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We use this protocol and it's working

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Wisecaver Lab DNAzol-based extraction of high molecular weight DNA from photosynthetic sea slugs

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ABSTRACT

Protocol for DNAzol-based extraction of high molecular weight DNA from photosynthetic sea slugs. This streamlined protocol takes less time compared to the lab's prior CTAB-based protocol while still yielding high quality of DNA for Illumina and Oxford Nanopore sequencing.

Prepare Station and Materials

1 Set the ThermoMixer to 35 °C

2 Get liquid nitrogen.

3 Wipe down your work area with 70% ethanol:

- Chemical fume hood
- Benchtop
- Rotors on the microcentrifuge and bench top spinner
- Pipettes

Prepare slug sample(s)

4 Freeze slug tissue in liquid nitrogen and pulverize using a Tissue Lyser II (Qiagen) or a mortar and pestle

5 Immediately add the DNAzol (see step 9) **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 environmental nucleases if allowed to sit at room temperature.

Homogenization and DNA extraction

6 Add 300 µL DNAzol. Pipet up and down to mix and transfer to a 1.5 mL centrifuge tube.

7 Add 5 µL RNAse A. Make sure the cap is closed securely, and mix well by vigorously shaking the tube for 15s 00:00:15.

8 Incubate on the ThermoMixer at 500 rpm, 35°C, 00:20:00. About half way through the incubation, take the tubes out and again mix well by vigorously shaking the tube for 15s 00:00:15. Place back in the ThermoMixer to complete the incubation step.

8.1 During incubation, prepare an aliquot of 100% ethanol. Prepare 300 µL per sample.

8.2 During incubation, prepare an ethanol and DNAzol wash by mixing 1 mL DNAzol with 750 µL ethanol.

8.3 During incubation, prepare a 75% ethanol wash using nuclease free water. Prepare 300 µL per sample.

9 Add 300 µL chloroform to each sample. Make sure the cap is closed securely, and mix well by vigorously shaking the tube for 00:00:15. 15s

All liquid 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 chloroform needs to be properly disposed of in the sharps bin inside the fume hood labeled 'Mutagen/Carcinogen Sharps Do Not Autoclave'.

10 Incubate on the Hula Mixer for 00:05:00 5m

11 Centrifuge at 12000 rpm, 00:10:00. Don't jostle samples when removing from the centrifuge. 10m

12 Transfer approximately 300 µL of the top aqueous phase to a fresh 1.5 mL tube. Pipet slowly from the top, being careful not to disrupt the interphase.

DNA Precipitation

13 Add 225 µL of 100% ethanol. Invert ten times to mix.

14 Incubate at room temperature for 00:05:00. 5m

15 Centrifuge at 5000 rpm, 00:04:00. Orient the centrifuge tube so that cap hinge is on the outer edge of the rotor. 4m

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.

16 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.

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.

17 Add 300 µL of previously prepared DNAzol wash and drag along the top of a tube rack to agitate the sample and dislodge the pellet.

18 Incubate at room temperature for 00:05:00. 5m

19 Centrifuge at 5000 rpm, 00:04:00. Orient the centrifuge tube so that cap hinge is on the outer edge of the rotor. 4m

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.

Be sure to avoid the hinge side of the tube where the sample is.

21 Add 300 µL 75% ethanol wash and vortex briefly. If the pellet does not dislodge at this step, that is okay.

22 Centrifuge at 5000 rpm, 00:04:00. Orient the centrifuge tube so that cap hinge is on the outer edge of the rotor. 4m

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.

24 Air dry the DNA pellet for 00:05:00 by placing the tube on its side inside a folded kim wipe. 5m

Do not over dry the DNA pellet as this will greatly decrease its solubility. However, also be sure that all the ethanol is removed as residual ethanol will also decrease solubility.

25 Resuspend sample in 50 µL TE Buffer.

26 Incubate on the ThermoMixer 500 rpm, 35°C, 00:05:00 .

27 Briefly spin down the tube using the little bench top spinner.

QC and clean up

28 Make sure all chloroform waste is properly disposed of in the chemical fume hood.
Wipe down the chemical fume hood and the benchtop with 70-75% ethanol.

29 Assess DNA integrity using the TapeStation. Make sure you reserve time on the TapeStation Teams calendar.

30 While the TapeStation is running, measure the DNA concentration using the Qubit.

31 If the TapeStation reports a high DNA integrity (DIN) score (≥ 7) and DNA concentration ≥ 20 ng/uL, then you have successfully isolated DNA for whole genome sequencing!

Cap your sample tube tightly. Make sure each tube is labeled on the cap and vertically on the side with the sample's unique identifier*. Wrap the cap with parafilm and place sample in the 'Novogene' box in the full sized

-20 freezer.

**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 this tube will be shipped off for DNA sequencing.*