

Standard Operating Procedure - Diagnostics Validation Experiment

Part II: Clinical Samples

Let's validate your Nanopore Sequencing

Note This validation experiment will be performed on different clinical samples. In duplicate, PathoSense will also analyse these clinical samples. All samples can be processed up to 'Wet lab part 1: Benzonase'. The benzonase treated duplicates can be sent to Pathsense on dry ice. All 12 samples can be loaded together on the same flow cell (= 'Wet lab part 5:ONT').

Sample nr	Sample	Animal Species
1	Faeces 1	Pig
2	Faeces 2	Pig
3	Faeces 3	Pig
4	BALF 1	Cattle
5	BALF 2	Cattle
6	BALF 3	Cattle
7	Organ swab 1	
8	Organ swab 2	
9	Organ swab 3	
10	Serum 1	
11	Serum 2	
12	Serum 3	

WET LAB

1. Collect **different sample types** (see proposal table above). Include 3 separate samples for each sample type. Use the **Pathsense sample kit** for sample collection.
2. Register the samples (not the duplicates) in the **PathoCloud** with the **barcodes** on the kits (or the dummy barcodes provided) > see 'DRY LAB' below.

3. Begin the PathoSense SOPs with "**Wet Lab 1: Benzonase**", ensuring all steps are performed in duplicate:

- Prepare materials and reagents for a total of 24 samples (12 in duplicate).
- Take 24 aliquots of HMB + IC (or HMB without IC for cat/dog samples).
- After filtration, transfer **2 × 200 µL** of the sample as follows:
 - **200 µL** into the aliquot HMB+IC designated for lab validation.
 - **200 µL** into the aliquot HMB+IC designated for duplicate testing (PathoSense validation).

4. After 'Wet lab part 1: Benzonase' send **duplicates** on dry ice to PathoSense.

5. Continue the PathoSense **SOPs**.

6. Fill in the Minknow software*:

- Experiment name : date_name-run
- Kit selection : SQK-RBK114.24
- Run duration : 9 hours
- Advanced options > time between pore scans : 1 hour + deselect reserve pores
- Basecalling > Edit options > basecalling model : super-accurate basecalling
- Barcoding > Edit options > select 'trim barcodes' and 'mid-read barcode filtering'
- Output location* : /path-to-/PathoSense_Diagnostics

*Path to shared folder

- Qscore : 1

7. Write the output to the PathoSense_Diagnostics folder*

* shared folder

8. After sequencing (9h), the data will be transferred to PathoSense via the cloud and will be further analysed at PathoSense.

*find below instruction on using MinKNOW and how to generate a sequencing run (file)

How to generate a sequencing run (file)

DRY LAB

Register the respective samples via <https://cloud.pathosense.com/analyses>

And register with your account (account will be provided by PathoSense)

1. Add New Analysis

Registered office
PathoSense BV
Pastoriestraat 10
2500 Lier, Belgium
VAT BE 0755 557 744

Laboratories
PathoSense BV, UGent
Entrance 24, 1st floor
Salisburylaan 133
9820 Merelbeke, Belgium

[Linkedin](#)
[Facebook](#)
[Instagram](#)
[Twitter](#)
info@pathosense.com



+ NEW ANALYSIS

2. Fill in all mandatory fields as shown below

Note add information about the validation run to the Lab Feedback

User*
Test DGZ

Existing Customer New customer

Customer*
DGZ

Status
Arrived

Purpose
Diagnostics

Animal*
Other

Animal Anamnesis

Lab Feedback

Validatie run 1 - dPBS

CLOSE DIALOG **SUBMIT ANALYSIS**

3. Add sample via ‘+’ sign

	Veterinarian	Customer	Animal
	Test DGZ	DGZ	Other
	+		

4. Fill in all fields as shown below

Note 1. Make sure the status of the sample is changed to ‘Arrived’
 2. Add information about the sample via the identifier field

Status
Arrived

Barcode
2-0000005

identifier
dPBS Manueel 1 WB PCR

Sample type*
Serum

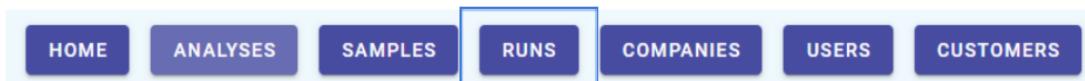
Animal Count
1

Sick

CLOSE DIALOG
SUBMIT SAMPLE

5. Repeat step 3 and 4 for each sample

6. Go to the '*Run*' page



7. Add *New Run*

Note First select amount of samples for the run in the '*Size of run*' field

8. A new run empty is created

Id	Title	Description	Protocol	Created										
664	No title			01:00 March 09, 2023										

9. Add a title to the run by double clicking on 'No title'

Note Fill in date and flow cell ID (to be found on flow cell) for traceability

Registered office
 PathoSense BV
 Pastoriestraat 10
 2500 Lier, Belgium
 VAT BE 0755 557 744

