Sanger Tree of Life HMW DNA Extraction: Automated MagAttract v.2

Authors

Graeme Oatley, Amy Denton, Caroline Howard

Abstract

This protocol is for the automated extraction and SPRI of HMW DNA from multiple different tissue samples from a variety of species intended for long-read sequencing using the Qiagen MagAttract HMW DNA extraction kit and the Thermo Fisher KingFisher™ Apex. This process is effective for a wide variety of taxonomic groups covered by the Tree of Life Programme, excluding plants and fungi. The output of this protocol is HMW DNA, which depending upon yield and genome size of the species, can be directed towards either HMW DNA Pooling, HMW DNA Fragmentation: Diagenode Megaruptor® 3 for LI PacBio or HMW DNA Fragmentation: g-Tube for ULI PacBio. This protocol was adapted from Sanger Tree of Life HMW DNA Extraction: Automated MagAttract v.1 to include a pre-shear SPRI of the HMW DNA extracted.

Safety Warnings

- The operator must wear a lab coat, powder-free nitrile gloves and safety specs to perform the laboratory procedures in this protocol. Cotton glove liners are strongly recommended when handling the samples on dry ice.
- 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 the lysis buffer master mix, prepare enough for n+1 samples to account for pipetting errors**.**
- Keep samples on dry ice to maintain temperature and prevent nucleic acid degradation until the lysis buffer is ready to be added to them.
- For the 0.45X SPRI, the DNA and AMPure beads should not sit together in the sample plate for more than 5 minutes before starting the KingFisher™ Apex.
- An experienced operator can expect to comfortably process up to 32 samples, with approximately 2-3 hours handling time over a start to finish period of 4-5 hours. This estimation includes the utilisation of the KingFisher™ Apex for both the extraction and SPRI protocols, and excludes subsequent QC checks.

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.

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

Before starting:

- Add 100% ethanol to the MW1 and PE wash buffers as per manufacturer's instructions.
- Remove the AMPure PB beads from the fridge 30 minutes before starting the 0.45X SPRI KingFisher™ Apex protocol to bring them to room temperature.

Laboratory Protocol:

Sample Lysis

1. Prepare a lysis buffer master mix:

Reagent	Volume per sample
Phosphate buffered saline (PBS)	200 µL
Proteinase K	20 µL
RNase A	4 µL
Buffer AL	150 µL

2. Set a heat block to 25 ºC.
3. For cryoprepped samples:
    a. Transfer 25 mg cryoprepped sample into a 2 mL microcentrifuge tube, then hold on dry ice to keep the sample frozen.
    b. Add 374µL of the lysis buffer master mix to sample, then homogenise the sample and master mix by gently pipetting 10 times with a wide bore pipette tip.
4. For PowerMashed samples (weight less than 25 mg):
    a. Transfer sample into a 1.5 mL BioMasher II tube and add 374 µL lysis buffer.
    b. Disrupt sample in lysis buffer using the Diagnocine PowerMasher II tissue disruptor and BioMasher pestle, until no large pieces remain or sample cannot be disrupted further (for more detailed instructions regarding powermashing, please refer to the Sanger Tree of Life Sample Homogenisation: Powermash protocol)
    c. Transfer the entire contents of the BioMasher tube to a 2 mL microcentrifuge tube using a wide-bore tip.

5. Centrifuge sample tubes briefly and incubate on the heat block at 25 ºC for 2 hours.

Loading and Running the KingFisher™ Apex for DNA Extraction

6. While samples are lysing, label nine 1 mL 96-well deep-well KingFisher™ plates and fill the number of wells required for the number of samples in each plate as follows:

Plate	Reagent(s) required
Tip plate	96-well tip comb (no reagent)
Elution 2	200 µL Buffer AE
Elution 1	200 µL Buffer AE
NFW Wash	500 µL Nuclease-Free Water
PE Wash 2	700 µL Buffer PE
PE Wash 1	700 µL Buffer PE
MW1 Wash 2	700 µL Buffer MW1
MW1 Wash 1	700 µL Buffer MW1
Sample plate	15 µL Suspension G magnetic beads 280 µL Buffer MB

7. Once samples have completed lysing, remove sample tubes from the heat block and briefly centrifuge to spin down.

8. Using a wide bore pipette tip, set the volume to 380 µL, transfer lysate from the sample tubes to individual wells in the sample plate, taking care not to transfer large pieces of debris if possible.

