Jan 28, 2022

DNA extraction from colonial tunicates

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dx.doi.org/10.17504/protocols.io.b33sqqne

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

DOI

dx.doi.org/10.17504/protocols.io.b33sqqne

Marta Wawrzyniak, Simon Blanchoud 2022. DNA extraction from colonial tunicates. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b33sqqne

DNA extraction, colonial tunicates, ascidians

protocol ,

Jan 20, 2022

Jan 28, 2022

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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.

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

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.

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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.

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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 Cl-IA solution.

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

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

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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.

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34 Add **200 µL** of cold **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.

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