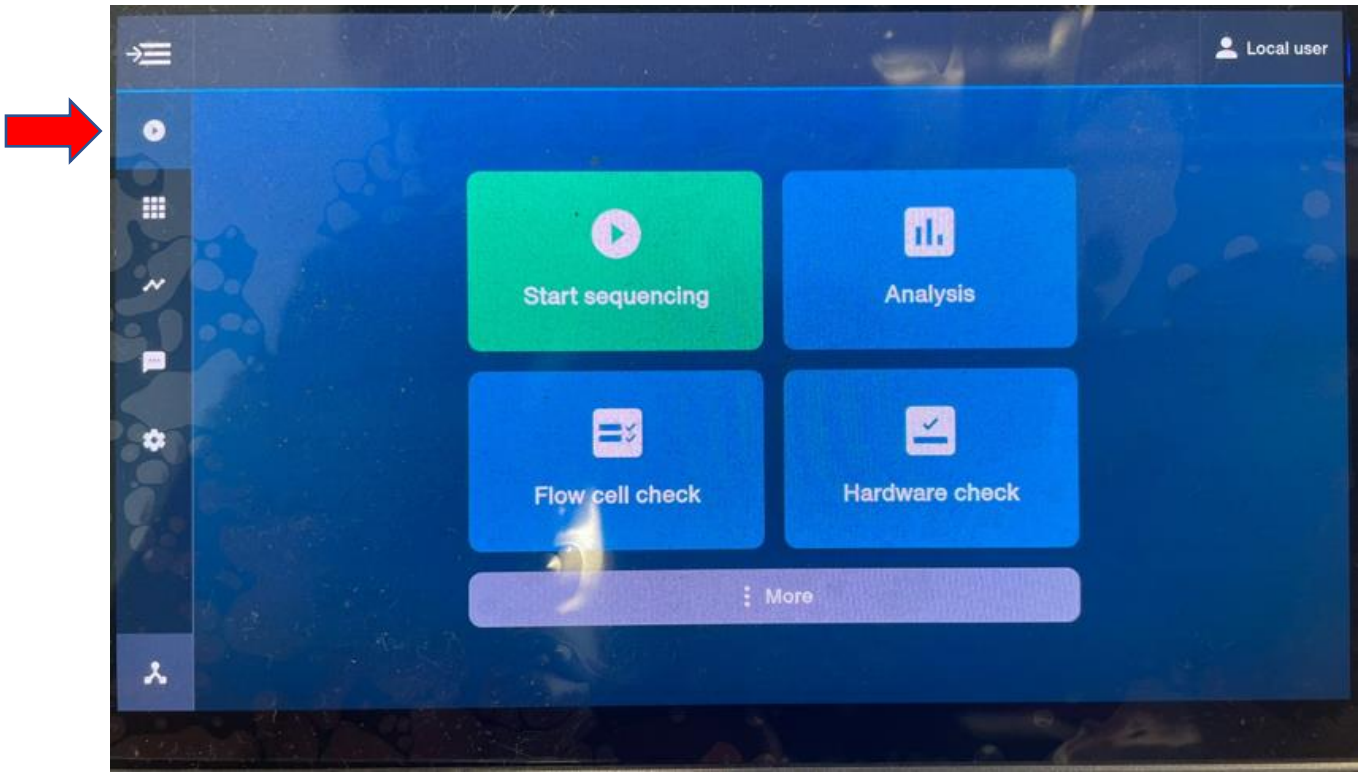
APHL Training: Reanalyzing on MINKOW, ONT (Updated November, 22, 2022)

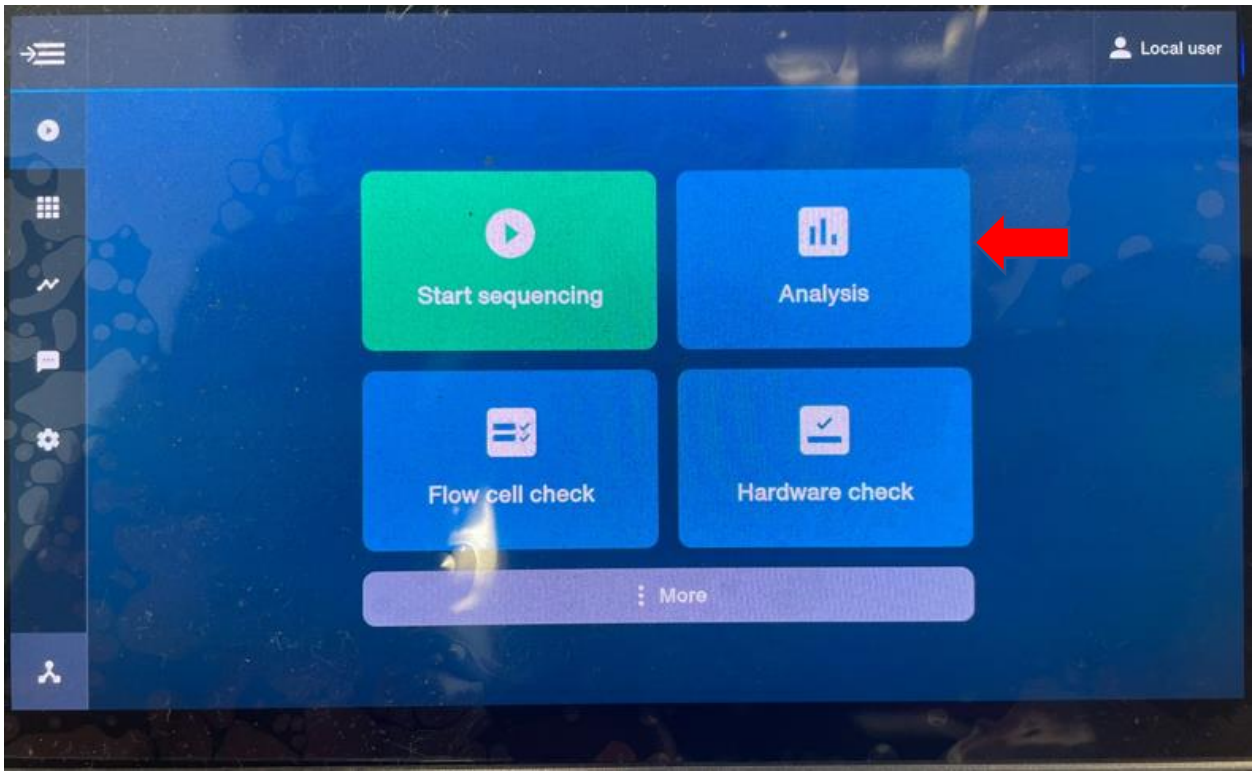
Purpose:

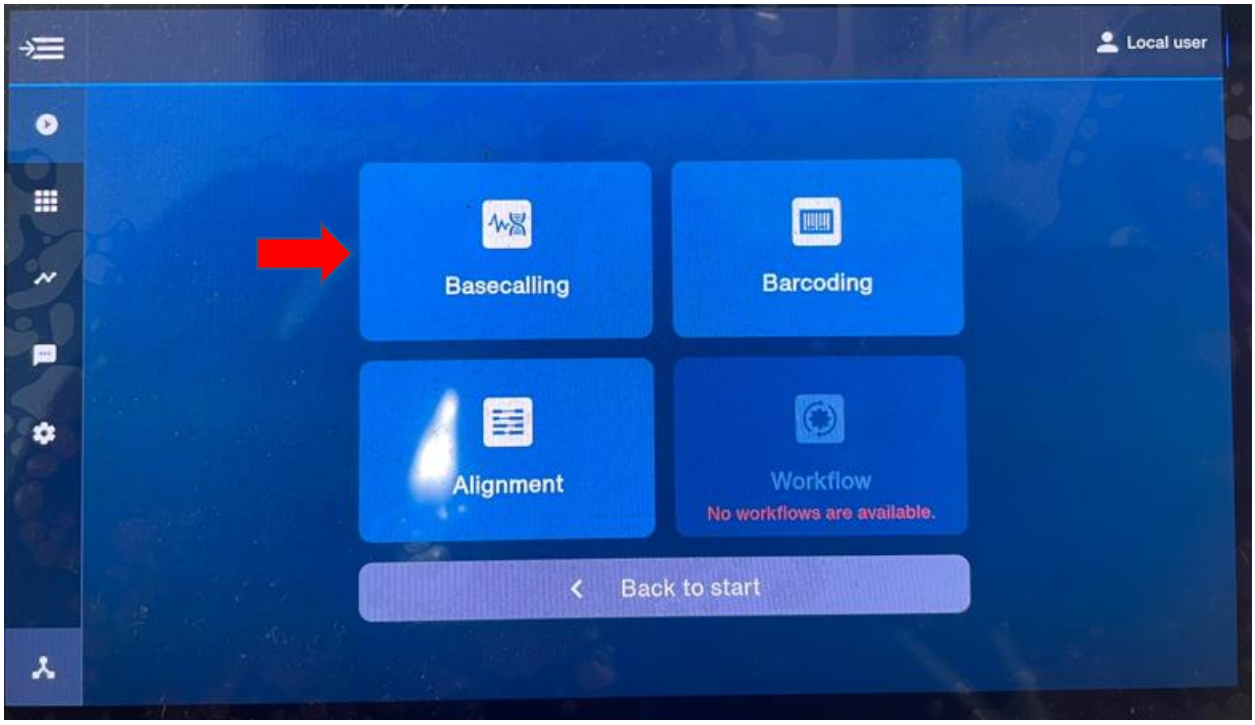
This procedure outlines how to reanalyze sequenced data using the Oxford Nanopore Technology (ONT) MINKNOW software.

