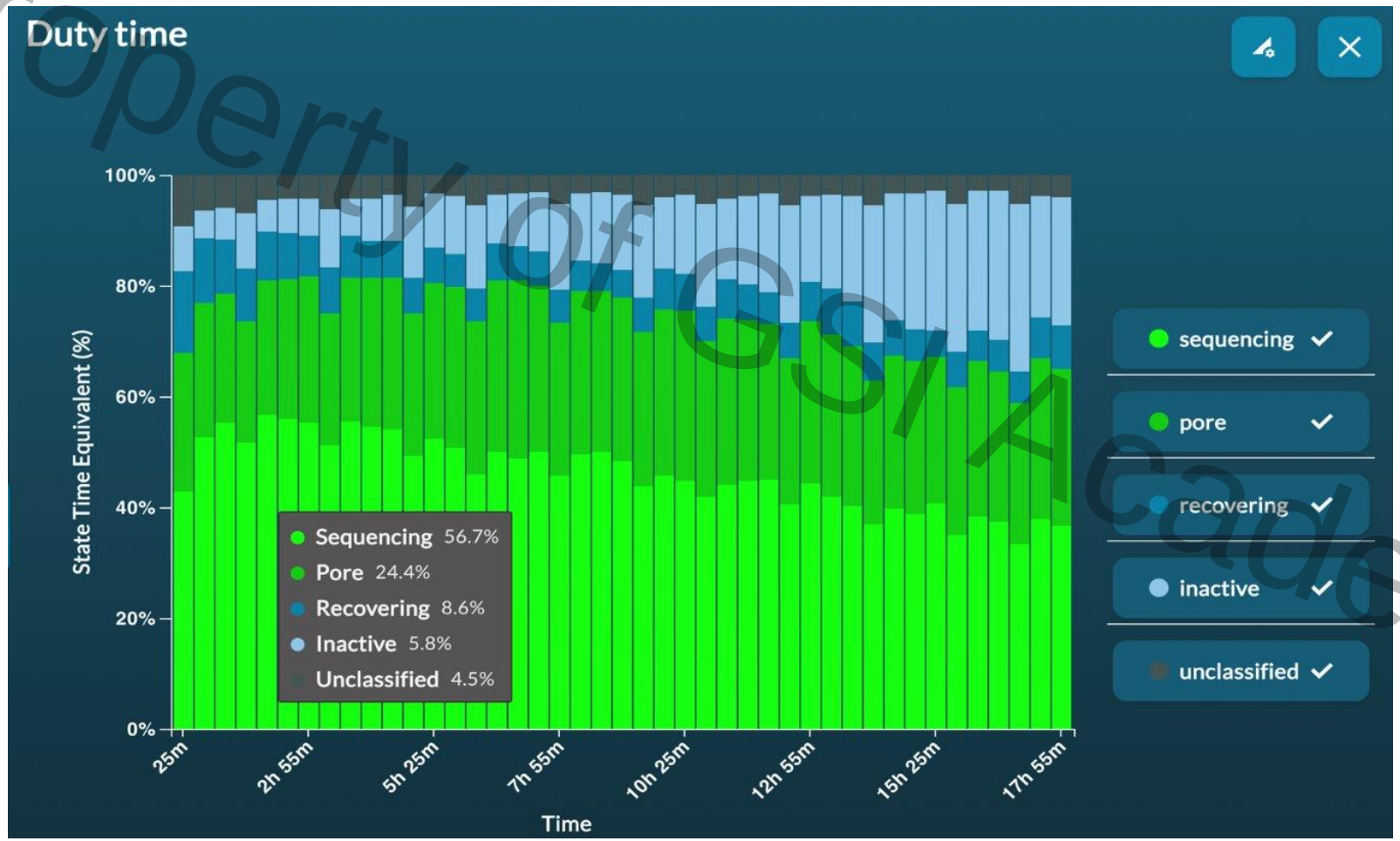
Open pore ready for sequencing

To give you real time state of the channels

# Monitoring Your Sequencing Run

Duty time plot



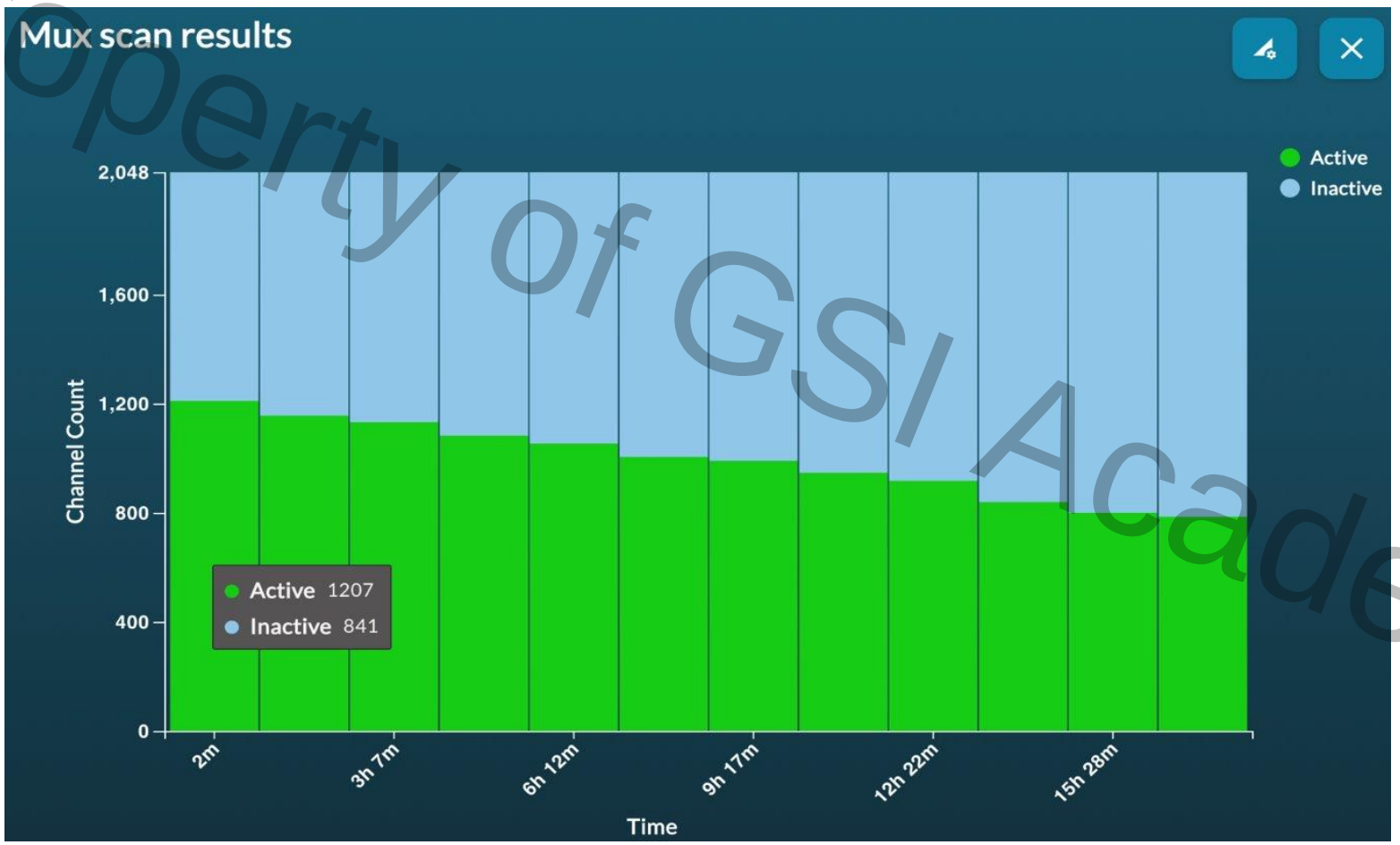



Channel states panel in different bucket of time

# Monitoring Your Sequencing Run

Mux scan






Classification of each nanopore every 1.5 hours

# Monitoring Your Sequencing Run

Barcode hits

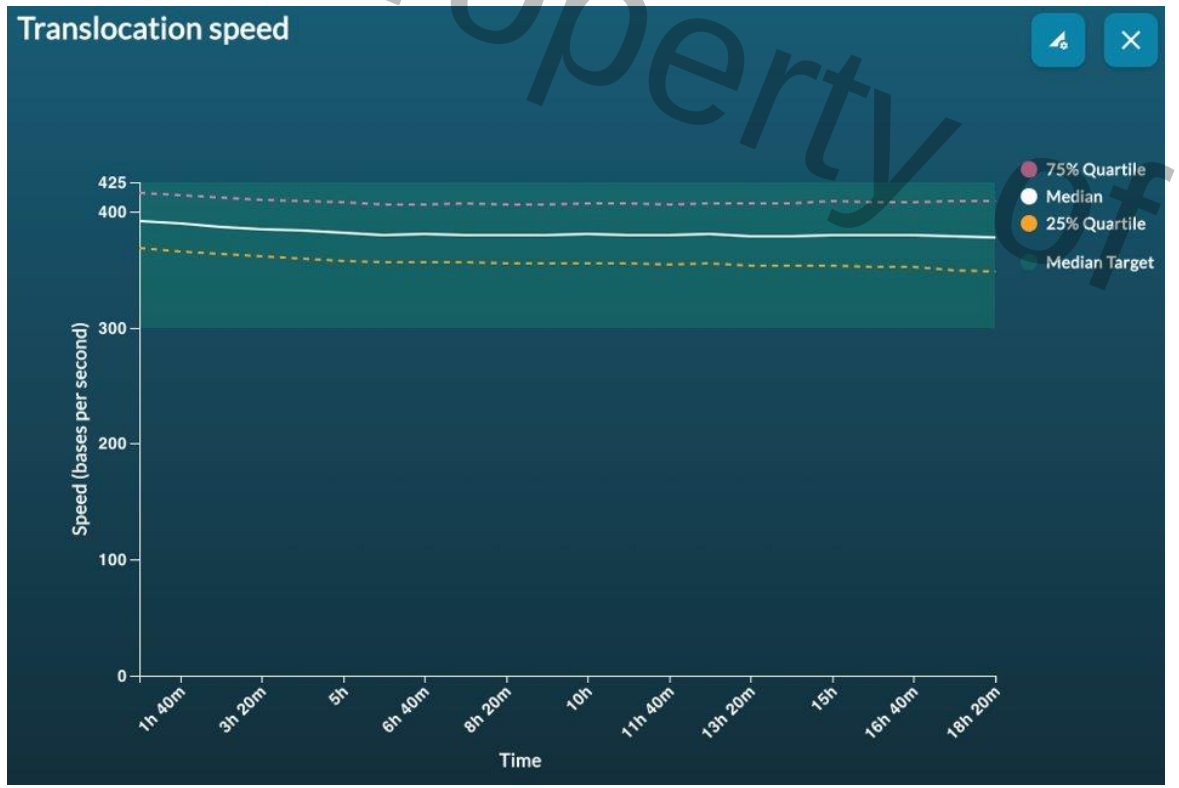


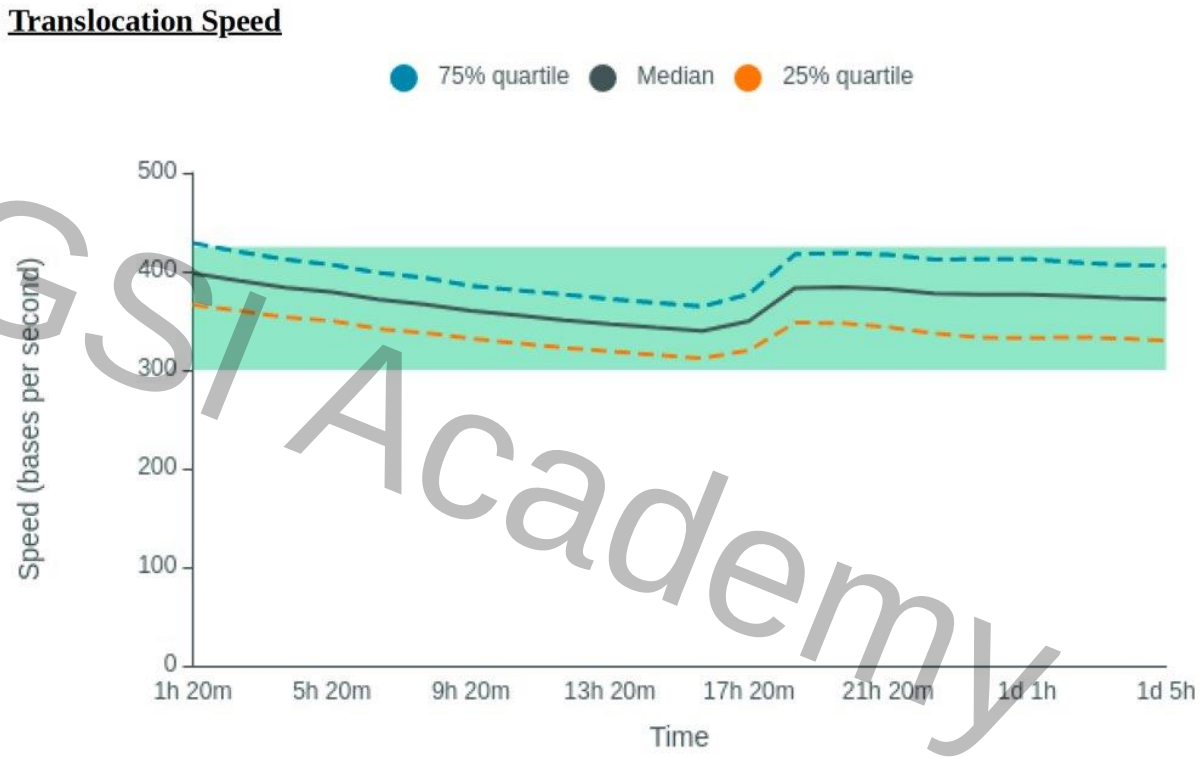

Read counts of every barcoded samples in the flow cell

