Feb 22, 2022

High Molecular Weight DNA extraction from tunicates

Marta Wawrzyniak[1], Simon Blanchoud[1]

[1]University of Fribourg

dx.doi.org/10.17504/protocols.io.b5axq2fn

Blanchoud lab, UNIFR

Marta Wawrzyniak
University of Fribourg

This protocol has been successfully used with *Botrylloides diegensis* and has been based on the following publication (with small changes):

https://febs.onlinelibrary.wiley.com/doi/pdf/10.1016/0014-5793(89)80446-6

DOI

dx.doi.org/10.17504/protocols.io.b5axq2fn

Marta Wawrzyniak, Simon Blanchoud 2022. High Molecular Weight DNA extraction from tunicates . **protocols.io** https://dx.doi.org/10.17504/protocols.io.b5axq2fn

high molecular weight DNA, tunicates, ascidians, DNA extraction

protocol ,

Feb 18, 2022

Feb 22, 2022

58423

from step 8 on, avoid vortexing or vigorous shaking; invert the tube gently to avoid breaking the DNA.

TE Buffer: 100 mM Tris-borate, pH 8.0 + 50 mM Na_2EDTA
14 % (w/v) SDS (sodium dodecyl sulfate)
10 mg/mL RNase
20 mg/mL Proteinase K
Phenol pH 8.0
Chloroform
1:1 (v/v) Phenol pH 8.0/Chloroform
Cold ethanol (-20 °C)
3M sodium acetate
RNase-free water

2mL tubes
sterile plastic pestle

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

2 Isolate a cleaned colony composed of approx. 20-30 zooids.

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

2.2 Remove the excess water.

3 Homogenize the sample in **500 µL** of TE buffer using a sterile plastic pestle.

4 Add **500 µL** of SDS and **4 µL** of RNase and mix by vortexing.

5 Incubate at **55 °C** for **00:10:00** . 10m

Citation: Marta Wawrzyniak, Simon Blanchoud High Molecular Weight DNA extraction from tunicates https://dx.doi.org/10.17504/protocols.io.b5axq2fn

6 Add **4 µL** of Proteinase K and mix by vortexing.

7 Incubate at **55 °C** for **00:15:00** . 15m

8 Add **1 mL** of phenol and mix well by inverting the tube until the phases are completely mixed.

9 Spin at **10000 rcf** for **00:05:00** and carefully transfer the **500 µL** of the upper phase to a new 2 mL tube. 5m

10 Add **500 µL** of (1:1 v/v) phenol/chloroform and mix well by inverting the tube.

11 Spin at **10000 rcf** for **00:05:00** and carefully collect the **300 µL** of upper phase to a new 2 mL tube. 5m

12 Precipitate the DNA by addition of **30 µL** 3 M sodium acetate and **600 µL** of ethanol. Mix gently by inverting the tube.

13 Spin at **10000 rcf** for **00:03:00** to pellet nucleic acids and carefully remove and discard supernatant. 3m

14 Wash in **1 mL** cold ethanol (**-20 °C**) and invert gently several times.

15 Spin at **10000 rcf** for **00:02:00** . 2m

Citation: Marta Wawrzyniak, Simon Blanchoud High Molecular Weight DNA extraction from tunicates
https://dx.doi.org/10.17504/protocols.io.b5axq2fn

16 Carefully remove and discard supernatant and place the tube up-side-down on a paper towel for **00:10:00** . 10m

17 Resuspend the pellet gently in RNase-free water at **37 °C** for **01:00:00** . 1h

18 Quantify the DNA concentration and quality.

19 Store at **-20 °C** or **-80 °C** (for longer storage).

Citation: Marta Wawrzyniak, Simon Blanchoud High Molecular Weight DNA extraction from tunicates
https://dx.doi.org/10.17504/protocols.io.b5axq2fn