1. Start the MinKnow Software and navigate to the main page by clicking on the play icon



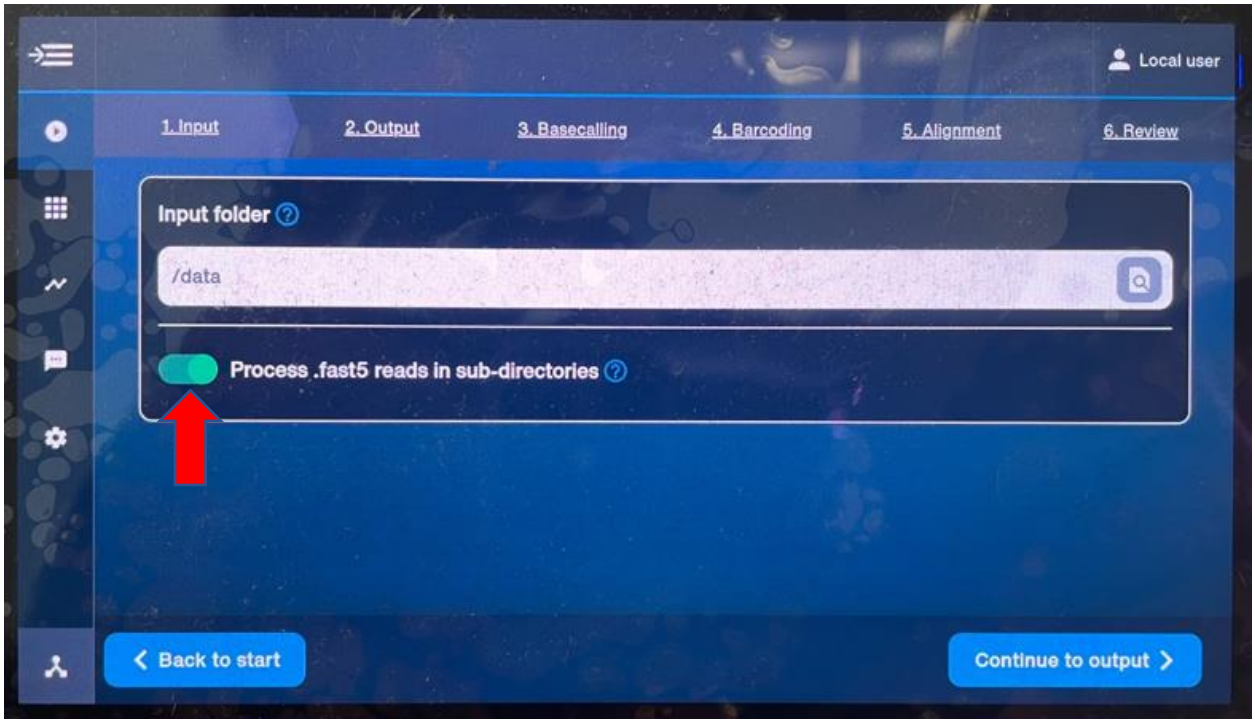
2. Click on Analyze



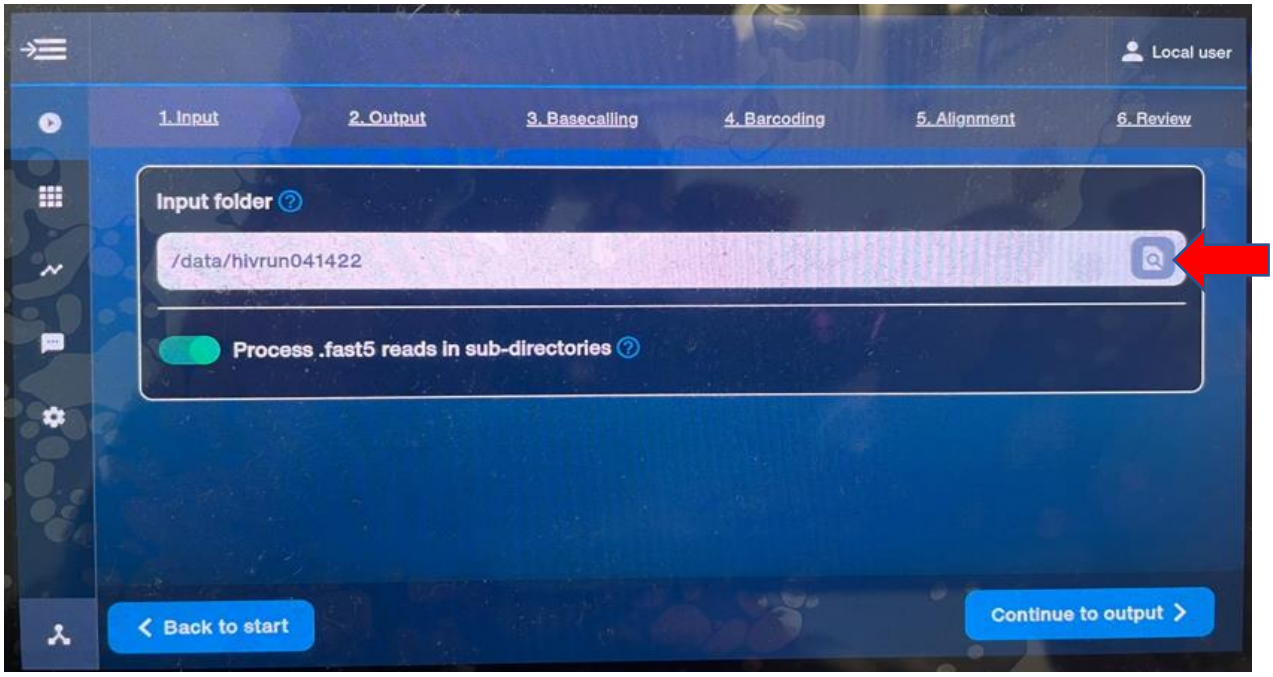


3. After clicking on Analyze, the next screen shows three options.
    a. Basecalling – For reanalysis using FAST5 data:

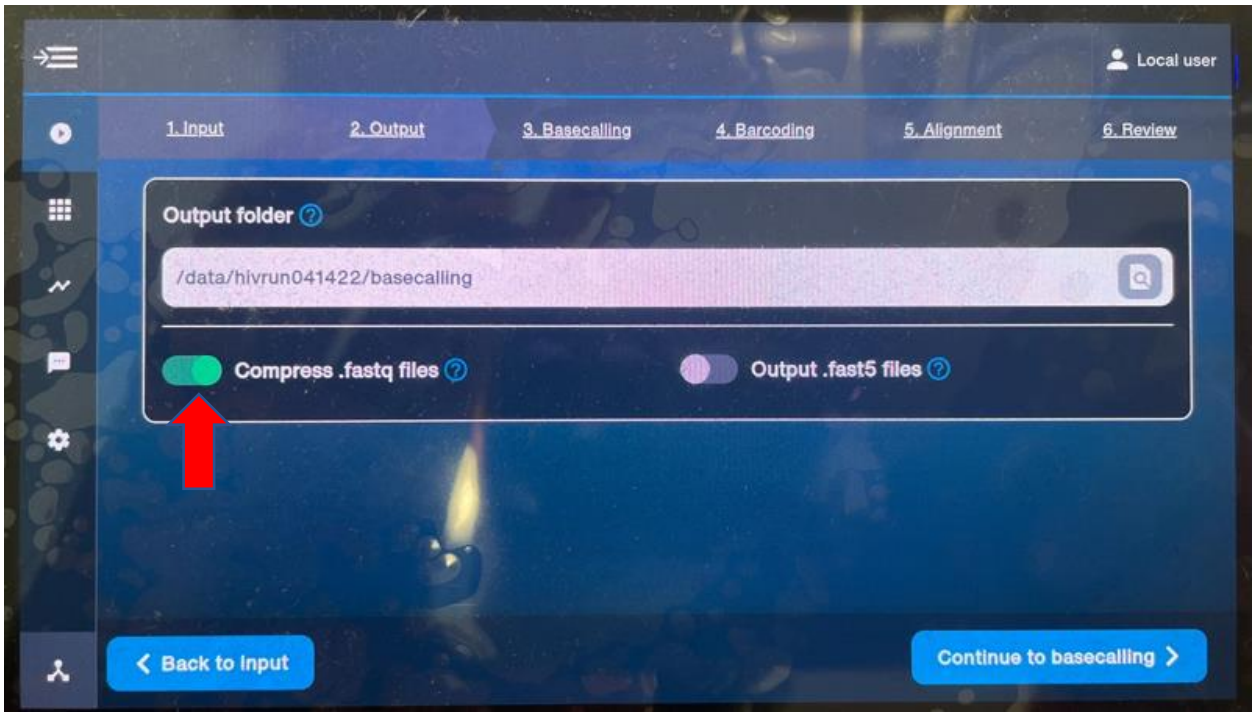
    i. Reanalyze FAST5 data to FASTQ
  b. Barcoding – For reassigning barcodes if the wrong kit was selected during innitial sequencing
    i. If the wrong kit was selected, most of the reads would be characterized as “unclassified”.
4. To reanalyze FAST5 to FASTQ, click on Basecalling



