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Wisecaver Lab algal RNA extraction protocol using Ambion TRI Reagent

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ABSTRACT

Modified protocol for extracting RNA from the haptophyte *Prymnesium parvum* using Ambion Tri Reagent.

Original protocol with toubleshooting **Tri_reagent.pdf**.

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EXTERNAL LINK

https://www.thermofisher.com/order/catalog/product/AM9738#/AM9738

PROTOCOL CITATION

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MATERIALS TEXT

TRI-Reagent
Chloroform
Chloroform:isoamyl alcohol
7.5 M ammonium acetate
100% ethanol
Nuclease-free water
RNase-free water
DNase Buffer
DNase

Prepare Station and Materials 20m

1 Set a large bench top centrifuge with 50 mL falcon tube compatibility, and a ThermoMixer to **4 °C** .

2 Get liquid nitrogen if starting from live cells.

3 Wipe down with RNAse away:
- Chemical fume hood. Remove the spill pad from the chemical fume hood if present and replace with a new pad.
- RNA clean work station
- Rotors on large microcentrifuge and bench top spinner
- ThermoMixer
- Plastic racks (in RNA clean drawer)
- Pipettes (in RNA clean drawer)

4 Get RNA clean tips out from the clean drawer. Place one set of 1 mL pipettes and tips inside the chemical fume hood. Place one set of 50 mL and 1.5 mL tube racks in the chemical fume hood.

5 Prepare 75% ethanol. Per sample, mix **1.5 mL** ethanol with **500 µL** nuclease free water. Scale up for how ever many samples you are processing.

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6 Using gloved hands freshly sprayed with RNAse away, get three 1.5 mL microcentrifuge tubes per sample from the bag in the RNA clean drawer. Cap each tube tightly and place the sealed bag back in the clean drawer. Label each tube vertically on the side with the sample's unique identifier. Write down the sample ID and description in your lab notebook.

The identifier should start with a letter and be no more than 12 characters long. Write it carefully using a fine tip permanent marker as one of these tubes will contain your final sample that will be shipped off for RNA sequencing.

7 Place two labeled 1.5 mL tubes per sample inside the chemical fume hood. Leave the third tube at the RNA clean workstation.

Pellet Cells 20m

8 Collect approximately 10 million cells growing exponentially in a centrifuge tube and centrifuge 10m **4000 x g, 4°C, 00:10:00** .

9 Pour off the supernatant into a *P. pavum* waste container, and flash freeze the cell pellet in liquid nitrogen.

10 ⚠

Immediately add the TRI Reagent (below) **or** store in **-80 °C** freezer.

It's important to work quickly here as the liquid nitrogen will have lysed many cells already. Material will be highly susceptible to RNAse if allowed to sit at room temperature.

Homogenization and RNA extraction (ALL WORK DONE IN THE CHEMICAL FUME HOOD) 1h

11 ☠

Warm the frozen centrifuge tube by cupping the area around the pellet in the palm of your hand. As soon as you see the edges of the pellet beginning to melt, add **1 mL** TRI Reagent. Pipet up and down to dislodge pellet, and transfer to a 1.5 mL centrifuge tube.

All liquid TRI Reagent and chloroform liquid waste should be ejected or decanted into the amber bottle labeled 'Phenol:Chloroform Waste' inside the fume hood. Any plasticware that comes into contact with either of these chemicals needs to be

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properly disposed of in the sharps bin inside the fume hood labeled 'Mutagen/Carcinogen Sharps Do Not Autoclave'.

12 Incubate for **00:05:00** at room temperature. 5m

13  15m

OPTIONAL

If you notice a lot of debris following incubation, centrifuge at **12000 x g, 4°C, 00:15:00** , and transfer the supernatant to a fresh 1.5 mL tube.

This optional centrifugation is only rarely required to remove insoluble material from homogenates that contain high amounts of protein, polysaccharide, or extracellular material. Centrifugation pellets extracellular membranes, polysaccharides, and high molecular weight DNA, leaving the RNA in the supernatant. With the cell concentrations and species that we are working, this step is typically skipped.

14  15s

Add **200 µL** chloroform. Make sure the cap is closed securely, and mix well by vigorously shaking the tube for **00:00:15** .

15 Incubate at room temperature for **00:15:00** . 15m

16  15m

Centrifuge at **12000 x g, 4°C, 00:15:00** , then transfer approximately **500 µL** of the aqueous phase to a fresh 1.5 mL tube. Pipet slowly from the top, being careful not to disrupt the interphase.

RNA remains exclusively in the aqueous phase whereas DNA and protein are in the interphase and organic phase. Centrifugation at temperatures > 8°C may cause some DNA to partition in the aqueous phase.

RNA Precipitation (ALL WORK DONE AT THE RNA CLEAN WORK STATION) 1h

17 Add **500 µL** of isopropanol, and vortex for **00:00:10** . The clear solution should turn cloudy. 10s

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18 Using the Eppendorf ThermoMixer, incubate at **4 °C** for **00:30:00** . 30m

19 Centrifuge at **12000 x g, 4°C, 00:08:00** . Orient the centrifuge tube so that cap hinge is on the outer edge of the rotor. 8m

Proper orientation of the tube helps to avoid the pelleted RNA when removing the supernatant in subsequent steps. Even if you can't see the pellet, you will know that it is located on the on the bottom corner under the hinge.

20 

Discard the supernatant by taking off the initial volume with a 1 mL pipette tip. Use a 200 mL pipette tip to get the last bit of liquid.

You may not see a pellet at this stage, but that is okay. Just be sure to avoid the hinge side of the tube.

This is the last supernatant and plastic waste that needs to be disposed of in the phenol:chloroform waste. All waste in the remaining steps can go in the standard lab 'look-alike' waste bin.

21 Add **1 mL** of 75% ethanol to wash the pellet.

You do not need to vortex or disrupt the pellet at this stage.

22 Centrifuge at **7500 x g, 4°C, 00:05:00** . Again orienting the tube hinge so that it is on the outside of the rotor. 5m

23 Discard the supernatant by taking off the initial volume with a 1 mL pipette tip. Briefly spin down the tube using the little bench top spinner, and then use a 200 mL pipet tip to get the last bit of liquid.

Again, even if you can't see the pellet, you will know that it is located on the on the bottom corner under the hinge. So be careful to avoid that side of the tube. Pipette away the supernatant by tipping the tube slightly towards you and pipetting the liquid from the bottom corner opposite the hinge.

3m

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24

Air dry the RNA pellet for **00:03:00** by placing the tube on its side inside a folded kim wipe.

Do not overdry the RNA pellet as this will greatly decrease its solubility.

Two major sources of RNase contamination are fingers and dust. Never leave the tube open and oriented up for dust to get into your sample.

25 Re-suspend the pellet in **50 µL** of nuclease-free water. Pipette up and down 10 times letting the water wash down the hinge side of the tube.

QC and clean up

26 Place a 5 mL aliquot of your RNA sample into a separate 1.5 mL tube for QC and store on the Eppendorf ThermoMixer at **4 °C** . Store the rest of your sample immediately in the **-80 °C** freezer.

27 Make sure all TRI Reagent and chloroform waste is properly disposed of in the chemical fume hood.
Place all RNA clean pipettes, racks and consumables (tips, tubes, water etc) back in the RNA clean drawer.
Wipe down the chemical fume hood and RNA clean workstation with 75% ethanol.

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