



## 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](mailto: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

↶

↷

B

I

U

☒

☒

<>

☰

☷

🔗

H1

H2

H3

P

—

Validatie run 1 - dPBS

CLOSE DIALOG

SUBMIT 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](mailto:info@pathosense.com)



3. Add sample via '+' sign

 Veterinarian	Customer	Animal
Test DGZ	DGZ	<span>Other</span>
<span>+</span>		

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

1 / 10

☐ Sick

CLOSE DIALOG

SUBMIT SAMPLE

5. Repeat step 3 and 4 for each sample

#### 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](mailto:info@pathosense.com)



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										
Empty Empty Empty Empty Empty Empty Empty Empty Empty Empty Empty Empty Empty Empty Empty														

Items per page 10 Page 1

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





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

id	Title	Description	Protocol	Created															
664	date_flowcell_run			10:05 March 09, 2023															
		Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty

Items per page: 10 Page: 1 |< - + >|

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

		Diagnostics
En Route		
Arrived		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

Generate a hostfile

Barcode	Slot
2-0000002	Barcode slot 01 02 03

CLOSE DIALOG

14. Download the run file (=host file)

Generate a hostfile

Barcode	Slot
2-0000002	Barcode slot 01

CLOSE DIALOG DOWNLOAD

#### 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](#)

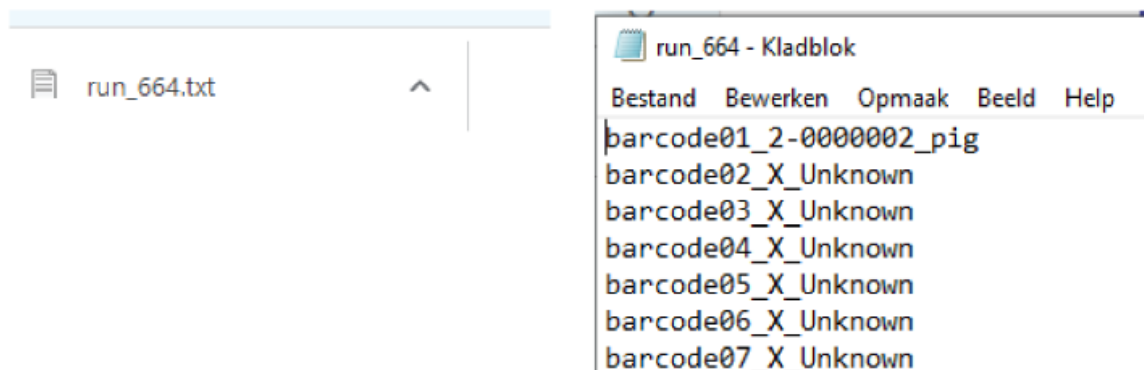
[Instagram](#)

[Twitter](#)

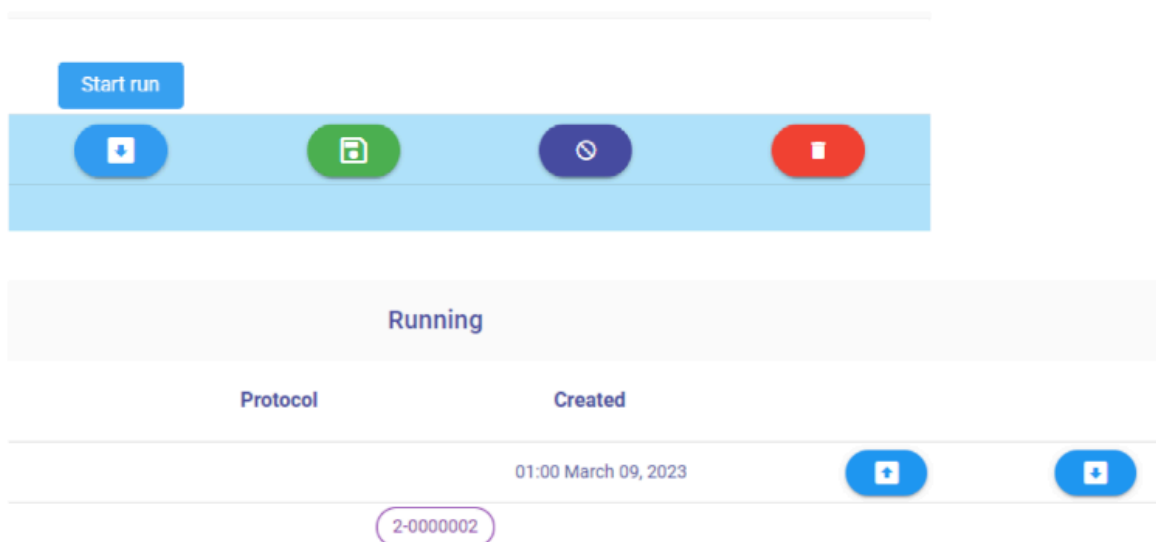
[info@pathosense.com](mailto:info@pathosense.com)