5. Choose the location where the FAST5 files are located. Usually this is defaulted to /data
6. Select “Process .fast5 reads in sub-directories”

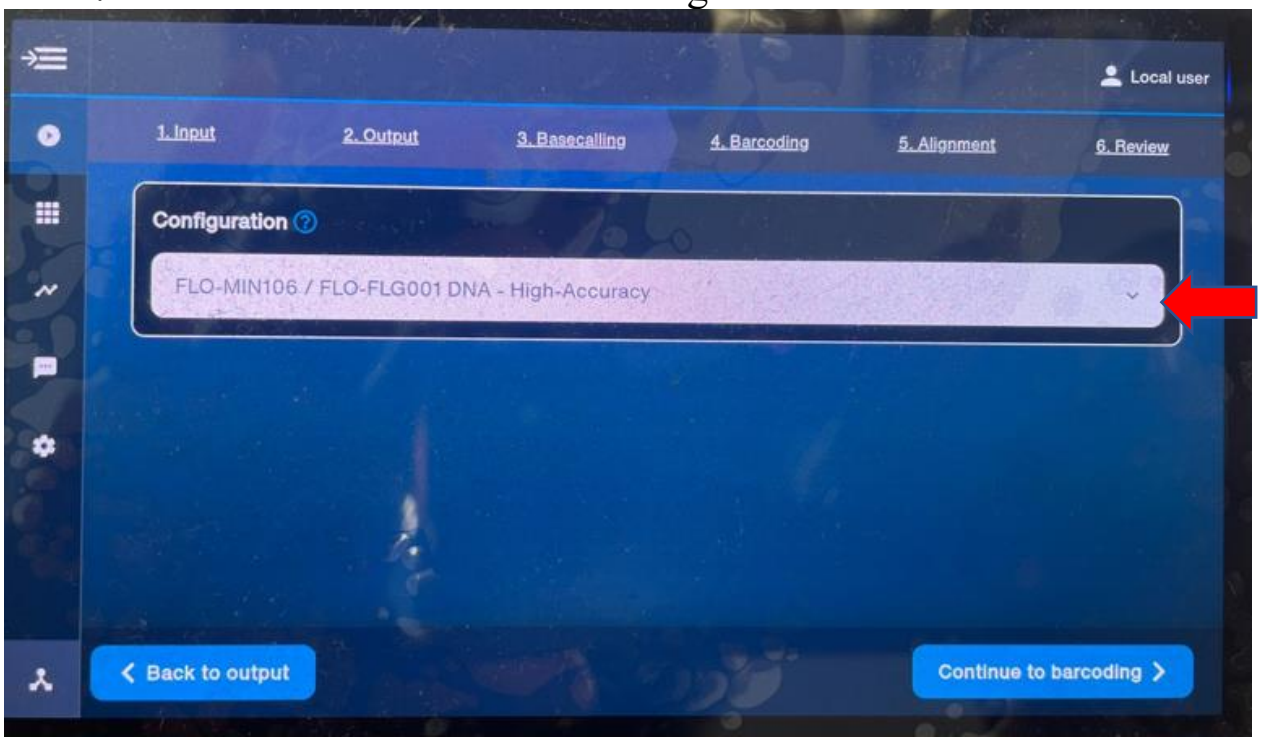


7. Navigate to the folder where the files are by clicking on search icon
8. Click “Continue to output”

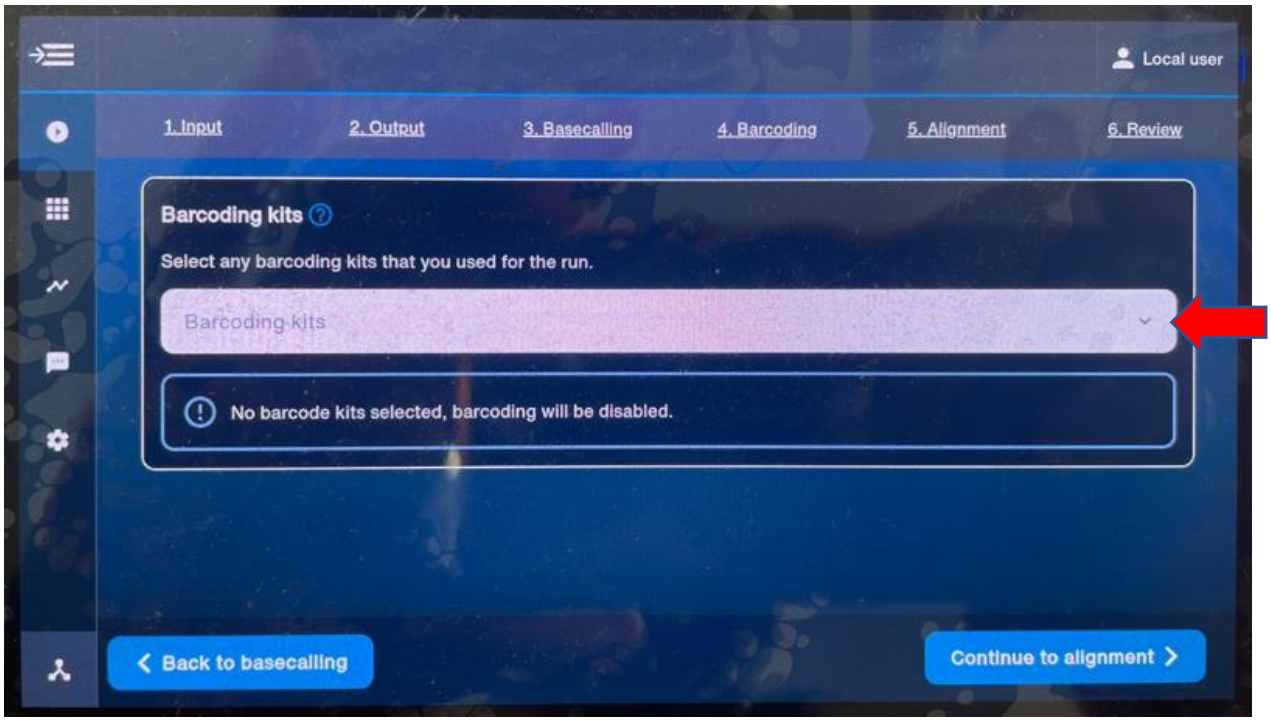


9. On the output section, choose the location where you would like the reanalyzed files to be outputted.
10. Select “Compress .fastq files.

