Sanger Tree of Life Fragmented DNA clean up: Automated SPRI v2

Authors

Graeme Oatley, Filipia Sampaio, Amy Denton & Caroline Howard

Abstract

This protocol is for the automated clean up and shorter fragment removal from fragmented DNA following any of 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 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

- 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.

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: Covaris 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 or the Sanger Tree of Life HMW DNA Fragmentation: Opentrons® OT-2 for PacBio LI protocol, use a ratio of 1X AMPure PB beads to DNA volume.
- The KingFisher™ Apex Automated SPRI Protocol: ‘Post-shear SPRI’ is recommended for DNA sheared used the Sanger Tree of Life HMW DNA Fragmentation: Diagenode Megaruptor® 3 for PacBio HiFi protocol, Diagenode Megaruptor® 3 for LI PacBio protocol or Covaris g-Tube for ULI PacBio protocol.
- The KingFisher™ Apex Automated SPRI Protocol: ‘Opentron post-shear SPRI’ is recommended for DNA sheared using the Sanger Tree of Life HMW DNA Fragmentation: Opentrons® OT-2 for PacBio LI protocol, due to this protocol being adapted for larger sample volumes, with an increased binding time to improve the DNA recoveries.

- 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, 70 µL of EB is added in step 13 (3–5 µL is for QC and 50 µL for sequencing), however any volume of EB buffer can be used to elute the sheared DNA.

Additional Notes:

- The KingFisher™ Apex protocol scripts and the KFX.files for both post-shear SPRI protocols are available – the KFX.files require 'BindIx software for KingFisher Apex' to enable viewing of the KingFisher™ Apex protocols on a PC or laptop. Alternatively, the files can be transferred directly onto a KingFisher™ Apex instrument with 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 just before use each time, using 100% absolute ethanol and nuclease-free water, to achieve optimal results.

Laboratory Protocol:

1. Normalise the volumes of all sheared DNA samples to the sample with the largest volume.
   Note: Samples sheared using the Sanger Tree of Life HMW DNA Fragmentation: Opentrons® OT-2 for PacBio LI protocol will all be 300 µL and therefore do not require normalising.
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	1 mL 80% ethanol (freshly made)
Elution plate	KingFisher™ Apex 200 µL standard 96-well plate	70 µL Buffer EB

3. Load the normalised samples into the 1 mL 96-well deep-well 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.
Note: Samples sheared using the Sanger Tree of Life HMW DNA Fragmentation: Opentrons® OT-2 for PacBio LI protocol will already be in a 1 mL 96-well deep-well plate and therefore do not require transfer.

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

5. Select the automated SPRI protocol recommended for your sheared DNA on the KingFisher™ Apex (details below in the KingFisher™ Apex Automated SPRI Protocols section/attached files).

6. For samples undergoing the 'Post-shear SPRI', modify the sample plate volumes on KingFisher protocol to reflect the volumes that you have loaded. Choose the pencil icon when the protocol is highlighted to 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.
This step is not required for samples undergoing the 'Opentrons post-shear SPRI'.

7. 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 for the 'Post-shear SPRI' protocol and around 45 minutes to complete for the 'Opentrons post-shear SPRI' protocol.

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 standard 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 Protocols:

Post-shear SPRI

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℃ 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

Opentron post-shear SPRI

1) Pick Up Tip - Tip Plate
2) Mix 1 - Sample Plate
   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 10 Count 30 Seconds
3) Mix 2 - Sample Plate
   Pre-collect beads: Off
   Release beads: Off
   Heating & Cooling: Off
   Mixing:
   1# 00:00:00

Postmix:	Off		
Collect beads:	On	10 Count	30 Seconds

4) Wash 1 - Ethanol Wash Plate

Pre-collect beads:	On		
Release beads:	Off		
Heating & Cooling:	Off		
Mixing	1#	00:00:30	Slow
Postmix:	Off		
Collect beads:	Off		

5) Wash 2 - Ethanol Wash Plate

Pre-collect beads:	Off		
Release beads:	Off		
Heating & Cooling:	Off		
Mixing	1#	00:00:30	Slow
Postmix:	Off		
Collect beads:	Off		

6) Dry - Ethanol Wash Plate

Duration:	00:01:00	Above well

7) Elute - Elution Plate

Pre-collect beads:	Off		
Release beads:	On	00:01:00	Slow
Heating & Cooling:	On	37℃	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	4 Count	30 Seconds

8) Leave Tip - Ethanol Wash Plate

Materials:

- 1.5 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030108051)
- 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 PB beads (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 (Thermo Fisher Cat. no. 5400930)
- Vortexer (Vortex Genie™ 2 SI-0266)

References:

[Procedure & Checklist – Using AMPure PB Beads for Size-Selection (pacb.com)]