1D^2 genomic DNA sequencing for the MinION™ device using SQK-LSK308 (1/3)

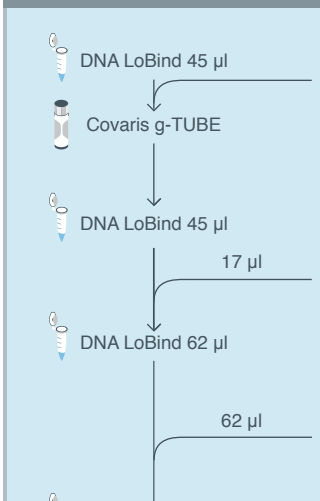
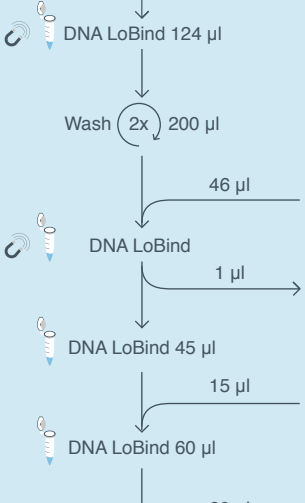
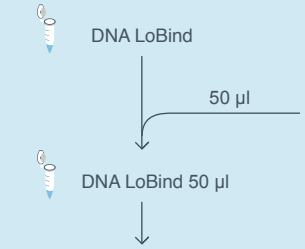
Oxford NANOPORE Technologies

Flow Cell Number .....

DNA Samples .....

Before start checklist

- ☐ 1D^2 Sequencing Kit (SQK-LSK308)
- ☐ Library Loading Beads Kit (EXP-LLB001)
- ☐ SpotON Flow Cell FLO-MIN107
- ☐ Vortexer and Hula mixer
- ☐ Pipettes P1000, P200, P100, P20, P10 and P2
- ☐ Pipette tips P2, P20, P100/200, P1000
- ☐ Approx 20 DNA LoBind Eppendorf 1.5 ml tubes
- ☐ Freshly-prepared 70% EtOH
- ☐ Covaris g-TUBE (optional)
- ☐ AMPure beads resuspended and at RT
- ☐ Magnet for bead separation
- ☐ Nuclease-free water (NFW)
- ☐ Laptop sleeptimer / update off
- ☐ Microfuge
- ☐ NEBNext FFPE Repair Mix (M66360) (optional)
- ☐ Run platform QC prior to library prep
- ☐ NEB Blunt / TA Ligase Master Mix
- ☐ 0.2 ml thin-walled PCR tubes
- ☐ Heat block at 37 °C capable of taking 1.5 ml tubes
- ☐ Thermal cycler at 20 °C and 65 °C
- ☐ NEBNext Ultra II End-repair / dA-tailing Module
- ☐ Update checks for MinKNOW™ completed
- ☐ Check that the MinKNOW output directory is on the SSD

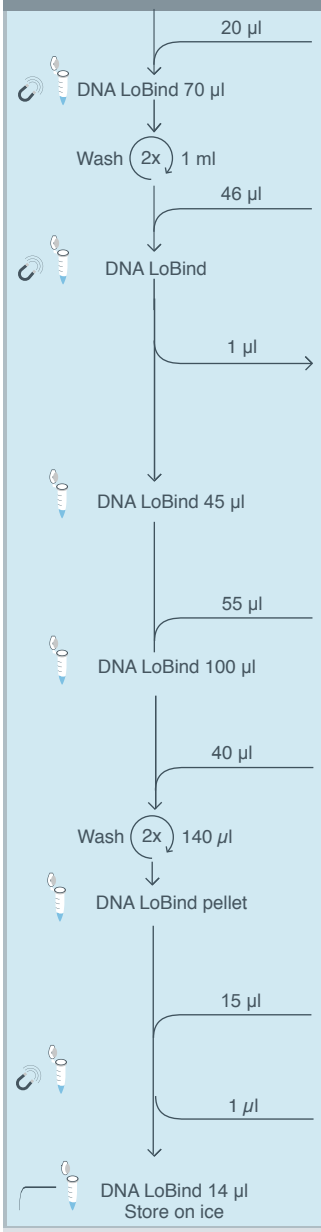
MASSFLOW	INSTRUCTIONS	NOTES / OBSERVATIONS	TIME / DATE
	**Genomic DNA** 1-1.5 µg Genomic DNA in 45 µl Shear in a Covaris g-TUBE Eppendorf 5424; 6000 rpm for 8 kb fragments. 2 x 1 minute (invert tube to collect) Keep processing time under 15 minutes	This step is optional and can be omitted if long fragments are required.	
	DNA repair **(optional)** Add from NEBNext FFPE RepairMix ☐ 8.5 µl NFW ☐ 6.5 µl FFPE Repair Buffer ☐ 2 µl FFPE Repair Mix		
	Mix by pipetting + spin down Incubate for 15 mins at 20 °C		
	☐ Add 62 µl resuspended AMPureXP beads to the end-prep reaction at room temperature and mix gently by pipetting ☐ Incubate on rotator for 5 mins, spin down and pellet on magnet		
	☐ 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 fluorometer - recovery aim < 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 NFW Mix by inversion + spin down Incubate for at 20 °C for 5 minutes and 65 °C for 5 minutes		
	☐ 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 25 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 fluorometer – recovery aim about 700 ng of material		
	☐ Thaw the 1D² Adapter ☐ Add 22.5 µl end prepped DNA (500 ng) ☐ Add 2.5 µl 1D² Adapter ☐ Add 25 µl Blunt/TA Ligase Master Mix Mix gently by inversion + spin down Incubate at RT for 10 minutes		