# Monitoring Your Sequencing Run

Translocation speed





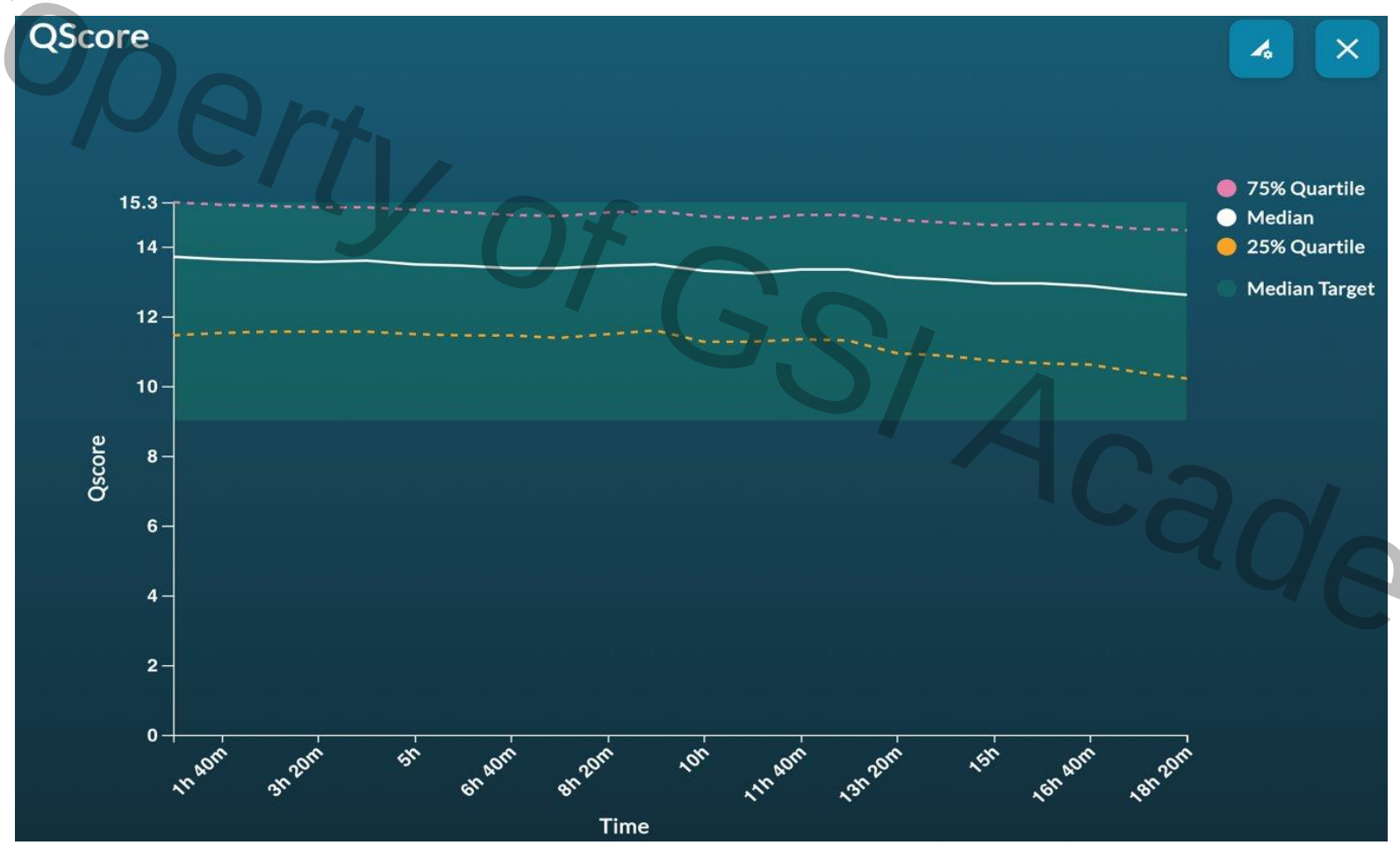




Speed of DNA or RNA when passing through the nanopore. Expected speed: 400 bases per second

# Monitoring Your Sequencing Run

QScore






Qscore monitoring: quality of your data

# Additional workflow

# Flow Cell Wash (After Experiment)



- To maximise flow cell usage, we could do sequential runs in the same flow cell
- ONT provides flow cell wash kit suitable for experiments where the amount of data per sample is achieved with a short run time
- This kit contains **Dnase I**, that could digest any remaining library on a flow cell
- Once the flow cell is washed, it can be re-used immediately or stored for later used


Prepare WMX (do not vortex) and thaw DIL (vortex)

↓

Prepare 1.5 uL microtube

↓

Mix 2 uL WMX with 398 uL DIL

↓

Priming via priming port

↓

Wait for 1 hour

↓

Dispose the waste liquid

↓

Insert 400 uL Storage Buffer (S)

# Refueling and Top Up

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## Refueling Flow Cell

In case of translocation speed drop

1. Thaw Flush Buffer to room temperature, vortex and spin down
2. Pause the sequencing run
3. Open priming port, check for bubble under the cover, in the priming port
4. Draw back a small volume of buffer to remove the bubble
5. Load 250 µl of the FB (priming buffer) into the flow cell via the priming port
6. Close priming port and GridION lid, resume sequencing run

## Library Top Up

In case of low pore occupancy

1. Prepare the library to load
2. Pause the sequencing run
3. Open priming port and SpotON port (sample port)
4. Add prepared library to the SpotON port in a drop-wise manner
5. Close the SpotON and priming port, resume the sequencing run

