Lambda control experiment for the MinION™ device (1/2)

Oxford NANOPORE Technologies

Flow Cell Number .....

DNA Samples .....

Before start checklist

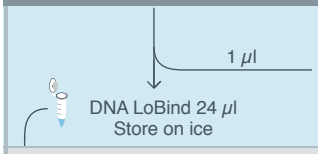
- ☐ Vortexer and Hula mixer
- ☐ Pipettes P1000, P200, P100, P20, P10 and P2
- ☐ Tips ready: 40 x 1000 µl, 30 x 200 µl, 20 x 10 µl
- ☐ Covaris g-TUBE
- ☐ AMPure beads resuspended and at RT
- ☐ MyOne C1 Streptavidin beads resuspended and at RT
- ☐ Magnet for bead separation
- ☐ Approx 20 DNA LoBind Eppendorf tubes
- ☐ LMD / BBB / ELB / DCS / HPT/ RBF1 on ice
- ☐ HPA / AMX in freezer until needed
- ☐ Freshly prepared 70% EtOH
- ☐ Nuclease free water (NFW)
- ☐ Notebook sleeptimer / update off
- ☐ 10mM Tris-HCl pH 8.5
- ☐ Microfuge
- ☐ Nanopore sequencing kit SQK-NSK007
- ☐ Update checks for MinKNOW™ and Metrichor™ completed
- ☐ 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 1.5ml tubes
- ☐ Thermal cycler AT 20°C & 65°C
- ☐ NEBNext Ultra II End-repair / dA-tailing Module

MASSFLOW	INSTRUCTIONS	NOTES / OBSERVATIONS	TIME / DATE
DNA LoBind 46 µl Covaris g-TUBE 46 µl → 1 µl	**Lambda control experiment** 1 µg Genomic DNA in 46 µl (20 µl LMD and 26 µl NFW) 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	LMD A PreCR step is not required when using the Lambda DNA contained in the kit as it is high quality DNA, however it is recommended with other gDNA samples	
	1 µl Agilent Bioanalyzer / Gel analysis		
DNA LoBind 45 µl 15 µl DNA LoBind 60 µl	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 3.6 kb (DNA CS – positive control) Mix by inversion + spin down Incubate for at 20 °C for 5 minutes and 65 °C for 5 minutes	DCS	
60 µl DNA LoBind 120 µl Wash 2x 200 µl 31 µl DNA LoBind	☐ 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	1 µl QuBit fluorimeter – recovery aim about 700 ng of material		
DNA LoBind 30 µl 70 µl DNA LoBind 100 µl	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 ☐ 8 µl NFW ☐ 10 µl Adapter Mix ☐ 2 µl HPA ☐ 50 µl NEB Blunt / TA Master Mix Incubate at RT for 10 minutes	AMX HPA	
1 µl DNA LoBind 101 µl	☐ 1 µl HPT Mix by inversion + spin down Incubate at RT for 10 minutes While incubating prepare MyOne C1 beads for purification of the adapted and tethered DNA	HPT	
DNA LoBind 50 µl Wash 2x 100 µl 100 µl DNA LoBind 100 µl	Prepare the MyOne C1 beads in DNA LoBind tube ☐ Vortex MyOne C1 beads to resuspend ☐ Transfer 50 µl of beads to DNA Lobind tube ☐ Pellet beads on magnet and remove supernatant ☐ Wash beads 2x with 100 µl BBB vortexing to resuspend. Pellet on magnet, pipette off and discard supernatant ☐ Resuspend washed beads in 100 µl BBB to be used for adapter ligation cleanup	BBB	
100 µl DNA LoBind 201 µl	Purifying the adapted DNA ☐ Add 100 µl washed MyOne C1 beads to the adapted, tethered DNA reaction on a rotator ☐ Incubate at RT for 5 minutes		
Wash 2x 150 µl DNA LoBind pellet	☐ Place on magnet, pellet and pipette off the supernatant ☐ Wash beads with library bound 2x with 150 µl BBB, pipette to resuspend. Pellet on magnet pipette off and discard supernatant ☐ Close the lid, spin down, replace on magnet for 1-2 minutes, open lid and pipette off any residual BBB	BBB	
25 µl	Elute the adapted library (Pre-sequencing Mix) ☐ Resuspend pelleted beads in 25 µl ELB and incubate at 37°C for 10 minutes ☐ Pellet beads on the magnet, remove the eluate and transfer to new DNA LoBind tube	ELB	

