

Sanger Tree of Life HMW DNA Extraction: Automated MagAttract v.3

Authors

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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 the majority 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: 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 MagAttract v.2 to include an overnight elution step in order to maximise the yield of DNA, as well as an updated pre-shear SPRI to improve recovery 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 ‘Bindx 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.
- Set a heat block to 25 °C.
- 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. For cryoprepped/bead beaten samples:
 - a. Transfer a 25 mg cryoprepped/bead beaten 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.
3. 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 Diagenode 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.
4. Centrifuge all sample tubes briefly and incubate on the heat block at 25 °C for 2 hours.

Loading and Running the KingFisher™ Apex for DNA Extraction

5. 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 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

6. Once samples have completed lysing, remove sample tubes from the heat block and briefly centrifuge to spin down.
7. 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.
8. 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.

9. Load the filled plates onto the instrument following the instructions provided on screen.
10. Once the final plate is loaded, the protocol will automatically begin; this takes approximately 40 minutes.
11. Once the protocol has completed, follow the on-screen instructions to remove plates from the instrument. Make sure to retain the Tip Plate containing the tip comb for future steps.
12. Seal the Elution Plate with a plate seal and leave overnight at room temperature to allow the DNA to elute.
13. The following morning, remove the plate seal and return to the KingFisher™ Apex in order to run the 'bead collection' protocol – select this protocol from the protocol list and load the Tip Plate and Elution Plate as prompted by the instrument.
14. Once the bead collection protocol has completed after approximately 5 minutes, follow the on-screen instructions to remove the plates from the instrument.
15. 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.
16. The Elution Plate is now the 'Sample Plate' for the 0.45X SPRI.

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

17. 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 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

18. 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.
19. Load the filled plates onto the instrument following the instructions provided on screen.
20. Once the final plate is loaded, the protocol will automatically begin; this will take approximately 50 minutes.
21. Once the protocol has completed, follow the on-screen instructions to remove plates from the instrument.
22. Using a wide-bore pipette tip, transfer the 130 µL of eluate from the elution plate into microcentrifuge tubes.
23. Perform the required QC to determine quantity and quality of the DNA extracted.
24. Store the DNA at 4 °C.

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

KingFisher™ Apex 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°C 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)
- 1.5 mL BioMasher II tubes and pestles (sterile) (Takara 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
- 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)
- Corning® CoolRack CF45 (Cat. no. 432051) or equivalent
- Diagenode 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 (Cat. no. SS-6050) or similar
- DynaMag™-2 magnetic rack (Cat. no. 12321D) (or similar)
- 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 MagAttract v.2](#) G. Oatley et al. (2023)