Nanopore sequencing workshop

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Learning objectives

- Understand the power of long read sequencing
- Be able to prepare a library and set up a sequencing run
- Look out for potential pitfalls in preparing samples
- Check the quality of sequencing reads and assemblies
- Know best practices for analyzing long read data
- Use the pathogen surveillance Nextflow pipeline to automate epidemiological analyses

The instructor team

- Dr. Zachary Foster (USDA-ARS)
- Dr. Arafat Rahman (OSU)
- Dr. Camilo Parada Rojas (OSU)
- Dr. Martha Sudermann (OSU)
- Dr. Jeff Chang (OSU)
- Dr. Nik Grünwald (USDA-ARS)
- Dr. Alexandra Weisberg (OSU)

- Oxford Nanopore generously provided library kits and flow cells for this workshop
- A. Weisberg was supported by ONT for travel to the conference

- Thanks to Katrina Olson, ONT representative

- https://nanoporetech.com/

Thank you!

Workshop schedule

- **Morning 1**: Nanopore library prep
  - coffee break
- **Morning 2**: loading flow cells and sequencing

- Lunch

- **Afternoon 1**: Data quality and analysis
  - coffee break
- **Afternoon 2**: Using the nextflow pipeline

Let’s get started!

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Contact information and survey

- Workshop feedback survey:

https://tinyurl.com/APSNanopore

- Other questions or comments? Contact me here: Alexandra.Weisberg@oregonstate.edu

Devices

Flow cell

MinION (open)

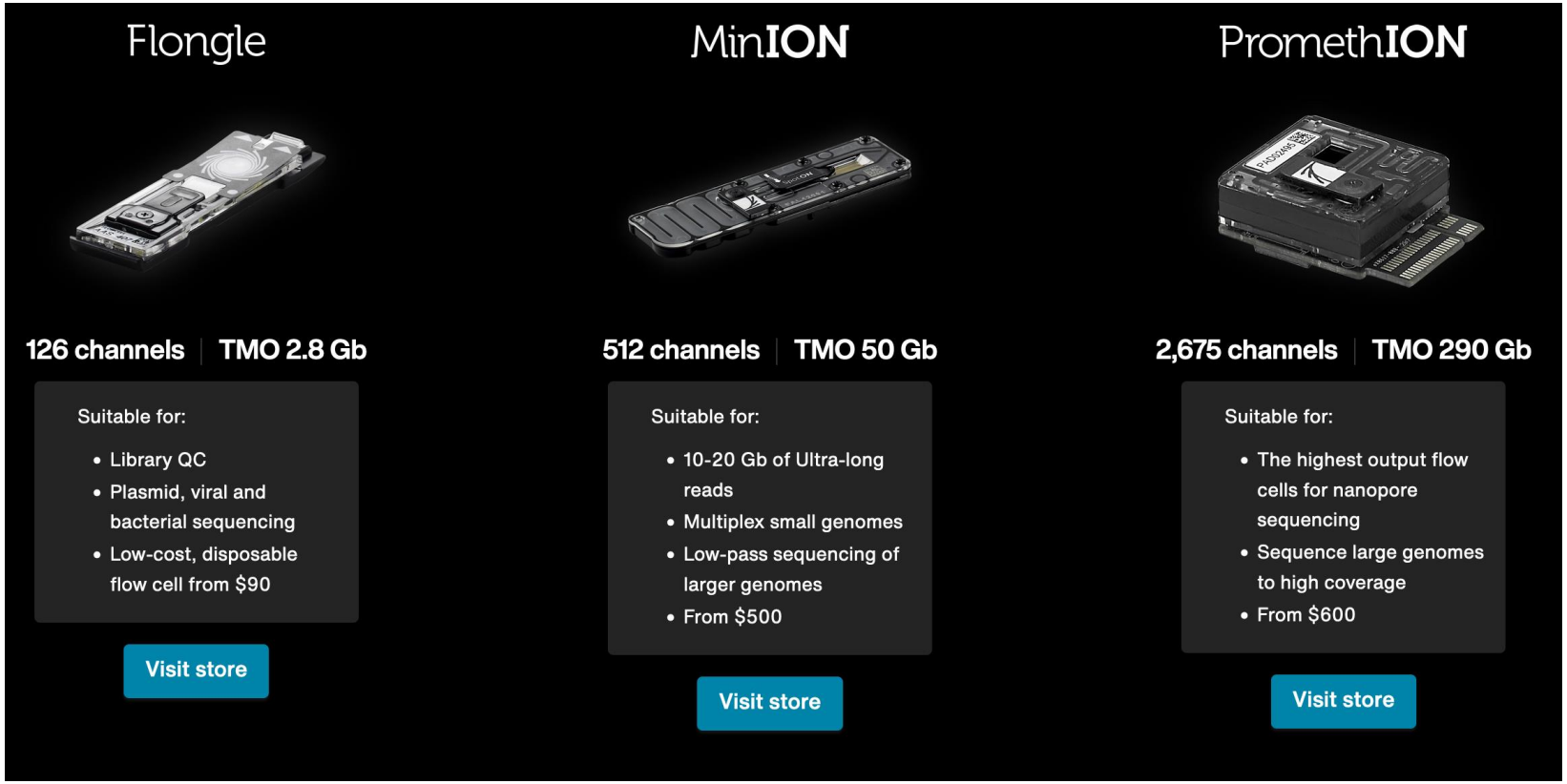
Flongle adapter for flongle flow cells ($60/each, 1-2 GB)
$1800 for adapter + 12 flow cells

