Introduction

This protocol describes a method for size selection of genomic DNA, using the BluePippin from Sage Science.

Materials

- 5 µg of HMW DNA in 30 µl
- Qubit Fluorometer
- Qubit dsDNA BR Assay Kit (ThermoFisher Scientific)
- Sage Science BluePippin
- Buffers and cassettes
- 70% ethanol in nuclease-free water
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- 1.5 ml Eppendorf DNA LoBind tubes
- SPRI beads (e.g. Agencourt AMPure XP)

Method

1. Take 5 µg of DNA and make up to 30 µl in TE buffer in a 1.5 ml Eppendorf DNA LoBind tube.
2. Prepare your gel cassette, following the manufacturers guide (the cassette used will depend on the length of the molecules that are being enriched/selected).
3. Add 10 µl loading dye to the DNA sample and mix gently but thoroughly.
4. Perform size selection: load sample onto prepared cassette and run the BluePippin instrument according to manufacturer guide (the run conditions used will depend on the length of molecules that are being enriched/selected).
5. Recover 40 µl of sample from the elution port and transfer it to a fresh 1.5 ml Eppendorf DNA LoBind tube.
6. *Optional step:* A secondary elution can be performed by adding an additional 40 µl of elution buffer to the elution port, incubating at room temperature for 30 min, before recovering the sample. This yields up to 200 ng additional sample. Combine with primary elution.
7. Prepare the AMPure XP beads for use; resuspend by vortexing.
8. Measure the recovered volume (should be ~40 µl or ~80 µl depending on whether a secondary elution was omitted or performed, respectively) and add 1x volume of the resuspended AMPure XP beads to the size selected DNA and mix gently. Incubate on a HulaMixer for 5 min at room temperature.
9. Spin down briefly and pellet on a magnet. Keeping the tube on the magnet, aspirate and discard the supernatant. Then wash the pelleted beads with 200 µl freshly-prepared 70% ethanol, without disturbing the pellet.
10. Keeping the tube on the magnet, aspirate and discard the ethanol wash buffer and add a further 200 µl freshly prepared 70% ethanol, without disturbing the pellet.
11. Aspirate and discard the ethanol wash buffer and then spin down the tube and place it back on the magnet. Pipette off any residual 70% ethanol and allow to air dry for 30 sec.
12. Remove the tube from the magnetic rack, and resuspend the pelleted beads in 50 µl of TE buffer. Incubate for 10 min at room temperature, gently agitating the tube every 2 min to aid resuspension of the pellet.
13. Pellet the beads on magnet until the eluate is clear and colourless. Transfer the 50 µl of eluate into a clean 1.5 ml tube.
14. Quantify using the Qubit dsDNA BR Assay Kit.

Sequencing performance

- BluePippin size selection was performed using settings to enrich for molecules >40 kb.
- Libraries were prepared using the Ligation Sequencing Kit (SQK-LSK109)
- Output from the flow cell may be increased by performing a flow cell wash step (at the point where the rate of data acquisition begins to deteriorate due to the accumulation of pores in the “unavailable” or “recovering” state) and then adding a new library.

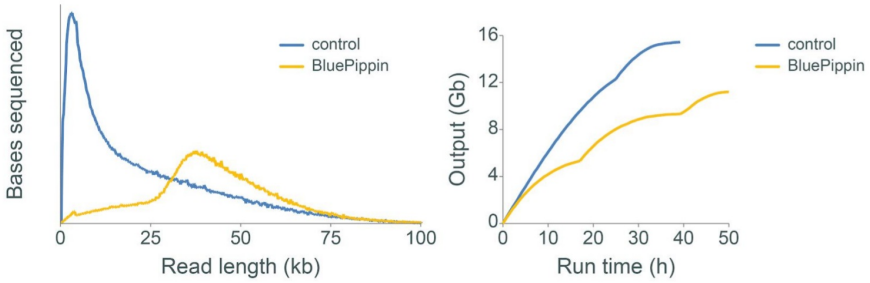

Figure 1. Read length distribution (left) and output (right) of libraries prepared with and without size selection and sequenced on MinION. The read length distribution for the library where size selection for molecules >40 kb was performed shows a striking depletion of short fragments fragments, with much less time spent sequencing shorter molecules, compared with the no size selection control. Output was reduced from the size selected library due to an accumulation of pores in the “unavailable” state: to maximise flow cell output a flow cell wash was performed after ~18 h and again at 42 h and fresh library load on to the flow cell.

Change log

Version	Change
v1, July 2019	Initial protocol publication