

Feb 04, 2020

Isolation of high-molecular weight (HMW) DNA from *Diplonema papillatum* V.1Matus Valach¹¹Université de Montréal, Montreal, Quebec, Canada

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Works for me

dx.doi.org/10.17504/protocols.io.bb4uiqww



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ABSTRACT

A simple protocol for high-molecular weight (HMW) DNA extraction from *Diplonema papillatum*. The material was used to prepare a Nanopore library using a standard ligation kit (SQK-LSK109) for a subsequent Nanopore sequencing run with satisfactory results (at a ~14 Gbp throughput: read N50 ~ 40 kbp, ~2,000 reads >100 kbp, 10 reads >200 kbp). The procedure was devised based on previously published protocols by Quick (DOI: 10.17504/protocols.io.mrxc57n), Jain *et al.* (DOI: 10.1038/nbt.4060), and Denis *et al.* (DOI: 10.1038/protex.2018.076).

MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
Chloroform		
Ethanol		
Tris(hydroxymethyl)aminomethane	327360010	Thermo Fisher Scientific
Isoamylalcohol		
EDTA		
Sodium Chloride	PubChem CID: 5234	
N-lauryl sarcosine	L5125-50G	Sigma Aldrich
Isopropanol		
Spermidine	85558	Sigma Aldrich
Ammonium acetate	AR0032.SIZE.500g	Bio Basic Inc.
Polyvinylpyrrolidone	PVP40	Sigma – Aldrich
Phenol Saturated (pH 6.6/7.9 Liq.)	BP1750I	Fisher Scientific
RNase	10109134001	Roche
Proteinase K	P2308	Sigma Aldrich

1 Prepare the cell lysis buffer:

100 mM Tris-HCl pH8.0

100 mM EDTA pH8.0

1.15 % polyvinylpyrrolidone (PVP)

5 mM spermidine



If highly quality HMW DNA is required, prepare the buffer freshly before use.

2 Harvest ~150 mg wet weight cells (~3×10⁹ *Diplonema* cells). Homogenize the cells in 1.5 mL (10 volumes) of the cell lysis buffer.



Up-and-down pipetting using a large-bore tip is preferable. Do not vortex to avoid damaging/breaking the DNA. Being a marine organism, *Diplonema* is sensitive to low salt conditions, so its cells start to lyse even before the addition of the detergent in the next step and the DNA is not protected in the cells anymore.



If working with a lower cell quantity, downscaling to keep the cells:buffer ratio at 1:10 is preferable (high DNA concentration facilitates DNA precipitation).

3 Add N-lauryl sarcosine (sarcosyl) to a final concentration of 0.5% and mix thoroughly, but gently. The solution should become very viscous.



SDS can be used instead of sarcosyl, but RNase digestion may not be as efficient.

4 Add 40 µL of RNase (5 mg/mL) and mix thoroughly, but gently (by inverting the tube or up-and-down pipetting using a large-bore tip). Incubate for 15–30 min at 37 °C with occasional gentle mixing by inverting the tube.

5 Add 100 µL proteinase K (10 mg/mL) and mix thoroughly, but gently (by inverting the tube or up-and-down pipetting using a large-bore tip). Incubate for 30–60 min at 50 °C with occasional gentle mixing by inverting the tube.

6 Add an equal volume of the Phenol:Chloroform:Isoamylalcohol (25:24:1) mixture. Mix well by inverting the tube until the suspension becomes white and less viscous than originally. Centrifuge (4,000 × g, 4 °C, 10 min) to extract the DNA into the aqueous phase.



For optimal DNA purity, use isoamylalcohol, even though is not a necessary component for the phenol/chloroform extraction to work.

- 7 Transfer the aqueous phase into a new tube using a large-bore tip. Add an equal volume of the Phenol:Chloroform:Isoamylalcohol (25:24:1) mixture. Mix well by inverting the tube several times and centrifuge ($4,000 \times g$, 4°C , 10 min) to re-extract the DNA into the aqueous phase.



If high quantity of HMW DNA has been extracted, the aqueous phase should be rather viscous. The re-extraction improves the purity of the HMW DNA. From this point on, use only large-bore tips (e.g., cut off the end of a tip to obtain 1.5–2 mm diameter at the very end of the tip).

- 8 Transfer the aqueous phase into a new tube. Add 2 volumes of a solution of 0.5 M ammonium acetate in pure ethanol. Mix thoroughly, but gently by inverting the tube. If the HMW DNA concentration is sufficient, a white filamentous precipitate will quickly start to form.



Alternatively, add a sodium chloride solution to a final concentration of 0.4 M, mix well, and then add an equal volume of isopropanol. Mix well by inverting the tube to initiate the formation of the precipitate.

- 9 Prepare a new tube filled with 0.5–1 mL of 80% ethanol. Use a glass hook/capillary to take out the filamentous precipitate and transfer it to the new tube.



