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.

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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

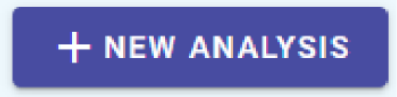
8. After sequencing (9h), the data will be transferred to PathoSense via the cloud and will be further analysed at PathoSense.

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

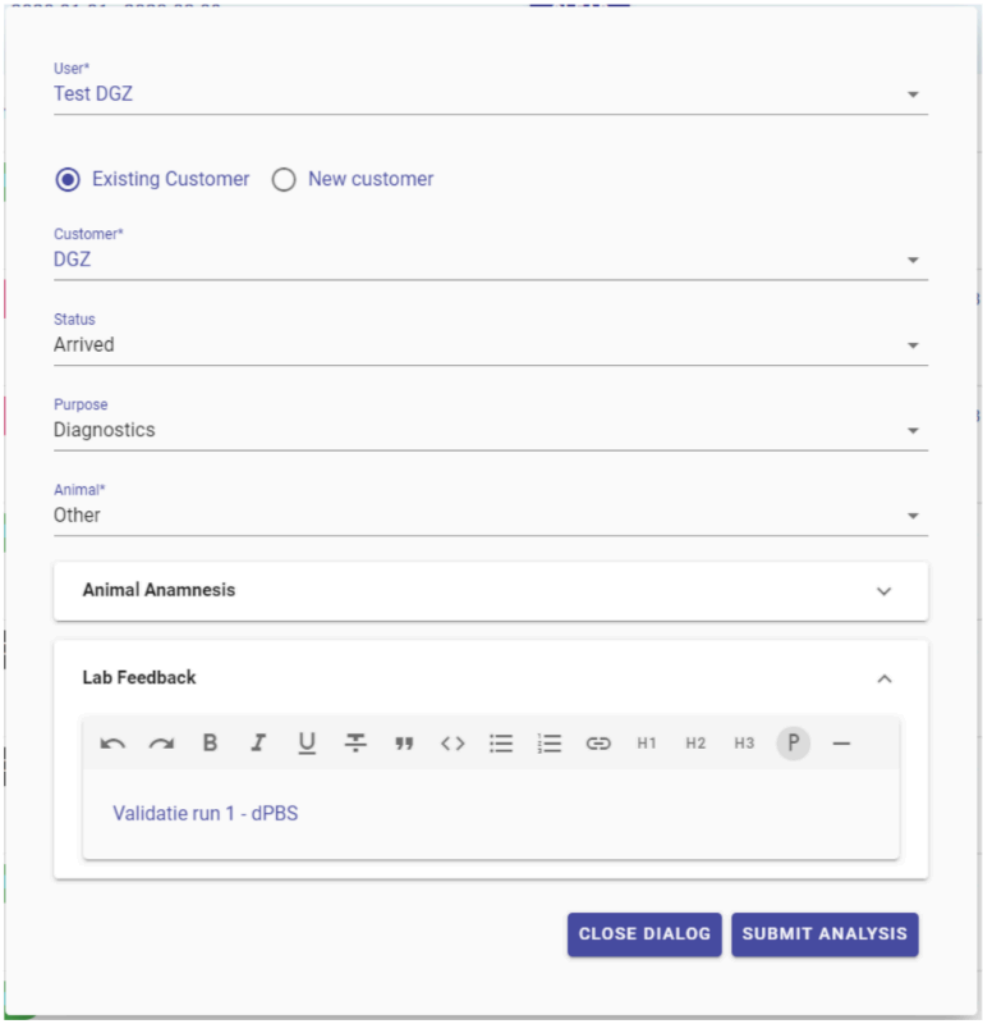
DRY LAB

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



2. Fill in all mandatory fields as shown below
Note add information about the validation run to the Lab Feedback



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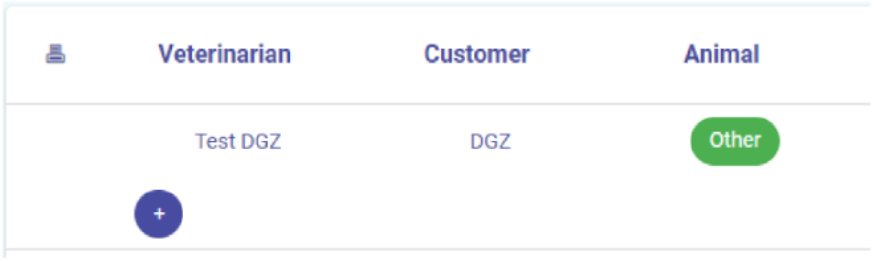
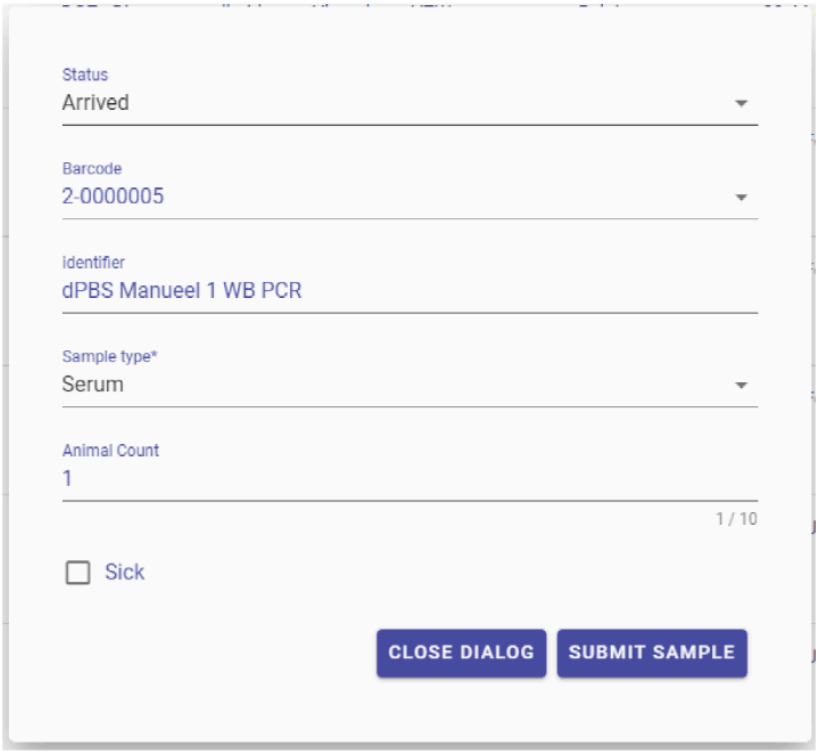
3. Add sample via ‘+’ sign