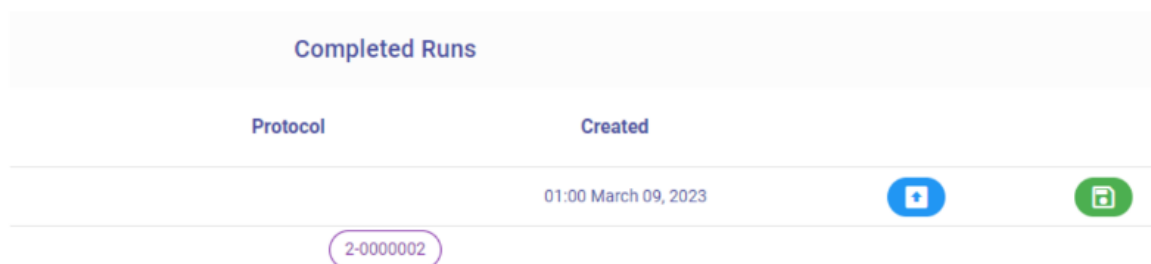
15. A run file (.txt) has been created, see example below



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



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

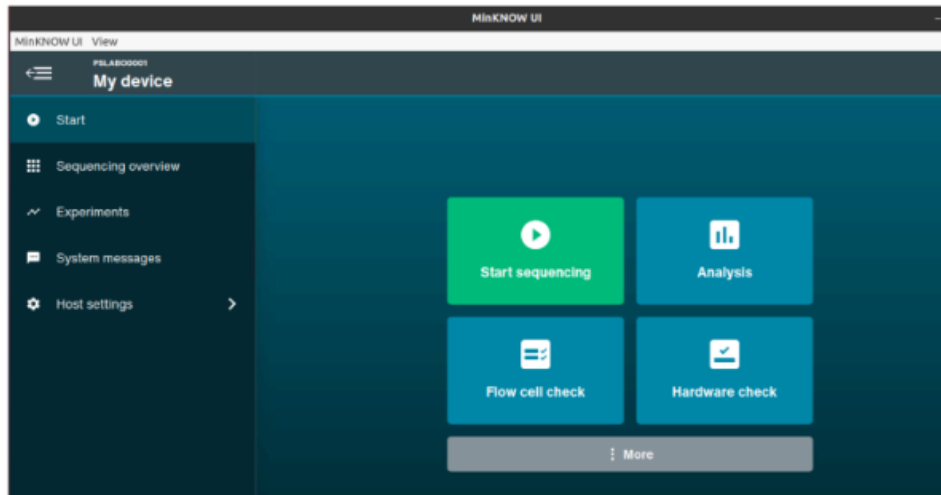




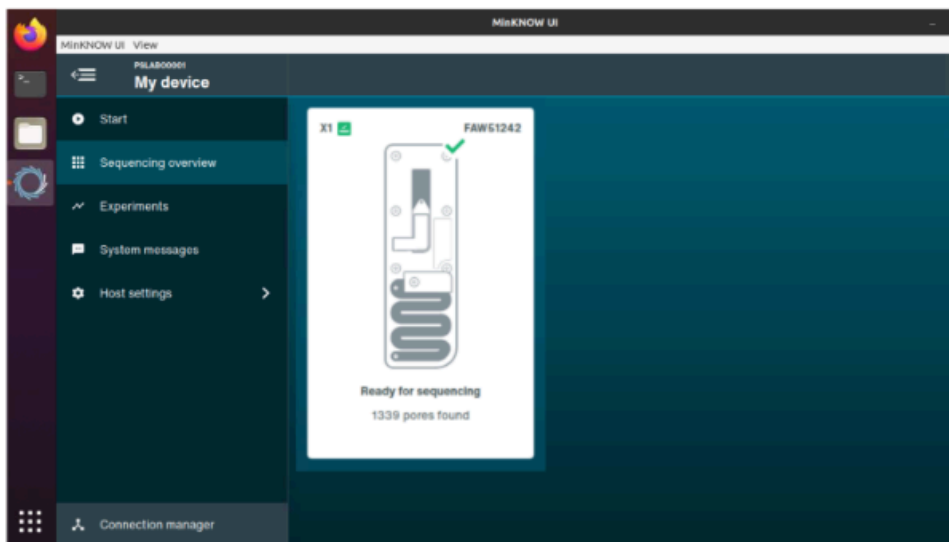


## Using MinKNOW to start a sequencing run

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



### 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](#)

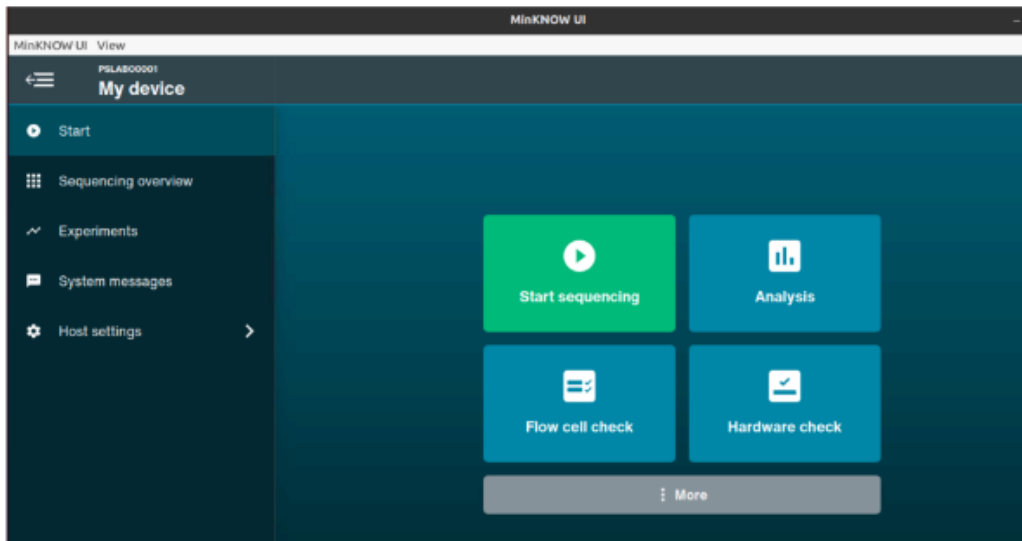
[Instagram](#)

[Twitter](#)

[info@pathosense.com](mailto:info@pathosense.com)