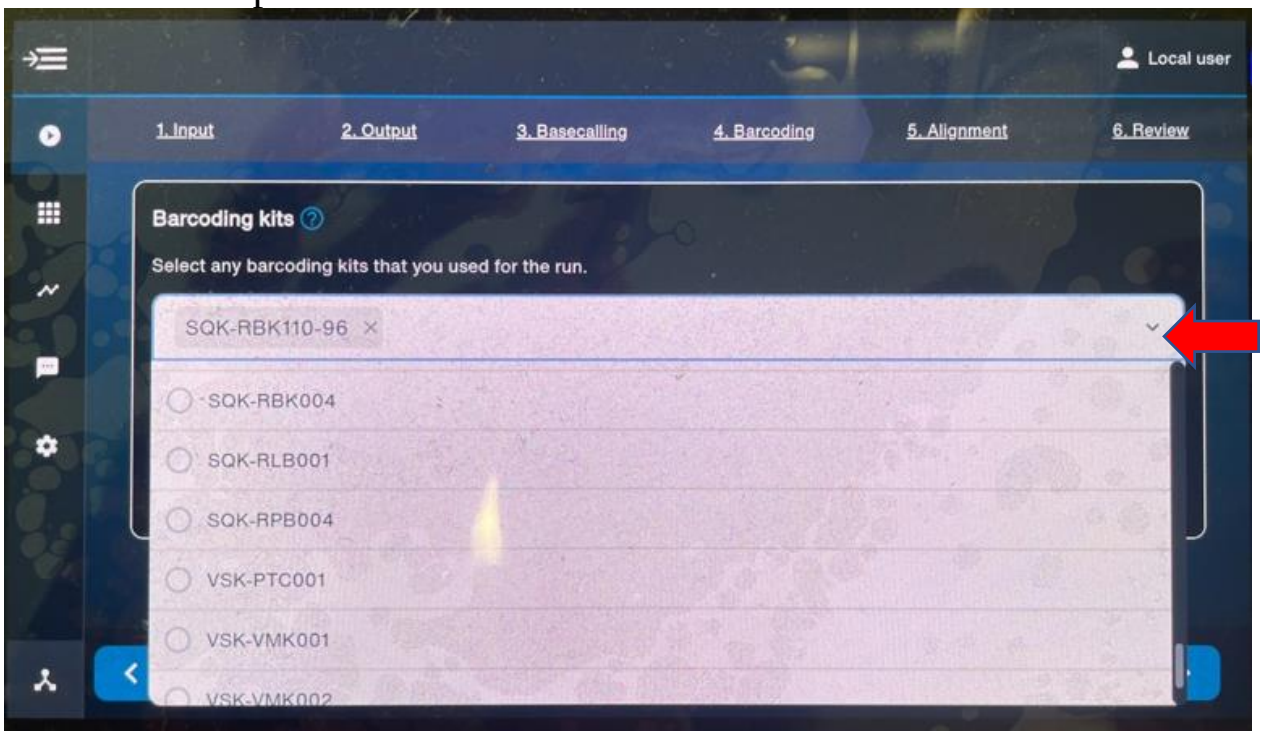
11. Click “Continue to basecaling”



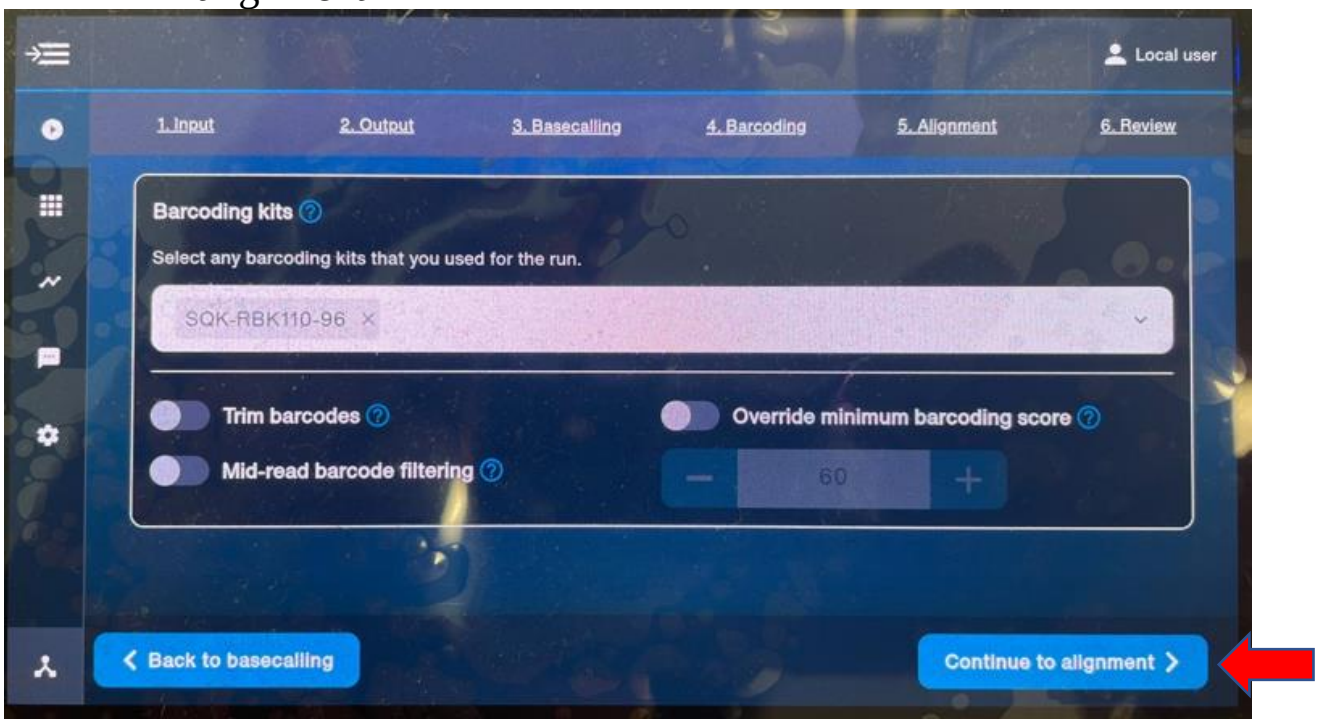
12. Under Basecalling select the level of accuracy.
    NOTE: The higher the accuracy the longer it will take for basecalling to complete. High-Accuracy on the MK1C will take about 40 hours to complete.
13. Click “Continue to barcoding”



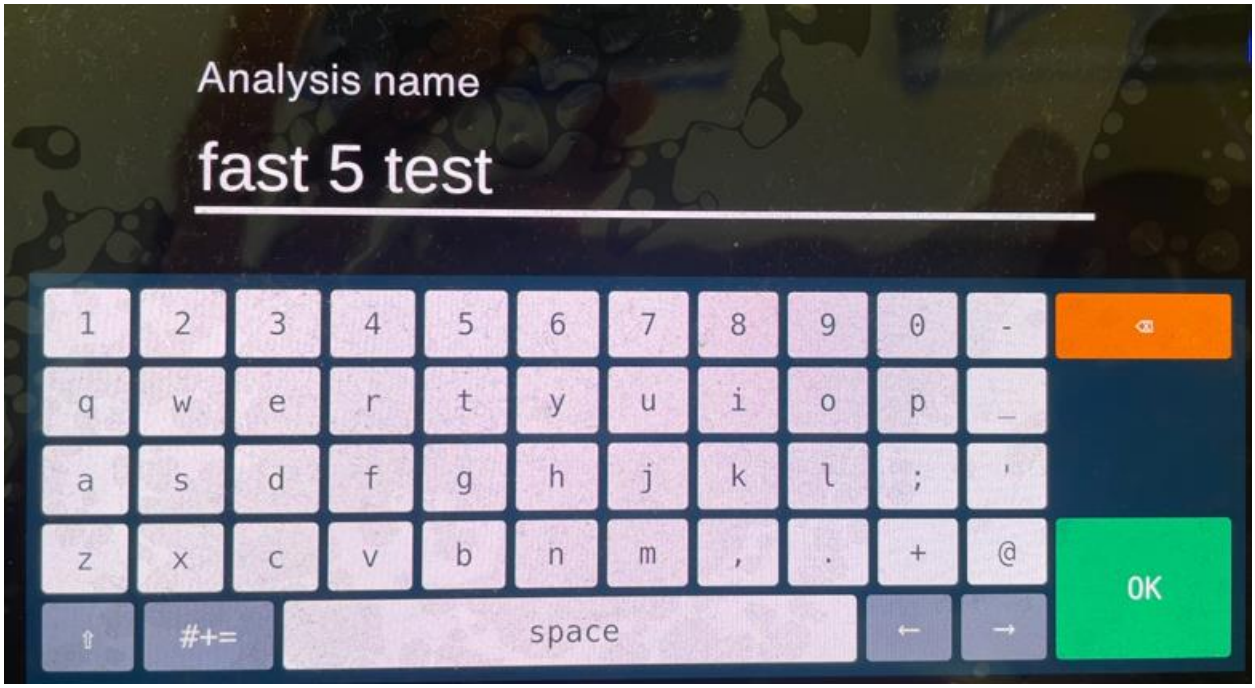
a. Select the barcoding kits used for sequencing by clicking on the drop down icon



