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Protocol status: Working
We use this protocol and it's working

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Sanger Tree of Life Fragmented DNA clean up: Manual SPRI

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ABSTRACT

This protocol describes the manual clean up of fragmented DNA following the Sanger Tree of Life HMW DNA Fragmentation protocols, using PacBio AMPure PB beads. This process is highly effective for the cleaning and removal of shorter fragments from sheared DNA from all of the taxonomic groups covered by the Tree of Life Programme. The output of this protocol is DNA which can be submitted for long read sequencing, including PacBio sequencing following Low Input (LI) or Ultra-Low Input (ULI) library preparation.

Acronyms
HMW: high molecular weight
SPRI: solid-phase reversible immobilisation
LI: low input
ULI: ultra-low input

GUIDELINES

- For DNA sheared using the Sanger Tree of Life HMW DNA Fragmentation: Diagenode Megaruptor® 3 for PacBio HiFi protocol, use a ratio of 0.6X AMPure PB beads to DNA volume.
- For DNA sheared using the Sanger Tree of Life HMW DNA Fragmentation: Diagenode Megaruptor® 3 for LI PacBio protocol, use a ratio of 1X AMPure PB beads to DNA volume.
- For DNA sheared using the Sanger Tree of Life HMW DNA Fragmentation: g-Tube for ULI PacBio protocol, use a ratio of 0.6X AMPure PB beads to DNA volume.
- To allow for QC to be performed and to meet internal requirements at Sanger for sequencing, 49 µL of EB is added in step 13 (3 µL is for QC and 45.4 µL for sequencing), however any volume of EB buffer can be used to elute the sheared DNA.

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Keywords: DNA clean up, solid phase reversible immobilisation, SPRI, manual SPRI, KingFisher, AMPure PB beads, reference genome, long read sequencing

MATERIALS

- 1.5 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030 108.051)
- AMPure beads PB (Pacific Biosciences Cat. no. 100-265-900)
- Buffer EB (Qiagen Cat. no. 19086)
- 100% absolute ethanol
- Nuclease-free water
- 15 mL or 50 mL centrifuge tubes

Equipment:

- Pipettes for 0.5 to 1000 µL and filtered tips
- Wide-bore tips (200 µL, filtered if available)
- DynaMag™-2 magnetic rack (Cat. no. 12321D) or similar
- Vortexer (Vortex Genie™ 2 SI-0266)
- Eppendorf ThermoMixer C (Cat. no. 5382000031) or similar
- Mini-centrifuge (Cat. no. SS-6050)
- Timer

Protocol PDF:

Sanger Tree of Life Fragmented DNA clean up_ Manual SPRI.docx.pdf

SAFETY WARNINGS

- The operator must wear a lab coat, powder-free nitrile gloves and safety specs to perform the laboratory procedures in this protocol.
- Waste needs to be collected in a suitable container (e.g. plastic screw-top jar or Biobin) and disposed of in accordance with local regulations.
- Liquid waste needs to be collected in a suitable container (e.g. glass screw-top jar) and disposed of in accordance with local regulations.

BEFORE START INSTRUCTIONS

- AMPure PB beads are stored in the fridge at 4 °C – take them out 30 minutes before use to allow beads to equilibrate to room temperature.
- Set the heat block to 37 °C.
- Prepare fresh 80% ethanol. This solution is hygroscopic and should be prepared fresh each time to achieve optimal results using 100% absolute ethanol and nuclease free water.
- Prepare 3 labelled 1.5 mL microcentrifuge tubes for each sample to be used in steps 1, 9 and 17.

Laboratory protocol

1 Using a standard pipette tip, measure the post-shearing sample volume and transfer DNA solution from Diagenode tube to a new labelled 1.5 mL microcentrifuge tube. Record the volume.

2 Calculate the volume of AMPure PB beads needed for each sample based on the sheared DNA volume and the ratio required.

3 Vortex the AMPure PB beads for 30 seconds, then immediately add the calculated volume of beads to the sheared DNA sample.

4 Mix the bead/DNA solution thoroughly by pipette mixing 15 times with a wide-bore pipette tip. Do not flick the tube.

5 Quickly spin down the tube (for 1 second) on a mini-centrifuge to collect the beads.

6 Incubate the mix on the bench top for 5 minutes at room temperature.

7 Spin down the tube (for 1 second) on a mini-centrifuge to collect the beads.

8 Place the tube in a magnetic bead rack and wait for the beads to pellet on the side of the tube. This can take approximately 1-5 minutes.

9 Slowly pipette off the cleared supernatant from the tube and save (in another labelled 1.5 mL microcentrifuge tube). Avoid disturbing the beads.

10 Wash the pelleted beads with freshly prepared 80% ethanol.
1. Do not remove the tube from the magnetic rack.
2. Use a sufficient volume of 80% ethanol to fill the tube (1.5 mL for 1.5 mL microcentrifuge tube).
3. Slowly dispense the 80% ethanol against the side of the tube opposite the beads, taking care not to disturb the pelleted beads.
4. After 30 seconds, pipette and discard the 80% ethanol.

11 Repeat step 10.

12 Spin down tubes on a mini-centrifuge for 1 second and return them to the magnetic rack, allowing the beads to pellet. Aspirate and dispose of any remaining ethanol.

13 Check for any remaining ethanol droplets in the tube. If droplets are present, repeat step 12.

14 Take tubes off the magnetic rack and add 49 µL of EB buffer to the beads. Gently mix by slowly pipetting 15 times with a wide-bore pipette tip. Do not flick the tube.

15 Incubate tubes at 37 °C for 15 minutes.

16 Briefly spin down the tubes (for 1 second) on a mini-centrifuge and place them in the magnetic rack. Allow beads to pellet.

17 Without disturbing the beads, transfer the supernatant to a new 1.5 mL DNA Lo-Bind microcentrifuge tube.

18 Perform QC as required.

19 Store samples at 4 °C.