1D Genomic DNA sequencing for the MinION™ device using SQK-LSK108

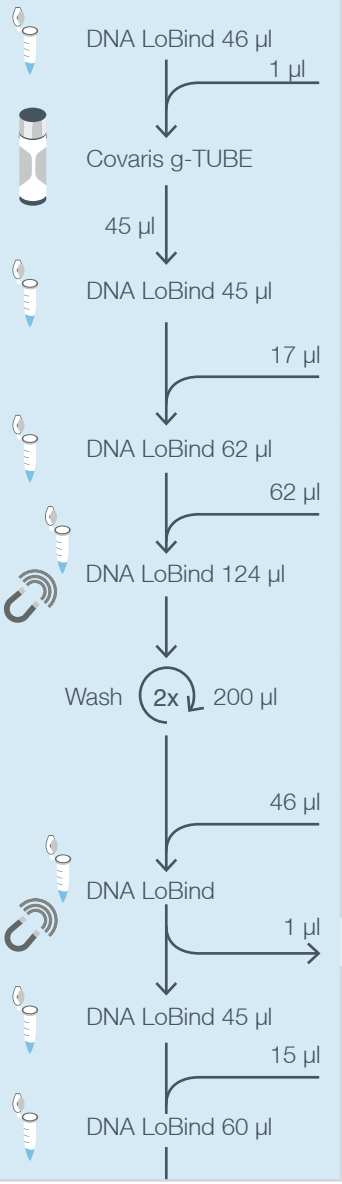
Flow Cell Number: ..... DNA Samples: .....

Before start checklist

- ☐ Ligation 1D Sequencing Kit SQK-LSK108 (AMX 1D in freezer until needed)
- ☐ AMPure beads
- ☐ Microfuge
- ☐ Vortexer and Hula mixer
- ☐ Pipettes P2, P20, P100/200, P1000
- ☐ Pipette tips P2, P20, P100/200, P1000
- ☐ Freshly prepared 70% EtOH
- ☐ MinION SpotON FLO-MIN106 or FLO-MIN107
- ☐ NEB Blunt/TA Ligase Master Mix
- ☐ Heat block for 1.5ml tubes at 37°C
- ☐ Thermal cycler at 20°C and 65°C
- ☐ Magnetic rack for bead separation
- ☐ Nuclease-free water (NFW) (not supplied)
- ☐ 10mM Tris-HCl pH8.5 (if used)
- ☐ Platform QC completed on flow cell
- ☐ Notebook sleeptimer/update off
- ☐ User adapt loading for (FLO-MIN104)
- ☐ NEBNext Ultra II End-repair/dA-tailing module
- ☐ Pre-chilled freezer pack for 0.2ml tubes at -20°C
- ☐ DNA LoBind Eppendorf tubes 1.5ml
- ☐ 0.2ml thin-walled PCR tubes
- ☐ 0.2ml thin-walled PCR tubes Latest versions of MinKNOW and Desktop Agent (check for updates)

Additions to checklist for this protocol:

Covaris g-TUBE (optional), NEBNext FFPE Repair Mix

MASSFLOW	INSTRUCTIONS	NOTES/ OBSERVATIONS	TIME/DATE
	**Genomic DNA** 1-1.5 µg Genomic DNA in 46 µl Shear in a Covaris g-TUBE Eppendorf 5424; 6000rpm for 8 kb fragments. 2 x 1 minute (invert tube to collect) Keep processing time under 15 minutes Analyse 1 µl for fragment size, quantity and quality (Agilent Bioanalyser)	This step is optional and can be omitted if long fragments are required.	
	DNA repair (optional) Add from NEBNext FFPE RepairMix (NEB M6630) ☐ 8.5 µl NFW ☐ 6.5 µl FFPE Repair Buffer ☐ 2 µl FFPE Repair Mix		
	Mix by inversion + spin down Incubate for 15 mins at 20 °C		
	☐ Add 62 µl resuspended AMPureXP beads to end-prep reaction at RT ☐ Incubate on rotator for 5 mins, spin down and pellet on magnet Discard the supernatant at RT		
	Keep on magnet wash 2x with 200 µl fresh 70% EtOH, ☐ do not disturb pellet ☐ Spin down, replace on magnet, pipette of residual wash Briefly allow to dry		
	☐ Resuspend pellet in 46 µl NFW, incubate for 2 mins at RT Pellet ☐ beads on magnet, remove eluate and transfer to fresh DNA LoBind tube		
	1 µl QuBit fluorimeter-recovery ≥ 1000 ng of material		
	Add from NEBNext Ultra II End-Repair / dA-tailing Module ☐ 7 µl Ultra II End-Prep buffer ☐ 3 µl Ultra II End-Prep enzyme mix ☐ 5 µl DNA CS 3.6kb (DNA CS – positive control) Mix by inversion + spin down Incubate for at 20 °C for 5 minutes and 65 °C for 5 minutes	DCS	

