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CTAB DNA Extraction Protocol for Mollusks

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

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Abstract

This is a comprehensive CTAB extraction protocol intended for DNA extraction of mollusks. It includes detailed methodology and several reagent preparation.

Protocol materials

- CTAB extraction solution **Teknova** **Catalog #**C2190
- Proteinase K
- Chloroform/Isoamyl Alcohol (24:1) **Acros Organics** **Catalog #**327155000
- Chloroform/Isoamyl Alcohol (24:1) **Acros Organics** **Catalog #**327155000
- RNase A
- Ethanol

Troubleshooting

Method

1 Slice tissue sample using a clean, sterile scalpel. Put sample into a 1.5 ml tube with 400 µL CTAB extraction solution **Teknova Catalog #**C2190 .

2 Add 10 µL Proteinase K [M] 20 mg/mL and invert tube to mix.

3 Incubate at 50 °C for 60 minutes until lysed (or longer depending on tissue thickness). Invert tube occasionally to mix sample.

4 Add 400 µL Chloroform/Isoamyl Alcohol (24:1) **Acros Organics Catalog #**327155000 . Gently shake tube to emulsify sample.

5 Leave at room temperature for 2 minutes and shake tube to mix.

6 Spin tube at 13 000 rpm for 2 minutes and transfer upper layer to a 1.5 ml tube.

7 Add 5 µL of RNase A [M] 10 mg/mL . Leave for 30 minutes at 37 °C .

8 Add 1 equal volume of Chloroform/Isoamyl Alcohol (24:1) **Acros Organics Catalog #**327155000 .

9 Spin tube at 13 000 rpm for 2 minutes and transfer upper layer to a new 1.5 ml tube containing 900 µL **CTAB dilution solution**. Gently invert tube to mix. Leave in fridge overnight **(optional)**.

10

Spin tube at 13 000 rpm for 10 minutes.

11

Discard supernatant with a micropipette and add 1000 µL **0.4 M NaCl in TE**. Invert tube to wash.

12

Spin tube at 13 000 rpm for 5 minutes and remove supernatant.

13

Add 300 µL **1.42 M NaCl in TE** to dissociate the CTAB from the DNA. Invert tube until pellet becomes transparent.

Note

May require gentle shaking at 37 °C

14

Add 600 µL Ethanol (previously left at -20 °C). Invert manually until precipitation appears.

Note

Leave precipitating overnight for a higher DNA concentration

15

Centrifuge at full speed for 8 minutes, then remove supernatant. Dry at room temperature or in heat block at 37 °C

16

Resuspend in 50 µL **EB buffer.** Use lesser volume of **EB buffer** if a higher concentration is required.

Notes

17 All centrifugations were performed using a benchtop centrifuge (Eppendorf - 5415 D)

18 The Nucleic acid quality is measured using a Nanodrop, and the A260/A230 ratio of 1.8-2.0 indicates good DNA purity. Qubit can be used to determine nucleic acid concentration.

19 Store DNA sample at -20°C until further use.

Reagent preparation

20 **CTAB Extraction Solution**

Note

11.69 g Sodium Chloride **Catalog #**PubChem CID: 5234
10 mL
Tris HCl Buffer 1M Solution, Sterile pH 7.5 **Bio Basic Inc. Catalog #**SD8124.SIZE.450ml
5 mL EDTA (0.5 M), pH 8.0 **Life Technologies Catalog #**AM9260G
3.33 g CTAB
Water to 100 mL

20.1 **CTAB Dilution Solution**

Note

10 mL
Tris HCl Buffer 1M Solution, Sterile pH 7.5 **Bio Basic Inc. Catalog** #SD8124.SIZE.450ml
4 mL EDTA (0.5 M), pH 8.0 **Life Technologies Catalog** #AM9260G
2 g CTAB
Water to 200 mL

20.2

0.4M NaCl in TE

Note

16 mL **5M** NaCl
2 mL
Tris HCl Buffer 1M Solution, Sterile pH 7.5 **Bio Basic Inc. Catalog** #SD8124.SIZE.450ml
400 µL EDTA (0.5 M), pH 8.0 **Life Technologies Catalog** #AM9260G
Water to 200 mL

20.3

1.42 M NaCl in TE

Note

24.8 mL **5M** NaCl
1 mL
Tris HCl Buffer 1M Solution, Sterile pH 7.5 **Bio Basic Inc. Catalog** #SD8124.SIZE.450ml
200 µL EDTA (0.5 M), pH 8.0 **Life Technologies Catalog** #AM9260G
Water to 100 mL