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HMW DNA extraction protocol for ferns V.1

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

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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.

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

- Sodium Hypochlorite Solution
- Liquid nitrogen
- Chloroform: Isoamyl Alcohol (24:1)
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- 3M sodium acetate
- 70% Ethanol

Safety warnings

Safety information

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

Safety information

Be careful when handling liquid nitrogen.

Before start

- 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

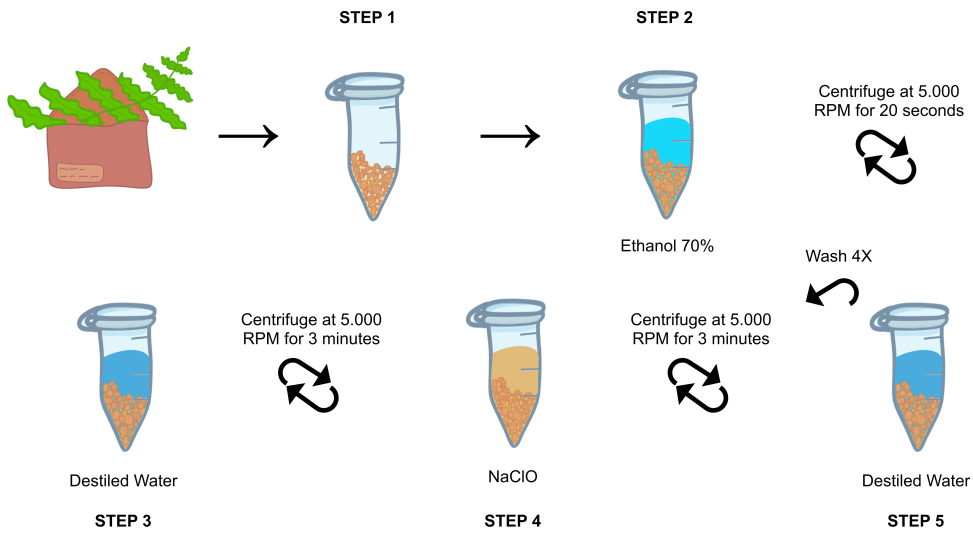

Figure 1. Step by step plant spore and sporangia sterilization. Illustration by Crix D'Oliveira.

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, homogenize by inversion for 00:00:20 and briefly centrifuge them. 20s

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 (active chlorine ~2%) at [M] 10 % (v/v), homogenize by inversion for 00:20:00, centrifuge at 5.000 rpm 00:03:00. 23m

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

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

Extraction of high-molecular-weight DNA 2h 31m

9

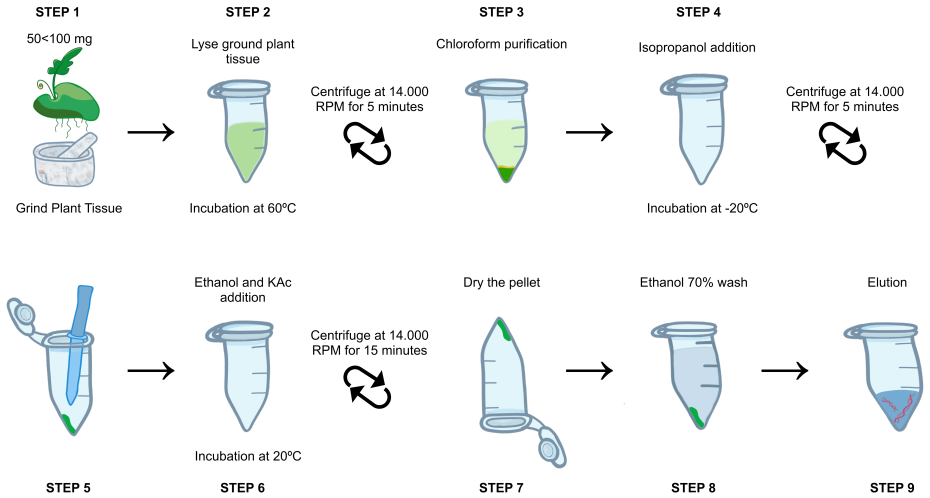

Figure 2. Step by step extraction of high-molecular-weight DNA illustrated protocol. Illustration by Crix D'Oliveira.

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

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

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) 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 -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 . Add 5 µL of 3M sodium acetate . 1m

21 Add 120 µL of iced Ethanol 100% (-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 , 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 the tube upside down. 10m

26 Elute in 50 µL volume in nuclease free water. (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.