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DNA extraction from colonial tunicates

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We use this protocol and it's working

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Abstract

This protocol has been successfully used with *Botrylloides diegensis* and was adapted to our needs based on the HotPhenol DNA extraction protocol.

Guidelines

Change gloves frequently, particularly as the protocol progresses from crude extracts to more purified materials. Use sterile tubes. Perform all steps on ice and use RNAse-free and DNase-free water unless otherwise stated.

Materials

Heat bath setup at 70 C (If working with fresh samples: glass beads 0.1mm and eppendorf thermal shaker)
phenol pH 8 (4 C)
Ph-Ch-IA: phenol:chloroform:isoamyl alcohol (25:24:1, best prepared fresh)
Ch-IA: chloroform:isoamyl alcohol (24:1)
SDS-Lysis buffer : 10mL Lysis buffer, 4mL SDS 10%
Lysis buffer 50mL: 12.3g 3M Sodium acetate (pH 5.2), 7.3g 0.5M EDTA , Nuclease-free water
12.3g 3M sodium acetate in 50mL Nuclease-free water
80% Ethanol
ultra pure water

Troubleshooting

1 This protocol was developed to extract both RNA and DNA in parallel (See **RNA extraction from colonial tunicates** , steps 1-7) using the same samples. However it could be run directly on fresh samples (steps 1.1-1.4).

1.1 Clean the slide from which you will take the colony of your interest. See **Cleaning colonial ascidians**.

1.2 Isolate a cleaned colony composed of approx. 20 zooids.

1.3 Transfer to a tube and spin at maximum speed for 00:02:00 . 2m

1.4 Remove the excess water.

Cell Lysis 4m 30s

2 Add 500 µL of Ph-Ch-IA solution and 350 µL of SDS-Lysis buffer.

3 Heat the tube at 70 °C for 00:02:00 . 2m

3.1 If working with fresh samples, add glass beads 0.1mm to the tube and shake on eppendorf thermal shaker at 900rpm.

4 Mix by vortexing at maximum speed for 00:00:45 . 45s

5 Cool for 00:01:00 on ice. 1m

6 Mix again by vortexing at maximum speed for 00:00:45 . 45s

7 Heat the tubes at 70 °C for 00:10:00 , mix regularly by inversion. 10m

7.1 If working with fresh samples, shake the tubes on eppendorf thermal shaker at 900rpm.

8 Mix again by vortexing at maximum speed for 00:00:45 . 45s

9 Cool for 00:01:00 on ice. 1m

10 Mix again by vortexing at maximum speed for 00:00:45 . 45s

DNA extraction 3m

11 Centrifuge at Room temperature at maximum speed for 00:03:00 . 3m

12 Transfer 400 µL of the upper aqueous phase to a new tube.

13 Add 400 µL of Ph-Cl-IA solution.

14 Shake the tube by inversion for 00:00:30 . 30s

15 Centrifuge at maximum speed for 00:03:00 . 3m

16 Transfer 300 µL of the upper aqueous phase to a new tube.

17 Add 300 µL of Ph-Cl-IA solution.

18 Shake the tube by inversion for 00:00:30 . 30s

19 Centrifuge at maximum speed for 00:03:00 . 3m

20 Transfer 200 µL of the upper aqueous phase to a new tube.

21 Add 200 µL of CI-IA solution.

22 Shake the tube by inversion for 00:00:30 . 30s

23 Centrifuge at maximum speed for 00:03:00 . 3m

24 Transfer aqueous phase to a new tube.

DNA precipitation 3h 30m

25 Add 2 volumes of [M] 100 % volume Ethanol (typically 300-400 µL).

26 Add 0.1 volume of [M] 3 Molarity (M) sodium acetate (typically 15-20 µL).

27 Mix by inversion.

28 Incubate at -20 °C for 03:00:00 . 3h

29 Centrifuge at maximum speed for 00:20:00 at 4 °C . 20m

30 Discard the supernatant.

31 Add 450 µL of cold [M] 80 % volume ethanol.

32 Centrifuge at maximum speed for 00:05:00 at Room temperature . 5m

33 Discard the supernatant.

34 Add 200 µL of cold [M] 80 % volume ethanol.

35 Centrifuge at maximum speed for 00:05:00 at Room temperature . 5m

36 Discard the supernatant.

37 Resuspend the pellet in ultra pure water (typically 20-100 µL).

38 Measure the DNA concentration using the NanoDrop.

39 Store at -20 °C for short storage or at -80 °C for long storage.