Sanger Tree of Life HMW DNA Extraction: Manual MagAttract v.3

Authors

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Abstract

This protocol is for 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 the majority of taxonomic groups covered by the Tree of Life Programme, excluding plants and fungi. 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 genome size of the species, can be directed towards either HMW DNA Pooling, HMW DNA Fragmentation: Opentrons® OT-2 for PacBio LI, HMW DNA Fragmentation: Diagenode Megaruptor® 3 for LI PacBio or HMW DNA Fragmentation: Covaris g-Tube for ULI PacBio. This protocol was adapted from Sanger Tree of Life HMW DNA Extraction: Manual MagAttract to include an overnight elution in order to maximise the yield of DNA, as well as an updated pre-shear SPRI to improve recoveries of HMW DNA.

Safety Warnings

- Operators should 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.

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 8 samples, with approximately 2 hours handling time over a start to finish period of 4 hours. This estimation excludes the overnight elution and subsequent QC checks.
- Whilst a manual 0.45X SPRI is detailed in this protocol, samples which undergo the manual extraction could alternatively undergo an automated 0.45X SPRI, as detailed in the Sanger Tree of Life HMW DNA Extraction: Automated MagAttract v3.

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.

Before starting:

- Add 100% ethanol to the MW1 and PE wash buffers as per manufacturer's instructions.
- Set a heat block to 25 °C.
- Remove the AMPure PB beads from the fridge 30 minutes before starting the 0.45X SPRI to bring them to room temperature.

Laboratory Protocol:

<u>Sample Lysis</u>

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 cryoprepped/beatbeaten samples:
    a. Transfer 25 mg prepped 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 all sample tubes briefly in a mini centrifuge, then incubate on the heat block at 25 ºC 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 new microcentrifuge tubes, whilst avoiding insoluble material.

7. Add 280 µL Buffer MB to each sample and 40 µL of Suspension G beads. Invert the tube 10 to 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 to 20 times to ensure the beads are suspended in the lysate.

11. Spin down tubes in a mini centrifuge for 1–2 seconds, then place the tubes back 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 & 11).

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

14. Spin down tubes in a mini centrifuge for 1–2 seconds, then 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 & 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 400 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 samples overnight at room temperature.

21. Following overnight incubation, pipette mix samples slowly 5 times with a wide bore pipette tip, before briefly centrifuging samples in a mini centrifuge and then placing them on a magnetic rack to allow bead capture.

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

23. Proceed with a 0.45X SPRI, either manually (as described below) or automated on the KingFisher Apex (following the 0.45X SPRI steps in protocol Sanger Tree of Life HMW DNA Extraction: Automated MagAttract v.3)

Manual 0.45X SPRI

24. Set the heat block to 37 °C and label two sets of 1.5 mL microcentrifuge tubes for each sample.

25. Vortex AMPure PB beads for 30 seconds.

26. Immediately add 180 µL of AMPure PB beads to the 400 µL of DNA.

27. Mix the beads/DNA thoroughly by pipette mixing 15 times with a wide-bore pipette tip. Do not flick the tube.

28. Spin down tubes in a mini centrifuge for 1–2 seconds to collect the beads.

29. Incubate the mix on the bench top for 5 minutes at room temperature.

30. Spin down tubes in a mini centrifuge for 1–2 seconds to collect beads.

31. Place the tubes in a magnetic bead rack and wait for the beads to pellet on the side of the tube. This may take up to 5 minutes or more depending upon the amount of DNA and beads within the sample.

32. Slowly pipette off cleared supernatant and save in the first labelled 1.5 mL microcentrifuge tube. Avoid disturbing the beads.

33. Wash beads with freshly prepared 80% ethanol.
    a. Do not remove the tube from the magnetic rack.

b. Use a sufficient volume of 80% ethanol to fill the tube – 700–1000 µL for 1.5 mL tube is usually sufficient.
c. Do not disturb the beads – slowly dispense the 80% ethanol against the side of the tube opposite the beads.
d. After 30 seconds, pipette and discard the 80% ethanol.

34. Repeat step 33 for a total of two ethanol washes.
35. Spin down tubes in a mini centrifuge for 1–2 seconds, then return them to the magnetic rack to allow the beads to pellet. Aspirate and dispose of any remaining ethanol.
36. Check for any remaining ethanol droplets in the tube. If droplets are present, repeat step 35.
37. Take tubes off the magnetic rack and add 135 µL of EB buffer to the beads. Gently mix by slowly pipetting 15 times with a wide bore pipette tip. Do not flick the tube.
38. Incubate tubes on the heat block at 37 °C for 30 minutes at 350 rpm.
39. Briefly spin down the tubes for 1–2 seconds in a mini centrifuge and then place them back on the magnetic rack. Allow the beads to pellet - this may take up to 5 minutes or more depending upon the quantity and quality of DNA within the sample.
40. Slowly pipette off cleared supernatant and save in the second labelled 1.5 mL microcentrifuge tube. Avoid disturbing the beads.
41. Proceed samples directly to QC. If extraction has been successful, the supernatants saved in the first set of labelled 1.5 mL microcentrifuge tubes can now be discarded.
42. Store the extracted gDNA sample at 4 ºC.

Materials:

- 2 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030108078)
- 1.5 mL BioMasher II tubes and pestles (sterile) (Takara Cat. no. 9791a)
- Qiagen MagAttract HMW DNA extraction kit (Qiagen Cat. no. 67563)
- Dry ice
- 1 x phosphate-buffered saline (PBS)
- EB buffer (Qiagen Cat. no. 19086)
- 100% absolute ethanol
- AMPure PB beads (Pacific Biosciences Cat. no. 100-265-900)
- 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)
- Diagnocine PowerMasher II tissue disruptor (Cat no. 891300)

- Corning® CoolRack CF45 (Cat. no. 432051) or equivalent
- 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) or similar
- DynaMag™-2 magnetic rack (Cat. no. 12321D) or similar
- Timer

References:

MagAttract HMW DNA Handbook: MagAttract HMW DNA Handbook - QIAGEN

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

Sanger Tree of Life HMW DNA Extraction: Manual MagAttract M. Strickland et al. (2023)