Sanger Tree of Life RNA Extraction: Automated MagMax™ mirVana

Authors

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Abstract

This protocol is for the automated extraction of RNA from multiple different tissue samples intended for RNA-Seq using the MagMax™ *mir*Vana total RNA isolation kit and the Thermo Fisher KingFisher™ Apex. This process is highly effective for the majority of taxonomic groups covered by the Tree of Life Programme, however challenging samples include corals, jellyfish and annelids. The output of this protocol is a highly concentrated RNA extract which can be diluted and submitted for RNA-Seq on Illumina Nova-Seq.

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

- All steps can be performed at room temperature unless stated otherwise.
- If samples are not going to proceed to sample lysis immediately, keep samples on dry ice to maintain temperature and prevent nucleic acid degradation..
- An experienced operator can expect to comfortably process up to 32 samples, with approximately 2 hours handling time over a start to finish period of 3 hours. This estimation 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 RNA storage, all routine RNA 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 ‘BindIx 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.

Before starting

- Add 10 mL of absolute isopropanol to Wash solution 1, mix well and store at room temperature.
- Add 48 mL of absolute ethanol to Wash solution 2, mix well and store at room temperature.

Laboratory Protocol:

Reagent Preparation

1. Prepare the TURBO DNase solution as described below, and once made, store on ice:

Component	Volume per sample (µL)
MagMAX TURBO DNase Buffer	60
Turbo DNase (stored in freezer)	2.5

2. Prepare the Binding Beads Mix as described below, and once made, store on ice:

Component	Volume per sample (µL)
RNA binding beads	12
Lysis/binding enhancer (stored in freezer)	12

Sample Lysis

3. Calculate the amount of lysis buffer required for the samples: 40 µL is required per 1 mg of tissue. 15 mg of sample is used for this protocol, so 600 µL per sample is required.

4. Create sufficient lysis buffer for your samples:

Component	Volume per sample (µL)
Lysis Buffer	1000
DTT	0.7

5. For samples that require powermashing, transfer 15 mg of tissue into a 1.5 mL BioMasher II tube and add 600 µL of lysis buffer. Disrupt sample in the lysis buffer using a PowerMasher II tissue disruptor and the 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).

6. For samples that have been cryoprepped, transfer the 15 mg of cryoprepped tissue into a 2 mL microcentrifuge tube and add 600 µL of lysis buffer. Pipette mix to homogenise the cryoprepped tissue and lysis buffer.

7. Incubate at room temperature for 30 seconds to 1 minute to allow samples to lyse. If samples will not immediately progress to Step 8, place samples on ice until ready to proceed.

Loading and Running the KingFisher™ Apex

8. Transfer 200 µL of each sample directly into individual wells of a Thermo Fisher KingFisher™ 1 mL 96-well deep-well plate.

9. Add 100 µL of isopropanol to each sample, seal the plate and shake at room temperature on a plate shaker for 2 minutes at 950 rpm.

10. Add 20 µL of the prepared binding beads mix to each sample, re-seal the plate and mix at room temperature on a plate shaker for 5 minutes at 950 rpm.

11. Prepare the remaining processing plates for the KingFisher™ Apex protocol:

Plate ID	Plate Position	Plate type	Reagent(s) required	Volume per well
Sample Plate	1	Deep-well	Sample + isopropanol + binding beads mix	200 µL sample + 100 µL isopropanol + 20 µL binding beads mix
Wash Plate 1	2	Deep-well	Wash solution 1	150 µL
Wash Plate 2	3	Deep-well	Wash solution 1	150 µL
DNase Plate	4	Deep-well	TURBO DNase solution	50 µL
Wash Plate 3	5	Deep-well	Wash solution 2	150 µL
Wash Plate 4	6	Deep-well	Wash solution 2	150 µL
Elution plate	7	Standard (200 µL)	Elution buffer	50 µL
Tip Comb	8	Deep-well	Place a tip comb in the plate	

12. On the KingFisher™ Apex, select the protocol (details below in the KingFisher™ Apex RNA Extraction Protocol section/attached file) on the protocols list and select using the play button.

13. Load the processing plates and the sample plate in the positions prompted by the instrument and then start the run; the full protocol will take approximately 50 minutes.

14. After 30 minutes, the protocol will pause and there will be a prompt to remove the DNase plate from the instrument, and add 50 µL of the rebinding buffer and 100 µL absolute isopropanol to each well containing sample as quickly as possible - do not premix these reagents and always add them separately to the wells.

