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Modified LSK109 ligation prep with needle shear and bead clean up

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.7emhjc6

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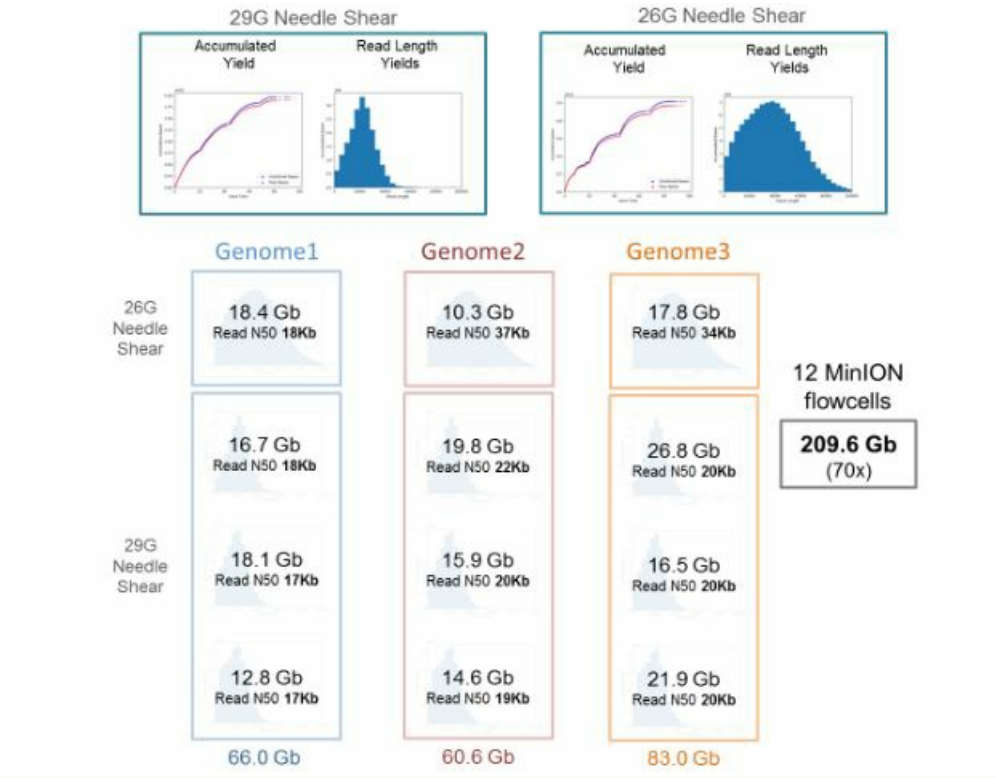
ABSTRACT

Modified LSK109 ligation prep using needle shear and bead purification

Using HMW DNA purified as above we have been performing 29G and 26G needle shears to produce fragmentation to around 18Kb and 34kb respectively (reported read N50's after basecalling). Also by going into the library preparations with 5-10ug of DNA have been able to produce enough material to provide up to 10 library loads from a single prep. We have not been performing the FFPE repair during the end-prep step, and have been diluting the sample at the cleanup points in the protocol with EB to prevent bead clumping during 0.4-0.5x AMPureXP purifications when DNA fragments are large and at high concentrations. Shown below are the results from a batch of 12 runs we performed using this approach.



EXTERNAL LINK

https://www.longreadclub.org/mountain-protocol/

MATERIALS TEXT

- HMW genomic DNA

- Buffer EB
- AMPureXP bead solution
- Qubit DNA HS kit
- 29G or 26G needle
- Ethanol
- Ultra II End-Prep Buffer (NEB)
- Ultra II End-Prep Enzyme mix (NEB)
- LNB Buffer
- AMX adapter
- Quick T4 DNA Ligase (NEB)
- LFB buffer

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

1 **10 µg** of HMW genomic DNA made up to **250 µl** with **EB** and sheared with either a *29G* or *26G* needle using 20 passes.