Promethion P2 solo

MinION Mk1C
MinION + MinIT + screen $4900

Different flow cell options

- Same libraries work on all
- Test part of library on flongle first etc.



1-2 Gb
~$60

10-20 Gb
$500

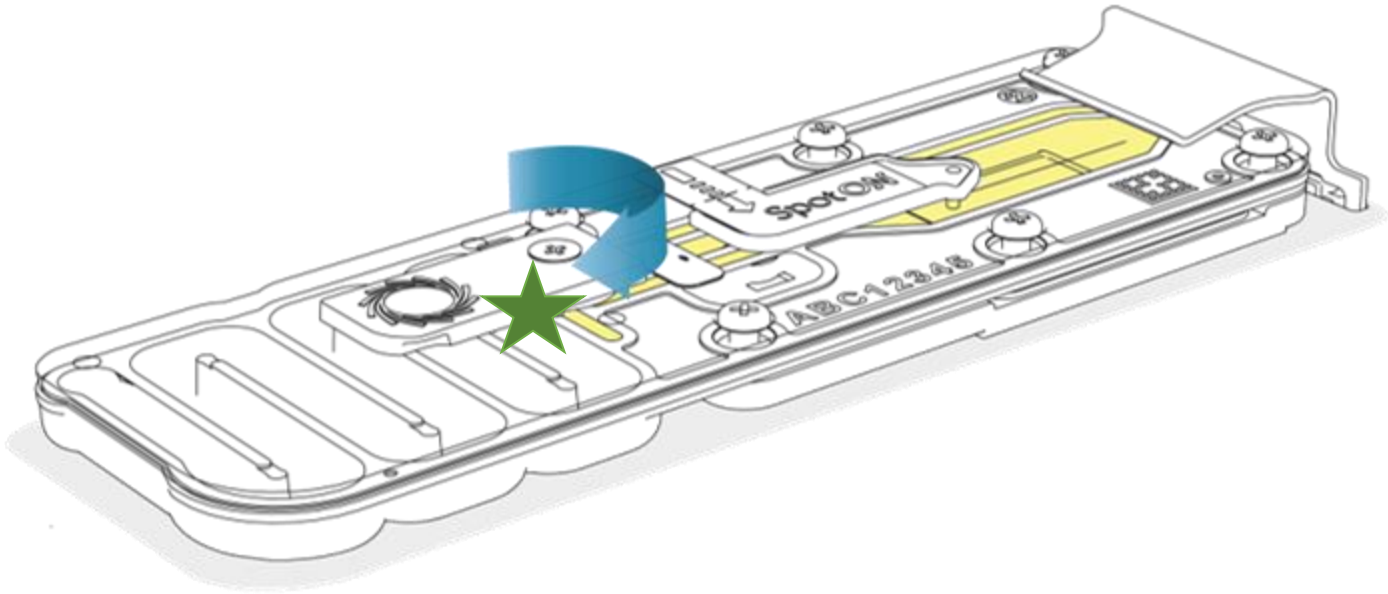
80-150 Gb
$900

Practice loading an old flow cell

Each group will either have a used PromethION flow cell or MinION flow cell at their workstations

