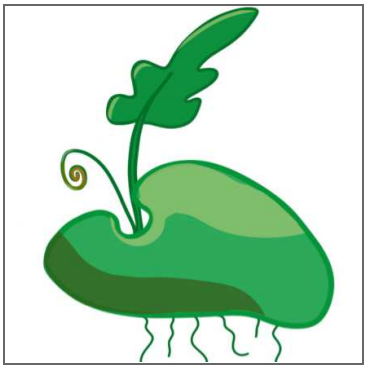

DEC 08, 2023

DOI:
dx.doi.org/10.17504/protocols.io.bp2l69kb1lqe/v1

Protocol Citation: Geferson Fernando Metz, Rafael Plá Matielo Lemos, Cristiane Barbosa D'Oliveira Matielo, Tiego Ferreira, Filipe Victoria 2023. HMW DNA extraction protocol for ferns. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l69kb1lqe/v1

MANUSCRIPT CITATION: Geferson Fernando Metz, Tiego Ferreira, Cristiane Barbosa D'Oliveira Matielo, Rafael Plá Matielo Lemos, Filipe de Carvalho Victoria. HMW DNA extraction protocol for ferns. protocols.io

HMW DNA extraction protocol for ferns

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ABSTRACT

Among the difficulties encountered in a laboratory, simple situations such as efficiently disinfecting fern spores and extracting large amounts of DNA with low tissue input in the protocol are one of the major impediments in carrying out sequencing of these plants in the axenic state. The objective of this work is to present a replicable, scalable and easy-to-execute protocol for work with ferns following the current generation of long-read sequencing. Our method is based on providing a disinfection protocol for spores and sporangia that guarantees growth free of contamination and, after this growth, DNA extraction using a low amount of material in order to obtain a good yield. The results are promising since, in up to 21 days, we obtained germinated plants. After their growth (in average 180 days) we were able to extract DNA in quantity and quality and perform the sequencing, emphasizing that our best N50 is 24 Kb.

IMAGE ATTRIBUTION

Illustration by Crix D'Oliveira.

Protocol status: Working
We use this protocol and it's working

Created: Aug 25, 2022

Last Modified: Dec 08, 2023

PROTOCOL integer ID: 69196

Keywords: Ferns, Nanopore, HMW, long reads, Plant DNA

Funders Acknowledgement:
Auxílio a Pesquisa GENO-ISLAND: Adaptações moleculares das plantas aos ambientes insulares
Grant ID: CNPQ 443237/2019-0
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil
Grant ID: CAPES 001

MATERIALS

- Liquid nitrogen
- Chloroform: Isoamyl Alcohol (24:1)
- Isopropanol
- TE Buffer
- 3M sodium acetate
- Ethanol 100%
- nuclease free water
- Sodium Hypochlorite Solution
- 70% Ethanol

β-mercaptoethanol
PVP 40
TRIS 1 M pH 8.0
EDTA 0.5 M pH 8
Sodium Metabisulfite
NaCl 2.5M
Sodium dodecyl sulfate (SDS) 20%
2 ml LoBind tubes
1.5 ml LoBind tubes
Mortar & pestle
Water Bath
Centrifuge

PROTOCOL MATERIALS

- nuclease free water — Materials, Step 26
- 3M sodium acetate — Materials, Step 20
- Sodium Hypochlorite Solution — Materials, Step 6
- Liquid nitrogen — Materials, Step 10
- Ethanol 100% — Materials, Step 21
- 70% Ethanol — In Materials and 2 steps
- Chloroform: Isoamyl Alcohol (24:1) — Materials, Step 15
- TE Buffer — Materials, Step 20
- Isopropanol — Materials, Step 18

SAFETY WARNINGS

Safety information

Work under fume hood when add β-mercaptoethanol and Chloroform.

Safety information

Be careful when handling liquid nitrogen.

BEFORE START INSTRUCTIONS

- Prepare the SDS Lysis Buffer on the day of the experiment;

	A	B	C
	Reagent	Reagent Stock Concentration	FINAL
	PVP 40	100%	1%
	Sodium Metabisulfite	1%	1%
	NaCl	5 M	0,5 M
	TRIS HCL pH 8	1 M	100 mM
	EDTA pH 8	0.5 M	50 mM
	DDH2O	-	~
	Sodium dodecyl sulfate (SDS)	20%	1,5%
	β-MERCAPTOETANOL	-	2% (v/v)

- When preparing the SDS Lysis Buffer, add SDS at last this will avoiding bubble formation.

