Run
Nanopore MinION sequencer
till “end of life” of flowcell

Version: March 2025

Before Nanopore sequencing:

- Use most recent Nanopore sequencing protocol on Nanopore website
    - Protocol for Rapid Barcoding Kit 96
    - Link for V14: Nanopore store: Rapid Barcoding Kit 96 V14

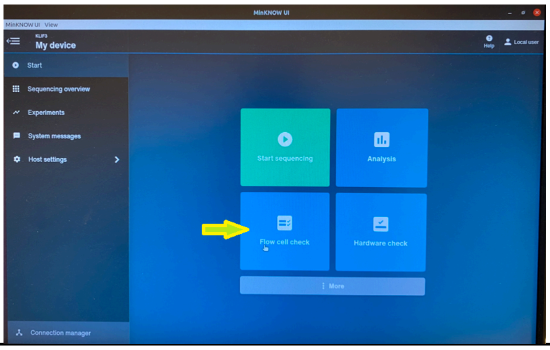
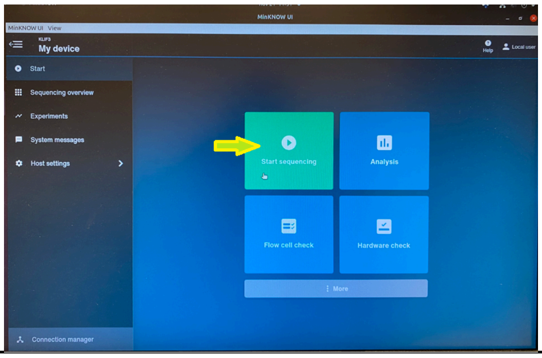
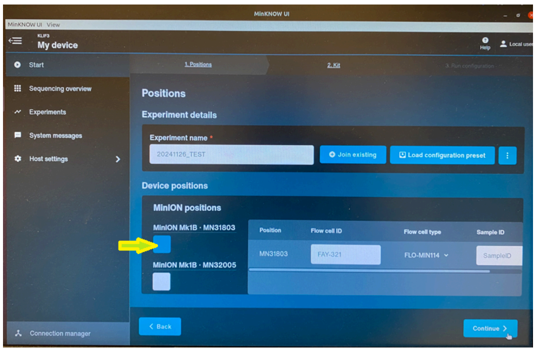
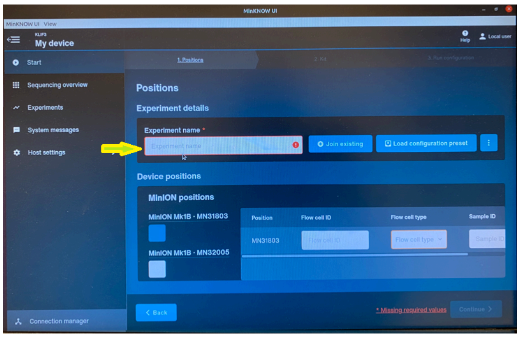
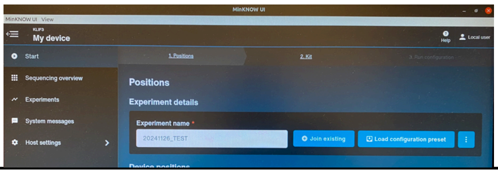
- Link how to load a Flow cell: Priming and loading your flow cell