Burn-In for the MinION™ device (2/2)

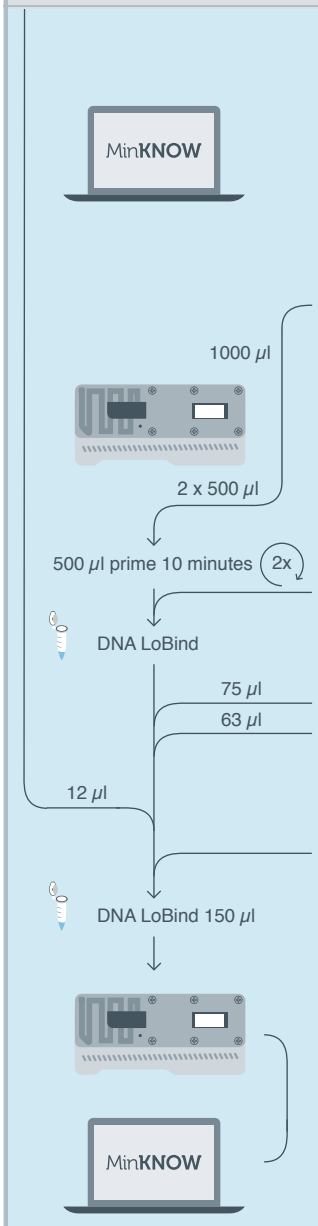
Flow Cell Number .....

DNA Samples .....

MASSFLOW	INSTRUCTIONS	NOTES / OBSERVATIONS	TIME / DATE
	1 µl QuBit fluorimeter – recovery aim ~250ng ng of material		

Before start checklist

- ☐ MinION™ connected to computer with MinION™ Flow Cell
- ☐ Run platform QC in parallel to library prep
- ☐ MinION with MinION Flow Cell
- ☐ Computer setup to run MinKNOW
- ☐ Metrichor Agent setup
- ☐ Run Name set
- ☐ Screws and Heat pad for original MinION
- ☐ Pre-sequencing Mix (PSM) at > 4 ng/µl
- ☐ PSM and RBF1 on ice
- ☐ NFW at RT
- ☐ Platform QC completed



Prepare the MinION for sequencing protocol This step can be run in parallel with the preparation of the library from genomic DNA to Presequencing 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_FLO_MIN104.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 Prepare priming buffer ☐ 500 µl 2x RBF1 ☐ 500 µl NFW Mix by vortexing and spin down	RBF1	
Prime the flow cell ☐ Open the sample port and using a 1000 µl pipette draw back the buffer in the flow cell to make sure there is continuous buffer flow from the sample port across the sensor array. ☐ Load 500 µl of the priming buffer using a vertical 1000 µl pipette and tip in a continuous flow avoiding introducing air bubbles or disturbing the sensor array. Wait 10 minutes, then repeat the loading and wait another 10 minutes		
Loading the prepared library ☐ Mix the Pre-sequencing Mix by inversion 10x, briefly spin down the library ☐ Add the reagents in the following order ☐ 75 µl RBF1 kept at RT ☐ 63 µl NFW kept at RT ☐ 12 µl Pre-sequencing Mix kept on ice ☐ Load 150 µl of the library loading mix via the sample port, close the cover and replace the MinION lid.	RBF1	
Starting the sequencing script in MinKNOW and the workflow in the Metrichor Agent ☐ Return to MinKNOW, name the run, select the NC_6Hr_Lambda_Burn_In_Run_FLO_MIN104.py and start using the start in the MinKNOW dialogue box ☐ Open the Metrichor Agent, select the latest version of the Lambda Control Experiment workflow, run the workflow and monitor the workflow using ☐ the visualisation options in details 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 System Ready**. Use the Stop in the Control Panel ☐ to finish the protocol. Quit the Metrichor Agent, close down MinKNOW and disconnect the MinION.		

After sequencing checklist

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

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