9. Select the required DNA extraction protocol in the protocol list on the KingFisher™ Apex (details below in KingFisher™ Apex DNA Extraction Protocol section/attached file) and select using the play button.

10. Load the filled plates onto the instrument following the instructions provided on screen.

11. Prior to loading the “Sample Plate”, the instrument will prompt to remove the “Tip Plate”. Once the final plate is loaded, the protocol will automatically begin; this takes approximately 50 minutes.

12. Once the protocol has completed, follow the on-screen instructions to remove plates from the instrument.

13. Inspect the elution plates for any magnetic beads in the wells. In the rare instance of magnetic beads remaining in the eluate (possible in viscous samples), these samples will need to be transferred to a 1.5 mL microcentrifuge tube and placed on a magnetic rack. Allow around 5 minutes for the beads to migrate and take the clear eluate containing the DNA using a wide bore pipette tip.

14. Using a 200 µL multi-channel pipette and wide bore tips, pipette eluates from Elution Plate 2 into Elution Plate 1, and gently pipette mix 5-10 times with wide bore tips to fully homogenise DNA in the eluate. Elution Plate 1 with the combined eluates is now the ‘Sample Plate’ for the 0.45X SPRI.

Loading and Running the KingFisher™ Apex for the 0.45X SPRI

15. Set-up the KingFisher™ plates for the 0.45X SPRI as detailed below:

Plate	Plate Type	Reagent(s) required
Tip Plate	1 mL Deep-well	96-well tip comb (no reagent)
Sample Plate (Elution Plate 1 from DNA Extraction Protocol)	1 mL Deep-well	380 µL DNA + 171 µL AMPure PB beads
Ethanol Wash Plate	1 mL Deep-well	1000 µL 80% EtOH (freshly made)
Elution Plate	200 µL standard	135 µL Buffer EB

16. Select the required 0.45X SPRI protocol in the protocol list on the KingFisher™ Apex (details below in KingFisher™ Apex 0.45X SPRI Protocol section/attached file) and select using the play button.

17. Load the filled plates onto the instrument following the instructions provided on screen.

18. Once the final plate is loaded, the protocol will automatically begin; this will take approximately 40 minutes.

19. Once the protocol has completed, follow the on-screen instructions to remove plates from the instrument.

20. Using a wide bore pipette tip, transfer the 130 µL of eluate from the elution plate into microcentrifuge tubes.

21. Incubate the DNA at room temperature overnight and perform the required QC the following morning.

22. Store the DNA at 4ºC.

KingFisher™ Apex DNA Extraction Protocol:

1) Pick Up Tip - Tip Plate
2) DNA Binding - Sample Plate
   Pre-collect beads: Off
   Release beads: Off
   Heating & Cooling: Off
   Mixing 1# 00:05:00 Fast
   Postmix: Off
   Collect beads: On 5 Count 2 Seconds
3) Collect Beads 1 - Sample Plate
   Collect beads: Count 5 Collect time 1 Second
4) Wash 1 - MW1 Wash 1 Plate
   Pre-collect beads: Off
   Release beads: On 00:00:10 Bottom mix
   Heating & Cooling: Off
   Mixing 1# 00:01:00 Fast
   Postmix: Off
   Collect beads: On 5 Count 1 Second
5) Collect Beads 2 - MW1 Wash 1 Plate
   Collect beads: Count 5 Collect time: 1 Second
6) Wash 2 - MW1 Wash 2 Plate
   Pre-collect beads: Off
   Release beads: On 00:00:10 Bottom mix
   Heating & Cooling: Off
   Mixing 1# 00:01:00 Fast
   Postmix: Off
   Collect beads: On 5 Count 1 Second
7) Collect Beads 3 - MW1 Wash 2 Plate
   Collect beads: Count 5 Collect time: 1 Second
8) Wash 3 - PE Wash 1 Plate
   Pre-collect beads: Off
   Release beads: On 00:00:10 Bottom mix
   Heating & Cooling: Off
   Mixing 1# 00:01:00 Fast