4. Fill in all fields as shown below

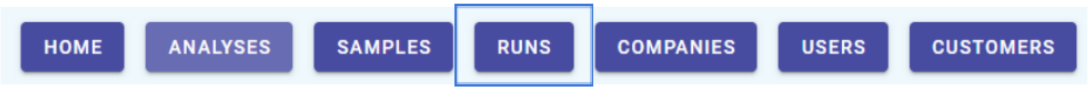
Note 1. Make sure the status of the sample is changed to ‘Arrived’

2. Add information about the sample via the identifier field



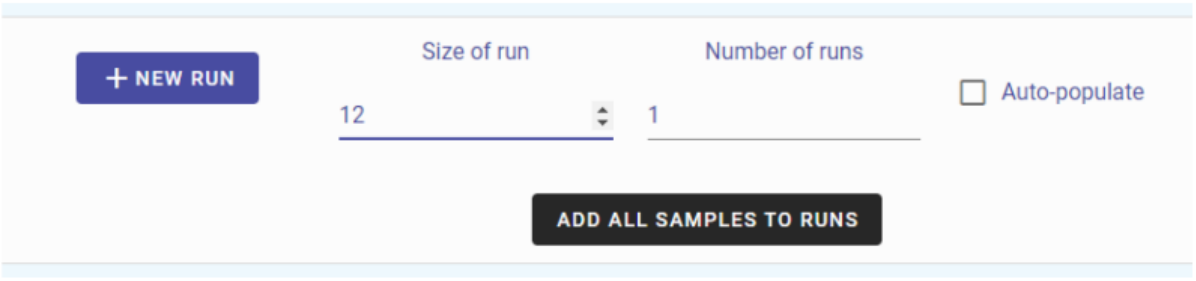
5. Repeat step 3 and 4 for each sample

6. Go to the '*Run*' page

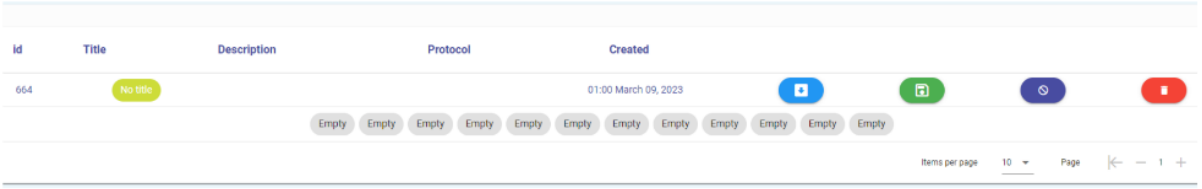


7. Add *New Run*

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

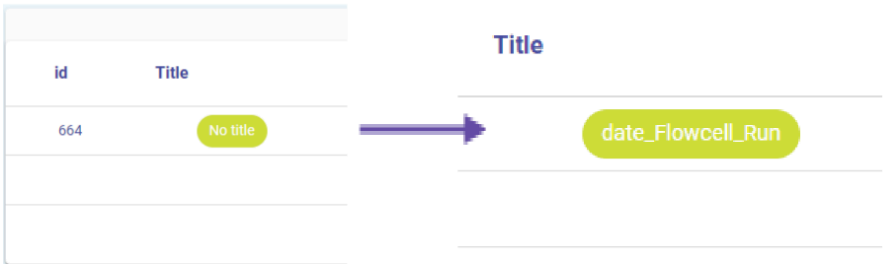


8. A new run empty is created



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