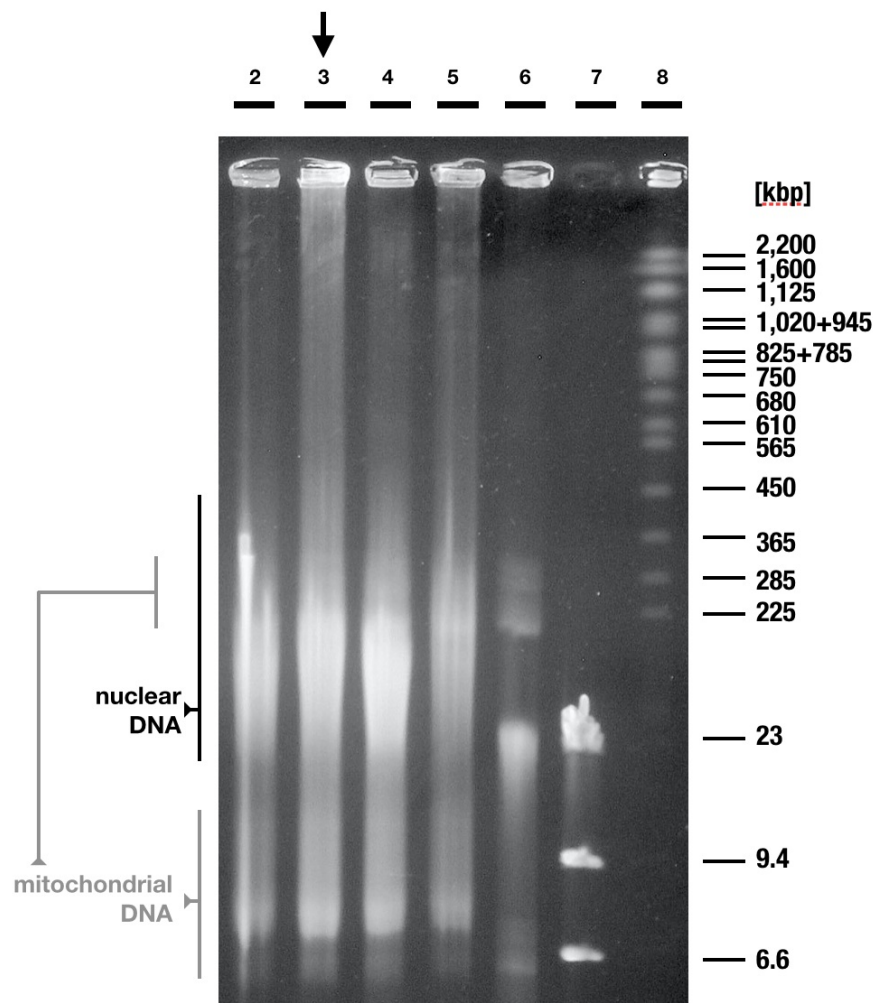
Using a glass tool is strongly recommended, because the precipitate will detach easily once in the 80% ethanol. In contrast, it strongly adsorbs to plastic (as well as to metal to a lesser extent).

- 10 Mix by gently inverting the tube several times and let the precipitate drop to the bottom. Carefully remove as much liquid as possible without touching the precipitate using a fine tip.
- 11 Add 0.5–1 mL of 80% ethanol, mix by gently inverting the tube several times. Once the precipitate has dropped to the bottom, carefully remove as much liquid as possible without touching the precipitate using a fine tip. Leave the tube open at room temperature until all remaining liquid has evaporated.
- 12 Add 30–40 μL of 10 mM Tris-HCl pH8.5 (or of the Qiagen Elution buffer) and let slowly hydrate and solubilize overnight at 4°C .
- 13 Resuspend the DNA by gently pipetting up and down the viscous solution several times using a large-bore tip. To spectroscopically measure the concentration and purity of the HMW DNA, dilute an aliquot 10 to 20 \times in the same resuspension buffer.




The concentration of the diluted sample should be 0.5–1 $\mu\text{g}/\mu\text{L}$, $A_{260}/A_{280} \sim 1.8\text{--}1.9$, and $A_{260}/A_{230} \sim 2.0\text{--}2.2$.)

14 Examples of the DNA isolated by the described procedure:



PFGE separation of HMW DNA samples extracted from *Diplonema papillatum*. **2**, the procedure as described, but using SDS instead of sarcosyl for the cell lysis; **3**, the procedure as described, Nanopore-sequenced sample; **4**, the procedure as described, but precipitation using NaCl+isopropanol; **5**, the procedure as described, half the starting amount of cells; **6**, DNA purified using a Qiagen gDNA column; **7**, λ DNA/HindIII; **8**, *S. cerevisiae* chromosomes (PFGE marker, BioRad).

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