Sanger Tree of Life HMW DNA Extraction: Automated Plant MagAttract v.5

Authors

Graeme Oatley, Amy Denton, Anna Kovalevskaia & Caroline Howard

Abstract

This protocol is for the automated extraction and SPRI of HMW DNA from cryoprepped tissue samples from plants and fungi 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 plant species covered by the Tree of Life Programme. 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: Opentrons® OT-2 for PacBio LI, HMW DNA Fragmentation: Diagenode Megaruptor® 3 for LI PacBio or HMW DNA Fragmentation: Covaris g-Tube for ULI PacBio. This protocol was adapted from Sanger Tree of Life HMW DNA Extraction: Automated Plant MagAttract v.4 to include an overnight elution in order to maximise the yield of DNA, as well as an updated pre-shear SPRI to improve recoveries of HMW DNA.

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 the overnight elution and 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 flash drive.

Before starting:

- Add 100% ethanol to the MW1 and PE wash buffers as per manufacturer's instructions.
- AMPure PB beads are stored in the fridge at 4 °C – take them out 30 minutes before starting the 0.45X SPRI KingFisher™ Apex protocol to bring them to room temperature.

Laboratory Protocol:

<u>Sample Lysis</u>

1. Prepare a lysis buffer master mix in a 50 mL centrifuge tube:

Reagent	Volume per sample
Phosphate-buffered saline (PBS)	200 µL
Buffer AL	150 µL

2. Place the lysis buffer on the 65 °C heat block and incubate at 400 rpm for at least 20 minutes. Keep at temperature until added to the sample.

3. Transfer 50 mg of cryogenically disrupted tissue from each sample to 2 mL microcentrifuge tubes.
    a. Ensure the disrupted tissue is completely disrupted into a fine powder; avoid matted/clumped powder. This is crucial for optimal DNA yield and integrity; poorly disrupted tissue drastically decreases lysis and extraction efficiency.
    b. Any samples containing poorly disrupted tissue 'chunks' should be further cryogenically disrupted.

4. Transfer the samples to a pre-chilled cold block on wet ice and incubate for 10 minutes to equilibrate temperature.

5. Add 20 µL Proteinase K (for n+1 samples) to the preheated lysis buffer immediately prior to initiating lysis, swirling the centrifuge tube to mix.

6. Add 370 µL of the preheated lysis buffer plus Proteinase K to each sample, immediately homogenising the lysate by mixing with 5 rapid pulse vortexes, and place on the 55 °C heat block at 600 rpm for 15 minutes.

7. After 5 minutes incubation, resuspend any severely aggregated samples by pipette mixing with a wide-bore pipette tip.

8. After the initial 15 minute incubation, add 4 µL RNase A to each sample and mix thoroughly by inversion until any aggregated, insoluble or sedimented tissue particles are resuspended.

9. Incubate samples for a further 45 minutes on the heat block at 55 °C at 600 rpm.

10. For the final 15 minutes of lysis, remove the 600 rpm mixing from the heat block to allow aggregated, insoluble or sedimented tissue particles to settle at the bottom of the tube - this will reduce gDNA loss during later centrifugation.

Loading and Running the KingFisher™ Apex for DNA Extraction

11. Whilst 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 plate	410 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	40 µL Suspension G magnetic beads

	280 µL Buffer MB

12. Once samples have completed lysing, remove sample tubes from the heat block and allow the lysate to settle to the bottom of the tube for 5 minutes.

13. Centrifuge the samples at 8000 rpm for 10 minutes at room temperature.

14. 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.

15. 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.

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

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

18. Once the protocol has completed, follow the on-screen instructions to remove plates from the instrument. Make sure to retain the Tip Plate which contains the tip comb for future steps.

19. Seal the Elution Plate with a plate seal and leave overnight at room temperature to allow the DNA to elute.

20. The following morning, remove the plate seal and return to the KingFisher™ Apex in order to run the ‘bead collection’ protocol. Select from the protocol list and load the Tip Plate and Elution Plate as prompted by the instrument.

21. Once the bead collection protocol has completed after approximately 5 minutes, follow the on-screen instructions to remove the plates from the instrument.

22. Inspect the Elution Plate 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. Return samples back to their original well following bead removal.

23. The Elution Plate is now the ‘Sample Plate’ for the 0.45X SPRI.

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

24. 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

25. 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.

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

27. Once the final plate is loaded, the protocol will automatically begin; this will take approximately 50 minutes.

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

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

30. Perform the required QC to determine the quantity and quality of the DNA extracted.

31. Store the DNA at 4 ºC.

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 - Elution Plate
    Pre-collect beads: Off
    Release beads: On 00:00:00
    Heating & Cooling: On 25°C Pre-heat: Off
    Mixing 1# 00:00:05 Medium Looping: 1
    2# 00:05:00 Slow
    Postmix: Off
    Collect beads: Off
15) Leave Tip - Tip Plate

Bead Collection Protocol:

1) Pick Up Tip - Tip Plate
2) Elute - Elution Plate
    Pre-collect beads: Off
    Release beads: On 00:00:00
    Heating & Cooling: On 25°C Pre-heat: On
    Mixing 1# 00:00:05 Medium Looping: 1
    2# 00:02:00 Slow
    Postmix: On 00:00:00 Medium
    Collect beads: On 3 Count 1 Second
3) Collect Beads - Elution Plate
    Collect beads: Count 5 Collect time: 1 Second
4) Leave Tip - Tip 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#	00:00:50	Medium
	2#	00:14:00	Slow
	3#	00:00:10	Fast
Looping: 2	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. 0030108051)
- 2 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030108078)
- Thermo Fisher KingFisher™ 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
- 1 x 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)
- Thermo Fisher KingFisher™ Apex instrument (Cat. no. 5400930)
- Corning® CoolRack CF45 (Cat. no. 432051) or equivalent

- Eppendorf ThermoMixer C (Cat. no. 5382000031)
- Eppendorf SmartBlock 2.0 mL (Cat. no. 5362000035)
- Eppendorf SmartBlock 50 mL (Cat. no. 5365000028)
- Eppendorf™ Centrifuge 5425/5425 R (Cat. no. 5405000263)
- Vortexer (Vortex Genie™ 2 SI-0266)
- Mini centrifuge (Cat. no. SS-6050)
- Timer

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)

Sanger Tree of Life HMW DNA Extraction: Automated Plant MagAttract v.4 B. Jackson & C. Howard (2023)