20190911_ MorexSamp62 _ Run 10
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Rapid Sequencing (SQK-RAD004) Guide Sheet

To print this document, navigate to:
https://gitprint.com/MorrellLAB/Nanopore/blob/master/SQK-RAD004/SQK-RAD004-protocol.md (https://gitprint.com/MorrellLAB/Nanopore/blob/master/SQK-RAD004/SQK-RAD004-protocol.md)

Input DNA Quality Control

- [X] Fill a large foam container with ice.
- [X] Put on a pair of lab gloves.
- [X] Fetch the size-selected DNA from inside a white foam container inside the lab refridgerator and place the tube on ice.
- [ ] skip If this is the first time using this DNA sample or the sample has been stored for a long period, you may want to check the quality of the input DNA using the Nanodrop. ONT recommends the following quality criteria:
  - OD 260/280 of 1.8 and OD 260/230 of 2.0-2.2
  - Concentration > 53.3 ng/uL (to be able to achieve 400 ng total in 7.5 uL)

Check the flow cell quality

- [X] Record today's date, the sample name, the run number, and your name at the top of this sheet.
  - Follow the convention "YYYYMMDD_SampleName_RunNumber" for the run number and sample name.
  - Example: 20190403_MorexSample2_Run3
- [ ] Check that the computer has at least 500 GB of free space (not counting any connected external drives). 665 GB available
- [ ] Fetch the flow cell from the lab refridgerator and record the flow cell information.
  - Flow cell type (FLO-MIN106 or FLO-MIN107): FLO-MIN106 R9
  - Flow cell label (Example: 11000540): 11000540
  - Flow cell barcode (Example: 2002540064): 2002525835
- [X] Plug the MinION into the computer.
- [X] Remove the configuration test cell and place it in the MinION box for safekeeping.
- [X] Insert the flow cell into the MinION.

- [X] Click "MinKNOW" in the dock in the upper left, then "About MinKNOW", and then record the MinKNOW version numbers.
    - MinKNOW version (Example: 19.06.7): 19.06.8
    - Core version (Example: 3.4.5): 3.4.8
    - Bream version (Example: 4.1.8): 4.1.9
    - GUI version (Example: 3.4.12): 3.4.5
    - Guppy version (Example: 3.0.4): 3.0.7
- [X] Select the flowcell, and change the flow cell type to match the flow cell being used.
- [X] Click "Check flow cells" at the bottom of the screen. Then click "Start test"
- [ ] Continue to library preparation while the test is running. When the test completes, make sure that the active pore count is > 800.
    - Number of active pores: 1560
    - Flow cell ID (Example: FAJ01802): FAH84690

Library preparation

- [X] Fetch the following reagents from the freezer and record their batch numbers.
    - FRA (brown) batch number: SK1311003
    - RAP (green) batch number: SK1241003
    - SQB (red) batch number: SK1281002
    - LB (pink) batch number: SK1271003
    - FLB (blue) (use only one vial) batch number: SK1291004
    - FLT (purple stiped) batch number: SK1301003
- [X] Thaw the SQB (red), LB (pink), FLB (blue), and FLT (purple) while keeping the other reagents on ice.
- [X] Briefly spin down all six reagents in the centrifuge, then immediately store on ice.
- [X] Sanitize a pair of lab scissors using ethanol, then cut the tip off of a 10 uL pipette tip.
- [X] Add 7.5 uL of sample DNA to a 0.2 mL thin-walled PCR tube using the cut pipette. Place the DNA sample tube back in the fridge, as it will not be used in further steps.
- [X] With a new pipette tip, mix the vial of FRA by pipetting up and down.
- [X] Add 2.5 uL of FRA (brown) to the PCR tube with DNA. Place the vial of FRA (brown) back on ice.
- [X] Gently mix the PCR tube by flicking, then spin down.
- [ ] Place the PCR tube in the middle of the thermocycler plate. Avoid the wells that are

- [X] Start the "Rapido1" cycle under the lilei username.
- [X] Incubate the tube at 30° C for 1 minute and then at 80° C for 1 minute.
- [X] When you hear the fan kick on, check to make sure the cycle is complete (holding at 4 C). Remove the PCR tube and place it on ice.

Adapter attachment

- [X] Mix the vial of RAP (green) by pipetting up and down.
- [X] Add 1 uL of RAP (green) to the PCR tube. Place the RAP (green) vial back on ice.
- [X] Gently mix the PCR tube by flicking, then spin down.
- [X] Wait 13 minutes at room temperature, then store the PCR tube on ice.

Priming the flow cell

- [X] Vortex the SQB (red) and FLB (blue) tubes, spin down, and return them to ice.
- [X] Open the priming port (not the SpotON sample port).
- [X] Set a 1000 uL pipette to 200 uL and insert the tip into the priming port.
- [X] Increase the pipette volume until you can see a small volume of buffer entering the pipette tip.
- [X] Visually check that there is continuous buffer from the priming port across the sensor array.
- [X] Use a pipette tip to mix the FLT (purple striped) tube by pipetting up and down.
- [X] Add 30 uL of FLT (purple striped) directly to the tube of FLB (blue) and mix by pipetting up and down. Return the tube of FLT (purple striped) to ice.

* used FLT from 2 different tubes

- [X] Load 800 µl of this mix into the flow cell via the priming port, avoiding the introduction of air bubbles.
- [X] Return the rest of the FLB (blue) mixture to ice.
- [X] Wait 5 minutes.

Loading the flow cell

- [X] Find a LoBind microfuge tube.
- [X] Using a pipette tip, mix the vial of SQB (red) by pipetting up and down.
- [X] Add 34 uL of SQB (red) to the LoBind tube, then return the SQB (red) to ice.

Need to mix 2 tubes of SQB to get 34ul

[X] Add 25.5ul LB (Pink) Mix immediately before use

- [X] Add 4.5 uL of nuclease-free water to the LoBind tube.
- [X] Sanitize a pair of lab scissors using ethanol, then cut the tip off of a 100 mL pipette tip.
- [X] Using the cut pipette tip, add all (11 uL) of the DNA library (PCR tube) to the LoBind tube.
- [X] Open the SpotON port. You will have to break the seal. Keep the removed cover in a secure location, as you will need to replace it later.
- [X] Load 200 µl of the priming mix (blue) into the flow cell via the priming port (not the SpotON sample port), avoiding the introduction of air bubbles.
- [X] Sanitize a pair of lab scissors using ethanol, then cut the tip off of a 100 mL pipette tip.
- [X] Using the cut pipette tip, mix the prepared library gently by pipetting up and down just prior to loading.
- [X] Add all (75 uL) of sample to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next.
- [X] Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port, close the priming port and replace the MinION lid.

Start the MinKNOW software

- [X] Click the "New Experiment" button at the bottom left of the GUI.
- [X] Follow the convention "YYYYMMDD_SampleName_RunNumber" for both the experiment name and the sample name.
    - Example: 20190403_MorexSample2_Run3
- [X] Select SQK-RAD004 as the sequencing kit.
- [X] Disable basecalling. Leave all other settings as the defaults.
- [X] Start the run.
- [X] Start the program "Amphetamine" and set the duration to "Indefinitely" to keep the machine awake.
- [X] Place all reagents back in the freezer (except the DNA sample, which should already be in the fridge).
- [X] Stick around for 10-15 minutes to make sure that reads are being collected.
- [X] Store this document in a safe place.