- Preheat the water bath; Keep the SDS Lysis Buffer at 65°C until the tissue powder is added.

- Washing solution (EtOH 70%), fresh

35 ml Ethanol 100% + 15 ml H2O

- Potassium Acetate 5M

Dissolve 4.9 gr KAc in 10 ml ddH2O (4.9 gr KAc + ≈ 7.5 ml H2O).
Adjust the pH with glacial acetic acid.

- Prepare TE buffer (10 mM Tris pH 8 and 1 mM EDTA pH 8)

Plant material sterilization

3d 0h 45m

1

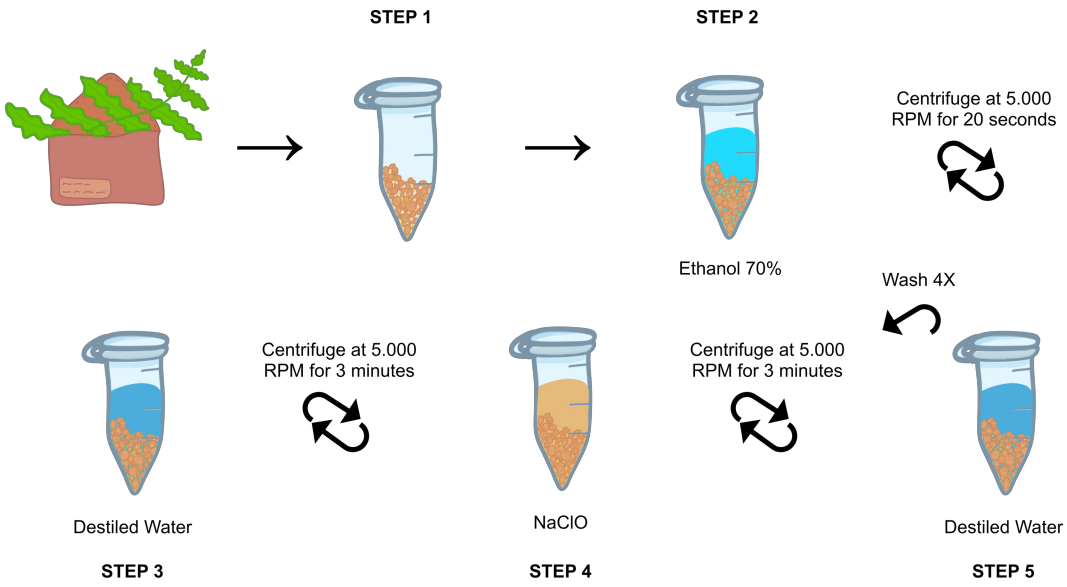

Figure 1. Step by step plant spore and sporangia sterilization. Illustration by Crix D'Oliveira.

2 Sample and store the leaf tissue (fronds) in paper envelopes for three days to induce dehiscence.

3 Recover and store the spores together with sporangia in 1.5 mL microtubes until one-third of the tube was filled and then stored at -20 °C until disinfection.

4 Add 1 mL 70% Ethanol **Contributed by users**, homogenize by inversion for 00:00:20 20s and briefly centrifuge them.

5 Discard the supernatant and wash by inversion with 1 mL of autoclaved purified water, briefly centrifuge and discard the supernatant. 2m

6 Add 1 mL of an Sodium Hypochlorite Solution **Contributed by users** (active chlorine ~2% 23m

[M] 10 % (v/v) , homogenize by inversion for 00:20:00 , centrifuge at 5.000 rpm 00:03:00 .

7 Discard the supernatant and wash by inversion with 1 mL of autoclaved purified water, centrifug 3m at 5 5.000 rpm 00:03:00 . Repeat this step 4 times.

8 Add 1 mL autoclaved purified water, homogenize and pipete into a Petri Dish with BCD medium 1m (MgS04.7H2O - 0.1 mM, KH2PO4 - 1.84 mM, KNO3 - 1M, FeSO4.7H2O - 4.5mM).

