

Sanger Tree of Life Fragmented DNA clean up: Automated SPRI

Authors

Graeme Oatley, Filipia Sampaio, Caroline Howard

Abstract

This protocol is for 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 either Low Input (LI) or Ultra Low Input (ULI) sequencing. This protocol was adapted from Sanger Tree of Life Fragmented DNA clean up: Manual SPRI to include automation for a higher throughput of samples.

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.
- Do not open the door of the KingFisher™ Apex instrument whilst it is in operation.

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 'Bindlx 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.

Before Starting:

- 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	KingFisher™ Apex 1 mL 96-well deep-well plate	KingFisher™ Apex 96-well tip comb
Sample plate	KingFisher™ Apex 1 mL 96-well deep-well plate	Sheared DNA - variable volume + AMPure PB beads - variable volume
Wash plate	KingFisher™ Apex 1 mL 96-well deep-well plate	800 µL 80% ethanol (freshly made)
Elution plate	KingFisher™ Apex 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 below in the KingFisher™ Apex Automated SPRI Protocol section/attached file).
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.

KingFisher™ Apex Automated SPRI Protocol:

- 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 - 55 µL EB Buffer
 - Duration: 00:01:00 Above well
- 6) Elute - Elution Plate
 - 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

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 - 1000 µL and filtered tips
- Thermo Fisher KingFisher™ Apex instrument (Cat. no. 5400930)
- Vortexer (Vortex Genie™ 2 SI-0266)

References:

[Procedure & Checklist – Using AMPure PB Beads for Size-Selection \(pacb.com\)](https://www.pacb.com/procedure-and-checklist-using-ampure-pb-beads-for-size-selection)