MinION DNA Library Prep (SQK-LSK109)

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

Protocol for ligation sequencing DNA library prep for MinION slightly modified from the ONT protocol.

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MATERIALS TEXT

MinION Flow Cell	
Sequencer	
Oxford Nanopore Technologies	**FLO-MIN106D**

MinION	
Sequencer	
Oxford Nanopore Technologies	**MinION 1B / MinION 1C**

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Magnetic Stand
Magnetic Stand
Thermo Scientific **MR02**
Any magnetic rack that fits your tubes will suffice.

Agencourt AMPure XP **Beckman Coulter Catalog #A63880**

Ethanol, pure **Omnipure Filter Company Catalog #4455**

Nuclease-free Water **Contributed by users**

NEBNext FFPE DNA Repair Mix - 24 rxns **New England Biolabs Catalog #M6630S** Step 9

NEBNext Ultra End Repair/dA-Tailing Module - 96 rxns **New England Biolabs Catalog #E7442L**

Qubit 4
Fluorometer
Invitrogen **Q33238**

Qubit dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #Q32851**

ThermoMixer
Benchtop Incubator
Eppendorf **5382000023**
Any heat block will suffice

Hula mixer
Mixer
Invitrogen **15920D**
Any rotator mixer

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Bead clean-up 1 15m

1 Make up **1 µg** of DNA sample in nuclease-free water to a volume of **49 µl** in an Eppendorf DNA LoBind tube.

Make **150 µl** of fresh 70% ethanol.

Place an 1.5 mL tube of nuclease-free water on a heat block set to **55 °C** .

2 5m

Agencourt AmPure XP beads **Contributed by**

Resuspend the **users Catalog #A63880** by vortexing.

Add **150 µl AMPure beads** to the DNA and gently flick the tube for **00:05:00** .

3 Place the tube on the magnetic rack and wait for the beads to pellet on the magnet.

Once the beads have pelleted, pipette off and discard the supernatant.

4 Without disturbing the pellet on the magnet, wash the beads with **50 µl 70% ethanol** .

Pipette off the ethanol and discard.

5

go to step #4

6 30s

Allow the bead pellet to dry on the magnet with the tube's lid open for about **00:00:30** , but do not allow the pellet to dry out completely.

7 5m

Remove the tube from the magnet. Elute the DNA with **50 µl nuclease-free water** at **55 °C** .

Gently flick the tube for **00:05:00** to resuspend the beads and replace the tube on the magnet.

Once the beads have pelleted, pipette off and retain **50 µl** of the eluate. Discard the beads.

Qubit 1 5m

8

Follow the Qubit DNA HS Assay protocol to quantify **1 µl** of clean DNA from the clean-up step.

DNA repair & end-prep 30m

9 Thaw DNA CS at **Room temperature** , mix by pipetting and place **On ice** .

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NEBNext FFPE DNA Repair Mix - 24 rxns **New England**

Thaw **Biolabs Catalog #M6630S** and

NEBNext® Ultra™ II End Repair/dA-Tailing Module **New England**

Biolabs Catalog #E7546 **On ice** .

10 

In a 0.2 mL PCR tube, mix:

- **1 µl DNA CS**
- **47 µl DNA sample**
- **3.5 µl NEBNext FFPE DNA Repair Buffer**
- **2 µl NEBNext FFPE DNA Repair Mix**
- **3.5 µl Ultra II End-prep Reaction Buffer**
- **3 µl Ultra II End-prep Enzyme Mix**

11 Mix by gently flicking the tube, then spin down. 25m

Incubate at **20 °C** for **00:20:00** , then at **65 °C** for **00:05:00** using a thermocycler.

Bead clean-up 2 10m

12 Agencourt AmPure XP beads **Contributed by**

Resuspend the **users Catalog #A63880** by vortexing.

Transfer the DNA mixture from the PCR tube to an Eppendorf DNA LoBind tube.

13 Add **60 µl AMPure beads** to the tube and flick the tube to mix.

14 Incubate on a rotator mixer or invert the tube by hand for **00:05:00** at **Room temperature** . 5m

Meanwhile, prepare **500 µl 70% ethanol** using nuclease-free water.

15 After the incubation, spin down the sample using a microfuge and place on the magnetic rack.

Once a pellet has formed, pipette off the supernatant, leaving tube on the magnet.

16 With the tube still on the magnet, wash the beads without disturbing the pellet with **200 µl 70% ethanol** .

Pipette off the ethanol and discard.