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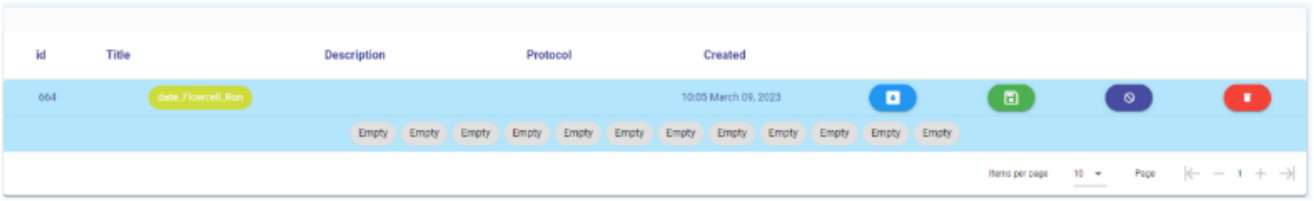
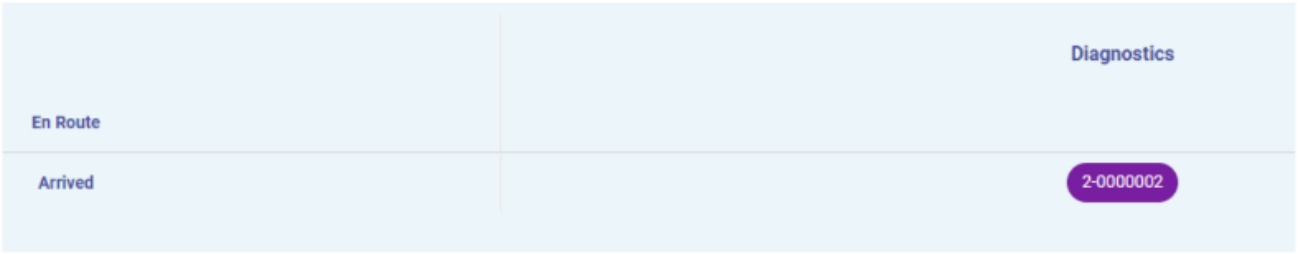
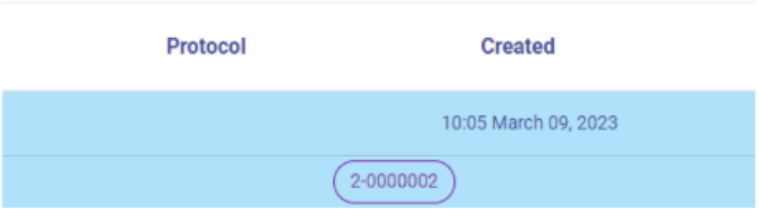
10. Select run by clicking next to the title
→ the run will turn blue when selected

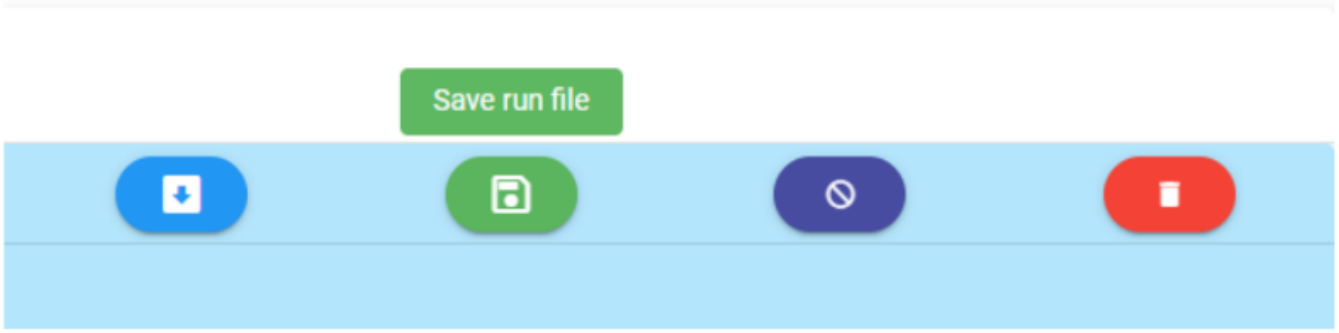


11. Click on the respective samples in the ‘arrived’ field to add the sample to the run





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



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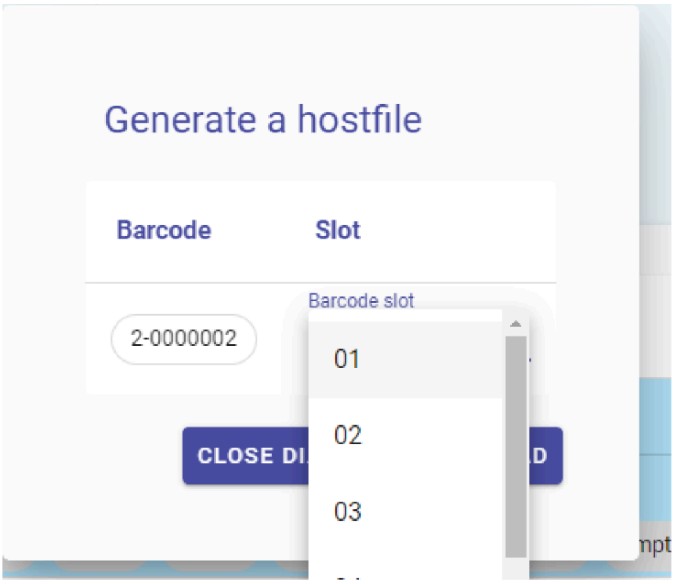
13. Select for each sample the correct corresponding barcode from the ONT kit