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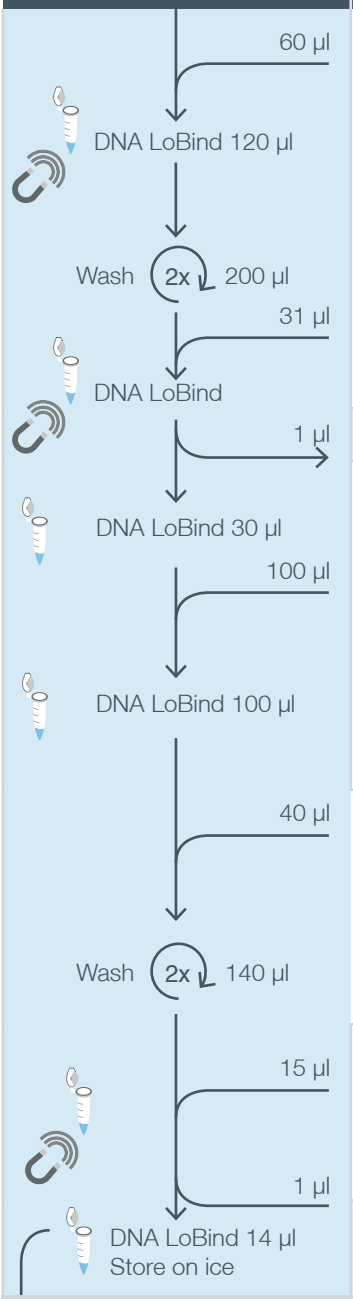	☐ Add 60 µl resuspended AMPure XP beads at RT ☐ Incubate on rotator for 5 minutes, spin down and pellet on magnet. Discard the supernatant. ☐ Keep on magnet, wash 2x with 200 µl fresh 70% EtOH, do not disturb pellet ☐ Briefly spin down, replace on magnet, pipette off residual wash. Briefly allow to dry ☐ Resuspend pellet in 31 µl Nuclease-free water, incubate at RT for 2 minutes ☐ Pellet beads on a magnet, remove eluate of End-Prepped DNA and transfer to fresh DNA LoBind		
	1 µl QuBit fluorimeter – recovery aim about 700 ng of material		
	NEB Blunt / TA Ligase Master Mix. When preparing the ligation reaction, mix by inversion between each addition. ☐ Check that the Master Mix is clear of any precipitate ☐ 30 µl end-prepped DNA ☐ 20 µl Adapter Mix ☐ 50 µl NEB Blunt / TA Master Mix Mix gently by inversion and spin down Incubate at RT for 10 minutes	AMX	
	☐ Vortex AMPure XP beads to resuspend ☐ Transfer 40 µl of beads into the adapter ligation reaction ☐ Mix by pipetting, incubate at RT on a rotator mixer for 5 mins ☐ Pellet beads on magnet and remove supernatant ☐ Add 140µl ABB to beads. Close tube lid, resuspend beads by flicking. Pellet beads on magnet and remove supernatant. Repeat	ABB	
	Elute the adapted library (Pre-sequencing Mix) ☐ Resuspend pelleted beads in 15 µl ELB and incubate at RT for 10 minutes ☐ Pellet beads on the magnet, remove the eluate and transfer to new DNA LoBind tube		
	1 µl QuBit fluorimeter		

