version 24.04.1

PathoSense

2024

CONFIDENTIAL

---

## Wet lab flow diagnostics PathoSense

# WET LAB VI - RUN SET-UP

### Set-up of a sequencing run on the MinKNOW software and generation of a sequencing run file

### Timing ~ 5 min

1. Open the MinKNOW software and click on '**Start sequencing**'
2. Fill in the **experiment name**
3. Click on the position of the correct flow cell
4. Copy the **experiment name** and paste it in the Sample ID
5. Click on '**Continue to kit selection**'
6. Select the used kit (Rapid Barcoding Kit 24, **SQK-RBK114.24**)
7. Click on '**Continue to run options**'
8. Click on '**Options**', change '**Run time**' to **'9' Hrs** and click on '**Apply rules**'
9. Click open '**Advanced options**', turn **off** '**Reserve pores**' and change '**Time between pore scans**' to **'1' Hours**

10. Click on ‘**Continue to analysis**’

11. In the section ‘Basecalling’, click on ‘**Edit options**’, change ‘**Basecalling model**’ to ‘**Super-accurate basecalling**’ and click on ‘**Apply**’

12. In the section ‘Barcoding’, click on ‘**Edit options**’, turn on ‘**Trim barcodes**’ and click on ‘**Apply**’

13. Click on ‘**Continue to output**’

14. Select the correct output location (**PathoSense_Diagnostics**)

15. Click on ‘**Options**’, change ‘**Qscore**’ to ‘**1**’ and click on ‘**Apply**’

16. Click on ‘**Continue to final review**’

17. Click on ‘**Start**’

18. While the flow cell is getting to temperature, go to cloud.pathosense.com and log in with your credentials.

19. On the Runs page, make a new run by setting the “**Size of run**” to the correct size and pressing “**+ New run**”

20. Select the run by clicking anywhere on the run and add the samples by clicking them
    ***Note:*** *only arrived samples can be added, if the samples are in “En route”, set the analysis to “Arrived” on the analysis page*

21. Click the green **save icon** and select the used ONT barcode for each of the samples

22. Press “**Download**” to download the run file

23. Press the blue “**Start run**” button to move the run to “**Running**”

24. When the flowcell has reached it’s temperature, the sequencing output folder should appear in the “**PathoSense_Diagnostics**” folder

25. Copy the downloaded run file and paste it in the sequencing folder, to the same directory that contains the “**fastq_pass**” and “**pod5_pass**” folders