14. Download the run file (=host file)

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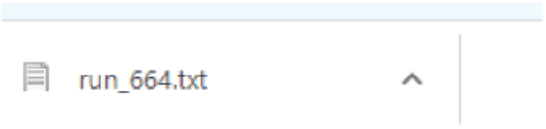
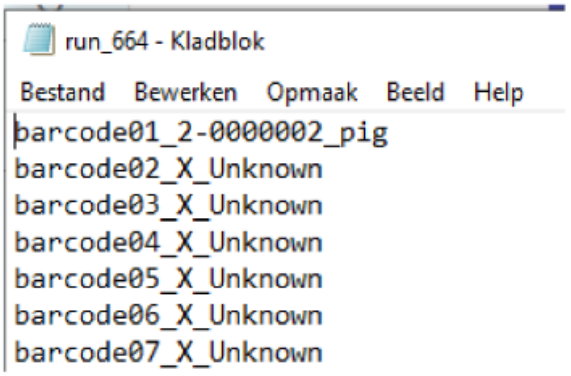
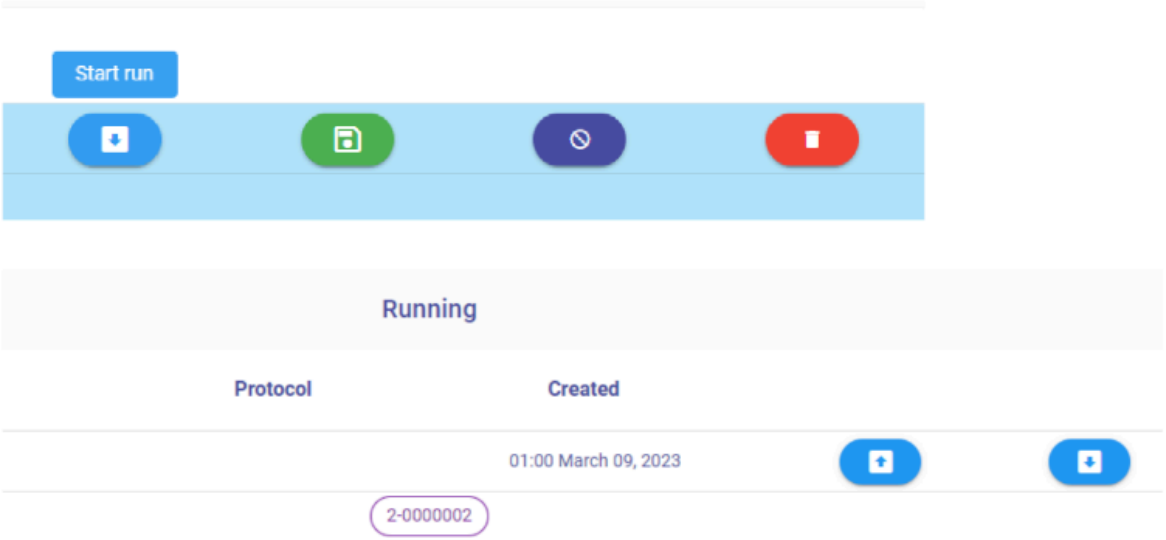
15. A run file (.txt) has been created, see example below

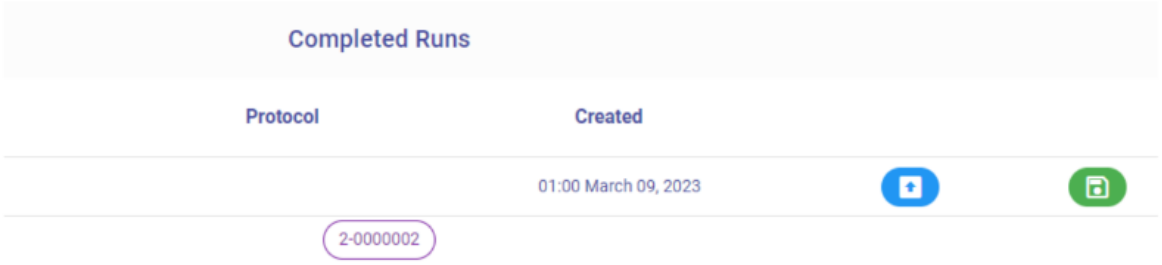




16. Click on '*Start run*' to move the run to '*Running*'



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



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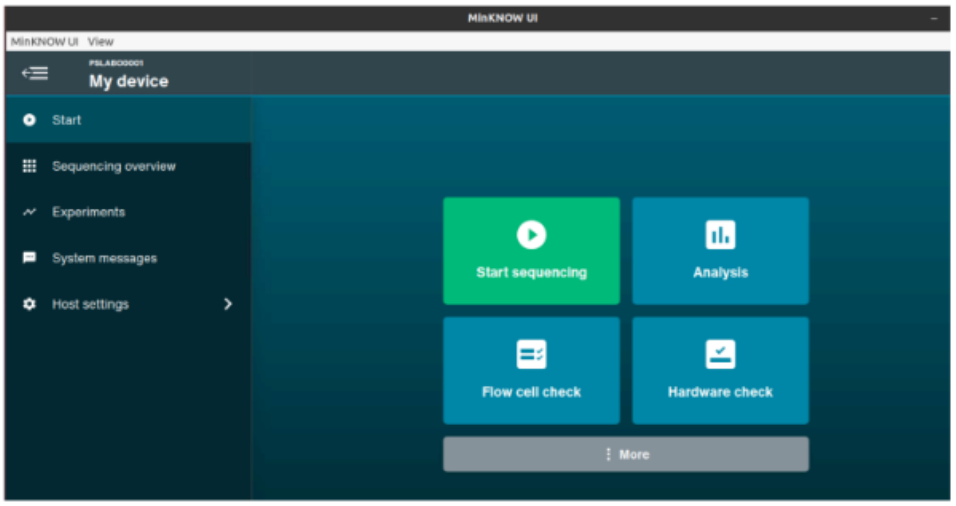
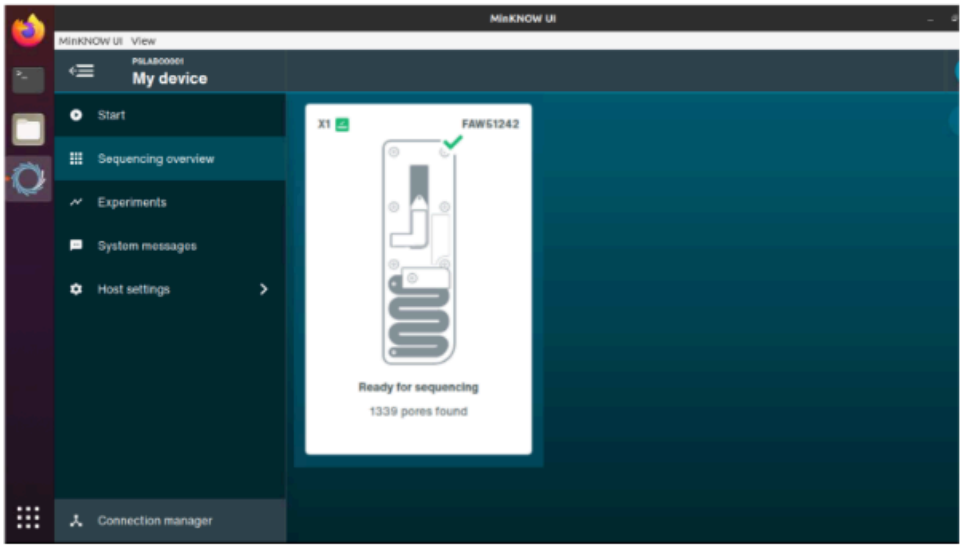
Using MinKNOW to start a sequencing run