Laboratories
 PathoSense BV, UGent
 Entrance 24, 1st floor
 Salisburylaan 133
 9820 Merelbeke, Belgium

[Linkedin](#)
[Facebook](#)
[Instagram](#)
[Twitter](#)
info@pathosense.com

10. Select run by clicking next to the title
 → the run will turn blue when selected

Id	Title	Description	Protocol	Created				
664	data_flowcell_Run	10:05 March 09, 2023						
Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty

11. Click on the respective samples in the 'arrived' field to add the sample to the run

En Route

Arrived

Diagnostics

2-0000002

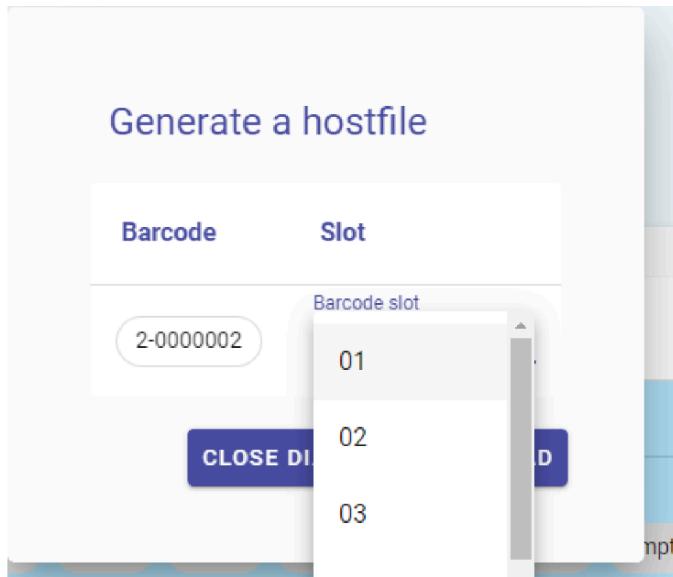
Protocol	Created
10:05 March 09, 2023	
2-0000002	

12. Click on the save run file button to create a run file

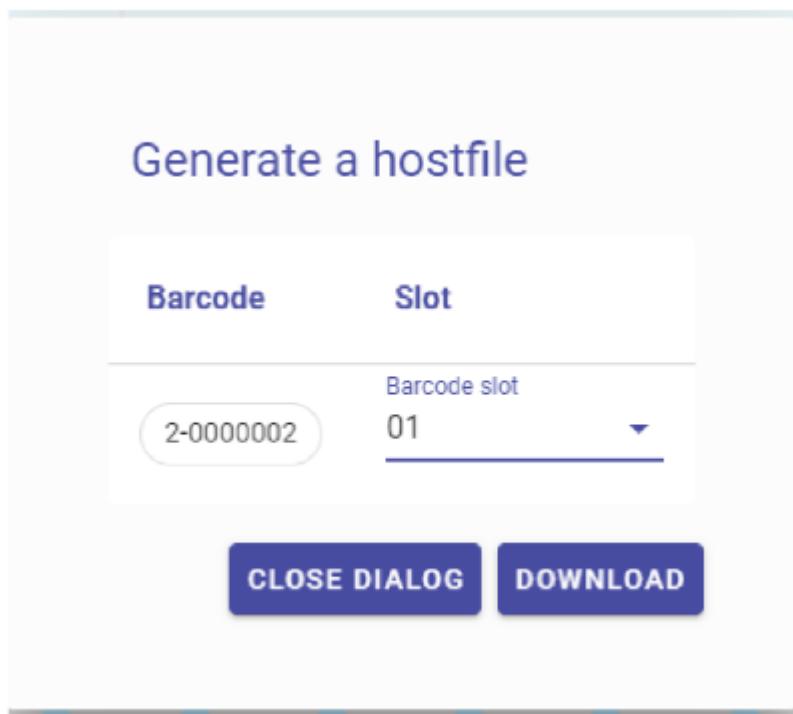
Save run file

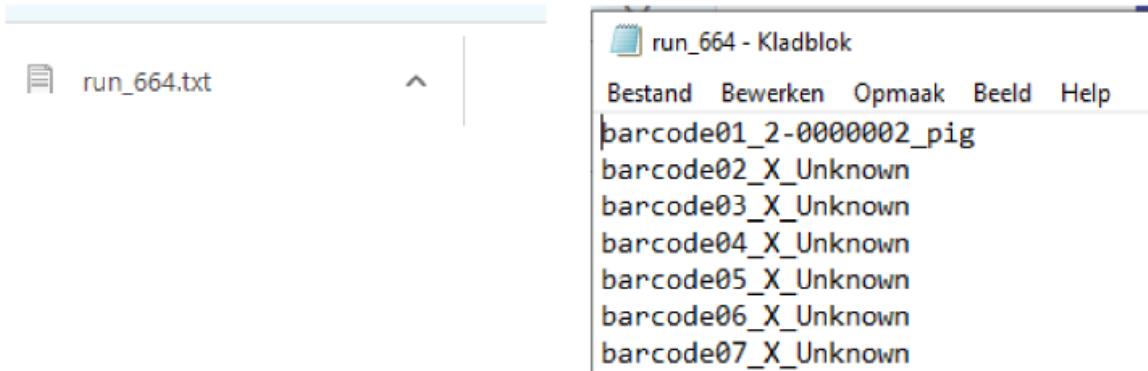
13. Select for each sample the correct corresponding barcode from the ONT kit



