

# Sanger Tree of Life HMW DNA Extraction: Automated Plant MagAttract v.2

## Authors

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## Abstract

This protocol is for the automated extraction of HMW DNA from cryoprepped tissue samples from plants and fungi intended for long-read sequencing using 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: Automated Plant MagAttract v.1 to improve sample lysis, and was updated to Sanger Tree of Life HMW DNA Extraction: Automated Plant MagAttract v.3 to include a pre-shear SPRI of the HMW DNA extracted.

## 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.

## 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 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.

**Before starting:**

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

**Laboratory Protocol:**

**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 55 °C.
3. Transfer 50 mg of cryoprepped 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 55 °C at 600 rpm for 1 hour.

**Loading and Running the KingFisher™ Apex**

6. While the 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 $\mu$ L Buffer AE
Elution 1	200 $\mu$ L Buffer AE
NFW Wash	500 $\mu$ L Nuclease-Free Water
PE Wash 2	700 $\mu$ L Buffer PE
PE Wash 1	700 $\mu$ L Buffer PE
MW1 Wash 2	700 $\mu$ L Buffer MW1
MW1 Wash 1	700 $\mu$ L Buffer MW1
Sample plate	15 $\mu$ L Suspension G magnetic beads 280 $\mu$ L 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  $\mu$ L, 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 below in KingFisher™ Apex DNA Extraction Protocol section/attached file) 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.

### KingFisher™ Apex DNA Extraction Protocol:

- 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 |                          |
| 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

**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 - 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

**References:**

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