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



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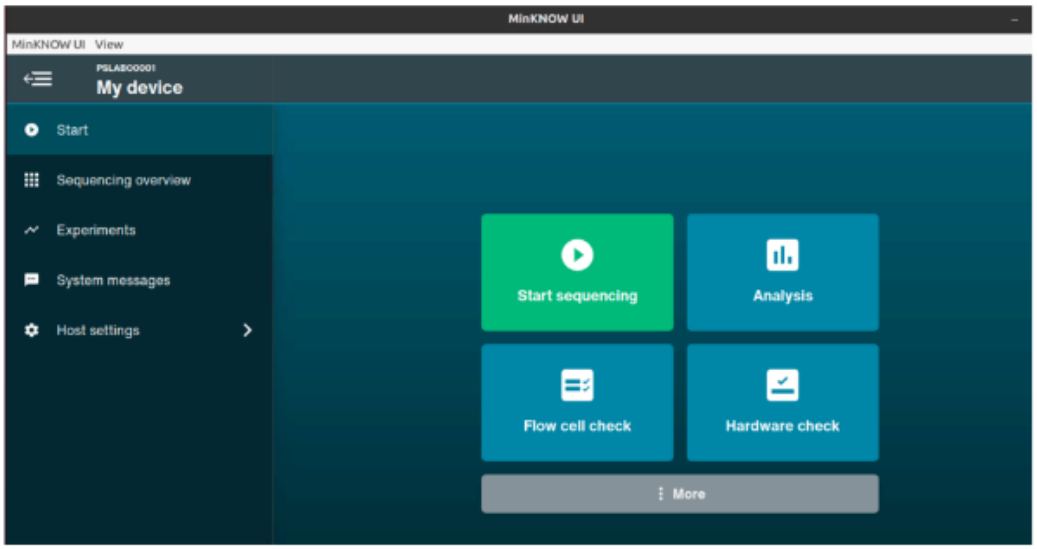
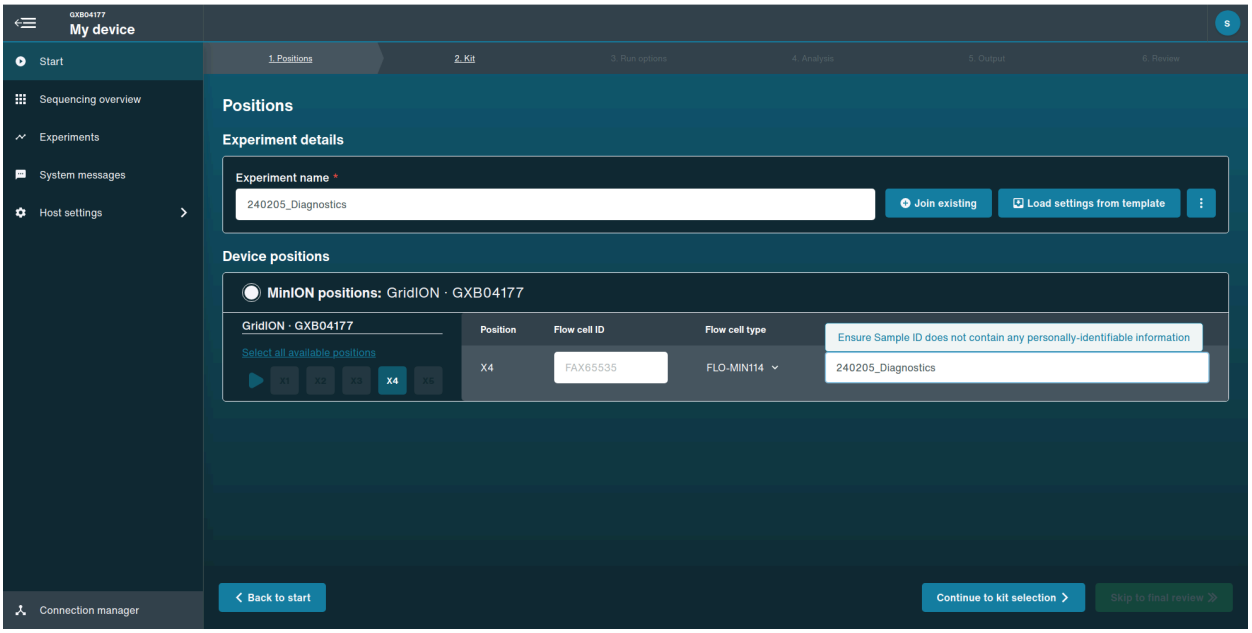
5. Go to the '**Start**' tab en press '**Start sequencing**'