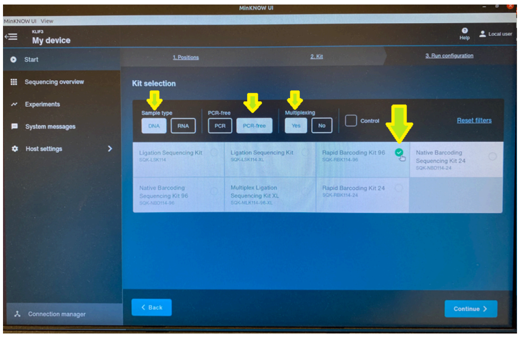
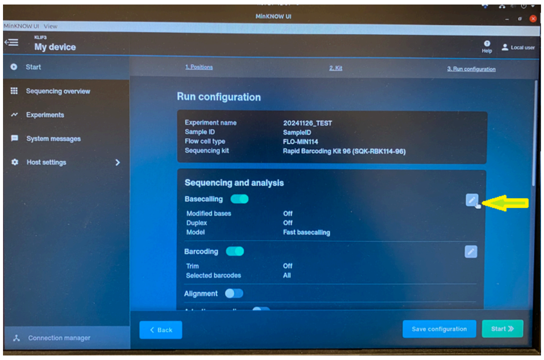
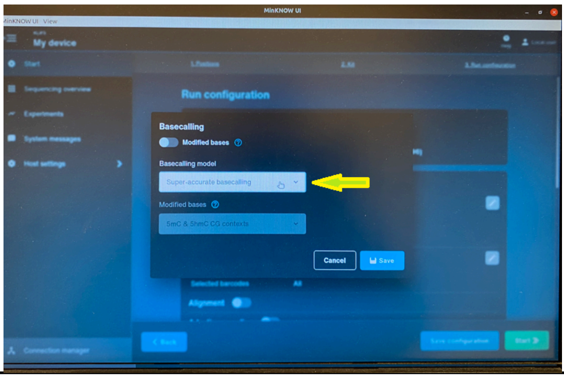
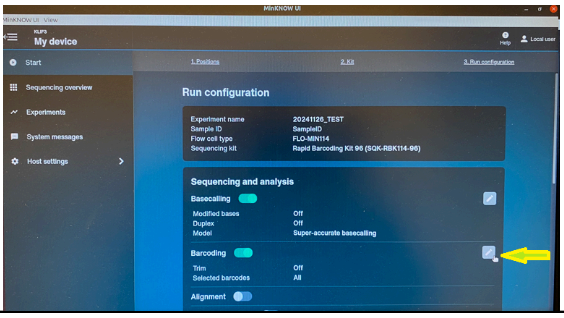
- Flowcell check: Check Flowcell at arrival, minimal 800 pores

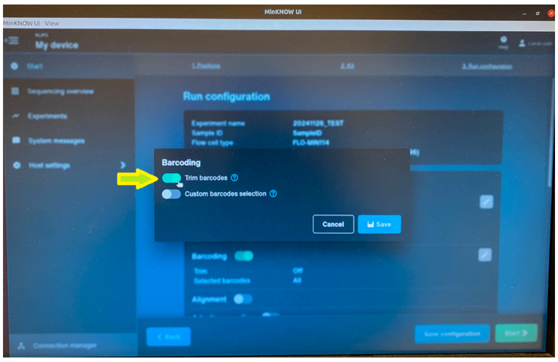
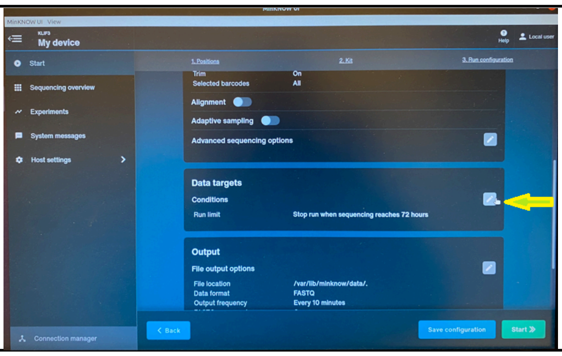
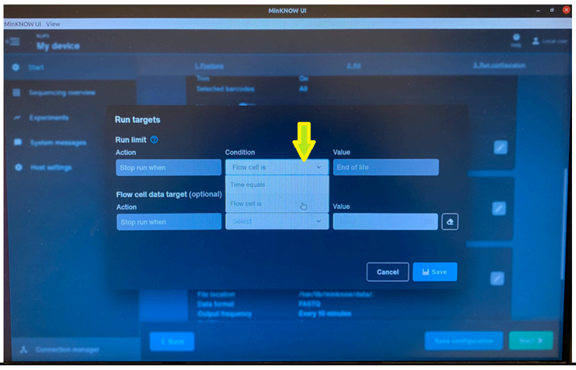
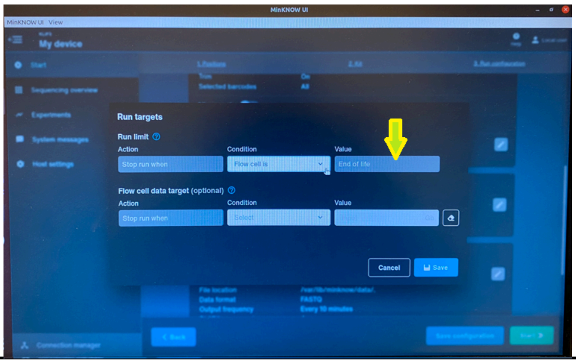
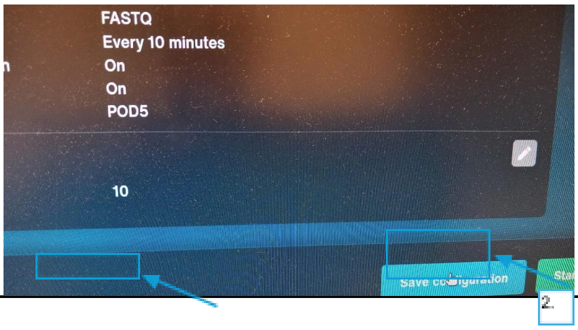
- Check disc space before starting the sequence run (1 TB SSD):
    - If data stored as fast5 and fastQ format: every 1 Gbase of sequence data takes up ~6,5 Gbytes of space
    - If data stored as fastQ only: every 1 Gbase of sequence data takes up ~0,5 gbytes of space