Practice loading an old flow cell

Turn priming port cover clockwise

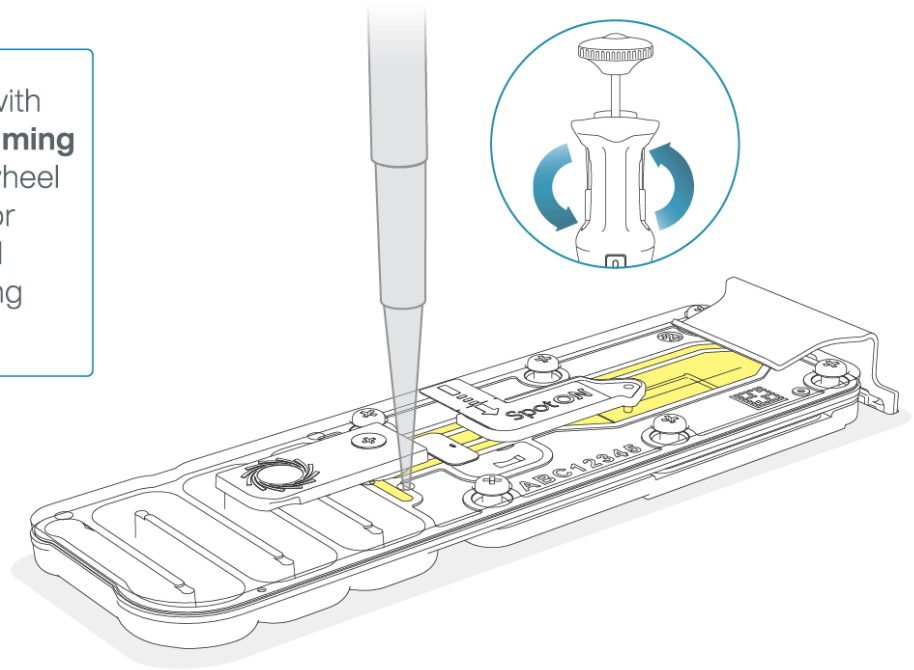


Practice loading an old flow cell

Before loading priming mix, check for bubbles under the cover

3

Insert a P1000 pipette with an empty tip into the **Priming port**. Turn the pipette wheel to draw back 20-30 µl or until you can see a small volume of buffer entering the pipette tip.

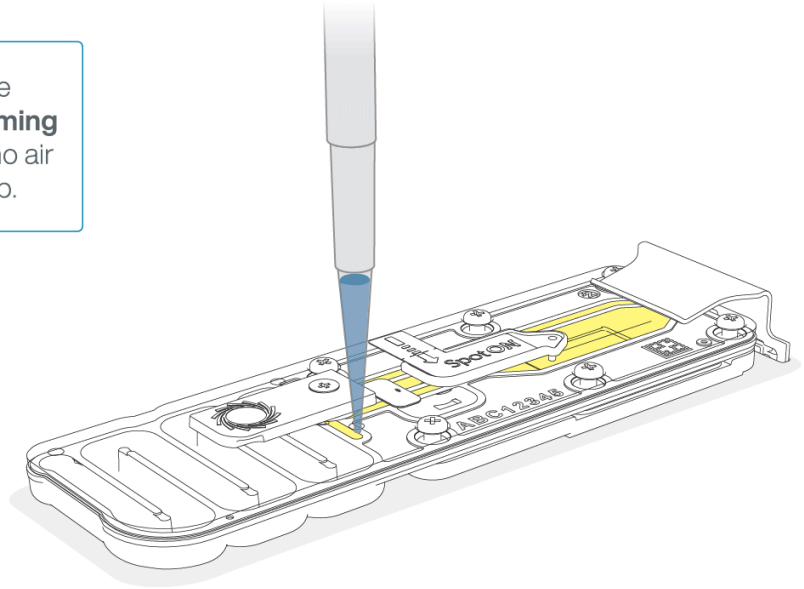


Practice loading an old flow cell

Slowly add first portion of priming into priming port

4

Slowly load 800 µl of the priming mix into the **Priming port**. Ensure there are no air bubbles in the pipette tip.



Wait 5 minutes before proceeding to the next step.