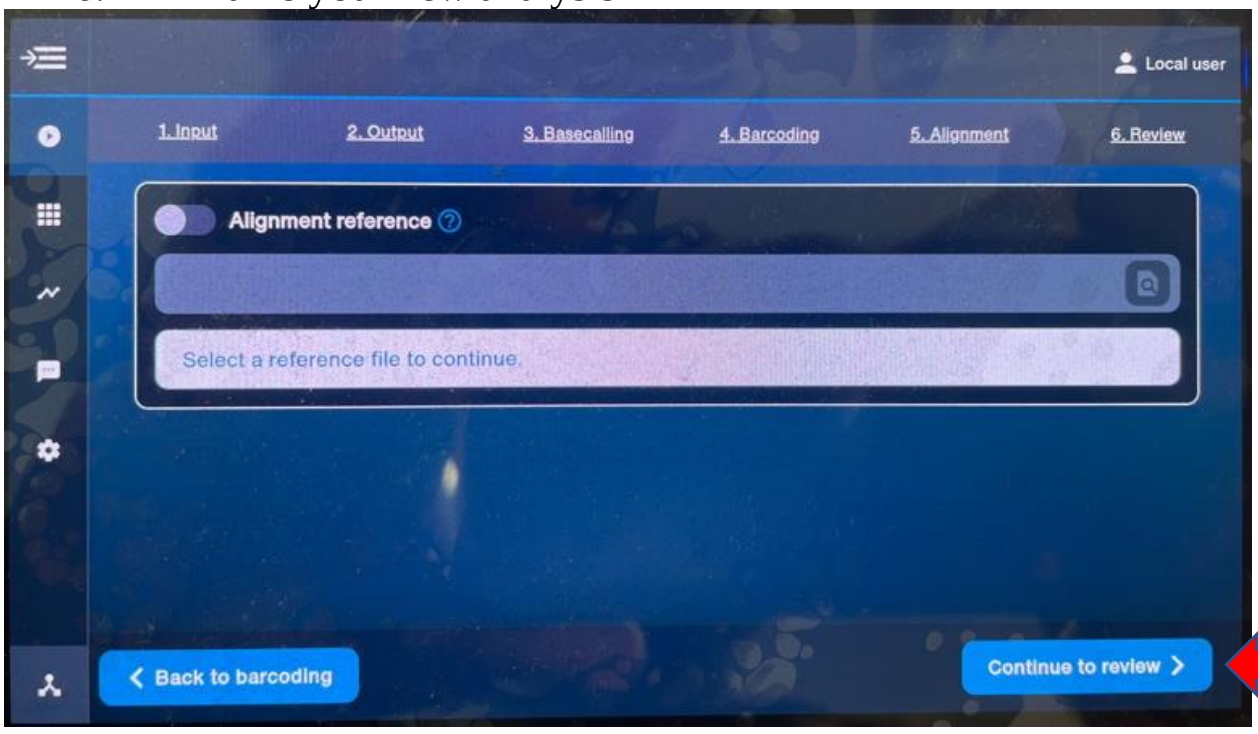
b. Once the right Barcoding kit is selected Click “Continue to alignment”



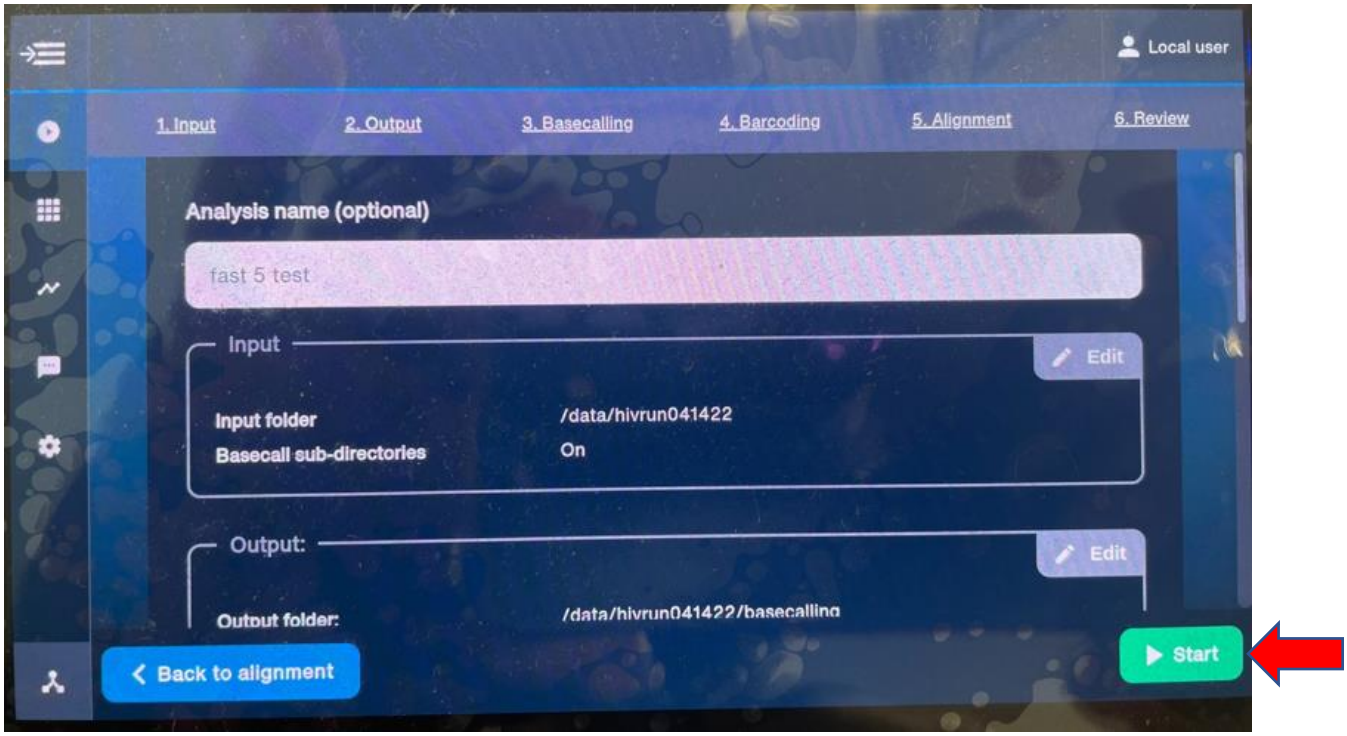
14. Select the parameters desired. These are optional.
15. Click “Continue to Alignment”