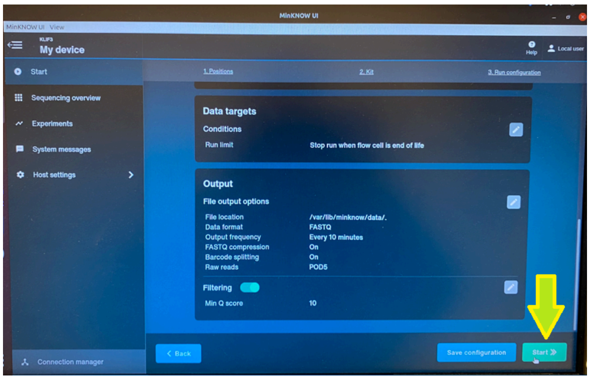
- Useful tips: Nanopore learning (account is necessary): Getting Started

Manual to run Nanopore MinION sequencer

Insert Flow cell in device Start software Select <**Flow cell check**> The flowcell should have minimal 800 pores	
Select <**Start Sequencing**>	
Select MinION device	Experiment name * 20241126_TEST MinION Mk1B · MN31803 MinION Mk1B · MN32005 Position: MN31803 · Flow cell ID: FAY-321 · Flow cell type: FLO-MIN114 · Sample ID: SampleID
Fill in your experiment name	
Select <continue>	Experiment name * 20241126_TEST

Select <**DNA**> Select <**PCR-free**> Select Multiplexing <**Yes**> Select <**Rapid Barcoding Kit 96 SQK-RBK114-96**> Select **<Continue>**	
Go to "Run Configuration" Go to "Sequencing and analysis"	Run configuration Experiment name 20241126_TEST Sample ID SampleID Flow cell type FLO-MIN114 Sequencing kit Rapid Barcoding Kit 96 (SQK-RBK114-96) Sequencing and analysis Basecalling Modified bases Off Duplex Off Model Fast basecalling Barcoding Trim Off Selected barcodes All Alignment
Go to Basecalling Model: Select <**Super Accurate**> instead of Fast basecalling	Basecalling Modified bases Basecalling model Super-accurate basecalling Modified bases 5mC & 5hmC CG contexts
Go to Barcoding in Run configuration	Run configuration Experiment name 20241126_TEST Sample ID SampleID Flow cell type FLO-MIN114 Sequencing kit Rapid Barcoding Kit 96 (SQK-RBK114-96) Sequencing and analysis Basecalling Modified bases Off Duplex Off Model Super-accurate basecalling Barcoding Trim Off Selected barcodes All Alignment

For Barcoding: Select Trim barcodes <**On**> **<Save>**	 Barcoding Trim barcodes Custom barcodes selection Cancel Save
Change Run limit/Action Go to Data targets	 Trim On Selected barcodes All Alignment Adaptive sampling Advanced sequencing options Data targets Conditions Run limit Stop run when sequencing reaches 72 hours Output File output options File location /var/lib/minknow/data/. Data format FASTQ Output frequency Every 10 minutes
For Data targets: Select Condition: stop run when…. <**Flow cell is**>	 Run targets Run limit Action: Stop run when Condition: Flow cell is Value: End of life Time equals Flow cell is Flow cell data target (optional) Action: Stop run when Condition: Select Cancel Save
At Run limit: Select Value: <**End of life**> <Save>	 Run targets Run limit Action: Stop run when Condition: Flow cell is Value: End of life Flow cell data target (optional) Action: Stop run when Condition: Select Cancel Save
Save configuration	 FASTQ Every 10 minutes On On POD5 10 Save configuration 2.

Select <**Start run**> Run will stop automatically when Flowcell is fully used. This will be approx. 3 days. Flowcell can’t be used again after it has run until ‘end of life’ (it is also no option to wash and use it again).	 Data targets Conditions Run limit: Stop run when flow cell is end of life Output File output options File location: /var/lib/minknow/data/. Data format: FASTQ Output frequency: Every 10 minutes FASTQ compression: On Barcode splitting: On Raw reads: POD5 Filtering Min Q score: 10