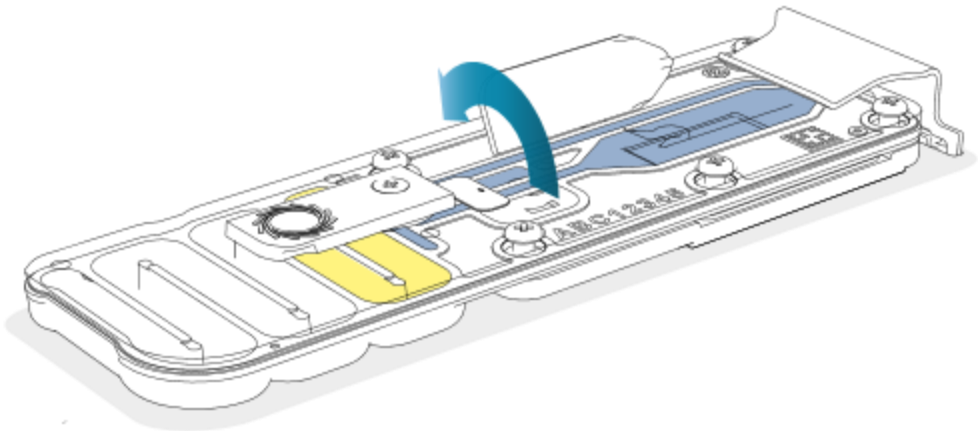
For best results, use the pipette wheel throughout the whole loading process

Practice loading an old flow cell

Finally, open SpotON sample port cover-but don’t load anything in that port

5

Gently flip open the SpotON sample port cover.

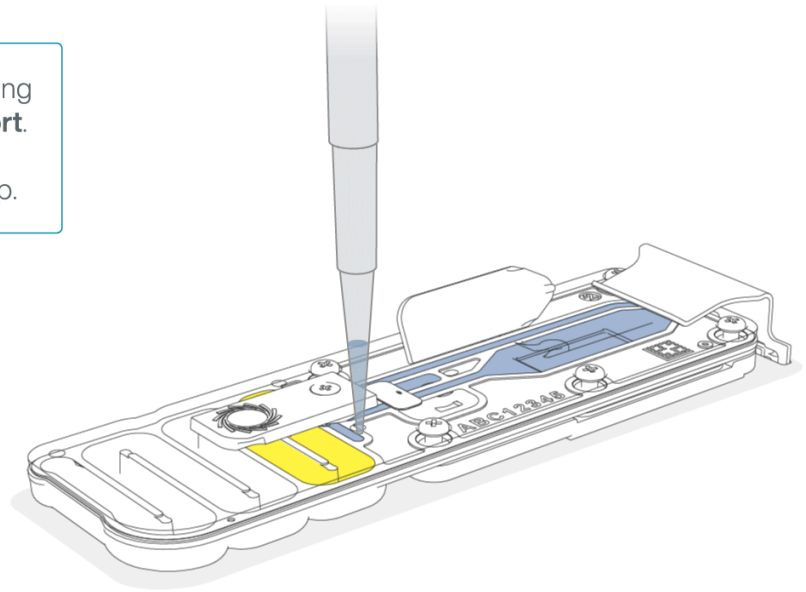


Practice loading an old flow cell

Load final portion of the priming mix to priming port (Not sample port even though it is now open!)

6

Load 200 µl of the priming mix into the **Priming Port**. Ensure there are no air bubbles in the pipette tip.