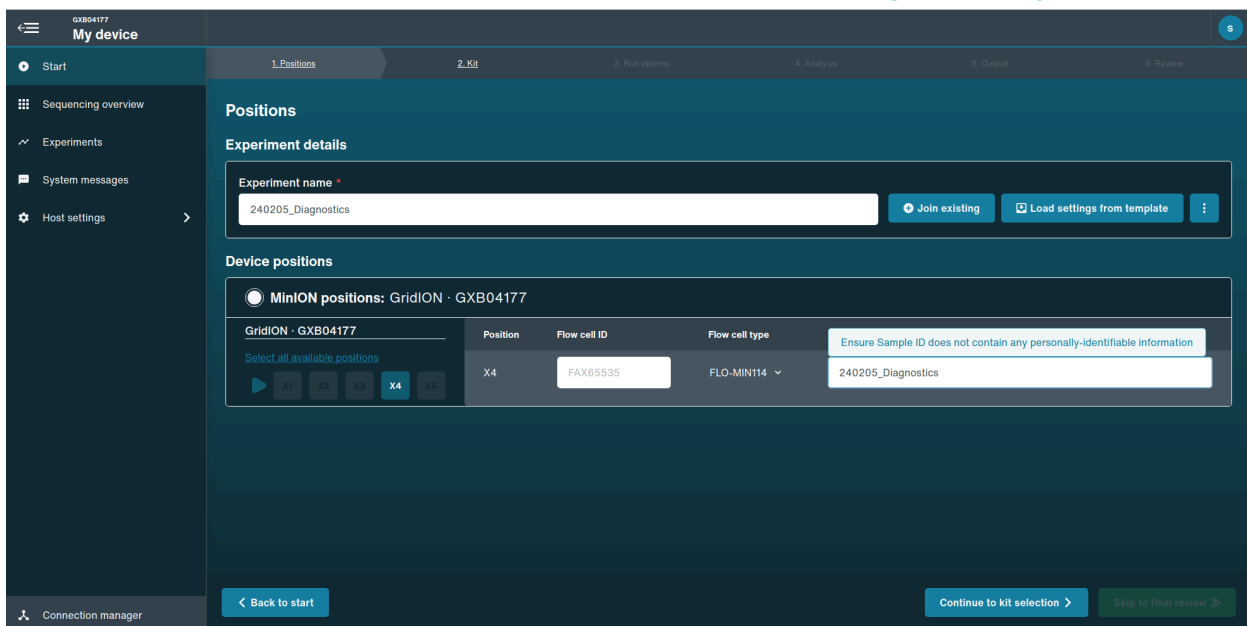


5. Go to the 'Start' tab and press 'Start sequencing'



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





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

MinKNOW UI View

63804177 My device

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

Sequencing overview Experiments System messages Host settings Connection manager

### Kit selection

Sample type:   PCR-free:   Multiplexing:   ☐ Control [Reset filters](#)

Ligation Sequencing Kit SQK-LSK114	Ligation Sequencing Kit SQK-LSK114-XL	Multiplex