Extraction of high-molecular-weight DNA

2h 31m

9

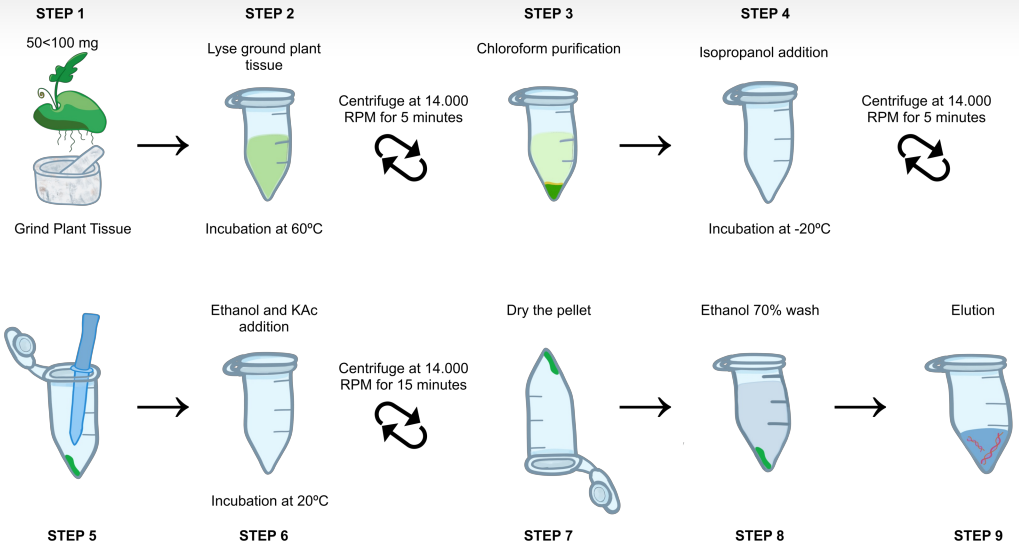

Figure 2. Step by step extraction of high-molecular-weight DNA illustrated protocol. Illustration by Crix D'Oliveira.

10 Weigh between 50 mg and 100 mg of leaf tissue. Grind must be done with a crucible and pe 7m (previously exposed to Liquid nitrogen **Contributed by users**) until a very fine powder is obtained.

11 Pre-heat the Lysis Buffer and aliquot 600 µL for each sample individually in 1.5 mL microtube. Wi 1m the aid of a spoon or spatula, transfer the macerate to the microtube.

12 Homogenize by inversion 10 times and incubate at 60 °C for 00:10:00. 10m

13 Add 4 µL of RNase A (100 mg/mL); add 4 µL of proteinase K (> 40 U/mg);

14 Homogenize by inversion 10 times and incubate at 60 °C for 00:20:00 gently homogenizing by inversion every 00:05:00. 25m

15 Add 600 µL of Chloroform: Isoamyl Alcohol (24:1) **Contributed by users** and homogenize by inversion for at least 00:03:00 until an off-white emulsion forms. 3m

16 Centrifuge at 14.000 rpm for 00:05:00 at room temperature. 5m

17 Carefully aspirate the upper phase of the tube and transfer to a new 1.5 mL microtube. 1m

18 Add 400 µL of Isopropanol **Contributed by users** -20 °C and incubate it for at least 01:00:00 at -20 °C. (Can be stored overnight) 1h

19 Centrifuge at 14.000 rpm for 00:05:00 at room temperature and discard the supernatant. 5m

20 Add 50 µL of TE Buffer Contributed by users. Add 5 µL of 3M sodium acetate Contributed by users. 1m

21 Add 120 µL of iced Ethanol 100% Contributed by users (-20 °C) and mix well by flicking the tube.

22 Store tubes at -20 °C for at least 00:20:00. 20m

23 Centrifuge at 14.000 rpm for 00:15:00 at room temperature and carefully discard the supernatant. 15m

24 Add 1 mL of freshly prepared 70% Ethanol Contributed by users, mix gently by inversion, centrifuge briefly and carefully discard the supernatant. 1m

25 50 µL Dry the pellet for approximately 00:10:00 at Room Temperature on the bench with th tube upside down. 10m

26 Elute in 50 µL volume in nuclease free water Contributed by users. (Elution volume can be adjusted for your needs) 1m

Quality Assessment

27 Quantify the DNA on a Qubit® fluorometer (dsDNA high sensitivity assay). DNA yield can be 500 - 1500 ng.

28 Check DNA integrity though electrophoresis in a 1'% Agarose Gel.

DNA Size Selection

29 Remove short DNA fragments with Circulomics® Short-Read Eliminator Kit.