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McGill Nanopore Ligation LibPrep Protocol SQK-LSK109

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1 Works for me dx.doi.org/10.17504/protocols.io.bpegmjbw

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ABSTRACT

This protocol is used to for both amplicon and high molecular weight DNA library preparations from genomic DNA using Nanopore ligation kit (SQK-LSK109). It contains no fragmentation and no PCR step. However, a fragmentation step is optional.

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ABSTRACT

This protocol is used to for both amplicon and high molecular weight DNA library preparations from genomic DNA using Nanopore ligation kit (SQK-LSK109). It contains no fragmentation and no PCR step. However, a fragmentation step is optional.

BEFORE STARTING

For ligation library preparations, it is highly recommended to check DNA's length, quantity and purity. Poor DNA reduces subsequent adapter ligation efficiency, increases the prevalence of chimeric reads, and facilitate pore blocking on sequencing.

DNA repair and end-prep 35m

1 Prepare 1.5 ug of genomic DNA into a 1.5 ml Eppendorf DNA LoBind tube. Adjust the volume to 48 uL with nuclease-free water. Mix by flicking the tube and spin down briefly.

1.5 ug genomic DNA is the default amount. We are aiming to have 5-50 fmol for MinION / PromethION loading and 3-20 fmol for Flongle loading. Depending on the fragment size, the amount of starting material may vary. If you

can, aim for 10x the amount of starting material than what is needed for flow cell loading.

2 Mix the following components in a 0.2 mL 8-strip tube;

Component	Volume
DNA	48 µl
NEBNext FFPE DNA Repair Buffer	3.5 µl
NEBNext FFPE DNA Repair Mix	2 µl
Ultra II End-prep reaction buffer	3.5 µl
Ultra II End-prep enzyme mix	3 µl
Total	60 µl

3 Mix gently by flicking the rube and spin down.

4 Incubate the reaction as follows: 35m

20 °C 00:30:00

65 °C 00:05:00

5 After the incubation, transfer the DNA to a clean 1.5 mL Eppendorf DNA LoBind tube.

6 Resuspend the SPRI beads by vortexing.

7 Add 60 uL of resuspended SPRI beads to the end-prep reaction an mix by flicking the tube.

8 Incubate the sample on a rotator mixer for 5 min at room temperature.

9 Prepare 500 uL of fresh 80% ethanol in nuclease-free water.

10 Spin down the sample and put on a magnet for 5 min. Keep the tube on the magnet and remove the supernatant.

11 Keep the tube on the magnet and wash the beads with 200 uL of fresh 80% ethanol without disturbing the beads for 30 seconds. Remove the ethanol and discard. Repeat the previous wash step.

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12 Quick spin the tube and place it back on the magnet rack. Pipette off the residual ethanol. Air-dry for 30 seconds, do not over dry the pellet.

13 Remove the tube from the magnet and resuspend the beads in 61 uL of nuclease-free water. Incubate for 10 min at 37 °C.

14 Quick spin the tube and place it on a magnet rack for 5 min.

15 Transfer 61 uL of elute DNA into a clean 1.5 mL Eppendorf DNA LoBind tube.

16 Quantify 1 uL of eluted DNA using a fluorimetric dsDNA assay.

Continue the end-repaired DNA into the adapter ligation step. It is also possible to store the sample at 4°C overnight at this point.

Adapter ligation step

17 Thaw Ligation buffer (LNB) and Elution buffer (EB) at room temperature, spin down and mix by pipetting. Place on ice immediately after thawing and mixing.

Due to viscosity, vortexing the LNB buffer is not effective, pipetting the buffer to mix well.

18 Spin down the Adapter Mix (AMX) and Quick T4 Ligase, and place on ice.

19 Thaw one tube of Long Fragment buffer (LFB) or Short Fragment buffer (SFB) at room temperature, mix by vortexing, spin down and place on ice.

Wash buffer LFB and SFB are designer to enrich DNA and purify all fragments equally after adapter ligation. LFB is used to enrich long DNA fragments of >3 kb and SFB is to retain DNA fragments of all size.

20 In a 1.5 mL Eppendorf DNA LoBind tube, mix the following order:

Component	Volume
End-repaired DNA from the previous step	**60 µl**
Ligation Buffer (LNB)	**25 µl**

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NEBNext Quick T4 DNA Ligase	10 µl
Adapter Mix (AMX)	5 µl
Total	100 µl

21 Mix gently by flicking the tube and spin down.

22 Incubate the reaction for 10 minutes at room temperature.

adapter ligated DNA purification

23 Resuspend the SPRI beads by vortexing.

24 Add 50 µL of resuspended SPRI beads to the ligation reaction and mix by flicking the tube.

25 Incubate the tube on a rotator mixer for 5 minutes at room temperature.

26 Spin down the sample and put on a magnet for 5 min. Keep the tube on the magnet and remove the supernatant.

27 Remove the tube from the magnet and wash the beads with 250 uL of Long Fragment Buffer (LFB) or Short Fragment Buffer (SFB). Flick gently to resuspend the beads, leave for 5 min at room temperature, then return the tube to the magnetic rack and let the beads to rebind. Remove the supernatant and discard.

28 Repeat the previous wash step with LFB or SFB.

29 Quick spin the tube and place it back on the magnet rack. Pipette off the residual ethanol. Air-dry for 30 seconds, do not over dry the pellet.

30 Remove the tube from the magnet and resuspend the beads in 25 uL of elution buffer (EB) for MinION and PromethION libraries and 15 ul for Flongle libraries. Incubate for 30 min at 37 °C to increase the recovery of long fragment DNA.

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