Rapid sequencing of genomic DNA for the MinION™ device using SQK-RAD002 (1/2)

Flow Cell Number .....
DNA Samples .....

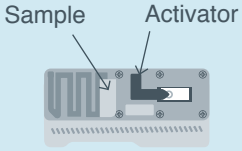
Before start checklist

- ☐ Rapid Sequencing Kit (SQK-RAD002 with EXP-LLB001)
- ☐ Pipettes and tips P1000, P200, P20, P10 and P2 1.5ml
- ☐ Eppendorf DNA LoBind tubes
- ☐ 0.2 ml thin-walled PCR tubes
- ☐ MinION SpotON Flow cell (FLO-MIN106)
- ☐ Nuclease-free water (NFW)
- ☐ NEB Blunt/TA Ligase Master Mix (MO367)
- ☐ Timer
- ☐ Microfuge
- ☐ Thermal cycler at 30 °C and 75 °C

MASSFLOW	INSTRUCTIONS	NOTES / OBSERVATIONS
0.2 ml PCR tube 10 µl 0.2 ml PCR tube	☐ Take 200 ng high molecular weight DNA in 7.5 µl ☐ Add 2.5 µl FRM Mix gently by inversion + spin down Incubate for 1 min at 30 °C then 1 min at 75 °C Spin down briefly	FRM
0.2 ml PCR tube 1.2 µl DNA LoBind 11.2 µl Store on ice	☐ Add 1 µl RAD ☐ Add 0.2 µl Blunt/TA Ligase Master Mix Incubate for 5 mins at RT The library preperation is complete and ready for loading onto the MinION	RAD

Before start checklist

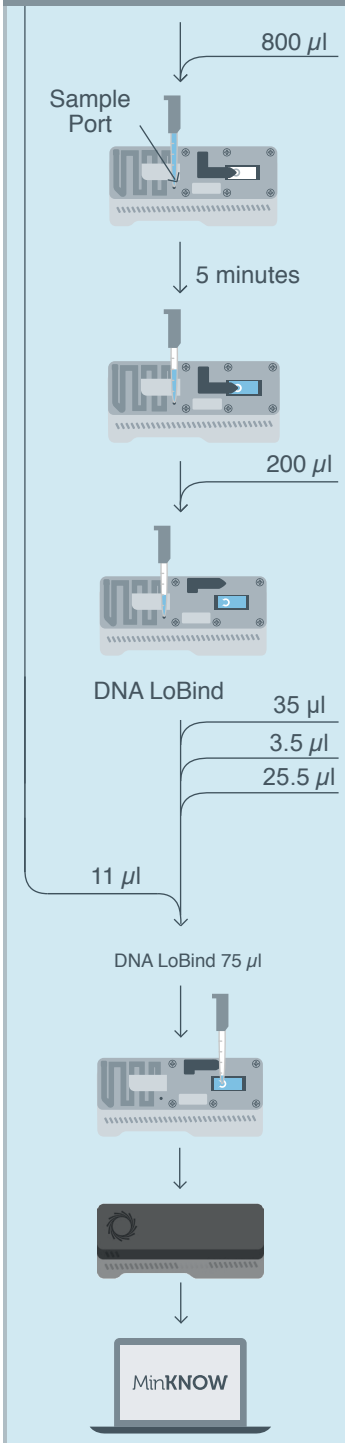
- ☐ MinION™ connected to computer with SpotON Flow Cell
- ☐ Run platform QC in parallel to library prep
- ☐ Computer set up to run MinKNOW
- ☐ Desktop Agent set up
- ☐ Run Name set
- ☐ Pre-sequencing Mix (PSM) at > 4 ng/µl
- ☐ PSM, RBF and LLB on ice
- ☐ NFW at RT
- ☐ Platform QC completed

MASSFLOW	INSTRUCTIONS	NOTES / OBSERVATIONS
Priming and loading the library  	Prepare the MinION for sequencing protocol This step can be run in parallel with the preparation of the library from genomic DNA to Pre-sequencing Mix ☐ Assemble the MinION and MinION Flow Cell ☐ Setup MinKNOW to run the Platform QC – name the run and start the protocol script – NC_Platform_QC.py ☐ Allow the script to run to completion and the number of active pores are reported	
	Prime the Flow Cell ready for the library to be loaded when library preparation is complete Prepare priming buffer ☐ 480 µl RBF ☐ 520 µl Nuclease-free water	RBF

Rapid sequencing of genomic DNA for the MinION™ device using SQK-RAD002 (2/2)

Flow Cell Number .....

DNA Samples .....

MASSFLOW	INSTRUCTIONS	NOTES / OBSERVATIONS
	**Prime the Flow Cell** Open the sample port. Draw back a few µls of buffer to make sure there is continuous buffer flow from the sample port across the sensor array. ☐ Load 800 µl of the priming buffer. Wait 5 minutes ☐ Gently lift the activator to make the SpotON port accessible ☐ Load 200 µl of the priming buffer as before	
	Prepare the library for loading ☐ 25.5 µl RBF kept on ice ☐ 12 µl NFW kept at RT ☐ 26.5 µl LLB kept on ice ☐ 11 µl Adapted and tethered library Mix by inversion and spin down	RBF LLB
	Loading the prepared library ☐ Add 75 µl of sample to the flow cell via the SpotON port in a dropwise fashion. Ensure each drop flows into the port before adding the next. ☐ Gently replace the activator, making sure the bung enters the SpotON port ☐ Close the sample port cover and replace the MinION lid.	
	Starting the sequencing script in MinKNOW ☐ Return to MinKNOW, name the run, select the NC_48Hr_Sequencing_FLO_MIN106_SQKLSK108_plus_Basecaller.py for live basecalling using the start in the MinKNOW dialogue box ☐ MinKNOW will report the number of pores available for sequencing before data collection begins. These may differ from those reported in the Platform QC. ☐ Allow the protocol to proceed until MinKNOW reports Finished Successfully. Use the Stop in the Control Panel to finish the protocol. ☐ Close down MinKNOW and disconnect the MinION If using Albacore for local basecalling please refer to the instructions in Albacore basecalling software	

Before start checklist

☐ Store washed flow cell at 4 °C or complete the returns form in the library prep

☐ Store MinION at RT
☐ Return reagents to the freezer

☐ Navigate to **www.metrichor.com** to review the full sequencing report