14. Download the run file (=host file)

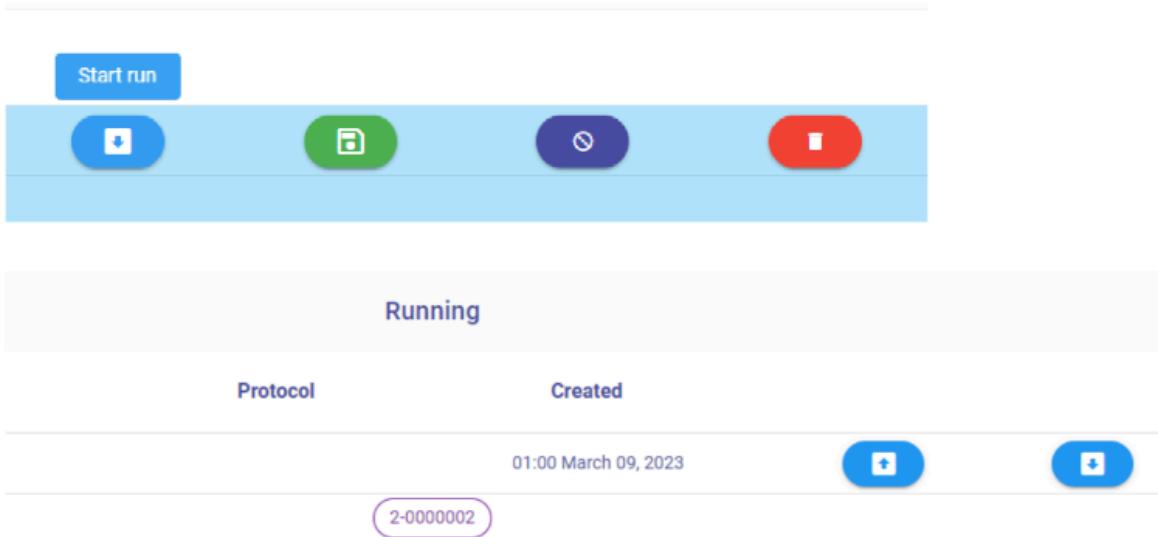


15. A run file (.txt) has been created, see example below



```
run_664 - Kladblok
Bestand Bewerken Opmaak Beeld Help
barcode01_2-0000002_pig
barcode02_X_Unknown
barcode03_X_Unknown
barcode04_X_Unknown
barcode05_X_Unknown
barcode06_X_Unknown
barcode07_X_Unknown
```

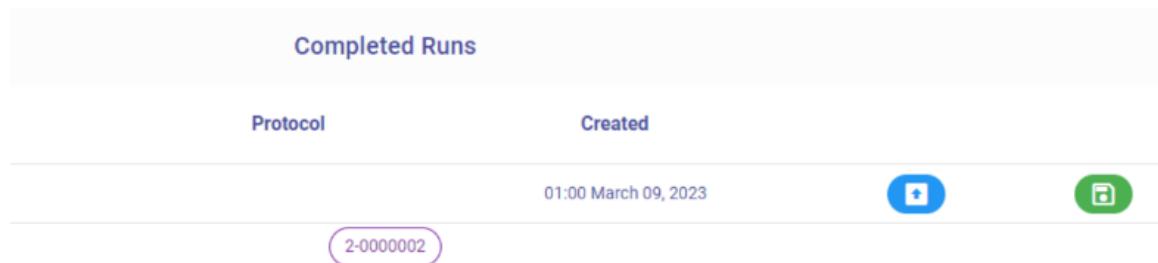
16. Click on 'Start run' to move the run to 'Running'



