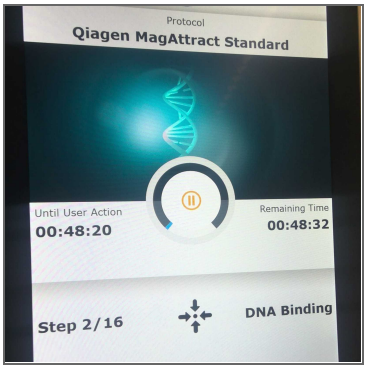

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Protocol status: Working
We use this protocol and it's working

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

Edel Sheerin[1], Filipa Sampaio[1], graeme oatley[1], Maja Todorovic[1], Michelle Strickland[1], Raquel Juliana Vionette do Amaral[1], Caroline Howard[1]

[1]Tree of Life, Wellcome Sanger Institute, Hinxton, Cambridgeshire, CB10 1SA

Tree of Life at the Wellcome Sanger Institute

Tree of Life Genome Note Editor

ABSTRACT

This protocol describes 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 ThermoFisher 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.

Acronyms
HMW: high molecular weight
SPRI: solid-phase reversible immobilisation
HiFi: high fidelity

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Keywords: HMW DNA extraction, magnetic bead extraction, MagAttract, automated DNA extraction, KingFisher, reference genome, long read sequencing

GUIDELINES

- For the lysis buffer master mix, prepare enough for n+1 samples to allow 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 'BindIx 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 flash drive.

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 to 1000 µL and filtered tips
- Wide-bore tips (200 µL and 1000 µL, filtered if available)
- Diagnocine 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

KingFisher™ Apex DNA Extraction Protocol Script:

KFX file: Qiagen MagAttract Standard.kfx

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

Protocol PDF:

Sanger Tree of Life HMW DNA Extraction_ Automated MagAttract v.1.pdf

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 while it is in operation.

BEFORE START INSTRUCTIONS

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

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 °C.

3 For cryoprepped samples:

1. Transfer 25 mg cryogenically disrupted sample into a 2 mL microcentrifuge tube, then hold on dry ice to keep the sample frozen.
2. 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.

4 For PowerMashed samples (weight less than 25 mg):
1. Transfer sample into a 1.5 mL BioMasher II tube and add 374 µL lysis buffer.
2. 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.)
3. 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 °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 in KingFisher™ Apex DNA Extraction Protocol Script/attached KFX file in the Materials section) 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 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.