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🌐 Sanger Tree of Life RNA Extraction: Automated MagMax™ mirVana

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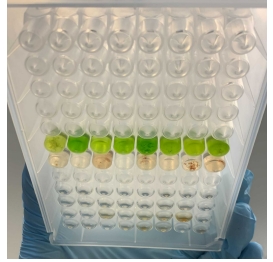
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We use this protocol and it's working

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Abstract

This protocol describes the automated extraction of RNA from multiple different tissue samples intended for RNA-Seq using the MagMax™ mirVana 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 NovaSeq.



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 '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.

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 to 1000 µL and filtered tips
- Diagenode 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)

KingFisher™ Apex RNA Extraction Protocol Script:

KFX File:  MagMax mirVana.kfx 2.8KB

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 + 100 µl Isopropanol
 - Dispense to plate: Isopropanol 100 µl
 - Rebinding Buffer 50 µl
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

Protocol PDF:  Sanger Tree of Life RNA Extraction... 90KB

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 in accordance with local regulations.
 - Liquid waste needs to be collected in a suitable container (e.g. glass screw-top jar) and disposed in accordance with local regulations.
 - Do not open the door of the KingFisher™ Apex instrument whilst it is in operation.

Before start

- 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.



Reagent Preparation

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

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

- 2 Prepare the Binding Beads Mix as described below, and once made, store on wet 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 cryoprepred, transfer the 15 mg of cryoprepred tissue into a 2 mL microcentrifuge tube and add 600 μ L of lysis buffer. Pipette mix to homogenise the cryoprepred 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 μ L of each sample directly into individual wells of a Thermo Fisher KingFisher™ 1 mL 96-well deep-well plate.
- 9 Add 100 μ L 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 μ L 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	Reagents(s) required	Volume per well
Sample Plate	1	Deep-well	Sample + isopropanol + binding beads mix	200 μ L sample + 100 μ L isopropanol + 20 μ L binding beads mix
Wash Plate 1	2	Deep-well	Wash solution 1	150 μ L
Wash Plate 2	3	Deep-well	Wash solution 1	150 μ L
DNase Plate	4	Deep-well	TURBO DNase solution	50 μ L
Wash Plate 3	5	Deep-well	Wash solution 2	150 μ L
Wash Plate 4	6	Deep-well	Wash solution 2	150 μ L
Elution plate	7	Standard (200 μ L)	Elution buffer	50 μ L
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 Script/attached KFX file in the Materials Section) 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 eluates into microcentrifuge tubes, perform the required QC, and then store eluates at –80°C.

Protocol references

Applied Biosystems MagMAX™ *mirVana*™ Total RNA Isolation Kit User Guide: **[Document Connect \(thermofisher.com\)](#)**