For best results use the pipette wheel throughout the whole loading process

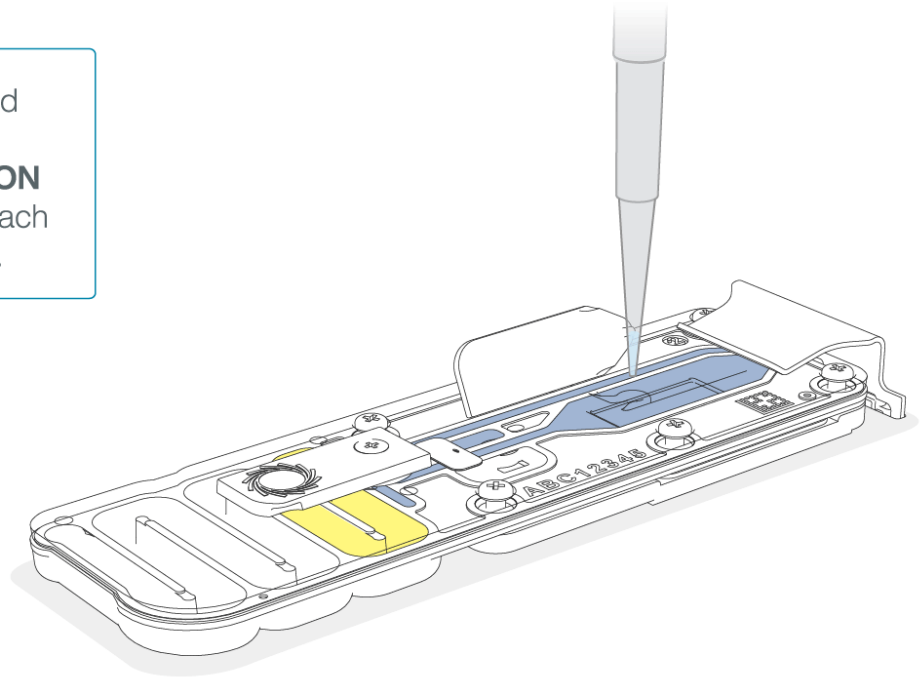
Practice loading an old flow cell

It is finally time to load sample

Remember to pipette sample and beads thoroughly before loading

7

Pipette mix the prepared library and load 75 µl dropwise into the **SpotON** sample port, ensuring each drop flows into the port.



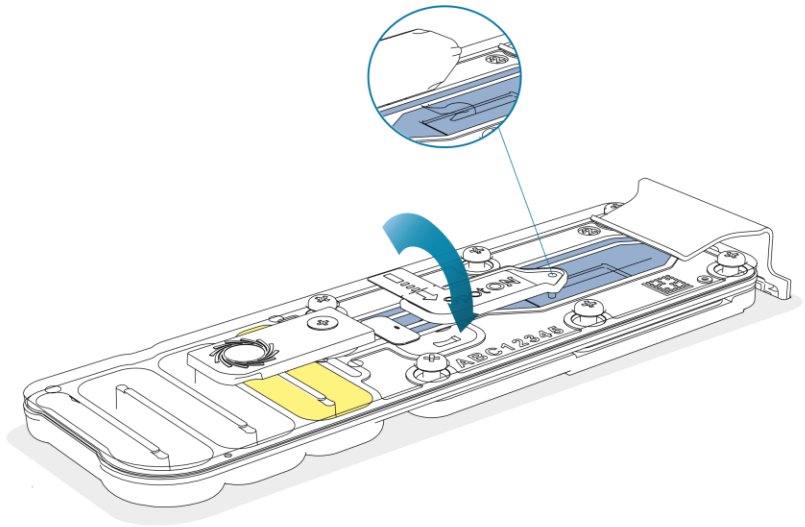
For best results, use the pipette wheel throughout the whole loading process, but you could also do a drop-wise approach

Practice loading an old flow cell

Remember to close ports before starting run

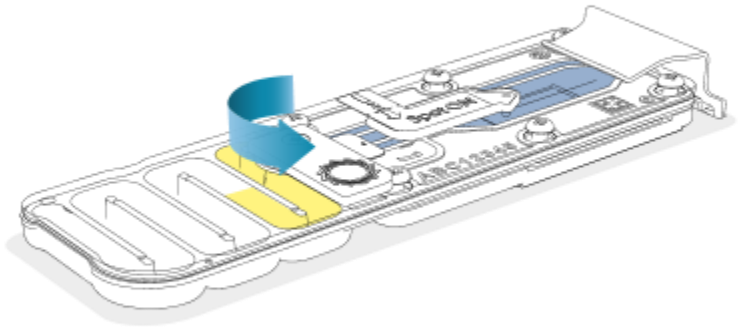
8

Gently replace the **SpotON** sample port cover.



9

Gently close the **Priming port**.



Flow cell loading activity

SpotOn sample port

Priming port

Instructions for loading MinION flow cell

1. Open the priming port, moving it clockwise

2. Load 800 of priming mix (water)

3. Open SpotOn sample port

4. At this point, you would combine your library with loading beads and buffer (for now we already have a tube labeled library)

5. Load 200 ul of priming mix into the priming port (again)

6. Then, finally, load 75 ul of library into the sample port

Both priming and sample port-all in one!

Instructions for loading PromethION flow cell

1. Open the only port, by moving it clockwise

2. Load 500 ul of priming mix (water)

3. At this point, library is combined with loading beads and buffer. We already have a tube labeled library

4. Load 500 ul of priming mix into the priming port (again)

5. Then, finally, load 200 ul of library into the sample port