

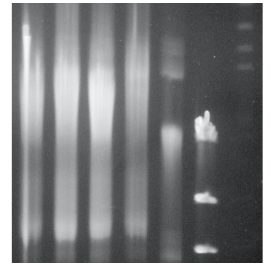
Feb 04, 2020

Version 2

🌐 Isolation of high-molecular weight (HMW) DNA from *Diplonema papillatum* V.2

DOI

dx.doi.org/10.17504/protocols.io.bb5eiq3e



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Protocol Citation: Matus Valach 2020. Isolation of high-molecular weight (HMW) DNA from *Diplonema papillatum*. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bb5eiq3e>

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Protocol status: Working

We use this protocol and it's working

Created: February 04, 2020

Last Modified: February 04, 2020

Protocol Integer ID: 32646


Keywords: high-molecular weight (HMW) DNA, Nanopore, protist, Diplonema, DNA extraction, dna extraction from diplonema papillatum, dna from diplonema papillatum, dna extraction, nanopore library, subsequent nanopore, diplonema papillatum, nanopore, dna, extraction,

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

MATERIALS

 Chloroform

 Ethanol

 Tris(hydroxymethyl)aminomethane **Thermo Fisher Scientific Catalog #327360010**

 Isoamylalcohol

 EDTA

 Sodium Chloride **Catalog #PubChem CID: 5234**

 N-lauryl sarcosine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L5125-50G**

 Isopropanol

 Spermidine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #85558**

 Ammonium acetate **Bio Basic Inc. Catalog #AR0032.SIZE.500g**

 Polyvinylpyrrolidone **Merck MilliporeSigma (Sigma-Aldrich) Catalog #PVP40**

 Phenol Saturated (pH 6.6/7.9 Liq.) **Fisher Scientific Catalog #BP1750I**

 RNase **Roche Catalog #10109134001**

 Proteinase K **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P2308**

Troubleshooting

1 Prepare the cell lysis buffer:

100 mM Tris- HCl pH8. 0
100 mM EDT A pH8. 0
1.15 % poly vinyl pyrr olido ne (PVP)
5 mM sper midi ne

Note

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

2 Harvest ~150 mg wet weight cells (~ 3×10^9 *Diplonema* cells). Homogenize the cells in 1.5 mL (10 volumes) of the cell lysis buffer.

Note

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.

Note

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.

Note

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

- 4 Add 40 μ L 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 μ L 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 \times g, 4 °C, 10 min) to extract the DNA into the aqueous phase.

Note

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.

Note

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 pre-chilled tube. Add 2 volumes of an ice-cold ethanol solution of 0.5 M ammonium acetate. Mix thoroughly, but gently by inverting the tube. If the HMW DNA concentration is sufficient, a white filamentous precipitate will quickly start to form.

Note

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 pre-chilled 80% ethanol (0–4 °C). Use a glass hook/capillary to take out the filamentous precipitate and transfer it to the new tube.

Note

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 pre-chilled 80% ethanol (0–4 °C), 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.

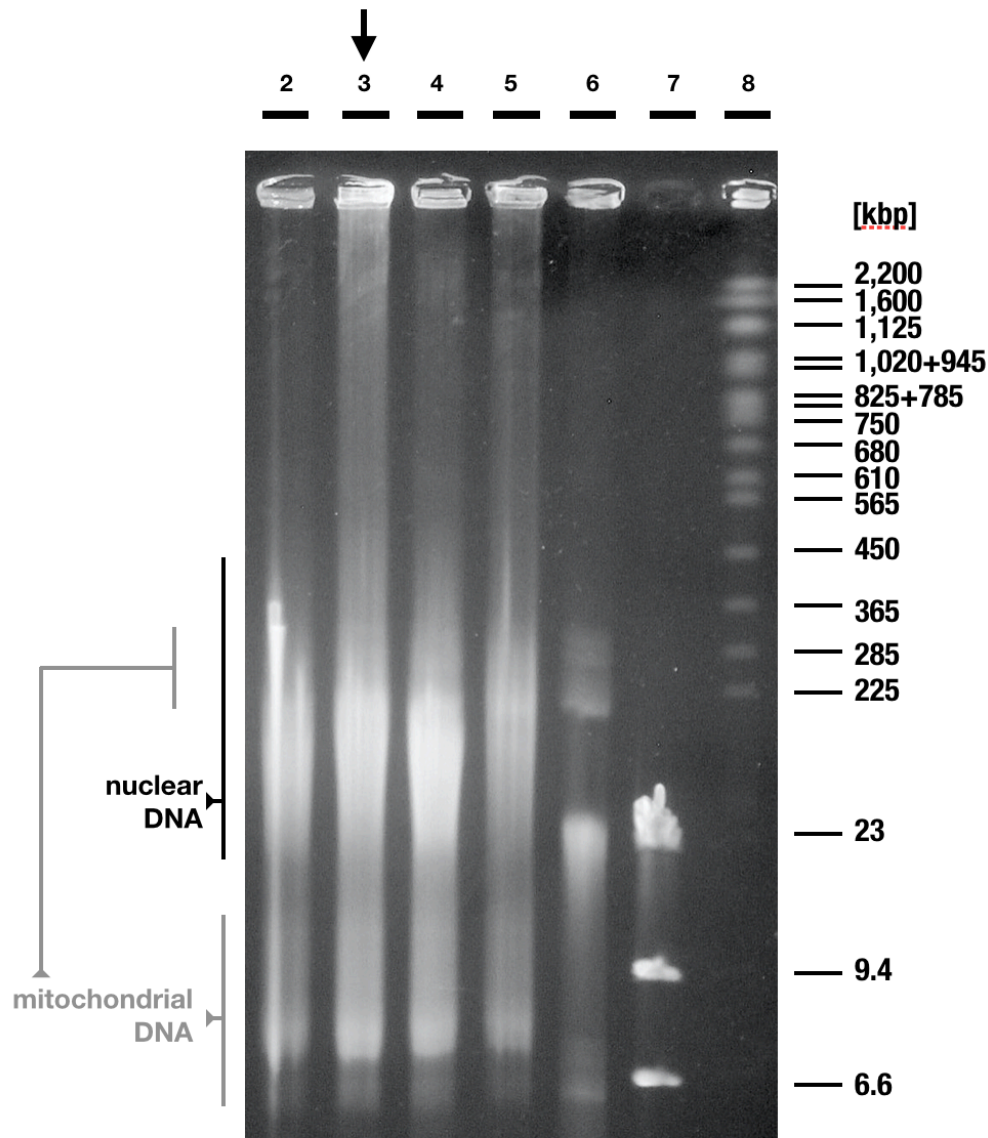


- 13 Resuspend the DNA by gently pipetting up and down the viscous solution several times using a large-bore tip. To spectroscopically measure the concentration and purity of the HMW DNA, dilute an aliquot 10 to 20× in the same resuspension buffer.

Note

The concentration of the diluted sample should be 0.5–1 µg/µL, A260/A280 ~ 1.8–1.9, and A260/A230 ~ 2.0–2.2.

- 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 (step #3); **3**, the procedure as described, Nanopore-sequenced sample; **4**, the procedure as described, but precipitation using NaCl+isopropanol (step #8); **5**, the procedure as described, half the starting amount of cells (i.e., 20 volumes of the cell lysis buffer added; step #2); **6**, DNA purified using a Qiagen gDNA column; **7**, λ DNA/HindIII; **8**, *S. cerevisiae* chromosomes (PFGE marker, BioRad).