Oct 12, 2022

CTAB DNA Extraction Protocol for Mollusks

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dx.doi.org/10.17504/protocols.io.e6nvwjpr2lmk/v1

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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.

DOI

dx.doi.org/10.17504/protocols.io.e6nvwjpr2lmk/v1

PROTOCOL CITATION

Lavanya M Vythalingam, Tim Regan, Tim Bean, Carolina Peñaloza 2022. CTAB DNA Extraction Protocol for Mollusks. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwjpr2lmk/v1

CREATED

Sep 06, 2022

LAST MODIFIED

Oct 12, 2022

PROTOCOL INTEGER ID

69614

Method

1 Slice tissue sample using a clean, sterile scalpel. Put sample into a 1.5 ml tube with

CTAB extraction solution **Teknova Catalog #C2190** **400 µL** .

2 Add **10 µL** Proteinase K **Contributed by users** **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 **Contributed by users** **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

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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.

> May require gentle shaking at **37 °C**

14 Add **600 µL** Ethanol **Contributed by users** (previously left at **-20 °C**). Invert manually until precipitation appears.

> 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**

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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**

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

20.1 **CTAB Dilution Solution**

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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 **Contributed by users**

Water to **200 mL**

20.2

0.4M Nacl in TE

16 mL **5M** NaCl **Contributed by users**

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

24.8 mL **5M** NaCl **Contributed by users**

1 mL

Tris HCl Buffer 1M Solution, Sterile pH 7.5 **Bio Basic Inc. Catalog #SD8124.SIZE.450ml**

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200 µL

EDTA (0.5 M), pH 8.0 **Life Technologies Catalog #AM9260G**

Water to **100 mL**

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