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# RNA Extraction Protocol for *Aurantiochytrium limacinum*

Michael Horowitz[1], Mariana Rius[2], Jackie Collier[2]

[1]State University of New York at Stony Brook, [2]Stony B

1 Works for me dx.doi.org/10.17504/protocols.io.bffgjjjw

Collier Lab

Michael Horowitz

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Liquid Nitrogen | | |
| TRIZOL reagent | | |
| Chloroform | | |
| Monarch Total RNA Miniprep Kit | T2010S | New England Biolabs |
| Ethanol | | |

SAFETY WARNINGS

Take safety precautions when using liquid nitrogen. Make sure to conduct Trizol and chloroform steps in fume hood.

## Prepare cells

1 Start a preculture 4 days prior to extraction by inoculating 5 ml of 790 with a colony of *Aurantiochytrium limacinum* (ATCC MYA-1381). Incubate overnight at 28 C 171 rpm.
Use preculture to inoculate 20 ml of 790 in a 250 ml flask. Culture for three days at 28 C, 171 rpm.

2 Determine volume of culture needed to reach 5*10^7 cells via cell count.

3 Place volume needed into a microcentrifuge tube and centrifuge down at 13000rpm for two minutes at room temperature. Discard supernatant.

4 Place pellet in liquid nitrogen for 3-5 minutes.

## Trizol Extraction

5 Resuspend pellet in 750ul Trizol lysis buffer and incubate on ice for 1 hour and 30 minutes.

6 Add 150ul chloroform in fume hood to the pellet and let sit for 2 minutes.

7 Vigorously shake the mixture and centrifuge at 13000rpm for 5 minutes at 4C.



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8 Pipette upper aqueous layer to a sterile microcentrifuge tube on ice.

9 Add an equal volume of >95% ethanol to the upper aqueous layer and mix well.

Monarch Kit RNA Extraction

10 Use NEB Monarch Total RNA Miniprep Kit from here. All centrifugation steps should be carried out at 16,000rcf.

11 Take an RNA purification column and collection tube. Place the mixture (the upper aqueous layer with ethanol) from the microcentrifuge tube to the purification column.

12 Centrifuge for 30 seconds and discard flow-through.

13 Add 500ul RNA Wash Buffer and spin for 30 seconds. Discard flow-through.

14 In a separate RNase-free microcentrifuge tube, combine 5ul DNase 1 provided in the Monarch Kit with 75ul DNase 1 Reaction Buffer. Pipette the mixture directly onto the column matrix.

15 Incubate for 15 minutes at room temperature.

16 Add 500ul RNA Priming Buffer and spin for 30 seconds. Discard the flow-through.

17 Add 500ul RNA Wash Buffer and spin for 30 seconds. Discard the flow-through.

18 Add another 500ul RNA Wash Buffer and spin for **two minutes**. Transfer the column to a new RNase-free microcentrifuge tube.

19 Add 30ul of Nuclease-free Water directly to the column matrix and spin for 30 seconds.

20 Pipette the flow-through back into the column matrix and spin again for 30 seconds.



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21 Remove column matrix, close the microcentrifuge tube, and label it. Store the eluted RNA at -20C for short-term storage or at -80C for long-term storage.
RNA yields for GPY grown cells are usually around a 1000ng/ul and for 790-grown cells are much lower around 50-100ng/ul. Lower yield for 790 cells are possibly due to excessive cell clumping.



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