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

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

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Abstract

Protocol for CTAB-based extraction of high molecular weight DNA from photosynthetic sea slugs. This protocol is optimized for samples with high mucopolysaccharide contents and is derived from:

Extraction of high molecular weight DNA from molluscs. 1993. Trends in Genetics.Vol 9: pg 407 https://doi.org/10.1016/0168-9525(93)90102-N.

Materials

Required sterile tools and disposables:

- 5 mL and 1.5 ml LoBind microcentrifuge tubes
- Mortar & Pestle
- Scalpel, if performing slug dissection
- Magnetic stand for Ampure beads
- Wide-bore pipette tips
- Water bath

Other required solutions/chemicals:

- TrisHCL pH 8
- NaCL
- Beta-mercaptoethanol
- EDTA
- proteinase K
- Chloroform:Isoamyl alcohol (24:1)
- Phenol:Chloroform:Isoamyl alcohol (25:24:21)
- Isopropanol
- 100% Ethanol
- RNaseA (100 mg/mL)
- TE buffer
- Ampure Beads

Troubleshooting

Safety warnings

At steps involving beta-mercaptoethanol and chloroform (**carcinogens**) should be performed in the chemical fume hood.

Prepare CTAB DNA extraction buffer

1 Prepare 100 mL CTAB buffer:

- 100 mL Ultrapure distilled water
- 1.576 g TrisHCL ph 8 (100 mM)
- 0.584 g EDTA (20 mM)
- 8.82 g NaCl (1.4 M)
- 10 mg Proteinase K (0.1 mg/mL)

Note: This stock buffer is sufficient for 9-10 extractions.

2 On the day of an extraction, per sample: add 20 µL beta-mercaptoethanol (BME) to 10 mL CTAB buffer.

Safety information

BME is a carcinogen.

ALL steps containing BME should be performed in the chemical fume hood.

All BME liquid waste should be ejected or decanted into the amber bottle labeled 'BME Waste' inside the fume hood. Any plasticware that comes into contact with BME needs to be properly disposed of in the sharps bin inside the fume hood labeled 'Mutagen/Carcinogen Sharps Do Not Autoclave'.

3 Use a water bath to preheat CTAB+BME buffer at 60 °C in a water bath.

Prepare equipment and reagents

4 On the day of the of extraction:

- Chill centrifuge to 4 °C
- Get liquid nitrogen
- Chill mortar and pestle in the -20 °C freezer
- Bring Ampure beads to room temperature (~ 20 °C)
- Prepare 5 mL of 70% Ethanol

5 Decide if performing DNA extraction using whole slugs or dissected tissue.

STEP CASE

Dissection of brain tissue 31 steps

Dissections must be performed using living slugs (i.e., not slugs that were previously frozen). Euthanize slugs by cutting of their head. Dissect the head and remove the brain (light colored tissue that does not contain any plastids). This minimizes contamination by gut contents.

6 Use mortar + pestle + liquid nitrogen to grind the sample(s), either whole/partial slug or dissected brain tissue) to a powder

Remove mucopolysaccharides

7 Transfer pulverized sample to a 50 mL centrifuge tube with 10 mL preheated CTAB+BME buffer

8 Incubate at 60 °C for 00:30:00 30m

9 Add 10 mL of chloroform:isoamyalcohol. Mix well.

Safety information

Chloroform is a carcinogen.

ALL steps containing Chloroform should be performed in the chemical fume hood.

All 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 Centrifuge at 7700 x g for 00:10:00 10m

11 Transfer 2.5 mL aqueous phase to a 5 mL lo-bind microcentrifuge tube.

Precipitate nucleic acids

5m

12 Add 2/3 volume (1.7 mL) isopropanol and gently invert tube for 00:05:00 at room temperature. 5m

13 Centrifuge for 0 mL at 10000 x g . Discard th supernatant.

14 Add 300 µL of 70% ethanol to the DNA pellet. Use a pipette tip to dislodge the pellet from the wall of the microcentrifuge tube.

15 Centrifuge for 00:05:00 at 10000 x g . Discard the supernatant. 5m

16 Use a pipette to remove the remainder of teh supernatant from the tube. Be careful not to disrupt the pellet.

17 Gently lay the tube on its side on a sterile work surface with the cap open. Allow the tube to air dry in this position for 00:10:00 . 10m

Degrade RNA and extract DNA

18 Resuspend the pellet in 120 µL of TE buffer.

19 Add DNAase-free RNase A to a final concentration of 1 ug/mL. Incubate at 37 °C for 00:30:00 . 30m

20 Add 120 µL phenol:chloroform:isoamylalcohol.

Safety information

Chloroform is a carcinogen.

ALL steps containing Chloroform should be performed in the chemical fume hood.

All 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'.

21 Centrifuge for 00:10:00 at 10000 x g. Transfer 60 µL of the aqueous phase to a clean 1.5 mL lo-bind microcentrifuge tube. Discard the remainder. 10m

Safety information

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

Bead-purify DNA

22 Resuspend the ampure beads by quickly vortexing. Add 108 µL ampure bead solution to the aqueous phase sample. Mix gently by pipetting up and down 10 times using a wide-bore pipette tip.

23 Incubate at room temperature (20 °C) for 00:05:00 . 5m

24 Place tube in the magnetic stand until the solution becomes clear (approx. 00:02:00). 2m

25 With the tube still in the magnetic stand, carefully discard the clear supernatant

26 Keeping the tube in the magnetic stand, add 200 µL of 70% ethanol. Incubate for 00:00:30. Gently remove the clear supernatant, being careful not to disturb the beads. 30s

Repeat this wash step three times.

27 Ensure that the ethanol is completely removed and leave the tube top open to dry for 00:05:00. Do not 'overdry' (i.e., to the point that the bead streak is cracking) 5m

28 Remove the tube from the magnetic stand. Add 60 µL TE to dissolve the DNA.

29 Mix gently by pipetting up and down 10 times using a wide-bore pipette tip.

30 Incubate at room temperature (20 °C) for 00:05:00 5m

31 Place tube in the magnetic stand until the solution becomes clear (approx. 00:01:00). 1m

32 Transfer the clear DNA suspension to a new tube. Discard the old tube with the beads.

QC and cleanup

33 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.

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

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

36 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.*