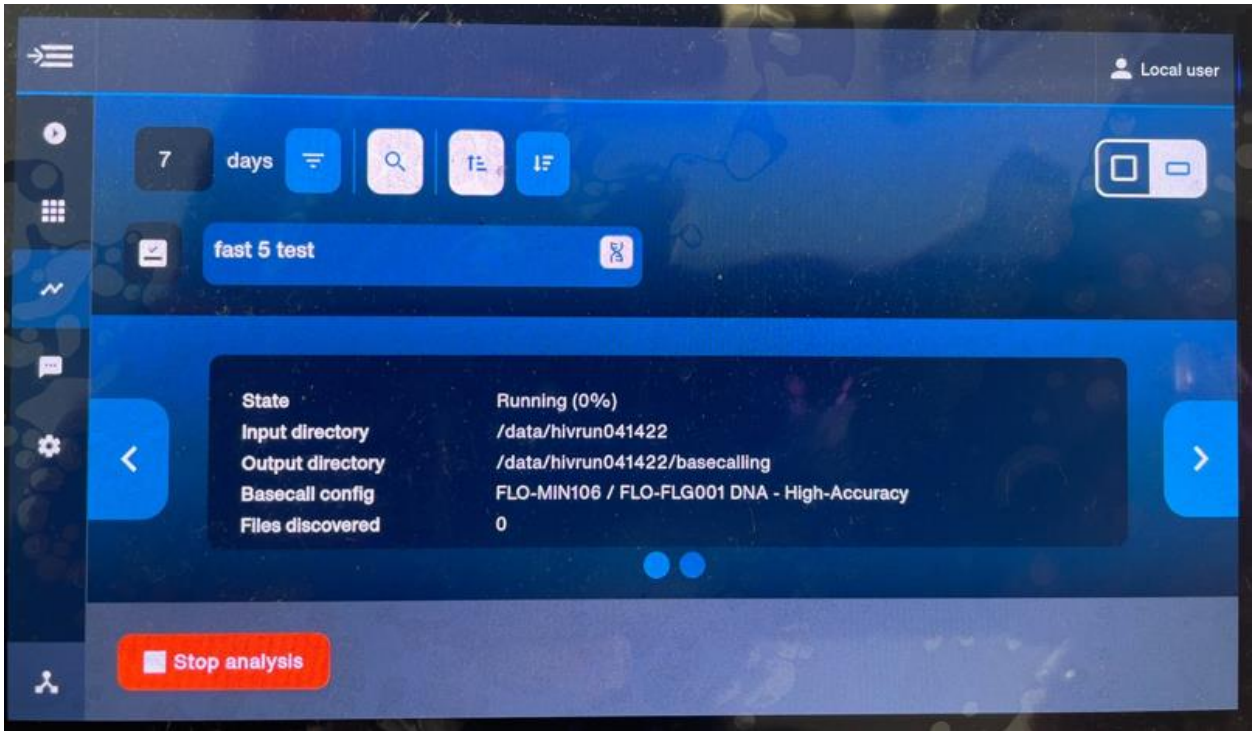
16. Name your new analysis



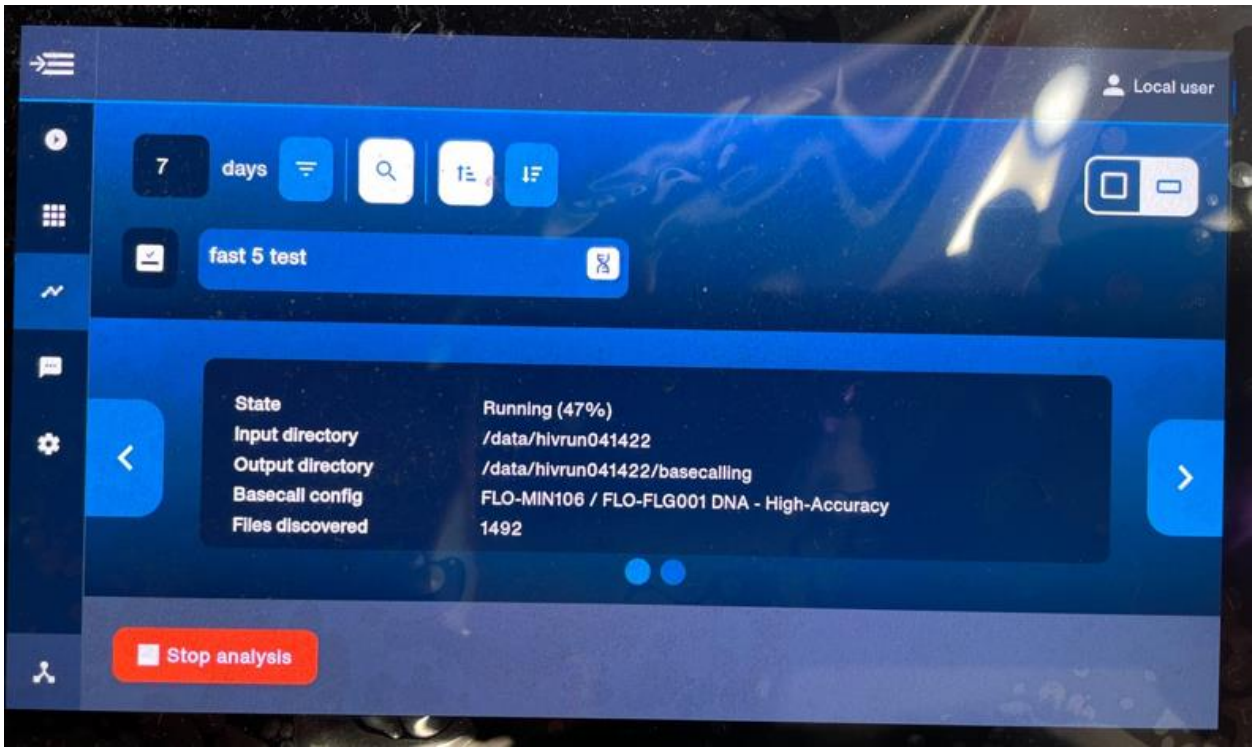
17. Alignment reference is optional. Leave this blank unless you have an alignment reference file.
18. Click “Continue to review”



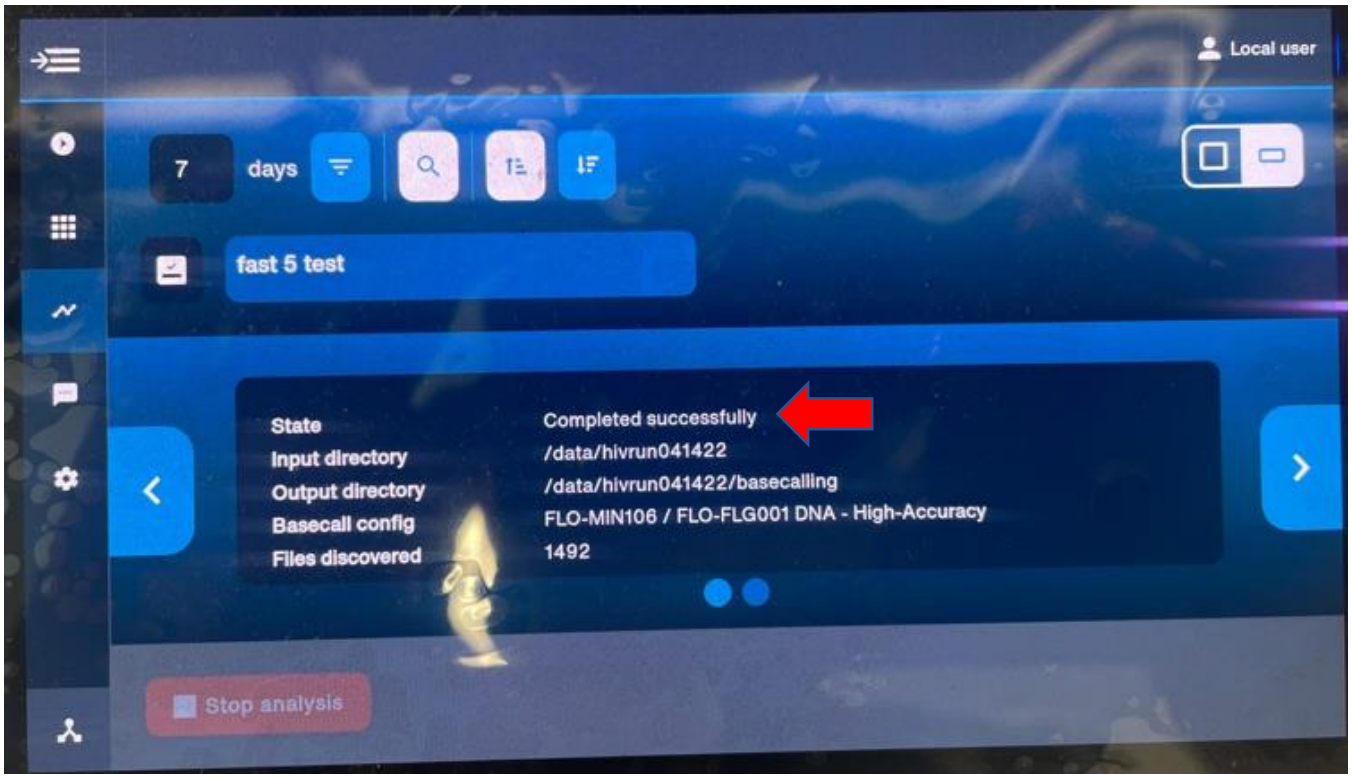
19. Review the submission
20. Click “Start”



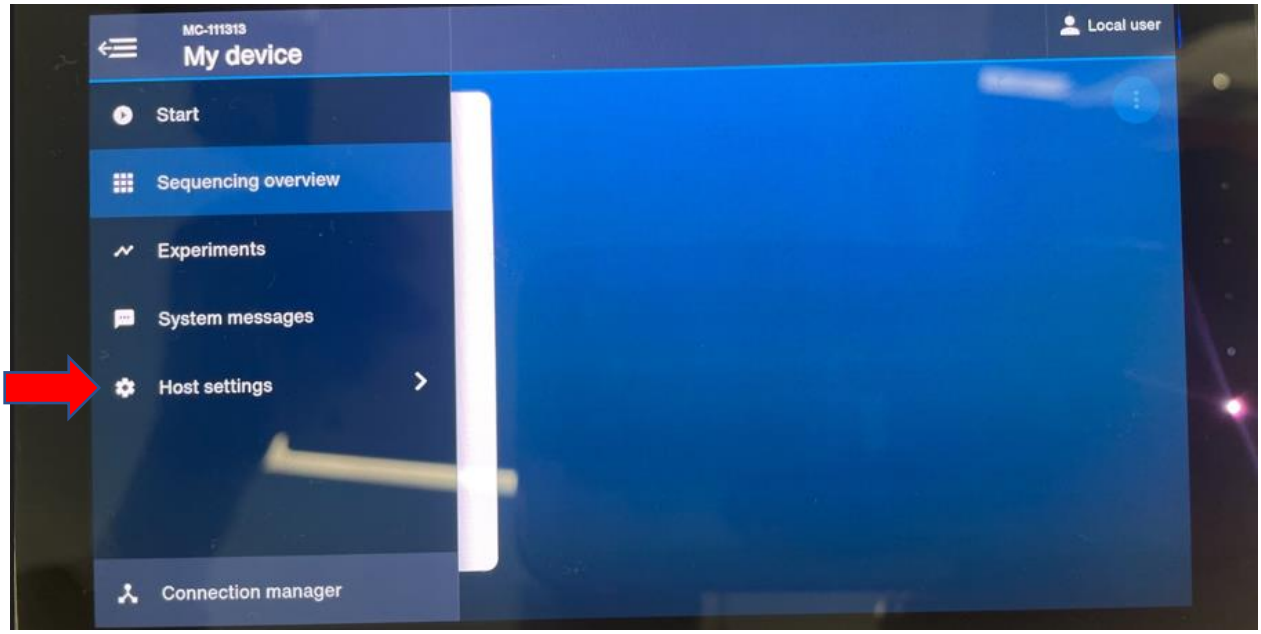
21. Once analysis starts, you can review the state of the run



