May 31, 2024

RNA extraction for Karlodinium veneficum PLY720

DOI
dx.doi.org/10.17504/protocols.io.n92ld9xe7g5b/v1

Will Lewis[1]

[1]Department of Biochemistry, University of Cambridge

Will Lewis
Department of Biochemistry, University of Cambridge

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.n92ld9xe7g5b/v1

Protocol Citation: Will Lewis 2024. RNA extraction for Karlodinium veneficum PLY720. **protocols.io https://dx.doi.org/10.17504/protocols.io.n92ld9xe7g5b/v1**

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: August 31, 2021

Last Modified: May 31, 2024

Protocol Integer ID: 52859

Keywords: rna extraction for karlodinium veneficum, rna extraction from karlodinium veneficum, rna extraction, karlodinium veneficum, rna, purposes of rna, cell harvesting, methodology for cell harvesting, extraction

Funders Acknowledgements:
Gordon and Betty Moore Foundation
Grant ID: GBMF9194

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

A methodology for cell harvesting and RNA extraction from Karlodinium veneficum for the purposes of RNA-seq.

Guidelines

Clean all pipettes and bench with ethanol and then RNase Zap before processing the collected cell pellets.

Regularly change gloves and spray with RNase Zap.

Ideally use newly opened boxes of filter tips.

Adapted from the TRIzol protocol available on the ThermoFisher website:
https://tools.thermofisher.com/content/sfs/manuals/trizol_reagent.pdf

Materials

Liquid N2 bath
TRIzol
Chloroform
Isopropanol
Ethanol
10 µl, 100 µl and 1000 µl pipettes and corresponding sterile RNase-free filter tips
50 ml centrifuge tubes
2 ml Eppendorf DNA LoBind Tubes
Tube racks
Invitrogen UltraPure DNase/RNase-Free Distilled Water

Centrifuge with adapter for 50 ml tubes
Centrifuge with adapter for 2 ml tubes

Troubleshooting

Safety warnings

Read MSDS for all reagents prior to performing protocol and follow appropriate safety precautions. Perform all steps involving TRIzol and chloroform in a fume cupboard and be sure to dispose of liquids and consumables contaminated with these reagents according to the department's waste disposal procedures

Before start

Cool centrifuges to 4°C.

Cell harvesting

20m

1

A culture of *Karlodinium veneficum* PLY720 ready for harvesting.

Grow 200 ml of *Karlodinium veneficum* PLY720 culture in L1 medium. Once cultures have grown to a density between 50,000 and 300,000 cells/ml (corresponding to exponential growth phase) they can be harvested.

2 For each culture being processed, gently invert the culture flask several times to homogenise the culture and then transfer four 50 ml aliquots of the culture to four 50ml centrifuge tubes.

3 Centrifuge the 50 ml tubes at 5000 x g for 5 minutes at 4°C. Decant and dispose of the supernatants from each tube by pouring, leaving the pellet in the bottom of the tubes. Inverting the tube in one smooth hand motion to remove the supernatant removes the majority of the liquid but should leave a small volume of liquid on the walls of the tubes. This is desirable as then once you reinvert the tube, the liquid will run back down to the pellet. This small volume of remaining liquid, which typically is approximately 500 µl in volume, can then be used to resuspend the pellet using a pipette.

4 Once the pellets are resuspended they need to be pooled by combining them using a 1000 µl pipette and transfering them to a 2 ml microcentrifuge tube.

5 Centrifuge the microcentrifuge tube at 5000 x g for 5 minutes at 4°C and then dispose of the supernatant using a 1000 µl pipette.

6 Flash-freeze the cell pellets by sealing the tubes and dropping them into a liquid N_2 bath for several minutes. The tubes can then be removed and stored at -80 °C until further processing for RNA extraction.

Cell lysis and phase separation

25m

7

Remove cells pellets from -80 °C storage and then process immediately. Work in a fume hood from this point forward whenever a tube is open. for each cell pellet, resuspend in 1 ml TRIzol reagent using a 1 ml pipette and pipette up and down to homogenise. This will lyse the cells and in doing so release their pigments, meaning that the mixture will be brown in colour.

8 Incubate the tube for 5 minutes at room temperature.

9 Add 200 µl chloroform to the tube and seal again.

10 Gently shake the tube to mix the chloroform with the lysed cells in TRIzol and then incubate at room temperature for 2-3 minutes.

11 Centrifuge all samples for 15 minutes at 12000 x g at 4°C.

The samples should separate into a lower phase, an interphase and an upper aqueous phase. Due to the presence of the brown colour pigments, the boundary between the upper aqueous phase and interphase might be slightly more difficult to see than for a non-pigmented organism, and therefore has to be recognised by a change in turbidity and shade, as opposed to a change in colour.

12 Using a 200 µl pipette, carefully transfer the upper aqueous phase containing the RNA to a sterile RNase-free 1.5 ml tube for each sample. Be conservative in doing this and leave some of the upper phase behind so as not to risk making contact with the DNA-containing interphase, thereby trying to minimise carry-over of DNA. Do not touch the sides of the tube with the pipette tip.

RNA precipitation 25m

13 Add 0.5 ml isopropanol to the upper aqueous phase and incubate for 10 minutes at room temperature.

14 Centrifuge the sample at 12,000 x g for 10 minutes at 4°C. The precipitated RNA should form a white or gel-like pellet at the bottom of the tube.

15 Remove and discard the supernatant using a 1 ml pipette for each sample.

Wash the RNA 25m

16 Resuspend each pellet in 75% ethanol. The pellet does not need to be broken-up by vigorous pipetting or vortexing, just dislodge the pellet from the bottom of the tube by pipetting up and down with the ethanol.

17 Centrifuge the resuspended RNA pellet samples at 7500 x g at 4°C.

18 Remove the supernatant with a 1 ml pipette.

19 Repeat the previous three steps at least a further two times, thereby washing the RNA pellet in total three times in 75% ehtnaol. This seems to reduce the amount of phenol and other contaminant carry-over, improving 260/280 and 260/230 ratios measured later with Nanodrop.

20 After the final wash remove as much of the supernatant as possible, first removing the majority with a 1ml pipette, then the remainder with a 10 µl pipette.

Dissolve the RNA

25m

21 Briefly centrifuge the tube to collect any remaining liquid and again remove this with a 10µl pipette. Repeat this once more to ensure all liquid is removed and the pellet is dry before immediately adding 100µl UltraPure™ DNase/RNase-Free Distilled Water to each sample. If the pellet stays dry for too long it may not redissolve. Pipette the water gently up and down to dislodge pellet from the bottom of the tube.

22 Place the tube containing the resuspended RNA pellet and water in a heat block set to 57 °C for 10 minutes

23 Visually confirm whether the RNA has dissolved. Gentle pipetting up and down may be required to see whether the RNA has dissolved fully and to ensure the sample is mixed well prior to quantitifcation. If the RNA concentration in the samples is high, then samples may need to be incubated for longer or another 50-100µl UltraPure™ DNase/RNase-Free Distilled Water added.

Quantification, quality control and storage

25m

24 Aliquot each sample, label and store at -80°C. I normally aliquot each sample in at least three tubes. This means that you do not need to thaw and then refreeze the entire sample, potentially compromising RNA integrity, and instead just thaw a single sample to perform quantification and other quality control analyses.

25 Analyse samples using Nanodrop to ensure the 260/280 ratio is ~2.0 and the 260/230 ratio is between 2.0 and 2.2.

26 Measure concentration of RNA and DNA using Qubit BR assay kits.