Ligation Sequencing Kit XL SQK-MLK114-96-XL	Native Barcoding Sequencing Kit 24 SQK-NBD114-24
Native Barcoding Sequencing Kit 96 SQK-NBD114-96	Rapid Sequencing Kit SQK-RAD114	Rapid Barcoding Kit 24 SQK-RBK114-24	Rapid Barcoding Kit 96 SQK-RBK114-96
Rapid PCR Barcoding Kit SQK-RPB114-24	Ultra-Long DNA Sequencing Kit SQK-ULK114		

< Back to position selection Continue to run options > Skip to final review >>

9. Continue to run options

#### 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

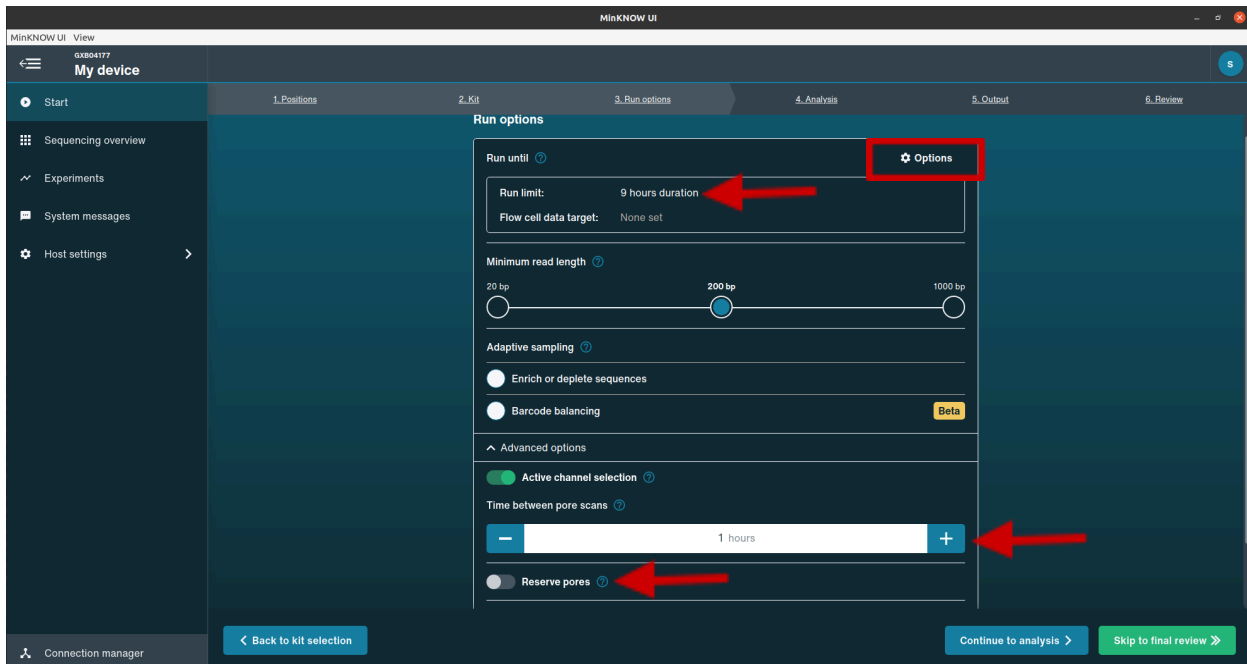
#### LinkedIn

[Facebook](#)