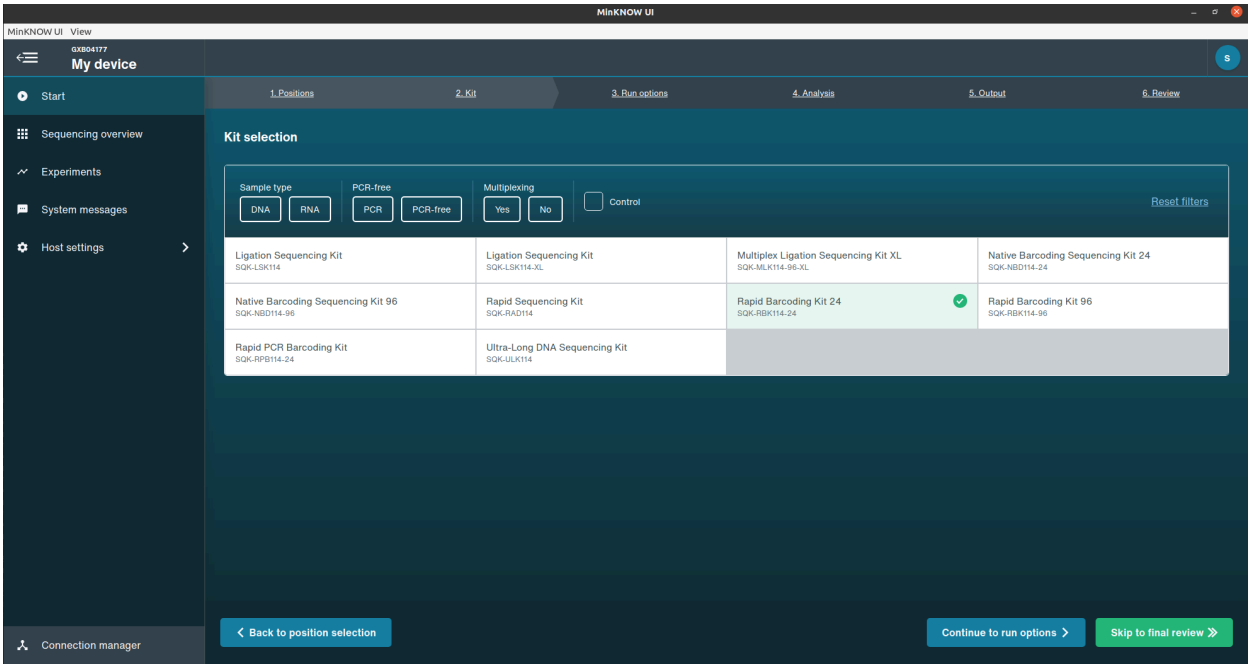
6. In the following screen, enter an experiment name.

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



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



9. Continue to run options

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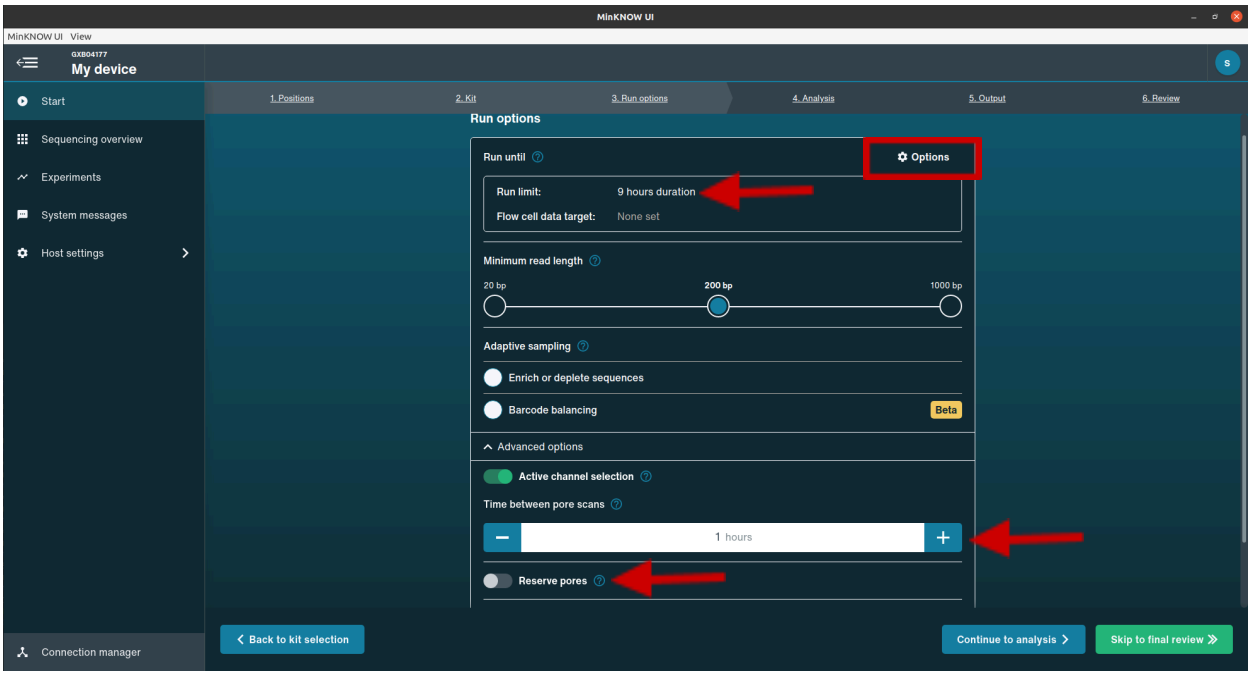

a. Adapt Run duration to **9 hours**
b. Select Advanced options > time between pore scans : **1 hour**
c. Select Advanced options > **deselect reserve pores**

