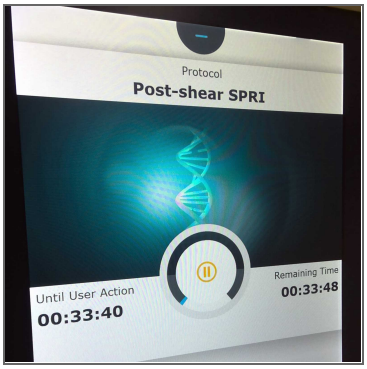

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Protocol status: Working
We use this protocol and it's working

Created: Sep 04, 2023

Sanger Tree of Life Fragmented DNA clean up: Automated SPRI

In 1 collection

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ABSTRACT

This protocol describes the automated clean up and shorter fragment removal from fragmented DNA following the Sanger Tree of Life HMW DNA Fragmentation protocols, using PacBio AMPure PB beads and the Thermo Fisher KingFisher™ Apex. This process is highly effective for 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. This protocol was adapted from Sanger Tree of Life Fragmented DNA clean up: Manual SPRI to include automation for a higher throughput of samples.

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

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PROTOCOL integer ID: 87307

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

GUIDELINES

- For DNA sheared using the Sanger Tree of Life HMW DNA Fragmentation: Diagenode Megaruptor® 3 for PacBio HiFi protocol or the Sanger Tree of Life HMW DNA Fragmentation: g-Tube for ULI PacBio protocol, use a 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 any evaporation occurring whilst on the KingFisher™ Apex, for QC to be performed and to meet internal requirements at Sanger for sequencing, 55 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.

Additional Notes:

- Both the KingFisher™ Apex protocol script and the KFX.file have been made available for this protocol - the KFX.file requires 'BindIx software for KingFisher Apex' to allow the KingFisher™ Apex protocol to be viewed on a PC or laptop. Alternatively, the file can be transferred directly onto a KingFisher™ Apex instrument using a USB.

MATERIALS

- 1.5 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030 108.051)
- Thermo Fisher KingFisher™ 1 mL 96-well Deep-well plates (Thermo Fisher Cat. no. 95040450)
- Thermo Fisher KingFisher™ 96 Deep-well Tip Comb (Thermo Fisher Cat. no. 97002570)
- Thermo Fisher KingFisher™ 200 µL standard 96-well plate (Thermo Fisher Cat. no. 97002084)
- 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
- Thermo Fisher KingFisher™ Apex instrument (Cat. no. 5400930)
- Vortexer (Vortex Genie™ 2 SI-0266)

KingFisher™ Apex Automated SPRI Protocol:

KFX file: Post-shear SPRI.kfx

1. Pick Up Tip - Tip Plate
2. Mix - Sample Plate - Variable volumes
   Pre-collect beads: Off
   Release beads: On 00:00:00
   Heating & Cooling: Off
   Mixing:
   1# 00:01:00 Slow
   2# 00:01:00 Medium
   3# 00:08:00 Paused
   Looping: 1 Tip position: Tip edge in liquid
   Postmix: Off
   Collect beads: On 6 Count 10 Seconds
3. Wash 1 - Ethanol Wash Plate - 800 µL 80% Ethanol
   Pre-collect beads: On
   Release beads: Off
   Heating & Cooling: Off
   Mixing 1# 00:00:30 Slow
   Postmix: Off
   Collect beads: Off
4. Wash 2 - Ethanol Wash Plate - 800 µL 80% Ethanol
   Pre-collect beads: Off
   Release beads: Off
   Heating & Cooling: Off
   Mixing 1# 00:00:30 Slow
   Postmix: Off
   Collect beads: Off
5. Dry - Ethanol Wash Plate - 800 µL 80% Ethanol
   Duration: 00:01:00 Above well
6. Elute - Elution Plate - 55 µL EB Buffer
   Pre-collect beads: Off
   Release beads: On 00:01:00 Slow
   Heating & Cooling: On 37°C Preheat: On
   Mixing: 1# 00:07:00 Slow
   2# 00:08:00 Paused
   Looping: 1 Tip position: Tip edge in liquid
   Postmix: Off
   Collect beads: On 3 Count 30 Seconds
7. Leave Tip - Ethanol Wash Plate

Protocol PDF:

Sanger Tree of Life Fragmented DNA clean up_ Automated SPRI.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.
- Do not open the door of the KingFisher™ Apex instrument whilst it is in operation.

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.
- Prepare fresh 80% ethanol – 80% EtOH is hygroscopic and should be prepared fresh each time to achieve optimal results using 100% absolute ethanol and nuclease free water.

Laboratory protocol

1 Normalise the volumes of all sheared DNA samples to the sample with the largest volume.

2 Set-up the KingFisher™ plates for the automated SPRI as detailed below:

	Plate name	Plate type	Reagent(s) required
	Tip plate	1 mL 96-well deep-well plate	96-well tip comb
	Sample plate	1 mL 96-well deep-well plate	Sheared DNA - variable volume + AMPure PB beads - variable volume
	Wash plate	1 mL 96-well deep-well plate	800 µL 80% ethanol (freshly made)

	Plate name	Plate type	Reagent(s) required
	Elution plate	200 µL standard 96-well plate	55 µL Buffer EB

3 Load the normalised samples into the 1 mL 96-well deep-well plate sample plate and ensure that the 80% ethanol and the EB buffer have been loaded into the same wells within the wash plate and elution plate respectively, corresponding to that of the samples.

4 Vortex the now room temperature AMPure PB beads and add the amount of beads required for the desired bead:sample ratio to the sample wells in the sample plate.

5 Select the automated SPRI protocol on the KingFisher™ Apex (details in the KingFisher™ Apex Automated SPRI Protocol Script/attached KFX file in the Materials section).

6 Modify the sample plate volumes on KingFisher protocol to reflect the volumes that you have loaded by choosing the pencil icon when the protocol is highlighted; this will allow the protocol to be edited. Navigate to protocol steps and select the sample plate in the mix step. Change the bead and sample volume as required to reflect the sample:bead volume and ratio used.

7 Once the sample plate volumes have been modified, use the play button to initiate the protocol and load the plates as prompted.

8 Once the final plate is loaded, the protocol will automatically begin; this will take around 35 minutes to complete.

9 Once finished, remove the elution plate from KingFisher™ Apex and follow the on-screen instructions to remove the plates from the instrument.

10 Using a wide-bore pipette tip, transfer the eluates from the elution plate into 1.5 mL DNA Lo-Bind

microcentrifuge tubes.

11 Perform QC as required.

12 Store samples at 4 °C.