- For *26G* shear add **250 µl** of **EB** (10mM Tris-Cl pH8.0) followed by **250 µl** **AMPureXP bead solution**.
- For *29G* shear add **125 µl** **AMPureXP bead solution**.

After shearing is a good time to get a reliable DNA quantification using the **Qubit DNA HS kit** etc.

2 Mix by flicking.

3 Incubate for **00:10:00** at **Room temperature** followed by pelleting on a magnet.

4 Remove supernatant and keeping on the magnet wash 2x with **300 µl** – **500 µl** **80 % ethanol** without disturbing the pellet.

5 Briefly spin, return to magnet and remove any residual **80 % ethanol** with a pipette before allowing the bead pellet to *air dry* for ~ **00:02:00** .

6 Removed tube from the magnet

7 Resuspend the bead pellet in **51 µl** **EB** by flicking.

8 Spin down.

9 

Allow DNA to elute by incubation at **37 °C** for **00:05:00** .

10 Place tube on magnet and remove eluted DNA sample to a fresh tube.

11 Quantify **1 µl** of the DNA sample using the **Qubit DNA HS kit** to confirm DNA recovery (~ **6 µg**).

12 Then set up the following End-Prep reaction:

- **50 µl** DNA sample
- **7 µl** **Ultra II End-Prep Buffer**
- **3 µl** **Ultra II End-Prep Enzyme mix**

13 

Incubate at **20 °C** for **00:30:00** followed by **65 °C** for **00:30:00** .

14 Add **120 µl** **EB buffer** followed by **72 µl** **AMPureXP bead solution**.

15 Mix by flicking.

16 

Incubate for **00:10:00** at **Room temperature** followed by pelleting on a magnet.

17 

Remove supernatant and keeping on the magnet wash 2x with **300 µl** **80 % ethanol** without disturbing the pellet.

18 Briefly spin.

19 Return to magnet and remove any residual **80 % ethanol** with a pipette before allowing the bead pellet to air dry for ~ **00:02:00** .

20 Remove tube from the magnet.

21 Resuspend the bead pellet in **67 µl** **EB** by flicking.

22 

Spin down and allow DNA to elute by incubation at **0 °C** for **00:05:00** with occasional flicking.

23 Place tube on magnet and remove eluted DNA sample to a fresh tube.

24 Quantify **1 µl** DNA sample using the **Qubit DNA HS kit** to confirm DNA recovery (~ **4 µg** – **5 µg**).

25 Then set up the following Ligation reaction:

- **66 µl** DNA sample
- **25 µl** **LNB buffer**
- **5 µl** **AMX adapter**
- **4 µl** **Quick T4 DNA Ligase**

26 

Incubate at **20 °C** / **Room temperature** for **01:00:00** .

27 Add **100 µl** **EB buffer** followed by **80 µl** **AMPureXP bead solution**.

28 Mix by flicking.

29 Briefly spin.

30 

Incubate for **00:10:00** at **Room temperature** followed by pelleting on a magnet.

31 Remove supernatant and keeping on the magnet wash 2x with **250 µl** of **LFB buffer** by removing from the magnet and flicking, briefly spin, and pelleting again on the magnet.

32 Remove any residual **LFB buffer** while on the magnet with a pipette after final pelleting.

33 Remove tube from the magnet.

34 Resuspend the bead pellet in **21 µl** **ONT-EB buffer** by flicking.

35 

Spin down and allow DNA to elute by incubation at **37 °C** for **00:05:00** .

36 Place tube on magnet and pellet beads.

37 Remove eluted DNA sample to a fresh tube.

38 Quantify **1 µl** DNA sample using the **Qubit DNA HS kit** to confirm DNA recovery (~ **2.5 µg**).

39 Library ready to be combined with SQB and LLB for loading onto flowcel (**200 ng** – **400 ng** per load).