Running

Protocol	Created
01:00 March 09, 2023	 
2-0000002	

17. When the run is complete, move the run to 'Completed runs'

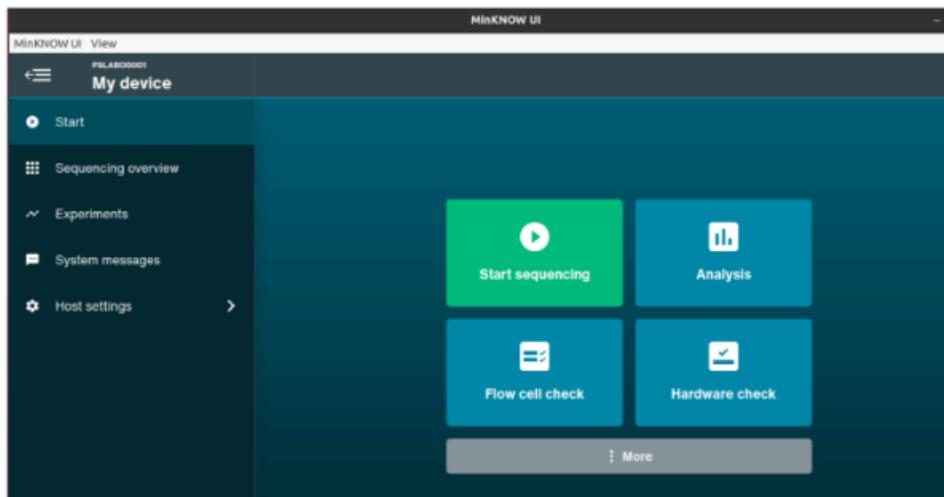


Completed Runs

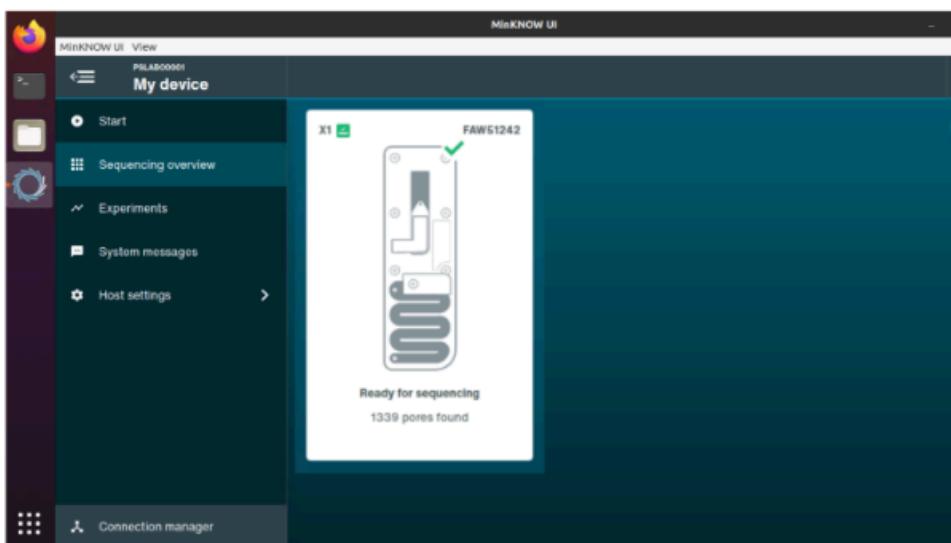
Protocol	Created
01:00 March 09, 2023	 
2-0000002	

Using MinKNOW to start a sequencing run

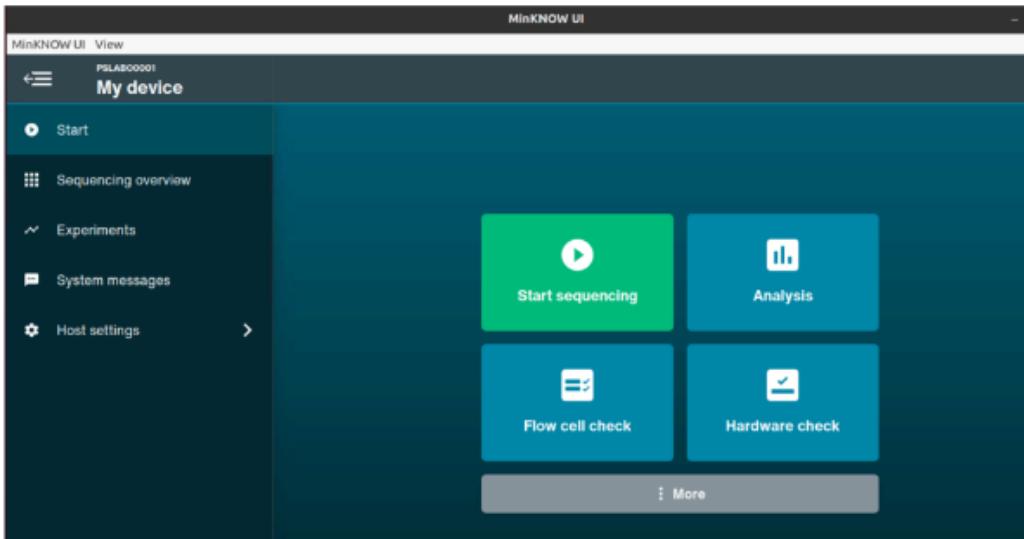
1. Connect the MinION to the computer.
2. Open MinKNOW
3. Insert your flow cell in the MinION or GridION and perform a flow cell check via **Start > Flow cell check**