Postmix: Off
Collect beads: On 5 Count 1 Second

9) Collect Bead 4 - PE Wash 1 Plate
Collect beads: Count 5 Collect time: 1 Second
10) Wash 4 - PE Wash 2 Plate
Pre-collect beads: Off
Release beads: On 00:00:10 Bottom mix
Heating & Cooling: Off
Mixing 1# 00:01:00 Fast
Postmix: Off
Collect beads: On 5 Count 1 Second
11) Collect Bead 5 - PE Wash 2 Plate
Collect beads: Count 5 Collect time 1 Second
12) Water Rinse - NFW Plate
Pre-collect beads: Off
Release beads: Off
Heating & Cooling: Off
Mixing 1# 00:00:00
Postmix: Off
Collect beads: On 5 Count 1 Second
13) Dry - NFW Plate
Duration: 00:01:00 Dry Type: Above Well
14) Elute 1 - Elution Plate 1 Plate
Pre-collect beads: Off
Release beads: On 00:00:00
Heating & Cooling: On 25°C Pre-heat: Off
Mixing 1# 00:01:00 Paused Looping: 1
2# 00:05:00 Slow Tip Position: Above Well
Postmix: Off
Collect beads: On 3 Count 1 Seconds
15) Elute 2 - Elution Plate 2 Plate
Pre-collect beads: Off
Release beads: On 00:00:00
Heating & Cooling: On 25°C Pre-heat: Off
Mixing 1# 00:01:00 Paused Looping: 1
2# 00:05:00 Slow Tip Position: Above Well
Postmix: Off
Collect beads: On 3 Count 1 Seconds
16) Leave Tip - NFW Plate

KingFisher™ Apex 0.45X SPRI Protocol:

1) Pick Up Tip - Tip Plate
2) Mix - 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) 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
4) 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
5) Dry - Ethanol Wash Plate
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 4 Count 30 Seconds
7) Leave Tip - Ethanol Wash Plate

Materials:

- 1.5 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030108418)
- 2 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030108078)
- 1.5 mL BioMasher II tubes and pestles (sterile) (Cat. no. 9791A)
- Thermo Fisher KingFisher™ 1 mL 96-well Deep-well plates (Thermo Fisher Cat. no. 95040450)
- Thermo Fisher KingFisher™ 200 µL standard 96-well plate (Thermo Fisher Cat. no. 97002084)
- Thermo Fisher KingFisher™ 96 Tip Comb (Thermo Fisher Cat. no. 97002570)
- Qiagen MagAttract HMW DNA extraction kit (Qiagen Cat. no. 67563)
- Dry ice
- 1x phosphate-buffered saline (PBS)
- 100% absolute ethanol
- 15 mL or 50 mL centrifuge tubes

- AMPure PB beads (Pacific Biosciences Cat. no. 100-265-900)
- Buffer EB (Qiagen Cat. no. 19086)

Equipment:

- Pipettes for 0.5 - 1000 µL and filtered tips
- Wide bore tips (200 µL and 1000 µL, filtered if available)
- Diagnocine PowerMasher II tissue disruptor (Cat. no. FNK-891300)
- Thermo Fisher KingFisher™ Apex instrument (Cat. no. 5400930)
- Eppendorf ThermoMixer C (Cat. no. 5382000031)
- Eppendorf SmartBlock 2.0 ml (Cat. no. 5362000035)
- Vortexer (Vortex Genie™ 2 SI-0266)
- Mini centrifuge
- Timer
- DynaMag™-2 magnetic rack (Cat. no. 12321D) (or similar)

References:

MagAttract HMW DNA Handbook: MagAttract HMW DNA Handbook - QIAGEN

Procedure & checklist - Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0 (pacb.com)