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

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We use this protocol and it's working

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Abstract

This protocol describes the automated extraction of HMW DNA from cryogenically disrupted plant and fungi tissue samples intended for long-read sequencing. It employs the Qiagen MagAttract HMW DNA extraction kit and the Thermo Fisher KingFisher™ Apex. This process is effective for a wide variety of plant species covered by the Tree of Life Programme. 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 or HMW DNA Fragmentation: Diagenode Megaruptor®3 for LI HiFi. This protocol was adapted from Sanger Tree of Life HMW DNA Extraction: Manual Plant MagAttract v.1 to include automation for a higher throughput of samples, and was updated to Sanger Tree of Life HMW DNA Extraction: Automated Plant MagAttract v.2 to improve sample lysis.

Acronyms

HMW: high molecular weight

SPRI: solid-phase reversible immobilisation

HiFi: high fidelity



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 up to 32 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 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 script and the KFX.file have been made available for this protocol – the KFX.file requires 'Bindlx software for KingFisher Apex' to allow the KingFisher™ Apex protocol to be viewed on a PC or laptop. Alternatively, the file 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. 0030 108.051)
- 2 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030 108.078)
- Thermo Fisher KingFisher™ 96-well Deep-well plates (Thermo Fisher Cat. no. 95040450)
- Thermo Fisher KingFisher™ 96 Tip Comb (Thermo Fisher Cat. no. 97002570)
- 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 to 1000 µL and filtered tips
- Wide-bore tips (200 µL and 1000 µL, filtered if available)
- Thermo Fisher KingFisher™ Apex instrument (Cat. no: 5400930)
- 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

KingFisher™ Apex DNA Extraction Protocol Script:

KFX file:  Qiagen MagAttract Standard.kfx 2KB

1. Pick Up Tip - Tip Plate
2. DNA Binding - Sample Plate
 - Pre-collect beads: Off
 - Release beads: Off
 - Heating & Cooling: Off
 - Mixing 1# 00:05:00 Fast
 - Postmix: Off
 - Collect beads: On 5 Count 2 Seconds
3. Collect Beads 1 - Sample Plate
 - Collect beads: Count 5 Collect time: 1 Second
4. Wash 1 - MW1 Wash 1 Plate
 - Pre-collect beads: Off
 - Release beads: On 00:00:10 Bottom mix
 - Heating & Cooling: Off
 - Mixing 1# 00:01:00 Fast



- Postmix: Off
Collect beads: On 5 Count 1 Second
5. Collect Beads 2 - MW1 Wash 1 Plate
Collect beads: Count 5 Collect time: 1 Second
6. Wash 2 - MW1 Wash 2 Plate
Pre-collect beads: Off
Release beads: On 00:00:10 Bottom mix
Heating & Cooling: Off
Mixing 1# 00:01:00 Fast
Postmix: Off
Collect beads: On 5 Count 1 Second
7. Collect Beads 3 - MW1 Wash 2 Plate
Collect beads: Count 5 Collect time: 1 Second
8. Wash 3 - PE Wash 1 Plate
Pre-collect beads: Off
Release beads: On 00:00:10 Bottom mix
Heating & Cooling: Off
Mixing 1# 00:01:00 Fast
Postmix: Off
Collect beads: On 5 Count 1 Second
9. Collect Bead 4 - PE Wash 1 Plate
Collect beads: Count 5 Collect time: 1 Second
10. Wash 4 - PE Wash 2 Plate
Pre-collect beads: Off
Release beads: On 00:00:10 Bottom mix
Heating & Cooling: Off
Mixing 1# 00:01:00 Fast
Postmix: Off
Collect beads: On 5 Count 1 Second
11. Collect Bead 5 - PE Wash 2 Plate
Collect beads: Count 5 Collect time: 1 Second
12. 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
13. Dry - NFW Plate
Duration: 00:01:00 Dry Type: Above Well
14. Elute 1 - Elution Plate 1 Plate
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

15. Elute 2 - Elution Plate 2 Plate

Pre-collect beads: Off

Release beads: On 00:00:00

Heating & Cooling:	On	25°C	Pre-heat: Off
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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

16. Leave Tip - NFW Plate

Protocol PDF:

 Sanger Tree of Life HMW DNA Extr... 88KB

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.
- Do not open the door of the KingFisher™ Apex instrument whilst it is in operation.

Before start

Add 100% ethanol to MW1 and PE wash buffers as per manufacturer's instructions.



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 Set a heat block to 50 °C.
- 3 Transfer 50 mg of cryogenically homogenised tissue from each sample to 2 mL microcentrifuge tubes and place on dry ice to keep the samples frozen.
- 4 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.
- 5 Centrifuge tube briefly to collect, then incubate on the heat block at 50 °C for 2 hours.

Loading and Running the KingFisher™ Apex

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

	Plate	Reagent(s) required
	Tip plate	96-well tip comb (no reagent)
	Elution 2	200 µL Buffer AE
	Elution 1	200 µL Buffer AE
	NFW Wash	500 µL Nuclease-free water
	PE Wash 2	700 µL Buffer PE
	PE Wash 1	700 µL Buffer PE

Plate	Reagent(s) required
MW1 Wash 2	700 µL Buffer MW1
MW1 Wash 1	700 µL Buffer MW1
Sample plate	15 µL Suspension G magnetic beads 280 µL Buffer MB

- 7 Once samples have completed lysing, remove sample tubes from the heat block and briefly centrifuge to spin down.
- 8 Using a wide-bore pipette tip, set the volume to 380 µL, transfer lysate from the sample tubes to individual wells in the sample plate, taking care not to transfer large pieces of debris if possible.
- 9 Select the required 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 50 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 the elution plate into microcentrifuge tubes, pipette mix with wide-bore tips to fully homogenise DNA in the eluate.
- 15 Perform required QC and then store the DNA at 4 °C.

Protocol references

MagAttract HMW DNA Handbook: [MagAttract HMW DNA Handbook - QIAGEN](#)