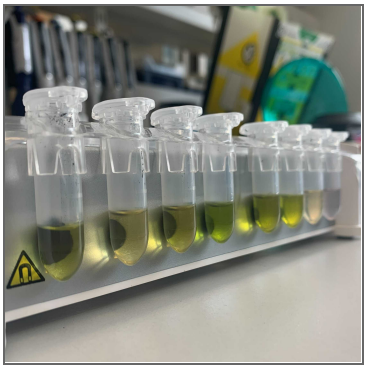

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Protocol status: Working
We use this protocol and it's working

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

In 1 collection

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ABSTRACT

This protocol describes the manual extraction of HMW DNA from plant or fungi tissue samples from a variety of species intended for long-read sequencing using the Qiagen MagAttract HMW DNA extraction kit. This process is effective for approximately 60% of the plant species covered by the Tree of Life Programme, but the resulting yield of CCS data from PacBio sequencing has been very variable. This protocol is particularly useful for samples with limited tissue availability, as it ensures the maximum amount of HMW DNA can be extracted and recovered. 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 HiFi, HMW DNA Fragmentation: Diagenode Megaruptor® 3 for LI PacBio or HMW DNA Fragmentation: g-Tube for ULI PacBio. This protocol was adapted from Sanger Tree of Life HMW DNA Extraction: Manual MagAttract for the lysis of plant and fungi samples, and has since been updated to Sanger Tree of Life HMW DNA Extraction: Manual MagAttract v.2/3 to improve sample lysis (v.2) and to include a pre-shear SPRI of the HMW DNA extracted (v.3).

Acronyms

HMW: high molecular weight
SPRI: solid-phase reversible immobilisation
HiFi: high fidelity
LI: low input
ULI: ultra-low input
CCS: circular consensus sequencing

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PROTOCOL integer ID: 86859

Keywords: HMW DNA extraction, magnetic bead extraction, MagAttract, automated DNA extraction, KingFisher, plant DNA extraction, 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 8 samples, with approximately 2 hours handling time over a start to finish period of 4 hours. This estimation excludes subsequent QC checks.
- For samples that are suitable for LI sequencing on PacBio, the extracts produced at the end of this protocol can undergo a 0.45X SPRI, using either the KingFisher™ Apex 0.45X SPRI Protocol detailed in the Sanger Tree of Life HMW DNA Extraction: Automated Plant MagAttract v.3, or following the Sanger Tree of Life Fragmented DNA clean up: Manual SPRI protocol using a bead:sample ratio of 0.45:1.

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.

MATERIALS

- 2 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030 108.078)
- 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, filtered if available)
- 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)
- DynaMag™-2 magnetic rack (Cat. no. 12321D)
- Timer

Protocol PDF:

Sanger Tree of Life HMW DNA Extraction_ Manual Plant MagAttract v1.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.

BEFORE START INSTRUCTIONS

- Add 100% ethanol to the MW1 and PE wash buffers as per manufacturer's instructions.
- Set one heat block to 50 °C and another to 25 °C.

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 Transfer 50 mg of cryogenically homogenised plant/fungi tissue from each sample to 2 mL microcentrifuge tubes and place on dry ice to keep the samples frozen.

3 Add 374 µL of the lysis buffer master mix to each sample, then homogenise sample and mastermix by gently pipetting 10 times with a wide bore pipette tip.

4 Centrifuge tube briefly to collect in a mini centrifuge, then incubate on the heat block at 50 °C for 2 hours.

DNA isolation

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

6 Using a wide-bore pipette tip, set the volume to 380 µL, transfer lysate to individual microcentrifuge tubes, whilst avoiding insoluble material.

7 Add 280 µL Buffer MB to each sample and 15 µL of Suspension G beads. Invert the tube 10–20 times to ensure the beads are suspended in the lysate. Allow 5 minutes for binding.

8 Briefly centrifuge the samples in a mini-centrifuge to collect at the bottom of the tube.

9 Place the tubes on the magnetic rack and allow 2–5 minutes for the beads to migrate (more viscous samples will take longer). Remove the supernatant and discard.

10 Remove the tubes from the magnetic rack and add 700 µL Buffer MW1 directly to the bead pellet, then invert the tube 10–20 times to ensure the beads are suspended in the lysate.

11 Place the tubes on the magnetic rack and allow 2-5 minutes for the beads to migrate (more viscous samples will take longer). Remove the supernatant and discard.

12 Repeat the MW1 wash for a total of two washes (steps 10 and 11).

13 Remove the tubes from the magnetic rack and add 700 µL Buffer PE directly to the bead pellet and invert 10–20 times to resuspend the beads.

14 Place the tubes on the magnetic rack and allow 2–5 minutes for the beads to migrate (more viscous samples will take longer). Remove the supernatant and discard.

15 Repeat the PE wash for a total of two washes (steps 13 and 14).

16 Briefly centrifuge the tubes in a mini centrifuge and place the sample back on the magnetic rack. Use a small micropipette to remove any residual wash buffer.

17 Pipette 700 µL nuclease-free water onto the side opposite of the beads in the microcentrifuge tubes whilst the tubes are on the magnetic rack. Do not pipette the nuclease-free water directly onto the bead pellet. Incubate for exactly 1 minute then slowly aspirate and discard water from the tubes.

18 Repeat step 17 for a total of two washes.

19 After exactly 1 minute, remove the water from the tubes, then take the samples from the magnetic rack and add 200 µL of Buffer AE directly to the bead pellet. Mix, either by gently flick mixing or using a wide-bore pipette tip in order to dislodge the pellet from the tube.

20 Incubate for 15 minutes at room temperature, with a gentle mix halfway through and again at the end.

21 Briefly centrifuge (spin down) the sample in a mini centrifuge and place on a magnetic rack, allowing 2–5 minutes for bead migration.

22 Using a 200 µL wide-bore pipette tip, carefully transfer the supernatant containing purified gDNA to a fresh microcentrifuge tube.

23 Remove the sample from the magnetic rack. Add 200 µL Buffer AE to the bead pellet. Incubate at 25 °C, shaking at 1000 rpm, for three minutes.

24 Centrifuge the tube briefly in a mini centrifuge and place it on a magnetic rack for 2–5 minutes for the beads to migrate.

25 Using a wide-bore pipette tip, carefully transfer the supernatant containing purified gDNA to the same microcentrifuge tube as step 22.

26 Store the extracted gDNA sample at 4 °C.