APR 28, 2020

RNA Extraction Protocol for *Aurantiochytrium limacinum*

Jackie

Michael Horowitz¹, Mariana Rius², Collier²

¹State University of New York at Stony Brook; ²Stony B

Collier Lab

Michael Horowitz

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bffgjijw

Protocol Citation: Michael Horowitz, Mariana Rius, Jackie Collier 2020. RNA Extraction Protocol for *Aurantiochytrium limacinum*. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bffgjijw>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Apr 22, 2020

Last Modified: Apr 28, 2020

PROTOCOL integer ID:
36040

MATERIALS

MATERIALS

✕ Liquid Nitrogen Contributed by users

✕ TRIZOL reagent Contributed by users

✕ Chloroform Contributed by users

✕ Monarch Total RNA Miniprep Kit New England Biolabs Catalog #T2010S

✕ Ethanol Contributed by users

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.
- 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.
- 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.