15. Load the DNAse plate back into the instrument and press run to resume the protocol.

16. At the end of the run, remove the elution plate and store on ice.

17. 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 RNA using a pipette tip.

18. Pipette the eluate into microcentrifuge tubes, perform the required QC, and then store eluates at -80 °C.

KingFisher™ Apex RNA Extraction Protocol:

1) Pick Up Tip - Tip Plate
2) Lysis & Bind - Sample Plate
   Pre-collect beads: Off
   Release beads: On 00:00:00
   Heating & Cooling: Off
   Mixing 1# 00:07:00 Medium
   Postmix: Off
   Collect beads: On 5 Count 2 Seconds
3) Wash 1 - Wash Plate 1
   Pre-collect beads: Off
   Release beads: On 00:00:00
   Heating & Cooling: On 4°C Preheat: Off
   Mixing 1# 00:01:00 Fast
   Postmix: Off
   Collect beads: On 5 Count 1 Second
4) Wash 2 - Wash Plate 2
   Pre-collect beads: Off
   Release beads: On 00:00:00
   Heating & Cooling: On 4°C Preheat: Off
   Mixing 1# 00:01:00 Fast
   Postmix: Off
   Collect beads: On 5 Count 1 Second
5) Dry 1 - Wash Plate 2
   Duration: 00:02:00 Dry Type: Outside Well
6) DNase Step - DNase Plate
   Pre-collect beads: Off
   Release beads: On 00:00:05 Bottom mix
   Heating & Cooling: Off
   Mixing 1# 00:15:00 Medium
   Postmix: Off
   Collect beads: Off
7) Dispense - DNase Plate
   Custom naming: Add 50µl Rebinding Buffer + 100µl Isopropanol
   Dispense to plate: Isopropanol 100µl
   Rebinding Buffer 50µl
8) Rebinding - DNase Plate
   Pre-collect beads: Off

Release beads:	On	00:00:05	
Heating & Cooling:	Off		
Mixing	1#	00:05:00	Medium
Postmix:	Off		
Collect beads:	On	5 Count	1 Second

9) Wash 3 - Wash Plate 3

Pre-collect beads:	Off		
Release beads:	On	00:00:00	
Heating & Cooling:	On	4°C	Preheat: Off
Mixing	1#	00:01:00	Fast
Postmix:	Off		
Collect beads:	On	5 Count	1 Second

10) Wash 4 - Wash Plate 4

Pre-collect beads:	Off		
Release beads:	On	00:00:00	
Heating & Cooling:	On	4°C	Preheat: Off
Mixing	1#	00:01:00	Fast
Postmix:	Off		
Collect beads:	On	5 Count	1 Second

11) Dry 2 - Wash Plate 4

Duration:	00:02:00	Dry Type: Outside Well

12) Elute - Elution Plate

Pre-collect beads:	Off		
Release beads:	Off		
Heating & Cooling:	On	60°C	Pre-heat: On
Mixing	1#	00:05:00	Medium
Postmix:	On	00:00:05	Fast
Collect beads:	On	5 Count	4 Seconds

13) Leave Tip - Tip Plate

Materials

- MagMAX mirVana Total RNA isolation kit (Boxes 1 & 2, Box 1 should be stored in the freezer and Box 2 at room temperature) (Thermo Fisher Cat. no. A27828)
- Thermo Fisher KingFisher™ 1 mL 96-well Deep-well Plates (Thermo Fisher Cat. no. 95040450)
- Thermo Fisher KingFisher™ 96 Deep-well Tip Comb (Thermo Fisher Cat. no. 97002570)
- Thermo Fisher KingFisher™ 200 µL standard 96-well Plate (Thermo Fisher Cat. no. 97002084)
- 1.5 mL BioMasher tubes and pestles (sterile) (Cat. no. 9791A)
- 2 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030 108.078)
- 15 mL or 50 mL centrifuge tubes
- Dry ice
- Ice
- 100% absolute ethanol
- 100% absolute isopropanol
- Dithiothreitol (DTT) (Cat. no A3668.0050)

Equipment

- Pipettes for 0.5 - 1000 µL and filtered tips
- Diagnocine PowerMasher II tissue disruptor (Cat. no. FNK-891300)
- Vortexer (Vortex Genie™ 2 SI-0266)
- Plate shaker/Thermomix (Thermo Fisher Cat. no. 88882006)
- Thermo Fisher KingFisher™ Apex instrument (Cat. no. 5400930)

References:

Document Connect (thermofisher.com)