4. When **>1100 pores** found, flow cell is ready for sequencing

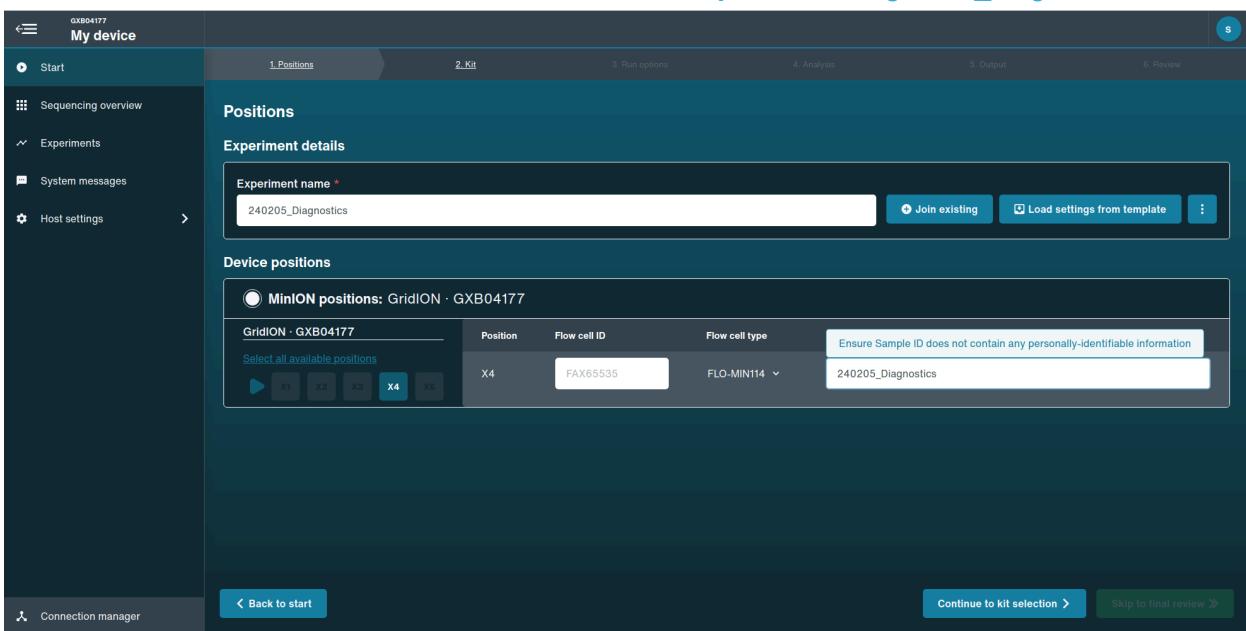


5. Go to the 'Start' tab en press 'Start sequencing'



6. In the following screen, enter an experiment name.

Note 1. This name will be given to the output directory and should not contain spaces or slashes
 2. This should be a **different name for each experiment**. E.g. date_diagnosen



Experiment details

Experiment name: 240205_Diagnostics

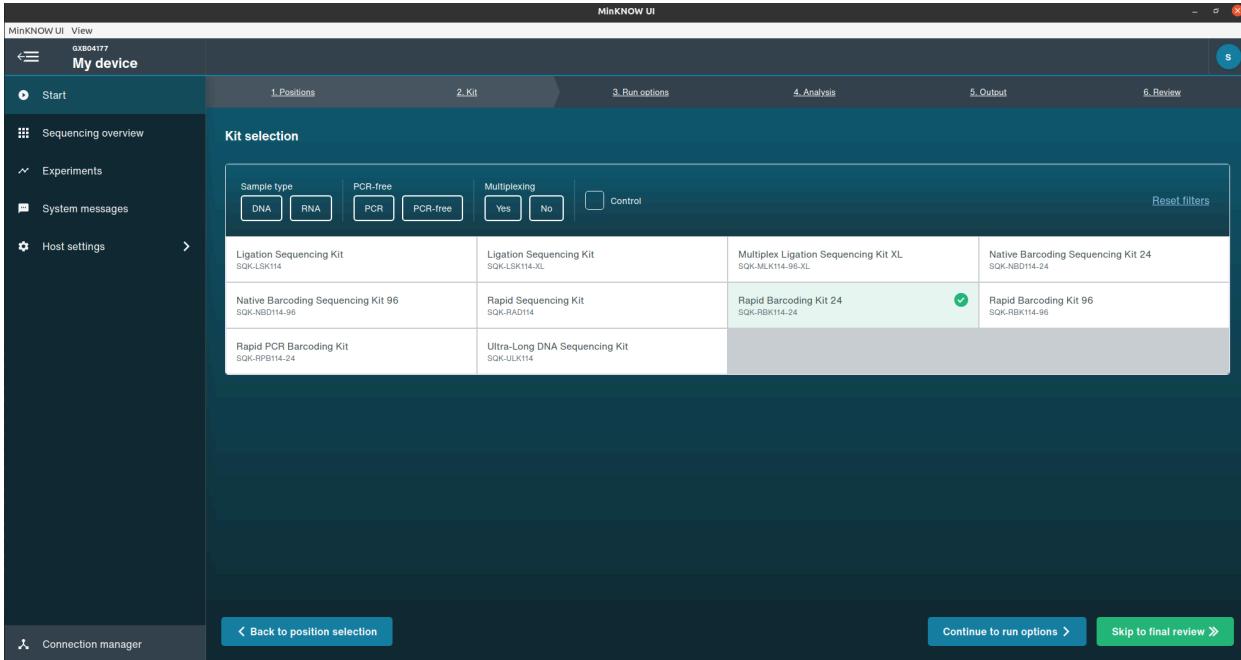
Device positions

MinION positions: GridION - GXB04177

GridION - GXB04177	Position	Flow cell ID	Flow cell type
Select all available positions	X4	FAX65535	Ensure Sample ID does not contain any personally-identifiable information FLO-MIN114 240205_Diagnostics

