Sanger Tree of Life HMW DNA Extraction: Manual Plant MagAttract v.4

Authors

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Abstract

This protocol is for 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, and the resulting yield of CCS data from PacBio sequencing has been good. 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 Megaruptor3 for LI HiFi, HMW DNA Fragmentation: Diagenode Megaruptor3 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 Plant MagAttract v.2/3 to further improve sample lysis. These improvements have been made through a combination of preheating the lysis buffer, delaying the addition of RNase A to later on in sample lysis and increasing centrifugation of the lysate, which have led to a reduction in tissue clumping, minimised oxidative damage of the DNA, reduced Proteinase K inhibition of RNase A and increased purity of the HMW DNA due to the exclusion of aggregated, insoluble or sedimented contaminants.

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.

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 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 v4, 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.

Before starting:

- Add 100% ethanol to the MW1 and PE wash buffers as per manufacturer's instructions.
- Set one heat block with a 50 mL SmartBlock to 65°C, another heat block with a 2 mL SmartBlock to 55°C and a third heat block with a 2 mL SmartBlock to 25°C.

Laboratory Protocol:

<u>Sample Lysis</u>

1. Prepare a lysis buffer master mix in a 50 mL centrifuge tube:

Reagent	Volume per sample
Phosphate buffered saline (PBS)	200 µL
Buffer AL	150 µL

2. Place the lysis buffer on the 65°C heat block and incubate at 400 rpm for at least 20 minutes. Keep at temperature until added to the sample.
3. Transfer 50 mg of cryogenically disrupted tissue from each sample to 2 mL microcentrifuge tubes.
    a. Ensure the disrupted tissue is completely disrupted into a fine powder; avoid matted/clumped powder. This is crucial for optimal DNA yield and integrity; poorly disrupted tissue drastically decreases lysis and extraction efficiency.
    b. Any samples containing poorly disrupted tissue 'chunks' should be flagged as requiring reprocessing and further cryogenically disrupted.
4. Transfer the samples to a pre-chilled cold block on wet ice and incubate for 10 minutes to equilibrate temperature.
5. Add 20 µL Proteinase K (for n+1 samples) to the preheated lysis buffer immediately prior to initiating lysis, swirling the centrifuge tube to mix.

6. Add 370 µL of the preheated lysis buffer plus Proteinase K to each sample, immediately homogenising the lysate by mixing with 5 rapid pulse vortexes, and place on the 55°C heat block at 600 rpm for 15 minutes.
7. After 5 minutes incubation, resuspend any severely aggregated samples by pipette mixing with a wide bore pipette tip.
8. After the initial 15 minute incubation, add 4 µL RNase A to each sample and mix thoroughly by inversion until any aggregated, insoluble or sedimented tissue particles are resuspended.
9. Incubate samples for a further 45 minutes on the heat block at 55°C at 600 rpm.
10. During this incubation, samples should be occasionally mixed (every 5–15 minutes) by inversion to resuspend sedimented particles.
    Do not mix the samples by inversion for the final 15 minutes of lysis, allowing aggregated, insoluble or sedimented tissue particles to settle at the bottom of the tube.

DNA Isolation

11. Once samples have completed lysing, remove sample tubes from the heat block and allow the lysate to settle to the bottom of the tube for 5 minutes.

12. Centrifuge the samples for 10 minutes, 8,000 rpm at room temperature.

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

14. 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.

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

16. 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.

17. 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.

18. 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.

19. Repeat the MW1 wash for a total of two washes (steps 17 & 18).

20. 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.

21. 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.

22. Repeat the PE wash for a total of two washes (steps 20 & 21).

23. 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.

24. 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.

25. Repeat step 24 for a total of two washes.

26. 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.

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

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

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

30. Remove the sample from the magnetic rack. Add 200 µL Buffer AE to the bead pellet. Incubate at 25°C, shaking at 1,000 rpm, for three minutes.

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

32. Using a wide-bore pipette tip, carefully transfer the supernatant containing purified gDNA to the same microcentrifuge tube as step 29.

33. Store the extracted gDNA sample at 4°C.

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)
- Eppendorf SmartBlock 50 mL (Cat. no. 5365000028)
- Vortexer (Vortex Genie™ 2 SI-0266)
- Mini centrifuge (Cat. no. SS-6050)
- DynaMag™-2 magnetic rack (Cat. no. 12321D)
- Timer

References:

MagAttract HMW DNA Handbook: MagAttract HMW DNA Handbook - QIAGEN