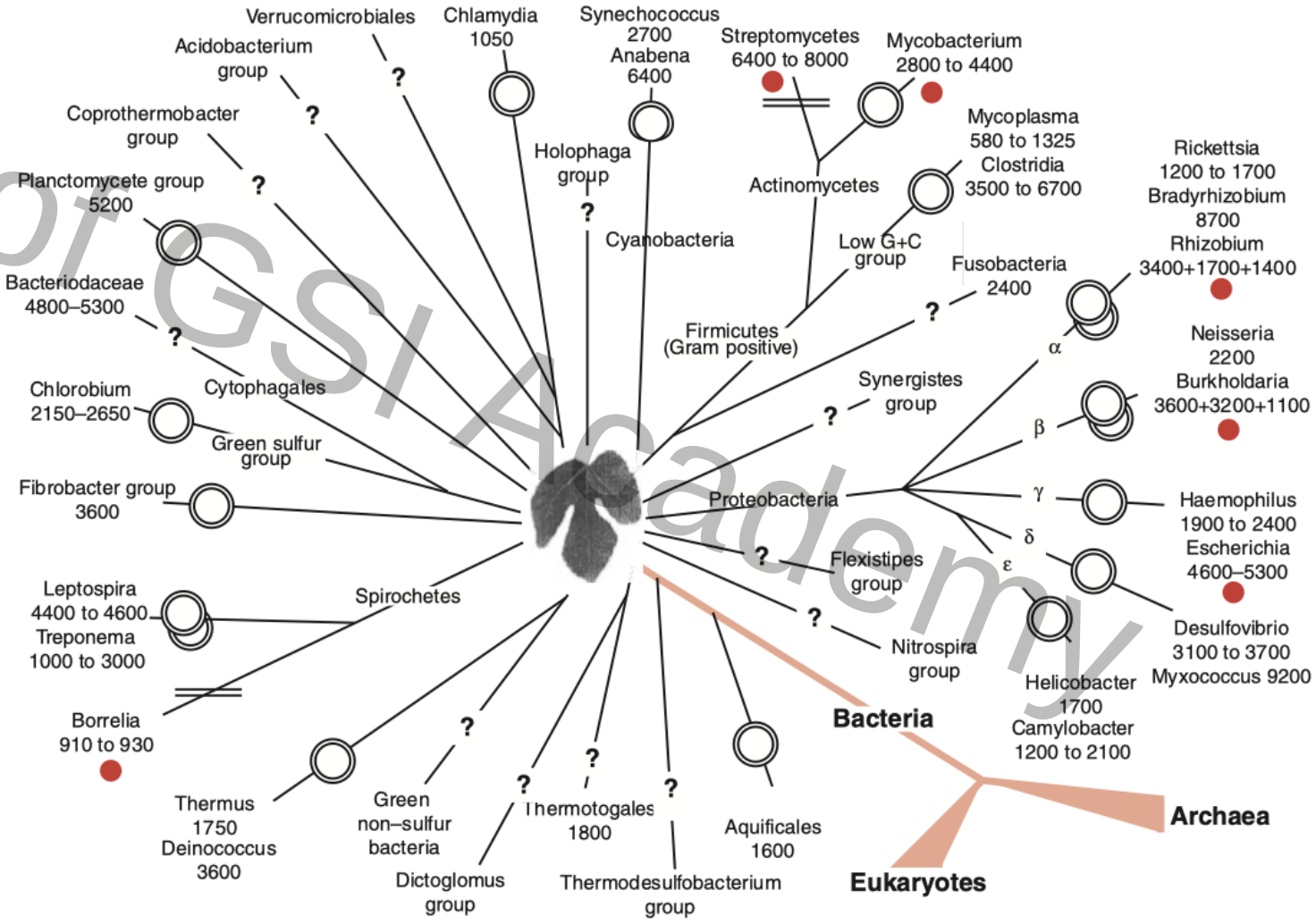
# INTRODUCTION TO 16S SEQUENCING

# Early Bacterial Identification



One area within the practice of clinical microbiology is the craft of putting scientific names to microbial isolates.

The historical method for performing this task is dependent on the comparison of an accurate morphologic and phenotypic description of type strains or typical strains with the accurate morphologic and phenotypic description of the isolate to be identified



# Isolating Bacteria: Is it possible?



Obtaining bacteria in pure culture is typically the first step in investigating bacterial processes. However, standard culturing techniques account for 1-2 % or less of the bacterial diversity in most environmental samples. The genomic study of natural communities has been largely driven by interest in the ~99% of microbes that are not easily isolated in culture