17 **go to step #16**

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18 Spin down the tube using a microfuge and replace on the magnet - pipette off residual ethanol and leave the lid open to allow the pellet to dry for around **00:00:30** . 30s

19 Remove the tube from the magnet and resuspend the pellet in **61 µl nuclease-free water** heated to **55 °C** . 2m

Incubate for **00:02:00** at **Room temperature** .

20 Replace the tube on the magnet to pellet the beads.

Remove and retain **61 µl** of the eluate in an Eppendorf DNA LoBind tube.

Qubit 2 5m

21 

Follow the Qubit DNA HS Assay protocol to quantify **1 µl** of DNA from the previous step.

Adapter ligation and clean-up 15m

22 Spin down AMX and NEBNext Quick T4 DNA Ligase. Place **On ice** .

Thaw LNB at **Room temperature** , spin down and mix by pipetting. Place **On ice** .

Thaw EB at **Room temperature** , vortex to mix and place **On ice** .

Thaw LFB **or** SFB at **Room temperature** , vortex to mix and place **On ice** .

23  10m

In an Eppendorf DNA LoBind tube, mix:

1. **60 µl DNA sample**
2. **25 µl LNB**
3. **10 µl NEBNext Quick T4 DNA Ligase**
4. **5 µl AMX**

Gently flick the tube to mix, then spin down.

Incubate the tube at **Room temperature** for **00:10:00** .

Bead clean-up 3 10m

24 Vortex the AMPure beads to resuspend.

Add **40 µl AMPure beads** to the reaction mix from the previous step. Gently flick the tube to mix.

25 Incubate the tube on a rotator mixer for **00:05:00** at **Room temperature** . 5m

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26 Spin down using a microfuge and place the tube on the magnetic rack.

Once a pellet has formed, pipette off and discard the supernatant.

27 Add **250 µl LFB or 250 µl SFB** to the tube. Gently flick the tube to resuspend the beads.

Spin the tube down briefly, then replace on the magnetic rack.

Once a pellet has formed, pipette off the supernatant and discard.

28 **go to step #27**

29 Using a microfuge, spin down the tube and replace on the magnet.

Pipette off any residual supernatant and discard.

30 Allow the pellet to dry with the lid open for about **00:00:30** . 30s

31 Remove the tube from the magnetic rack. 10m

Add **15 µl EB** and gently flick the tube to resuspend the pellet. Incubate for **00:10:00** at **37 °C** .

32 Spin down very briefly using a microfuge and replace the tube on the magnet.

Once a pellet has formed slowly pipette off and retain **15 µl** of the eluate and place **On ice** .

Qubit 3 5m

33

Follow the Qubit DNA HS Assay protocol to quantify **1 µl** of the DNA library from the previous step.

Priming the flow cell 15m

34 Thaw SQB, LB, FLT and a fresh tube of FB at **Room temperature** . Once thawed, place FB **On ice** .

Mix SQB, FB and FLT by vortexing, spin down using a microfuge and place **On ice** .

35 Open the lid of the MinION. Slide the MinION SpotON flow cell under the MinION clip.

35.1 Check the number of pores on the flow cell by navigating to and selecting **Flow Cell Check** on the **MinKNOW** software. This should take approximately **00:10:00** . 10m

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36 Rotate open the priming port cover.

Place a P1000 pipette tip (pipette set to 200 µL) upright in the priming port.

Slowly turn the volume dial to ~220 µL. There should be a small volume of yellow buffer in the pipette tip.

Still holding the pipette in an upright position, swiftly and smoothly lift the pipette out of the priming port.

37 Add **30 µl FLT** directly to a fresh tube of **FB** to make the flow cell priming mix.

Vortex to mix.

38 Load **800 µl** of the priming mix into the priming port: 5m

Hold the pipette upright in the priming port and turn the dial to gradually to force the priming mix into the flow cell. (To avoid the introduction of air, you may need to turn the dial a small amount in the opposite direction to draw up some of the flow cell buffer and establish liquid-to-liquid contact between the buffer and priming mix.)

Wait for **00:05:00** .

Preparing the loading library 5m

39 Thoroughly mix LB to resuspend the beads by pipetting.

40 

In an Eppendorf LoBind tube, mix:

- **37.5 µl SQB**
- **25.5 µl LB** (freshly mixed)
- **12 µl DNA library**

Loading the flow cell 5m

41 Lift the **SpotON sample port** cover and load an additional **200 µl** of the priming mix into the still open **priming port**.

42 Mix the loading library by slowly pipetting to resuspend the loading beads.

43 Slowly, drop **75 µl** loading library into the SpotON sample port.
Make sure the priming port is still open during this step and the library should easily fall into the sample port.

44 Close the priming port and replace the SpotON sample port cover.

Close the lid of the MinION device.

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Start the sequencing experiment on the MinKNOW software.

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