10. Continue to analysis

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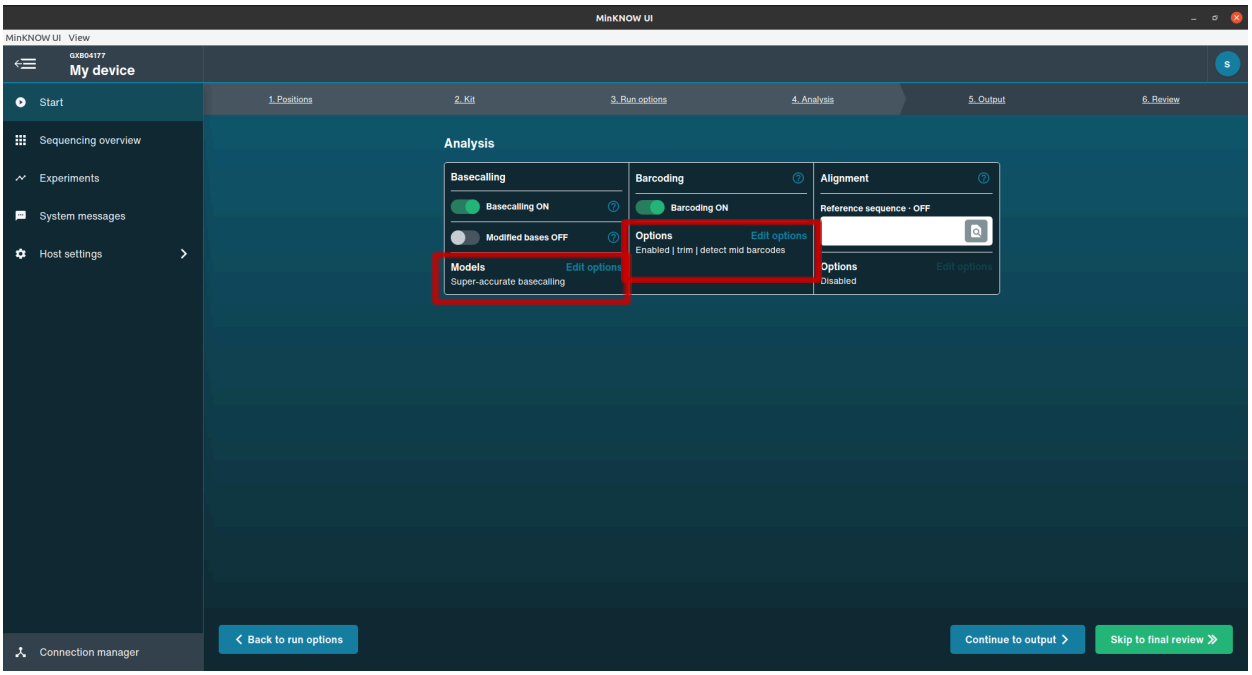

a. **Basecalling** > edit options > **Super-accurate basecalling** > save
b. **Barcoding** > Edit options >
Select '**Trim barcodes**' and '**Mid-read-barcode filtering**' > save

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.

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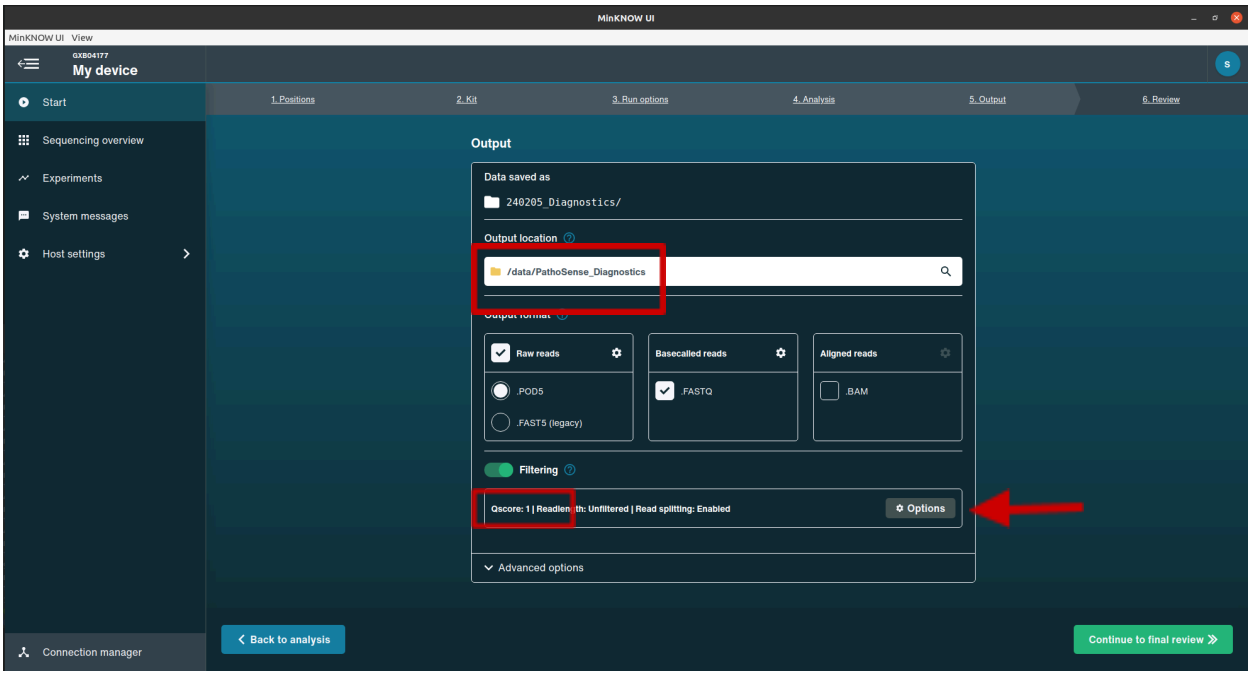