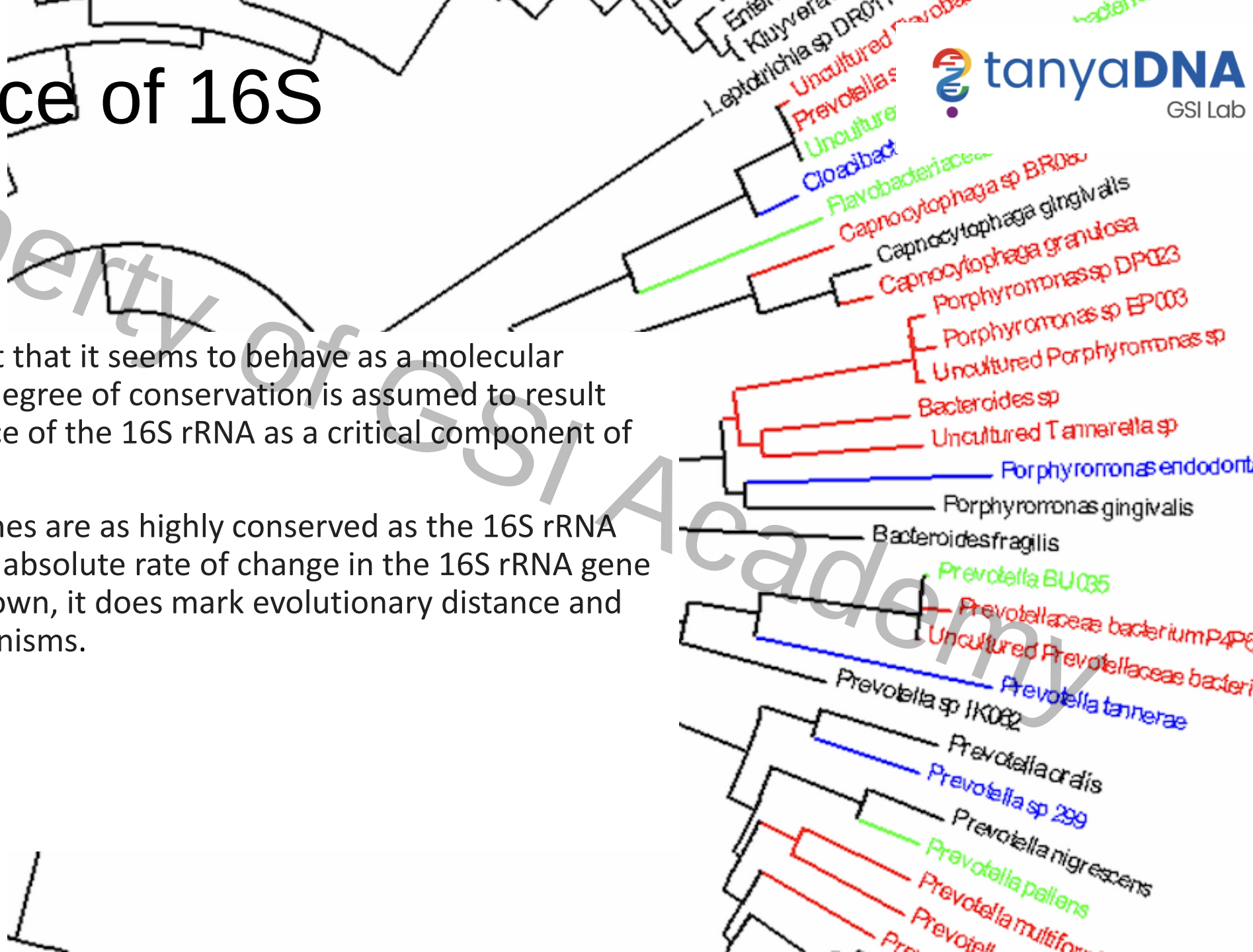
# Classification of Bacteria



We will now consider the classification of bacteria and archaea by six different criteria:

(1) Morphology
(2) genome size
(3) Lifestyle
(4) relevance to human disease
(5) molecular phylogeny using rRNA

