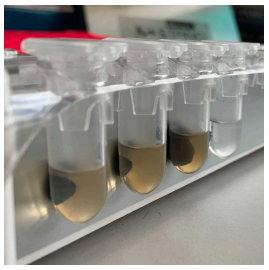
Sep 30, 2023

Sanger Tree of Life HMW DNA Extraction: Manual MagAttract



In 3 collections

DOI
dx.doi.org/10.17504/protocols.io.6qpvr33novmk/v1

Michelle Strickland[1], Robin Moll[2], Clare Cornwell[1], Michelle Smith[2], Caroline Howard[1]

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

Tree of Life at the Wellc... | Earth BioGenome Project

Tree of Life Genome Note Editor
Wellcome Sanger Institute

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.6qpvr33novmk/v1

Protocol Citation: Michelle Strickland, Robin Moll, Clare Cornwell, Michelle Smith, Caroline Howard 2023. Sanger Tree of Life HMW DNA Extraction: Manual MagAttract. **protocols.io https://dx.doi.org/10.17504/protocols.io.6qpvr33novmk/v1**

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

Created: August 23, 2023

Last Modified: September 30, 2023

Protocol Integer ID: 86858

Keywords: HMW DNA extraction, magnetic bead extraction, manual DNA extraction, solid phase reversible immobilisation, reference genome, long read sequencing, sanger tree of life hmw dna extraction, hmw dna extraction, life hmw dna extraction, manual extraction of hmw dna, qiagen magattract hmw dna extraction kit, hmw dna fragmentation, hmw dna pooling, hmw dna, dna extraction, more dna from these smaller sample, acronyms hmw, manual extraction, taxonomic group, genome size of the species, read sequencing, yielded more dna, genome, dna, sanger tree of life, tree of life programme, manual magattract this protocol, high molecular weight spri, sanger tree, genome size

Funders Acknowledgements:
Wellcome Trust
Grant ID: 218328
Wellcome Trust
Grant ID: 206194
Gordon and Betty Moore Foundation
Grant ID: GBMF8897

Abstract

This protocol describes the manual extraction of HMW DNA from multiple different tissue samples from a variety of species, excluding plants and fungi, intended for long-read sequencing using the Qiagen MagAttract HMW DNA extraction kit. This process is effective for a wide variety of taxonomic groups covered by the Tree of Life Programme. This protocol is particularly useful for samples with limited tissue availability, as it has consistently yielded more DNA from these smaller samples than the equivalent Automated method. The output of this protocol is HMW DNA, which depending upon yield and the genome size of the species, can be directed towards 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.

Acronyms
HMW: high molecular weight
SPRI: solid-phase reversible immobilisation
HiFi: high fidelity
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.
- 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 MagAttract v.2, 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)
- 1.5 mL BioMasher II tubes and pestles (sterile) (Cat. no. 9791a)
- 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)
- Diagnocine PowerMasher II tissue disruptor (Product no. 891300)
- 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

Protocol PDF: Sanger Tree of Life HMW DNA Extr... 82KB

Troubleshooting

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

- Add 100% ethanol to the MW1 and PE wash buffers as per manufacturer's instructions.
- Set a heat block 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 For samples which have been prepared by cryoPREP:
a) Transfer 25 mg sample into a 2 mL microcentrifuge tube, then hold on dry ice to keep the sample frozen.
b) When ready, remove sample from the dry ice and add 374 µL of the lysis buffer master mix to sample, then homogenise 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 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).
c) Transfer the entire contents of the BioMasher tube to a 2 mL microcentrifuge tube using a wide-bore tip.

4 Centrifuge sample tubes briefly in a mini-centrifuge, then incubate on the heat block at 25 °C for 2 hours.

DNA Isolation

5 Once samples have completed lysing, remove sample tubes from the heat block and briefly centrifuge samples 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 Remove 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 sample on the heat block at 25 °C, shaking at 1,000 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.

Protocol references

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