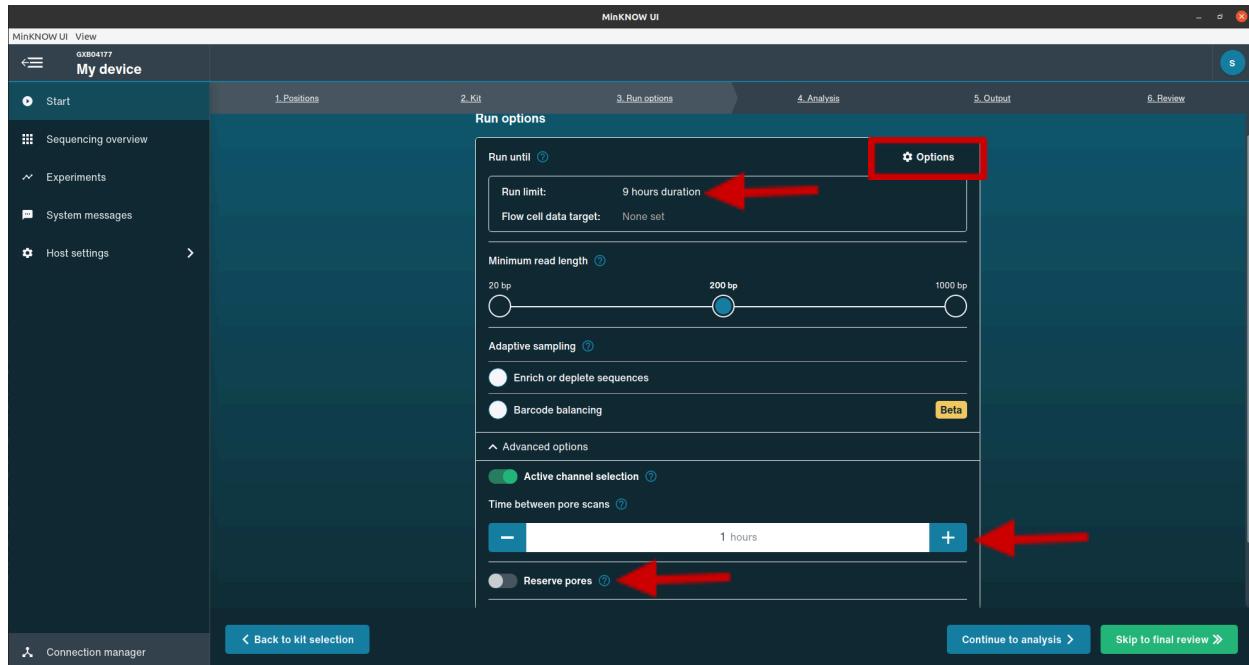
7. Enter the same name as chosen for the experiment to the **sample ID** field
8. Continue to kit selection and select the used kit