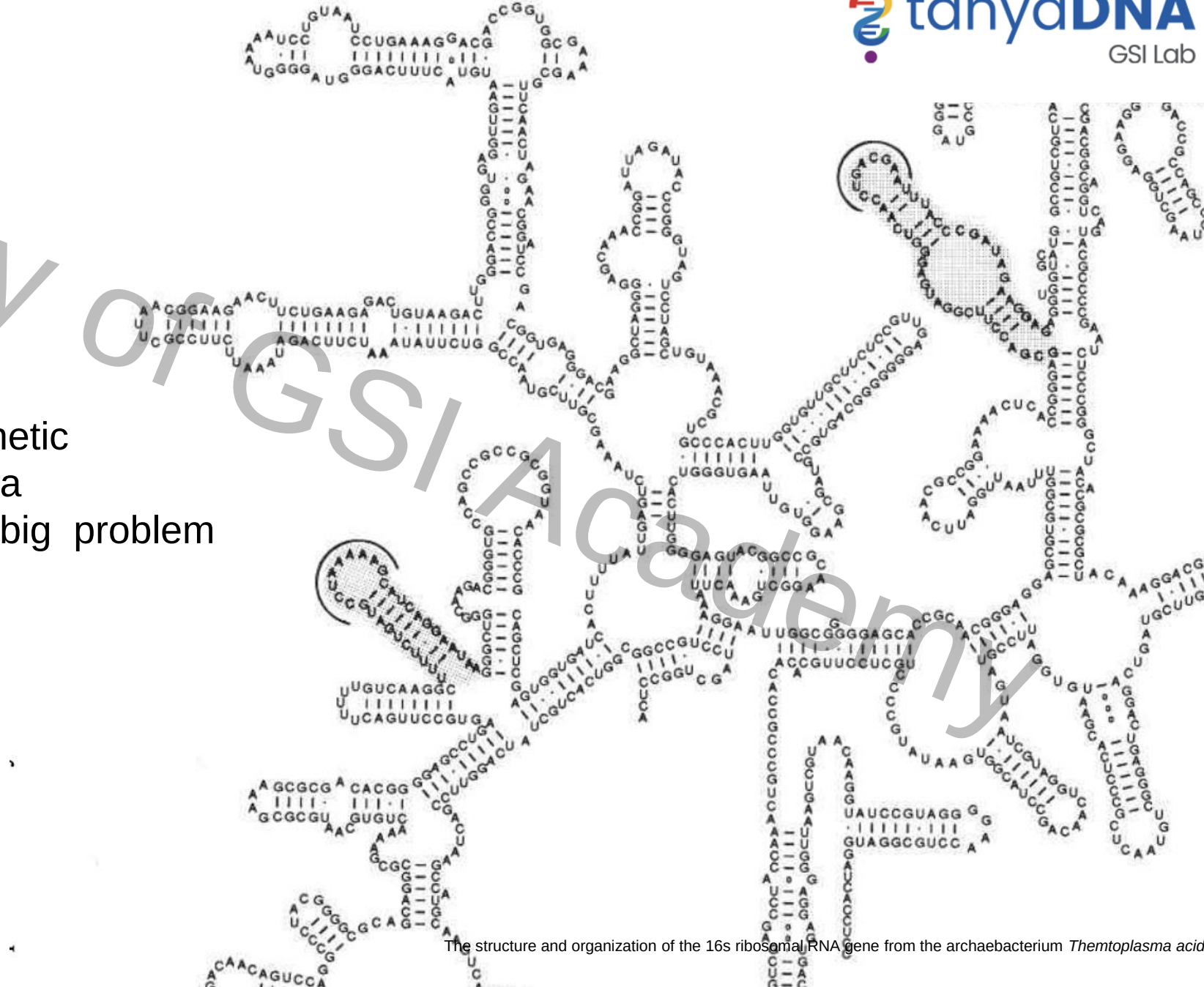
# Importance of 16S rRNA




- Foremost is the fact that it seems to behave as a molecular chronometer. The degree of conservation is assumed to result from the importance of the 16S rRNA as a critical component of cell function
- Thus, few other genes are as highly conserved as the 16S rRNA gene. Although the absolute rate of change in the 16S rRNA gene sequence is not known, it does mark evolutionary distance and relatedness of organisms.