Before start checklist

- ☐ MinION™ connected to computer with SpotOn Flow Cell
- ☐ Platform QC completed (can be done in parallel to library prep)
- ☐ Computer setup to run MinKNOW
- ☐ Desktop Agent setup
- ☐ Run Name set
- ☐ Prepared library > 4 ng/µl
- ☐ RBF and library on ice
- ☐ NFW at RT

Priming and loading the library 	Prepare the MinION for sequencing protocol The platform QC should be run prior to library preparation beginning ☐ 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		

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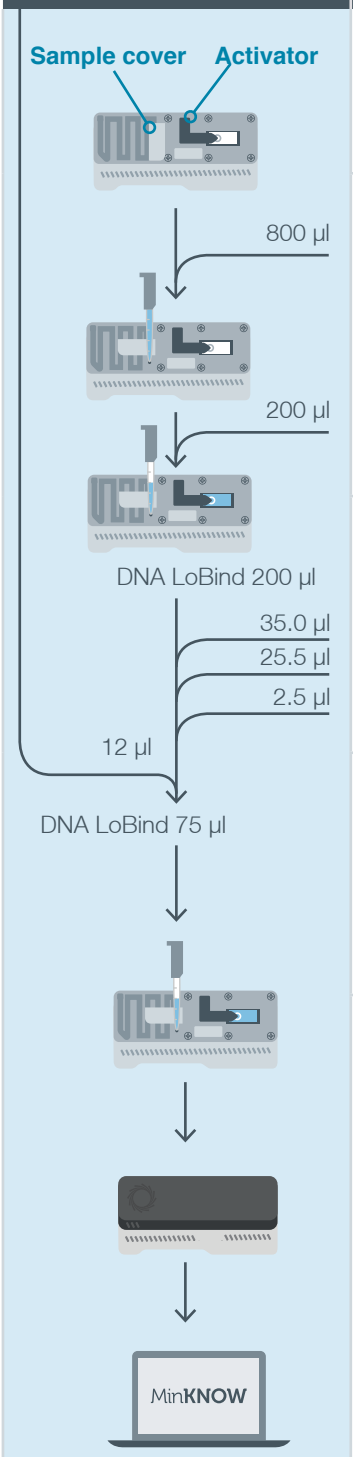	Prime the flow cell ready for the library to be loaded when library preparation is complete Prepare priming buffer ☐ 480 µl RBF ☐ 520 µl Nuclease-free water		
	Prime the flow cell ☐ Open the sample port. Draw back a few µls of buffer to make sure there is continuous buffer flow from the sample port across the sensor array. ☐ Load 800µl of the priming buffer. Wait 5 minutes ☐ Gently lift the activator to make the SpotON port accessible ☐ Load 200µl of the priming buffer through the sample port	RBF	
	Prepare the library for loading ☐ 35.0 µl RBF kept at RT ☐ 25.5 µl LLB kept at RT ☐ 12.0 µl Adapted and tethered library ☐ 2.5 µl Nuclease-free water Mix gently by pipetting	RBF LLB	
	Loading the prepared library ☐ Add 75µl of sample to the flow cell via the SpotON port in a dropwise fashion. Ensure each drop flows into the port beforeadding the next. ☐ Gently replace the activator, making sure the bung enters the SpotON port ☐ Close the sample port cover and replace the MinION li		
	Starting the sequencing script in MinKNOW and the workflow in the **Desktop** Agent ☐ Return to MinKNOW, name the run, select the NC_48Hr_Sequencing_Run_FLO_MIN106_SQK-LSK108 _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. ☐ Quit the Desktop Agent, close down MinKNOW and disconnect the MinION After sequencing **If using Albacore for local basecalling please refer to the instructions in Albacore basecalling software**		

After sequencing checklist

- ☐ Store washed flow cell at 4°C or complete the returns form in the Nanopore Community
- ☐ Return reagents to the freezer
- ☐ Navigate to **www.metrichor.com** to review the full sequencing report
- ☐ Store MinION at RT