Review page when running is complete

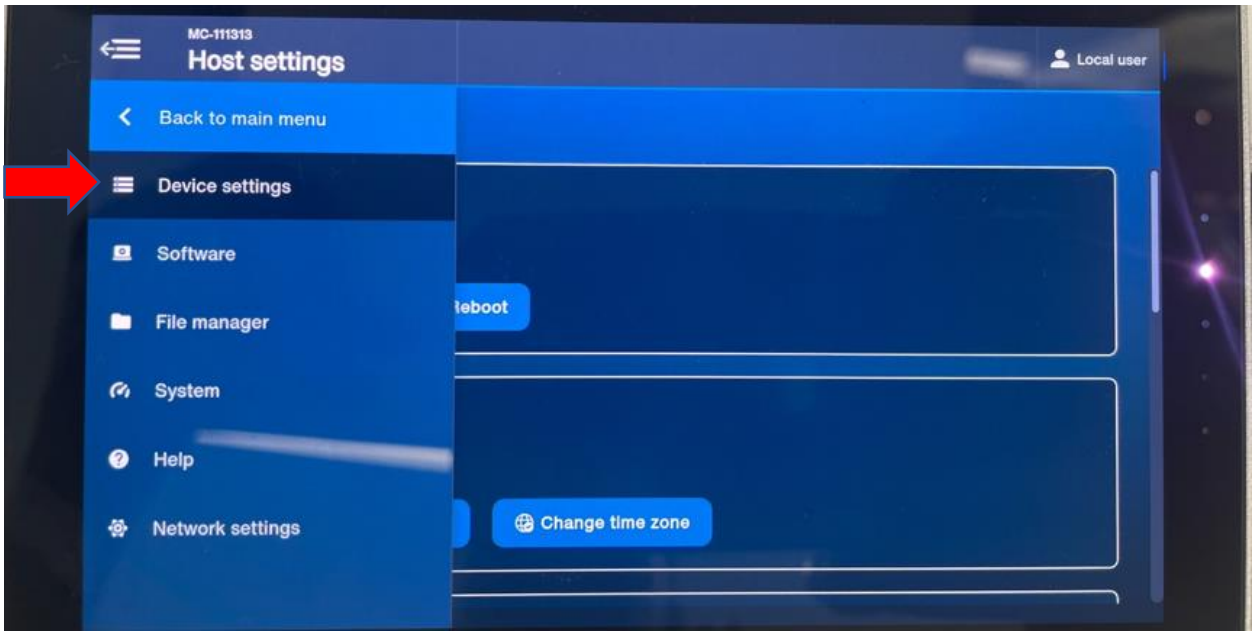


Reviewing Reanlyzed Results and Data on MINKNOW when Analysis is Complete

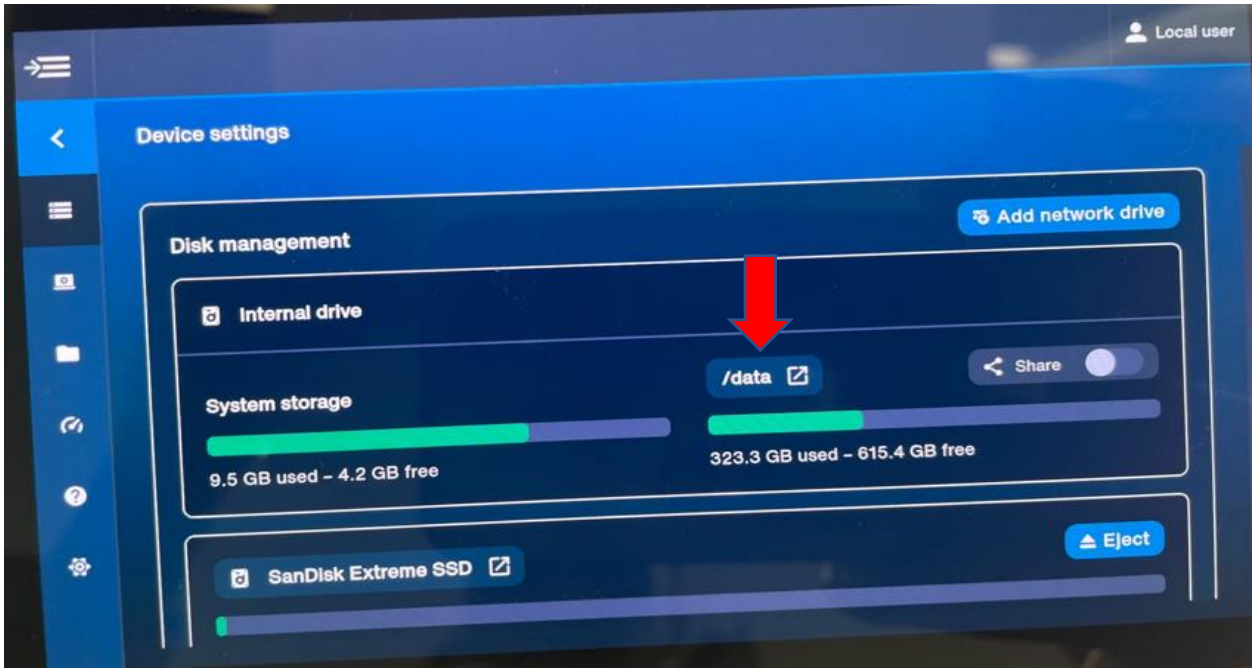


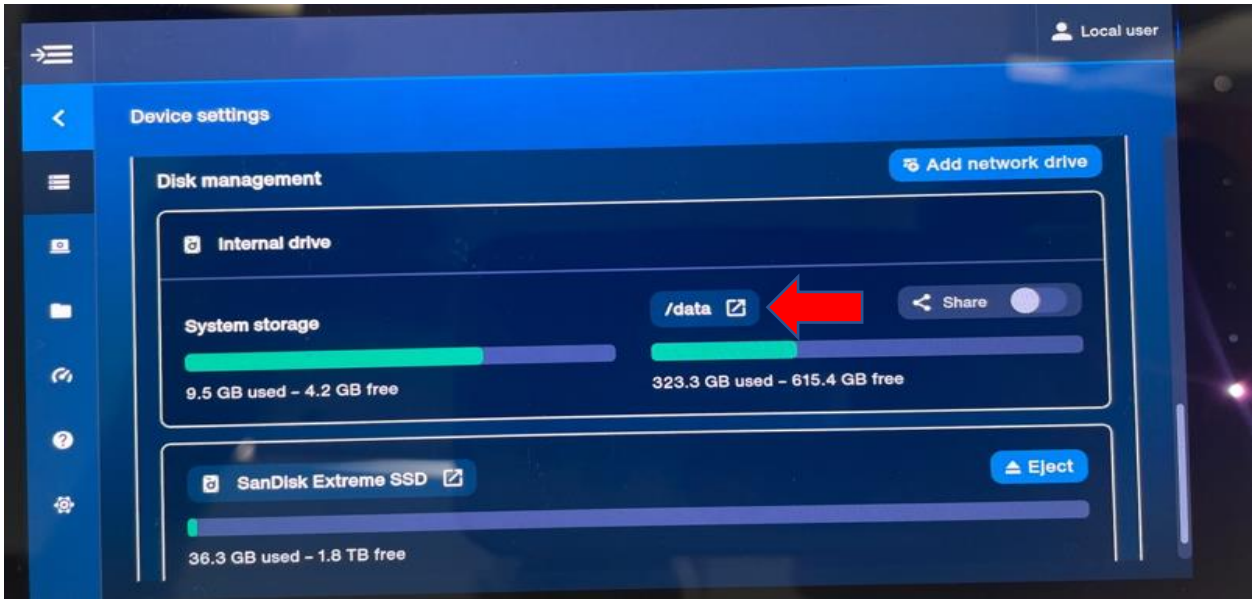
1. Navigate to My Device on the main user page
2. Click on Host Setting

(NOTE: After a successful reanalysis, MINKNOW, may need to be restarted before reviewing the data). Sometimes, “Host Settings” does not show up with appropriate features unless restarted after reanalysis.



