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DNA extraction from colonial tunicates

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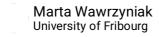
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This protocol has been successfully used with *Botrylloides diegensis* and was adapted to our needs based on the HotPhenol DNA extraction protocol.

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DNA extraction, colonial tunicates, ascidians

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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

- This protocol was developed to extract both RNA and DNA in parallel (See <u>RNA extraction from colonial tunicates</u>, 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

2m

1.4 Remove the excess water.

Cell Lysis 4m 30s

2 Add **3500 μL** of Ph-Ch-IA solution and **350 μL** of SDS-Lysis buffer.

3 Heat the tube at **§ 70 °C** for **© 00:02:00**.

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 ③ 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 s at 900rpm.	haker		
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 8 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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	23	Centrifuge at maximum speed for © 00:03:00 .	3m
	22	Shake the tube by inversion for $\circlearrowleft \mathbf{00:00:30}$.	30s
	21	Add ⊒200 µL of Cl-IA solution.	
	20	Transfer $\ \Box 200 \ \mu L$ of the upper aqueous phase to a new tube.	
	19	Centrifuge at maximum speed for \bigcirc 00:03:00 .	3m
	18	Shake the tube by inversion for \circlearrowleft 00:00:30 .	30s
	17	Add ⊒300 μL of Ph-Cl-IA solution.	
	16	Transfer ■300 µL of the upper aqueous phase to a new tube.	
	15	Centrifuge at maximum speed for $© 00:03:00$.	3m
	14	Shake the tube by inversion for $© 00:00:30$.	30s
	13	Add □400 µL of Ph-Cl-IA solution.	

24 Transfer aqueous phase to a new tube.

DNA precipitation 3h 30m 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 $\Box 15-20 \mu L$). 27 Mix by inversion. 3h 28 Incubate at 8-20 °C for (303:00:00). 20m 29 Centrifuge at maximum speed for **© 00:20:00** at **§ 4 °C**. 30 Discard the supernatant. 31 Add 450 µL of cold [M]80 % volume ethanol.

33 Discard the supernatant.

32

5m

Centrifuge at maximum speed for © 00:05:00 at & Room temperature .

- 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.
- Resuspend the pellet in ultra pure water (typically $\square 20-100 \ \mu L$).
- 38 Measure the DNA concentration using the NanoDrop.
- 39 Store at & -20 °C for short storage or at & -80 °C for long storage.