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WBDC355_RUN5
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Rapid Sequencing (SQK-RAD004) Guide Sheet

1. Input DNA Quality Control
2. ☒ Fill a large foam container with ice.
3. ☒ Fetch the size-selected DNA from inside a white foam container inside the lab refridgerator and place the tube on ice.
4. ~~☐~~ 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: DID NOT CHECK
   - 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)
5. Check the flow cell quality
6. ~~☐~~ ☒ Record today's date, the sample name, the run number, and your name at the top of this sheet.
7. ☒ Check that the computer has at 500 GB of free space (not counting any connected external drives).
8. ☒ Fetch the flow cell from the lab refridgerator and record the flow cell type (FLO-MIN106 or FLO-MIN107).
   - Flow cell type: FLO-MIN106 LABEL: 11000540 BARCODE: 2002540064
9. ☒ Plug the MinION into the computer.
   X REMOVE CONFIGURATION TEST CELL
10. ☒ Insert the flow cell into the MinION.
11. ☒ Start MinKNOW, select the flowcell, and change the flow cell type to match the flow cell being used. MINKNOW VERSION 19.06.7 core 3.4.5 GUI 3.4.12 BREAM 4.1.8 GUPPY 3.0.4
12. ☒ Click "Check flow cells" at the bottom of the screen. Then click "Start test"
13. ~~☐~~ ☒ 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: 1538 FLOW CELL FAJ01502
14. Library preparation
15. ☒ Thaw each of the following reagents, briefly spin down in the centrifuge, then immediately store on ice.

- FRA (brown) NOT FROZEN SK1311003
- RAP (green) NOT FROZEN SK1241003
- SQB (red) SK1281002
- LB (pink) SK1271003
- FB (blue) - use only one vial SK1291004 (FLB)
- FLT (purple stiped) NOT FROZEN SK 1301003

16. ☒ Sanitize a pair of lab scissors using ethanol, then cut the tip off of a 10 mL (red) pipette tip.
17. ☒ 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 on ice.
18. ☒ With a new (red) pipette tip, mix the vial of FRA by pipetting up and down.
19. ☒ Add 2.5 uL of FRA to the PCR tube with DNA. Place the vial of FRA back on ice.
20. ☒ Gently mix the PCR tube by flicking, then spin down.
21. ☒ Incubate the tube at 30° C for 1 minute and then at 80° C for 1 minute. Briefly put the tube on ice to cool it down.
22. Adapter attachment
23. ☒ Using a (red) pipette tip, mix the vial of RAP by pipetting up and down.
24. ☒ Add 1 uL of RAP to the PCR tube. Place the RAP vial back on ice.
25. ☒ Gently mix the PCR tube by flicking, then spin down.
26. ☒ Wait 13 minutes at room temperature, then store the PCR tube on ice.
27. Priming the flow cell
28. ☒ Vortex the SQB and FB tubes, spin down, and return them to ice.
29. ☒ Open the priming port (not the SpotON sample port).
30. ☒ Set a 1000 uL pipette to 200 uL and insert the tip into the priming port.
31. ☒ Increase the pipette volume until you can see a small volume of buffer entering the pipette tip.
32. ☒ Visually check that there is continuous buffer from the priming port across the sensor array. ???
33. ☒ Use a (green) pipette tip to mix the FLT tube by pipetting up and down.
34. ☒ Add 30 uL of FLT directly to the tube of FB and mix by pipetting up and down. Return the tube of FLT to ice.
35. ☒ Load 800 µl of this mix into the flow cell via the priming port, avoiding the introduction of air bubbles.

36. [x] Wait 5 minutes.
37. Loading the flow cell
38. [x] Find a LoBind microfuge tube.
39. [x] Using a (green) pipette tip, mix the vial of SQB by pipetting up and down.
40. [x] Add 34 uL of SQB to the LoBind tube, then return the SQB to ice.
41. [x] Vortex the LB tube, then immediately add 25.5 uL of LB to the LoBind tube. Return the LB tube to ice.
42. [x] Add 4.5 uL of nuclease-free water to the LoBind tube.
43. [x] Sanitize a pair of lab scissors using ethanol, then cut the tip off of a 100 mL (green) pipette tip.
44. [x] Using the cut pipette tip, add all (11 uL) of the DNA library (PCR tube) to the LoBind tube.
45. [x] Open the SpotON port. You will have to break the seal.
46. [x] Load 200 µl of the priming mix (from step 5) into the flow cell via the priming port (not the SpotON sample port), avoiding the introduction of air bubbles.
47. [x] Sanitize a pair of lab scissors using ethanol, then cut the tip off of a 100 mL (green) pipette tip.
48. [x] Using the cut pipette tip, mix the prepared library gently by pipetting up and down just prior to loading.
49. [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.
50. [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.
51. Start the MinKNOW software
52. [x] Click the "New Experiment" button at the bottom left of the GUI.
53. [x] Follow the convention "YYYYMMDD_SampleName_RunNumber" for both the experiment name and the sample name.
    - Example: 20190403_MorexSample2_Run3
54. [x] Select SQK-RAD004 as the sequencing kit.
55. [x] Disable basecalling. Leave all other settings as the defaults.
56. [x] Start the run.
57. [x] Start the program "Amphetamine" and set the duration to "Indefinite" to keep the machine awake.

58. ☒ Place all reagents back in the freezer. Place the DNA sample back in the refridgerator.

59. ☒ Stick around for 10-15 minutes to make sure that reads are being collected.