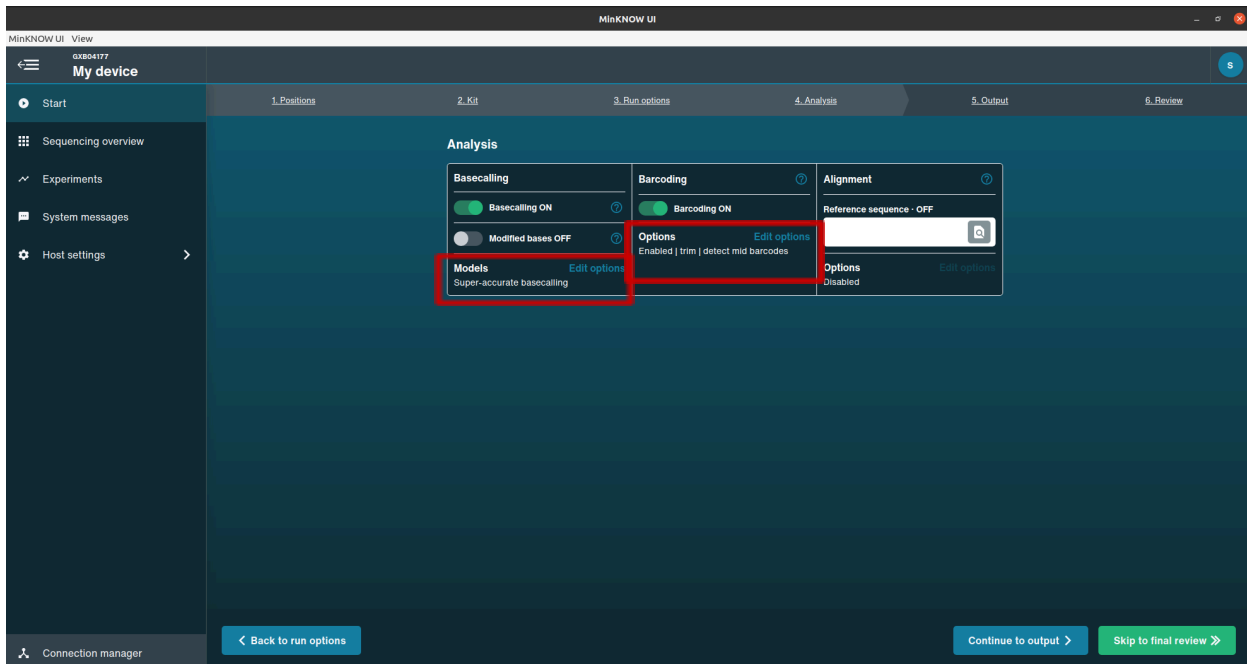
[Instagram](#)

[Twitter](#)

[info@pathosense.com](mailto:info@pathosense.com)



- a. Adapt Run duration to **9 hours**
  - b. Select Advanced options > time between pore scans : **1 hour**
  - c. 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.