12. Continue to final review

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

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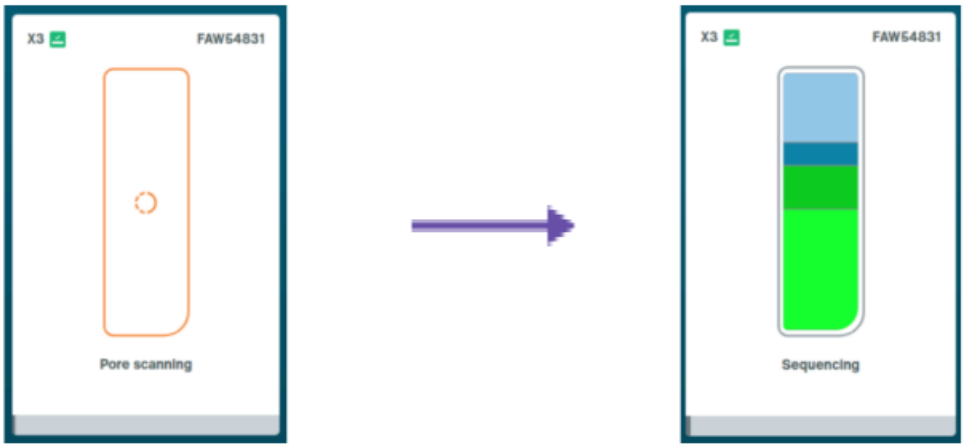
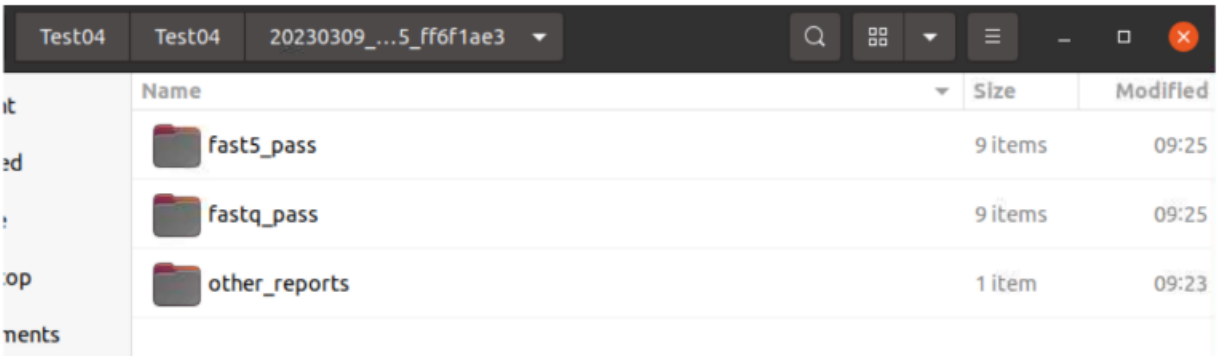


19. The **output directory**
(named after your experiment name: date_diagnosen_firstbarcode_lastbarcode)
will be automatically created in the output location* **/home/Pathosense_Diagnostics**
*Path to shared folder

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

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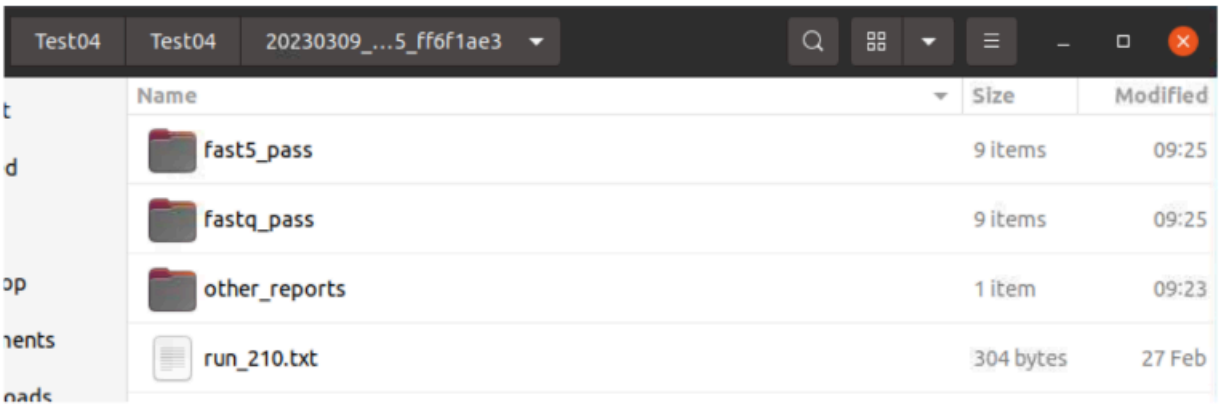

Everything is set up now!
When the sequencing is done, the data will be automatically sent to the cloud for analysis.

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