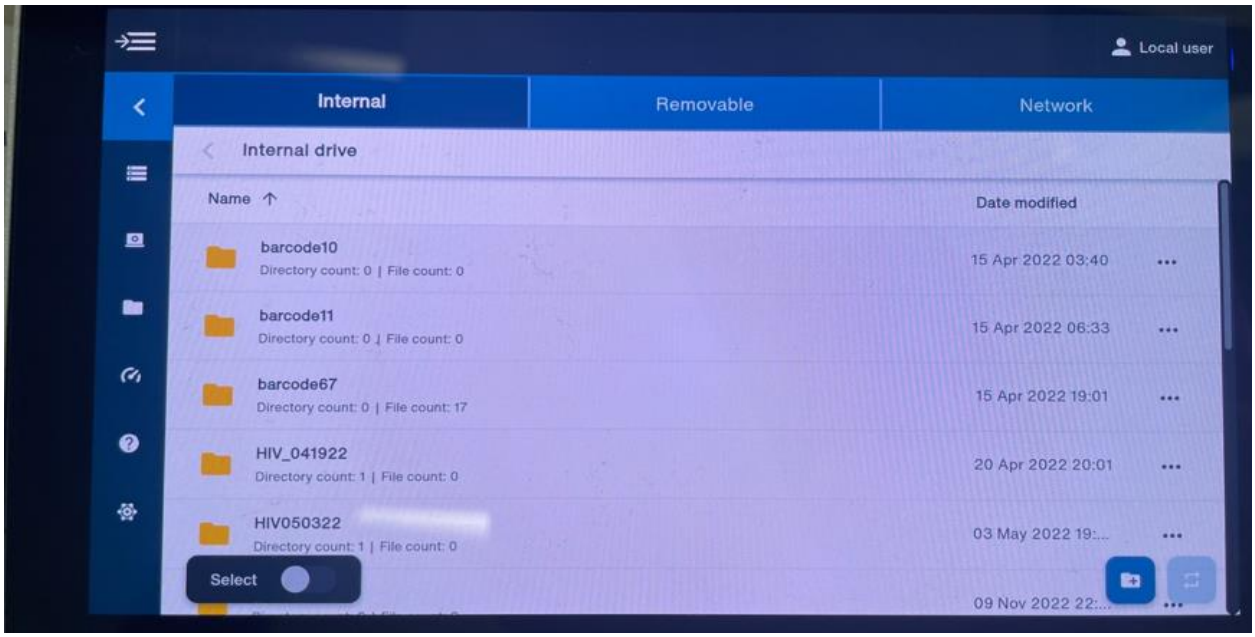
3. Under Host Settings, you can scroll down to Device Setting
4. Under Device Settings, there will be Disk Management options
    a. System Storage
    b. /data (This is where all sequencing results are saved)



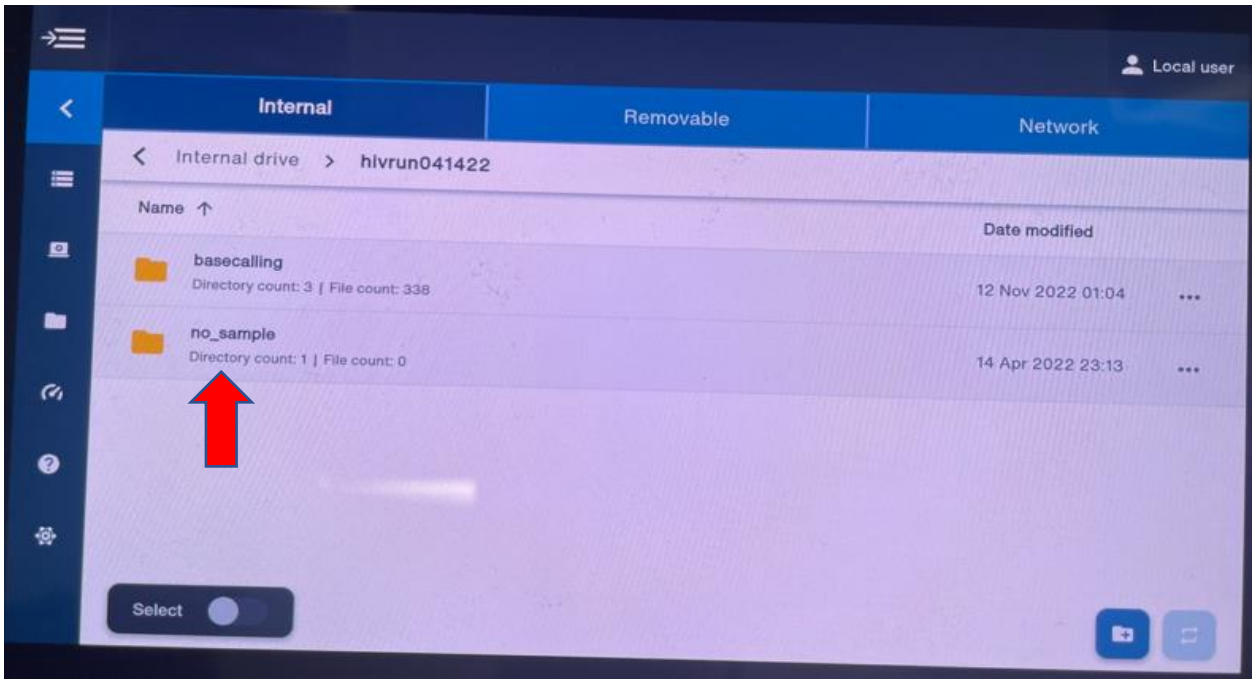


5. Click on /data icon (This will open the drive)
6. Click on Internal drive

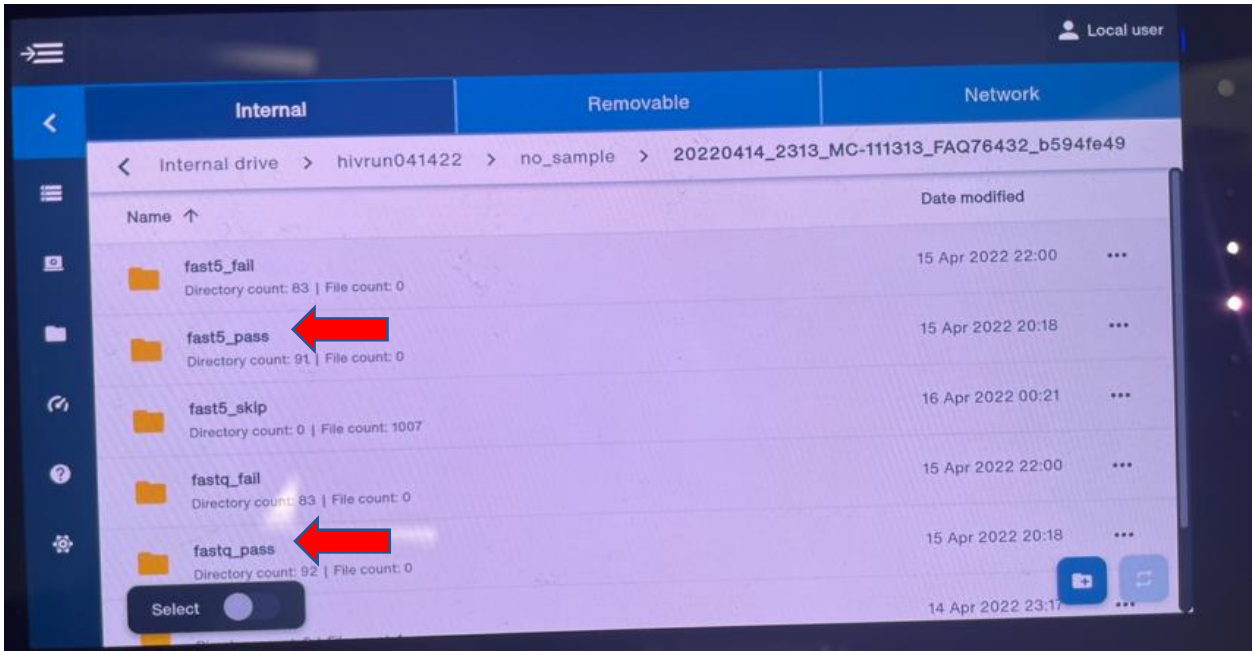
7. Under Internal drive, you should see all the folders of all the sequencing runs done on the instrument



8. Click on the folder that contains the data you want to access



9. Select no_sample (it contains all the subfolders for analyzed results)



10. Under the folder no_samples, you will see all FASTQ and FAST5 files