# 16S Sequencing Advantages

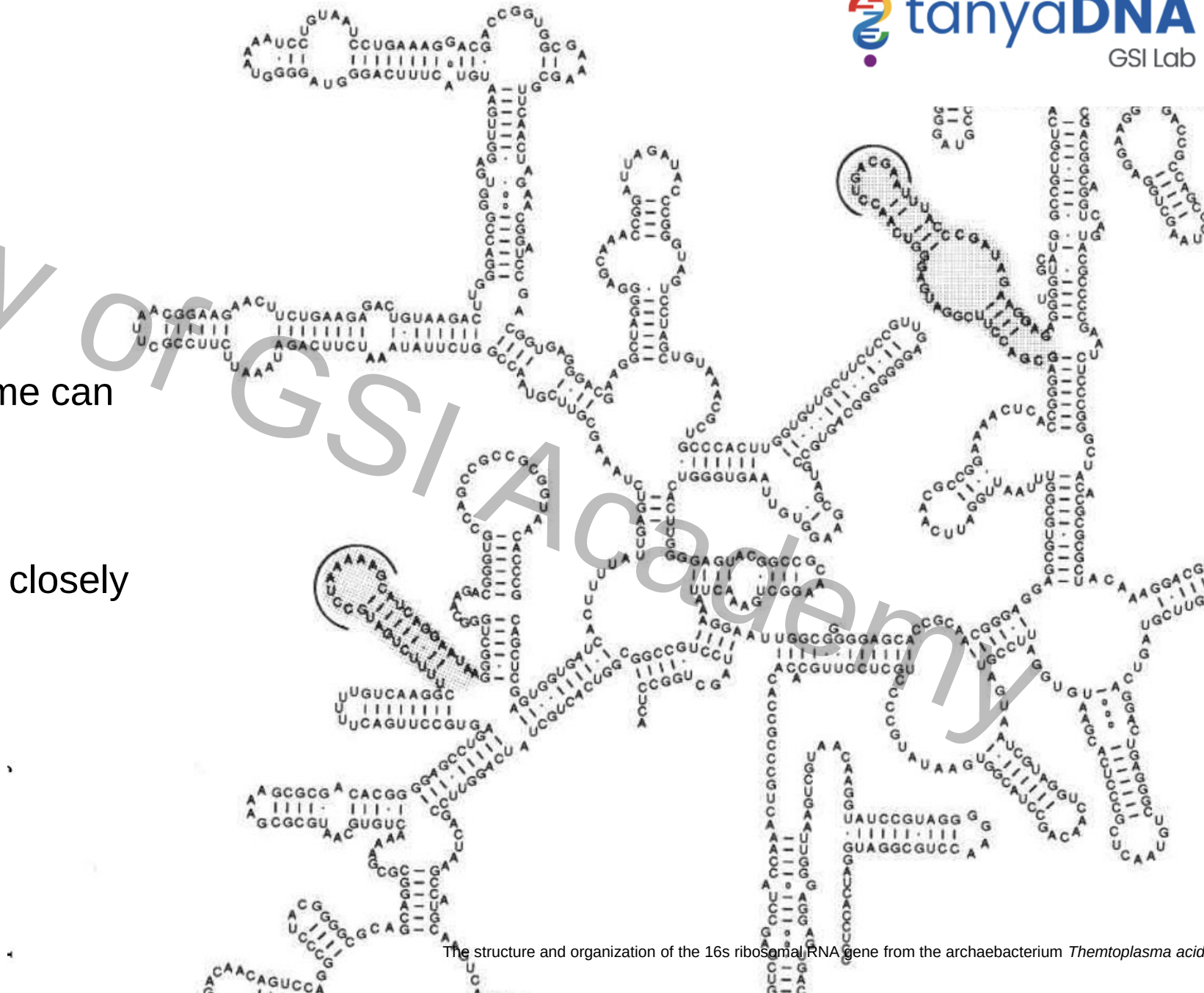
- Universally distribute
- Capability to measure phylogenetic relationship across different taxa
- Horizontal gene transfer isn't a big  problem
- Affordable cost



The structure and organization of the 16s ribosomal RNA gene from the archaebacterium *Themtoplasma acid*

# 16S Sequencing Disadvantages

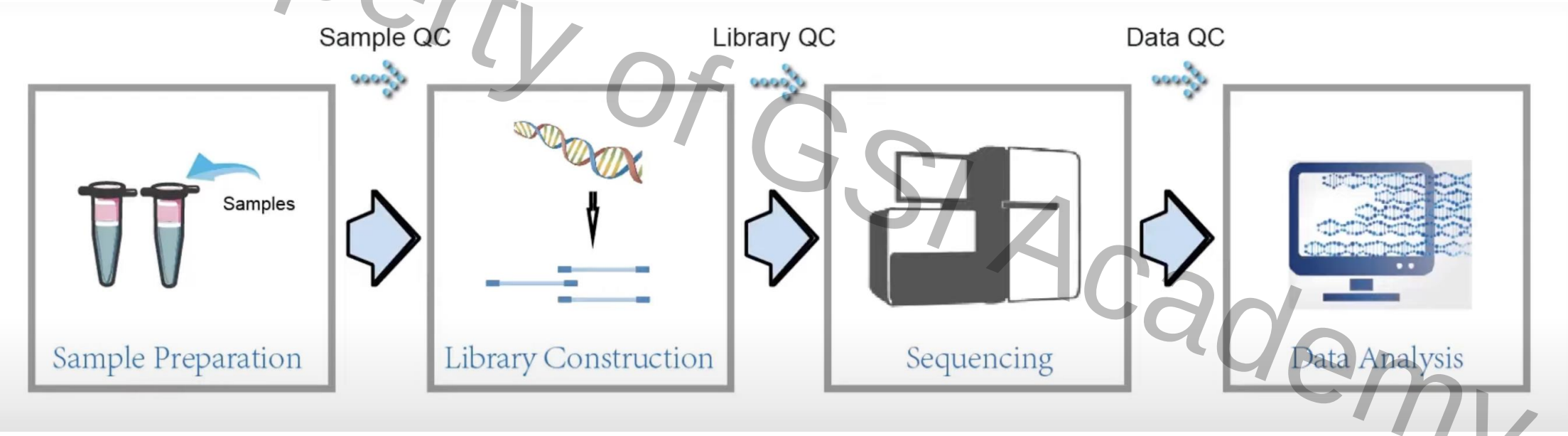
- Copy number variant per genome can vary
- PCR amplification biases
- Overestimate diversity
- Unable to differentiate between closely related species



The structure and organization of the 16s ribosomal RNA gene from the archaebacterium *Themtoplasma acid*

# General Sequencing Workflows





# THANK YOU AND STAY HEALTHY



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