Note For R10 this will be SQK-RBK114.24



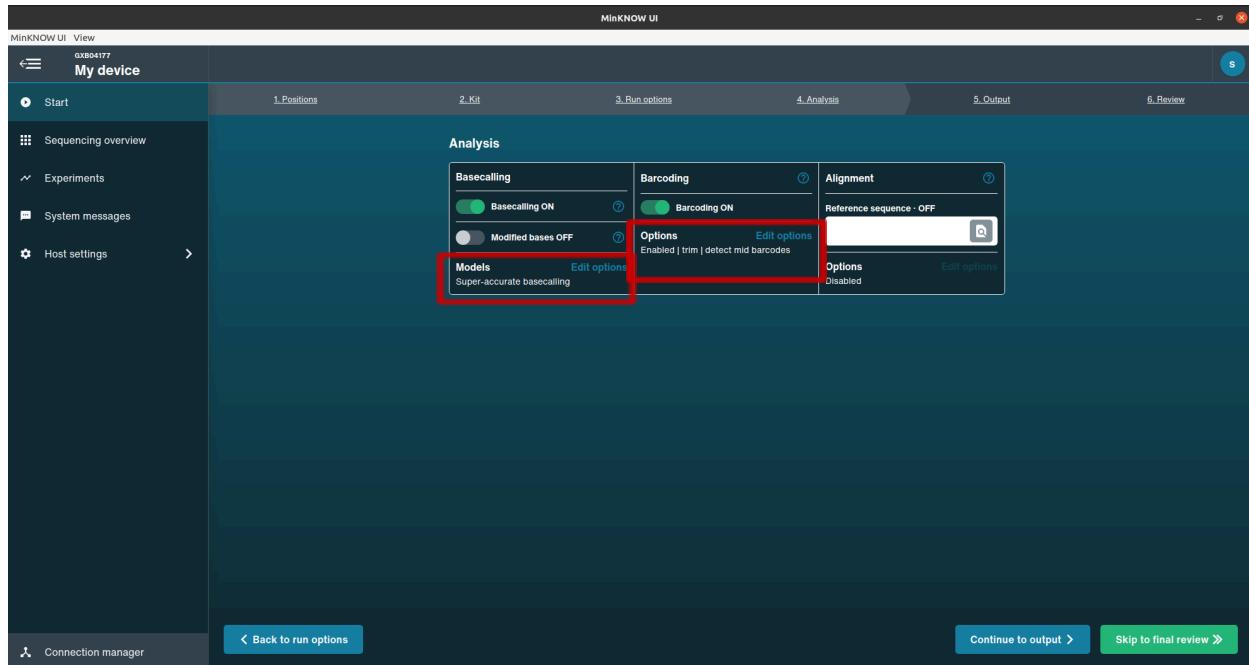
Sample type	PCR-free	Multiplexing	Control	
DNA	PCR	Yes	<input checked="" type="checkbox"/>	Reset filters
SQK-LSK114	SQK-RA0114	SQK-MLK114-96-XL	SQK-NBD114-24	
Ligation Sequencing Kit	Ligation Sequencing Kit	Multiplex Ligation Sequencing Kit XL	Native Barcoding Sequencing Kit 24	
SQK-LSK114	SQK-RA0114	SQK-MLK114-96-XL	SQK-NBD114-24	
Native Barcoding Sequencing Kit 96	Rapid Sequencing Kit	Rapid Barcoding Kit 24	Rapid Barcoding Kit 96	
SQK-NBD114-96	SQK-RA0114	SQK-RBK114-24	SQK-RBK114-96	
Rapid PCR Barcoding Kit	Ultra-Long DNA Sequencing Kit			
SQK-RBK114-24	SQK-ULK114			

9. Continue to run options



- Adapt Run duration to **9 hours**
- Select Advanced options > time between pore scans : **1 hour**
- Select Advanced options > **deselect reserve pores**

10. Continue to analysis



a. **Basecalling** > edit options > **Super-accurate basecalling** > save

b. **Barcoding** > Edit options >

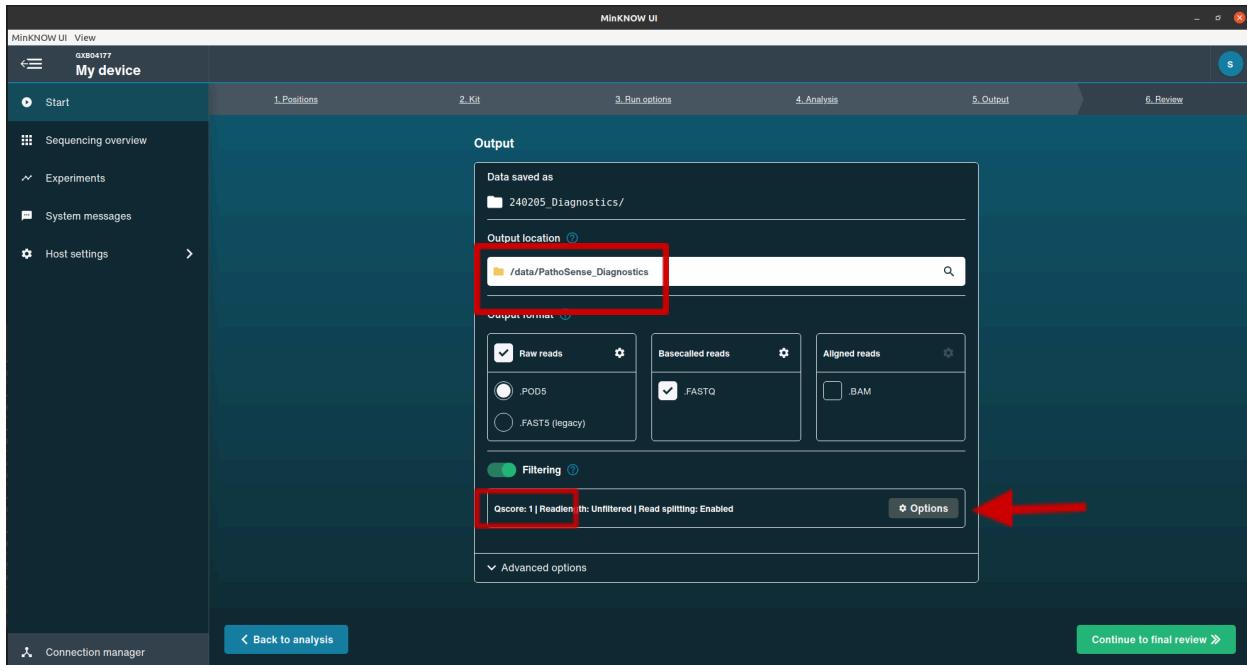
Select '**Trim barcodes**' and '**Mid-read-barcode filtering**' > save

11. Continue to output

a. Change the output location* to **/home/Pathosense_Diagnostics**.