## 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

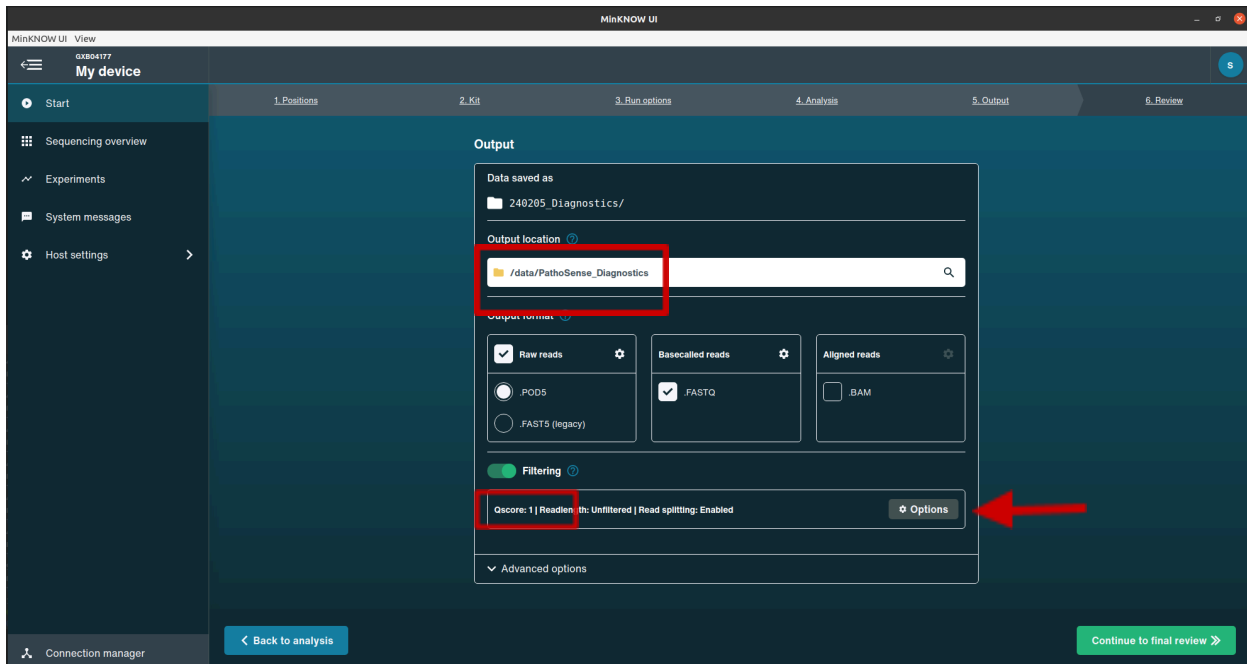
## Linkedin

[Facebook](#)

[Instagram](#)

[Twitter](#)

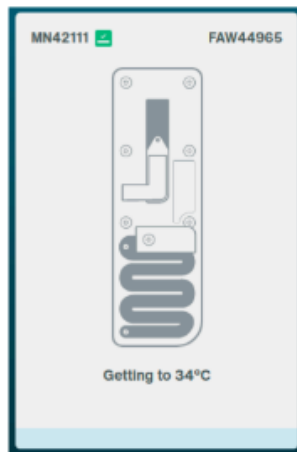
[info@pathosense.com](mailto:info@pathosense.com)



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

#### 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

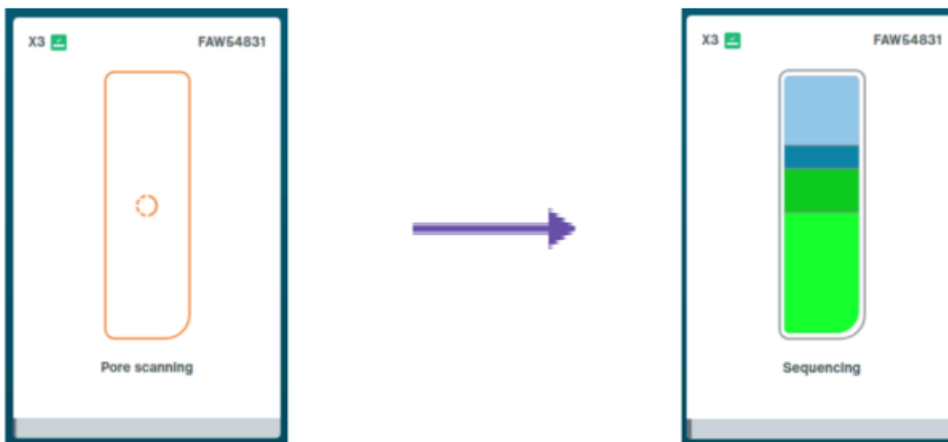
#### Linkedin

[Facebook](#)

[Instagram](#)

[Twitter](#)

[info@pathosense.com](mailto:info@pathosense.com)



## 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.

Name	Size	Modified
fast5_pass	9 items	09:25
fastq_pass	9 items	09:25
other_reports	1 item	09:23

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

### 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](mailto:info@pathosense.com)



Test04	Test04	20230309_...5_ff6f1ae3	Q						
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.

### 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](mailto:info@pathosense.com)