

Sanger Tree of Life HMW DNA Extraction: Automated MagAttract v.1

Authors

Edel Sheerin, Filipa Sampaio, Graeme Oatley, Michelle Strickland, Maja Todorovic, Raquel Juliana Vionette Do Amaral, Caroline Howard

Abstract

This protocol is for the automated extraction 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 or HMW DNA Fragmentation: Diagenode Megaruptor® 3 for LI HiFi. This protocol was adapted from Sanger Tree of Life HMW DNA Extraction: Manual MagAttract to include automation for a higher throughput of samples, and has since been updated to Sanger Tree of Life HMW DNA Extraction: Automated MagAttract v.2 to include a pre-shear SPRI of the HMW DNA extracted.

Safety Warnings

- 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.
- 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 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 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:

- Add 100% ethanol to the MW1 and PE wash buffers as per manufacturer's instructions.

Laboratory Protocol:

Sample Lysis

- 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

- Set a heat block to 25 °C.
- For cryoprepped samples:
 - Transfer 25 mg cryoprepped sample into a 2 mL microcentrifuge tube, then hold on dry ice to keep the sample frozen.
 - Add 374 µL 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.
- For PowerMashed samples (weight less than 25 mg):
 - Transfer sample into a 1.5 mL BioMasher II tube and add 374 µL lysis buffer.
 - 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)
 - Transfer the entire contents of the BioMasher tube to a 2 mL microcentrifuge tube using a wide-bore tip.
- Centrifuge sample tubes briefly and incubate on the heat block at 25 °C for 2 hours.

Loading and Running the KingFisher™ Apex

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 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 μ L multi-channel pipette and wide bore tips, pipette eluates from the elution plate into microcentrifuge tubes, pipette mix with wide bore tips to fully homogenise DNA in the eluate.
15. Perform required QC and then 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 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

Materials:

- 1.5 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030 108.051)
- 2 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030 108.078)
- 1.5 mL BioMasher II tubes and pestles (sterile) (Cat. no. 9791a)
- Thermo Fisher KingFisher™ 96-well Deep-well plates (Thermo Fisher Cat. no. 95040450)
- 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

Equipment:

- Pipettes for 0.5 - 1000 µL and filtered tips
- Wide bore tips (200 µL and 1000 µL, filtered if available)
- Diagenocine PowerMasher II tissue disruptor (Product no. 891300)
- Thermo Fisher KingFisher™ Apex instrument (Cat. no. 5400930)
- Eppendorf ThermoMixer C (Cat. no. 5382000031) (or similar)
- Eppendorf SmartBlock 2.0 mL (Cat. no. 5362000035)
- Vortexer (Vortex Genie™ 2 SI-0266)
- Mini centrifuge (Cat. no. SS-6050)
- DynaMag™-2 magnetic rack (Cat. no. 12321D)
- Timer

References:

MagAttract HMW DNA Handbook: [MagAttract HMW DNA Handbook - QIAGEN](#)