*Path to shared folder

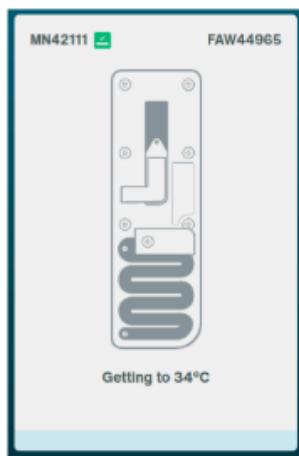
d. In the '**Filtering**' section, press 'options' and change the Qscore to 1.



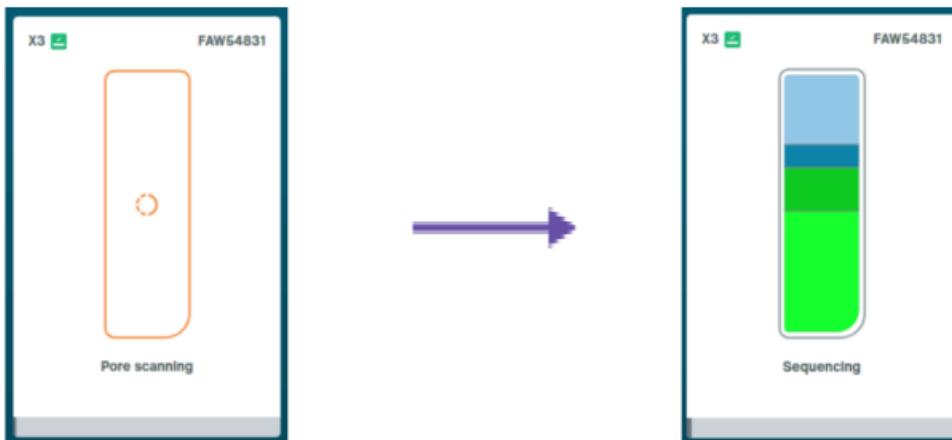
12. Continue to final review

16. Press “Start”

17. You should see the MinION getting to temperature and start the mux scan. This will take about 5 to 10 minutes.



18. After 10 minutes the sequencing run will start

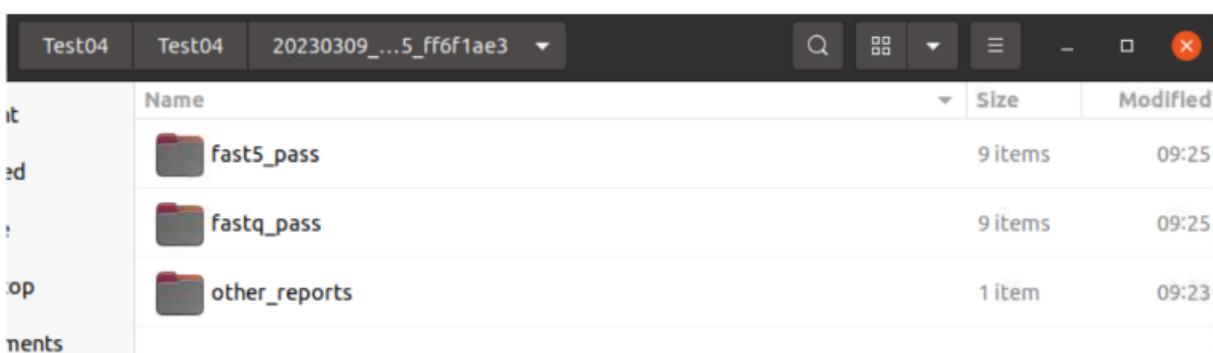


19. The output directory

(named after your experiment name: date_diagnosen_firstbarcode_lastbarcode)
will be automatically created in the output location* **/home/Pathosense_Diagnostics**

*Path to shared folder

20. Click through the subdirectories inside the experiment directory until you see the 'other_reports'/'fast5_pass'/'fastq_pass' directories.



21. Copy paste the generated run.txt file from downloads in this directory like shown below



Test04	Test04	20230309_...5_ff6f1ae3	▼	Q	grid	▼	≡	-	□	✖
t	d	op	ments	cards	Name	▼	Size	Modified		
					fast5_pass		9 items	09:25		
					fastq_pass		9 items	09:25		
					other_reports		1 item	09:23		
					run_210.txt		304 bytes	27 Feb		

Everything is set up now!

When the sequencing is done, the data will be automatically sent to the cloud for analysis.