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

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Sanger Tree of Life HMW DNA Extraction: Pooling

In 1 collection

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ABSTRACT

This protocol describes the method of combining multiple DNA extractions from the same individual in order to improve the concentration of HMW DNA for PacBio library preparation. This process is highly effective for DNA extracts from all of the taxonomic groups covered by the Tree of Life Programme, especially samples which are of high quality but low quantity following any of the Sanger Tree of Life DNA extraction protocols. The output of this protocol is a DNA extract which can be directed towards either HMW DNA Fragmentation: Diagenode Megaruptor® 3 for LI PacBio or HMW DNA Fragmentation: g-Tube for ULI PacBio.

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

GUIDELINES

- In order to generate a reference genome, the DNA pooled in this procedure must be from extractions obtained from a single individual.
- Either AMPure XP or AMPure PB magnetic beads can be used for this procedure.

Additional Notes:

- FluidX tubes are used throughout the Tree of Life programme in order to track samples, therefore rather than the microcentrifuge tubes which have been mentioned in this protocol for DNA storage, all routine DNA extracts are stored in FluidX tubes.

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Keywords: HMW DNA pooling, bead-based DNA pooling, reference genome, long read sequencing

MATERIALS

- 2 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030 108.078)
- AmPure XP beads (Pacific Biosciences Cat. no. 100-265-900) or AMPure PB beads (Pacific Biosciences Cat. no. 102-182-500)
- 100% absolute ethanol
- Nuclease-free water (NFW)
- Buffer AE (from Qiagen MagAttract HMW DNA Kit; Cat. no. 67563)

Equipment:

- Pipettes for 0.5 - 1000 µL and filtered tips
- Wide-bore pipette tips (200 µL, filtered if available)
- Mini centrifuge (Cat. no. SS-6050)
- DynaMag™-2 magnetic rack (Cat. no. 12321D) (or similar)
- Eppendorf ThermoMixer C (Cat. no. 5382000031) or similar
- HulaMixer Sample Mixer (Cat. no. 15920D)
- Timer

Protocol PDF: Sanger Tree of Life HMW DNA_ Pooling.pdf

SAFETY WARNINGS

- 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

- Remove AMPure XP or AMPure PB magnetic beads from the fridge to allow them to equilibrate to room temperature; this should take approximately 30 minutes.
- Set a heat block to 37 °C.
- Prepare enough 70% ethanol for your samples (4 mL per sample) using absolute ethanol and nuclease-free water.

Procedure

1 Gather all the DNA eluates that have been produced from multiple DNA extractions from the same individual.

2 In a 2 mL microcentrifuge tube, add all DNA to be pooled and pipette mix 5 times with a wide bore pipette. The combined volume of the DNA eluates going into the tube for pooling should not exceed 1000 µL; if it does, then multiple 2 mL microcentrifuge tubes will be required.

3 Vortex the Ampure XP or AMPure PB beads for 30 seconds and then immediately add 0.8 X volume of beads to the pooled DNA (e.g. for 100 µL DNA, add 80 µL beads).

4 Place the pooled DNA sample on a rotating mixer and incubate, rotating gently (10 rpm) at room temperature for 10 minutes.

5 Using a mini centrifuge, spin down the 2 mL microcentrifuge tube containing the pooled DNA for 1–2 seconds, then transfer to a magnetic rack and allow the magnetic beads to pellet.

6 Gently remove the supernatant and transfer to another 2 mL microcentrifuge tube to be retained until subsequent QC checks confirm the pooling procedure was successful.

7 Add 2 mL of freshly prepared 70% ethanol to the microcentrifuge tube on the magnetic rack, slowly dispensing the ethanol against the side of the tube opposite the beads. Incubate for 30 seconds, then aspirate ethanol and dispose.

8 Repeat step 7 for a total of two washes.

9 Using a mini-centrifuge, spin down the microcentrifuge tube containing the magnetic beads and return to the magnetic rack. Aspirate and dispose of any remaining ethanol.

10 Remove the microcentrifuge tube from the magnetic rack and add 400 µL buffer AE. In instances where you have split samples into multiple tubes, elute with volumes that will combine to 400 µL (e.g. if using two tubes, add 200 µL buffer AE to each tube). Gently mix by pipetting 1–2 times with a wide-bore pipette tip.

11 Incubate tube on a heat block at 37 °C for 15 minutes.

12 Briefly spin down the tube in a mini-centrifuge for 1-2 seconds, then transfer to the magnetic rack and allow the beads to pellet.

13 Using a wide-bore pipette tip, transfer eluate to a microcentrifuge tube for storage.

14 Perform QC as required; dispose of the retained supernatant and bead tubes if DNA has been successfully eluted.

15 Store the pooled gDNA at 4 °C.