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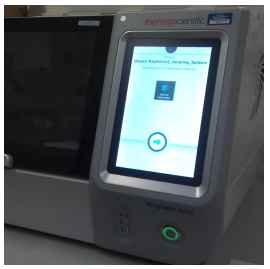
Sanger Tree of Life HMW DNA Extraction: Automated MagAttract for Small Arthropods



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Amy Denton[1], Abitha Thomas[2], Caroline Howard[1]

[1]Tree of Life, Wellcome Sanger Institute, Hinxton, Cambridgeshire, CB10 1SA; [2]Wellcome Sanger Institute

Amy Denton
Tree of Life, Wellcome Sanger Institute, Hinxton, Cambridges...

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We use this protocol and it's working

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Abstract

This protocol describes the automated extraction and SPRI of HMW DNA from small arthropod samples intended for long-read sequencing using the Qiagen MagAttract HMW DNA extraction kit and the Thermo Fisher KingFisher™ Apex. This protocol is primarily effective for the class Arachnida and order Isopoda within Arthropoda covered by the Tree of Life Programme. This protocol has resulted in successful extractions for a number of species including *Linyphia triangularis, Anilocra frontalis, Lekanesphaera levii, Cymodoce truncata*, *Porcellio scaber*, *Idotea baltica* and *Ligia oceanica*, as well as a reference genome generation for *Troglohyphantes excavatus.*

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 primarily adapted from the 'HMW DNA Extraction of Jumping Spiders v.4' protocol; this was developed through R&D by the Meier/Jiggin group for jumping spider tissue, which uses elements of the Qiagen MagAttract protocol and the Lawniczak Lab extraction v.6 for small insects. This protocol also incorporates the pre-shear SPRI element of the 'Sanger Tree of Life HMW DNA Extraction: Automated MagAttract v.2' protocol.

Acronyms
HMW: high molecular weight
SPRI: solid-phase reversible immobilisation
LI: low input
ULI: ultra-low input

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.
- 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 24 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 flash drive.

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
- 1 x phosphate-buffered saline (PBS)
- 100% absolute ethanol
- Nuclease-free water
- 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 to 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)

KingFisher™ Apex DNA Extraction Protocol Script:

KFX file: Qiagen MagAttract_Jumping_Spiders… 2KB

1. Pick Up Tip - Tip Plate
2. DNA Binding - Sample Plate
   Pre-collect beads: Off
   Release beads: Off
   Heating & Cooling: Off
   Mixing 1# 00:10:00 Medium Looping: 1
   2# 00:05:00 Slow
   Postmix: Off
   Collect beads: On 5 Count 5 Seconds
3. Wash 1 - MW1 Wash 1
   Plate Pre-collect beads: Off

Release beads: On 00:00:10 Bottom mix
Heating & Cooling: On 25°C Pre-heat: Off
Mixing 1# 00:01:00 Fast
Postmix: Off
Collect beads: On 5 Count 5 Second

4. Wash 2 - MW1 Wash 2 Plate
   Pre-collect beads: Off
   Release beads: On 00:00:10 Bottom mix
   Heating & Cooling: On 25°C Pre-heat: Off
   Mixing 1# 00:01:00 Fast
   Postmix: Off
   Collect beads: On 5 Count 5 Second
5. Wash 3 - PE Wash 1 Plate
   Pre-collect beads: Off
   Release beads: On 00:00:10 Bottom mix
   Heating & Cooling: On 25°C Pre-heat: Off
   Mixing 1# 00:01:00 Fast
   Postmix: Off
   Collect beads: On 5 Count 5 Second
6. Wash 4 - PE Wash 2 Plate
   Pre-collect beads: Off
   Release beads: On 00:00:10 Bottom mix
   Heating & Cooling: On 25°C Pre-heat: Off
   Mixing 1# 00:01:00 Fast
   Postmix: Off
   Collect beads: On 5 Count 5 Second
7. 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
8. Dry - NFW Plate
   Duration: 00:01:00
   Dry Type: Above Well
9. Elute 1 - Elution Plate 1
   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

10. Elute 2 - Elution Plate 2
    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
11. Leave Tip - NFW Plate

KingFisher™ Apex 0.45X SPRI Protocol Script:

KFX file: Pre-shear 0.45X SPRI.kfx 2KB

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#	00:07:00	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

Protocol PDF: Sanger Tree of Life HMW DNA Extra… 114KB

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.

Before start

- 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.

Sample Lysis

1 Prepare a lysis buffer mastermix:

Reagent	Volume per sample
Phosphate buffered saline (PBS)	100 µL
Proteinase K	10 µL
RNase A	2 µL
Buffer AL	75 µL

2 Set a heat block to 25 °C.

3 For PowerMashed samples (weight less than 25 mg):
1. Transfer sample into a 1.5 mL BioMasher II tube and add 187 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. Try to be as gentle as possible when using the PowerMasher – if samples are very small, you may wish to manually disrupt the tissue using the BioMasher pestle by hand. (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.

4 For samples which have been cryogenically homogenised via cryoPREP:
1. Transfer 25 mg of the sample into a 2 mL microcentrifuge tube, then hold on dry ice to keep the sample frozen.
2. Add 187 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.

5 Centrifuge sample tubes briefly in a mini-centrifuge and then incubate at 25 °C for 3 hours.

Loading and Running the KingFisher™ Apex for DNA Extraction

6 While samples are lysing, label seven 1 mL 96-well deep-well KingFisher™ plates and two 200 µL 96-well standard plates, then fill the number of wells required for the number of samples in each plate as follows:

Plate	Plate Type	Reagent(s) required
Tip Plate	1 mL	96-well tip comb (no reagent)
Elution 2	200 µL	100 µL Buffer AE
Elution 1	200 µL	100 µL Buffer AE
NFW Wash	1 mL	350 µL Nuclease-free water
PE Wash 2	1 mL	350 µL Buffer PE
PE Wash 1	1 mL	350 µL Buffer PE
MW1 Wash 2	1 mL	350 µL Buffer MW1
MW1 Wash 1	1 mL	350 µL Buffer MW1
Sample Plate	1 mL	15 µL Suspension G magnetic beads 140 µL Buffer MB

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

8 Using a wide bore pipette tip, set the volume to 200 µL, transfer lysate to the appropriate well in the sample plate taking care not to transfer large pieces of debris.

9 Select the required 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 66 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 1 and Elution Plate 1 into a new 1 mL 96-well deep-well KingFisher™ plate and gently pipette mix 5–10 times with wide-bore tips to fully homogenise DNA. This new 1 mL 96-well deep-well KingFisher™ plate 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 (from Step 13)	1 mL deep-well	180 µL DNA + 81 µL AMPure PB beads
	Ethanol Wash Plate	1 mL deep-well	1000 µL 80% EtOH (freshly prepared)
	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 in KingFisher™ Apex 0.45X SPRI Protocol Script/attached KFX file in the Material section) 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 4 °C.

Protocol references

MagAttract HMW DNA Handbook: **MagAttract HMW DNA Handbook - QIAGEN**

ChromiumTMGenome Reagent Kits User Guide pages 6-8 (https://support.10xgenomics.com/genome-exome/library-prep/doc/user-guide-chromium-genome-reagent-kit-v2-chemistry).

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