1D^2 genomic DNA sequencing for the MinION™ device using SQK-LSK308 (2/3)

Oxford NANOPORE Technologies

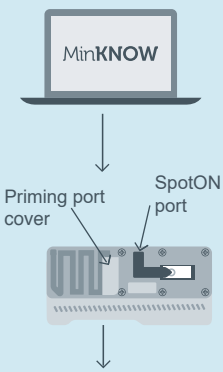
Flow Cell Number .....

DNA Samples .....

MASSFLOW	INSTRUCTIONS	NOTES / OBSERVATIONS	TIME / DATE
	Add 20 µl resuspended AMPure XP beads to the DNA sample ☐ Incubate on rotator for 10 minutes, spin down and pellet on magnet. Discard the supernatant. ☐ Keep on magnet, wash 2x with 1 ml fresh 70% EtOH, do not disturb pellet ☐ Briefly spin down, replace on magnet, pipette off residual wash. Briefly allow to dry ☐ Resuspend pellet in 46 µl nuclease-free water, incubate at RT for 2 minutes ☐ Pellet beads on a magnet, remove eluate of barcoded DNA and transfer to fresh DNA LoBind		
	1 µl Qubit fluorometer		
	Blunt/TA Ligase Master Mix When preparing the ligation reaction, mix by inversion between each addition ☐ 45 µl of 1D² adapted sample 5 µl ☐ BAM ☐ 50 µl Blunt/TA Ligase Master Mix Mix gently by inversion and spin down Incubate at RT for 10 minutes	BAM	
	☐ Vortex the AMPure XP beads to resuspend ☐ Transfer 40 µl of beads to the adapter ligation reaction ☐ Mix by pipetting, incubate at RT on a rotator mixer for 10 mins ☐ Pellet beads on magnet and remove supernatant ☐ Add 140 µl 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	ELB	
	1 µl Qubit fluorometer – recovery aim about 200 ng of material		

Before start checklist

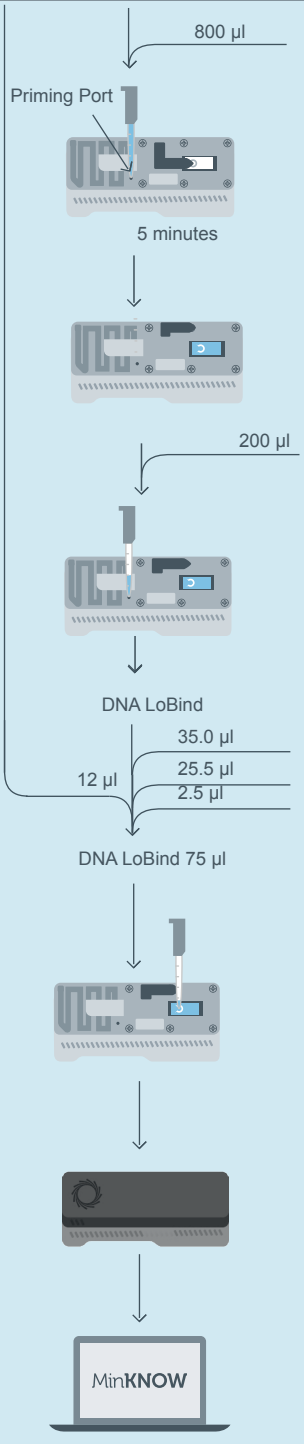
- ☐ MinION™ connected to computer with SpotON Flow Cell
- ☐ Run platform QC in parallel to library prep
- ☐ Computer setup to run MinKNOW
- ☐ Albacore downloaded
- ☐ Run Name set
- ☐ Pre-sequencing Mix (PSM) at > 4 ng/µl
- ☐ RBF and PSM on ice
- ☐ NFW at RT
- ☐ Platform QC completed

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		
	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	

1D^2 genomic DNA sequencing for the MinION™ device using SQK-LSK308 (3/3)

Flow Cell Number .....

DNA Samples .....

MASSFLOW	INSTRUCTIONS	NOTES / OBSERVATIONS	TIME / DATE
	Prime the flow cell using the priming port ☐ Open the priming port. Draw back a few µls of buffer to make sure there is continuous buffer flow from the priming port across the sensor array. ☐ Load 800µl of the priming buffer. Wait 5 minutes ☐ Gently lift the SpotON cover to make the SpotON port accessible ☐ Load 200 µl of the priming buffer through the sample port	The aim of the draw back step is to remove any small air bubbles that have developed in the channel between the priming port and the sensor array window. This should be achieved by removing just a few µls of buffer before the air bubble is taken up into the pipette tip. Avoid removing large volumes that result in the sensor array no longer being covered by buffer.	
	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 75 µlµl 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 sample port cover, 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_Run_FLO-MIN107_SQK-LSK308.py and start 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. ☐ Open Albacore, and run the 1D^2 basecalling workflow		

Oxford Nanopore Technologies thank Dirk Woortman, Technische Universitat Munchen for assistance in developing this tool