Size selection of HMW DNA by semi-selective DNA precipitation

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This protocol describes a method for the depletion of short fragments (<10 kb) from preparations of genomic DNA, using a buffer which promotes the precipitation of HMW DNA (>10 kb). This protocol is based on the work 'DNA clean-up and size selection for long-read sequencing V.4' by Benjamin Schwessinger, with some modifications. The method can increase read N50 of the subsequent sequencing library by 10–25 kb depending on sample quality.

Materials

- 3-10 µg of HMW DNA
- 2X “size selection buffer” (2.5% w/v PVP 360000 1.2 M NaCl, 20 mM Tris.HCl pH 8)
- Qubit dsDNA BR Assay Kit (ThermoFisher Scientific)
- 70% ethanol in nuclease-free water
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8)
- 1.5 ml Eppendorf DNA LoBind tubes
- Incubator or water bath set at 37°C
- Qubit fluorometer

Method

Step 1

Take 3–10 µg of DNA and dilute in 60–100 µl of TE buffer in a 1.5 ml Eppendorf DNA LoBind tube, for a final concentration of ~30–150 ng/µl.

Step 2

Add an equal volume of the 2x “size selection buffer” and mix thoroughly by either pipetting or briefly vortexing.

Step 3

Place the tube in the centrifuge and note the orientation of the tube within the rotor. Centrifuge the sample at 10,000 x g at room temperature for 30 minutes. Aspirate and discard the supernatant, taking care not to disturb the pellet.

Note: The pellet may not be visible, but it will be located on the side of the tube that was facing outwards during centrifugation.

Step 4

Add 200 µl of 70% ethanol to the tube, without disturbing the pellet.

Step 5

Centrifuge the sample at 10,000 x g for 3 minutes. Discard the supernatant.

Step 6

Repeat steps 4–5.

Step 7

Add 50 µl of TE buffer to the DNA pellet and mix by pipetting ~5 times. Incubate the tube at 37°C for 30 minutes. Gently agitate the solution every 5 minutes to aid with resuspension.

Step 8

Gently mix the tube contents by pipetting up and down using a wide-bore tip.

Step 9

Quantify 3x using the Qubit dsDNA BR Assay Kit, ensuring that replicate Qubit measurements are consistent before continuing to library preparation. If the Qubit measurements are not consistent, this could indicate that the DNA has not been homogeneously resuspended – increasing the elution time of warming the DNA at 50°C may aid with resuspension of the DNA pellet.

Results

- **DNA recovery:** Sample recovery will depend on the quality of the starting material. If the sample consists of predominantly short molecules, it is likely that the recovery will be poor (as most of the sample is actively depleted during the size selection process). We have observed that with DNA samples extracted using the QIAGEN Genomic-tip and QIAGEN Gentra Puregene extraction kits, a recovery of ~40-60% is typical. It is advised that the recommended sample volume (60–100 µl) is not exceeded. We have observed decreases in DNA recovery when the equivalent mass of DNA is processed in a higher volume.
- **Size selection:** It has been observed that the use of the size selection can increase the measured read N50 of sequencing libraries, 10–25+ kb by enriching for longer fragments (Figure 1A), without significant impact on sequencing throughput (Figure 1B). The enrichment for long fragments is robust to a range of input masses into the size selection, e.g. 3–10 µg.

Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit (SQK-LSK110):

- Typical output: ★★★ (8+ Gb in 48 h on FLO-MIN106D)
- Read length profile:

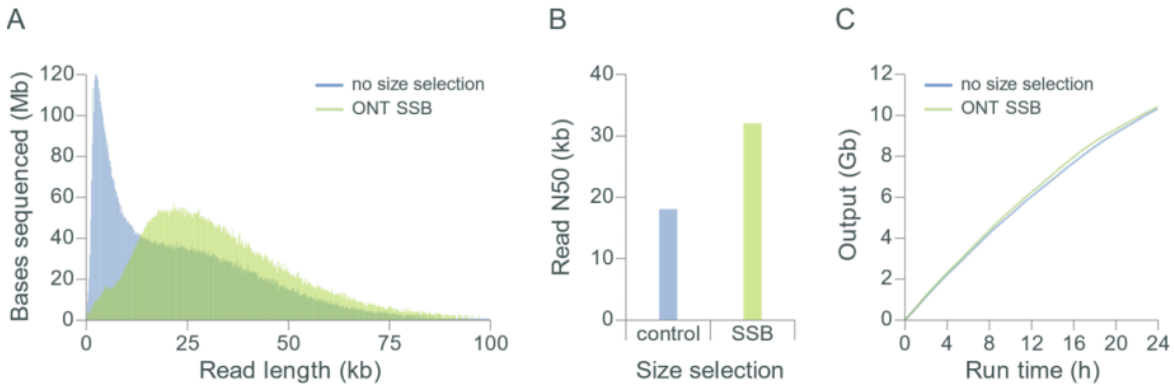

Figure 1. Read length distribution (A), read N50 (B) and output (B) of libraries prepared with and without size selection and sequenced on MinION. Panel A: the read length distribution for the library where size selection was performed shows a striking enrichment for long fragments, with much less time spent sequencing shorter molecules, compared with the no size selection control. Panel B: there is a marked increase in read N50 for the library that underwent size selection. Panel C: output does not appear to be significantly impacted by performing size selection prior to library preparation.