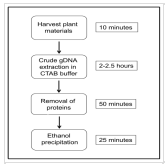

May 02, 2021

High-Molecular-Weight gDNA extraction protocol from moss gametophytes

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1 Works for me dx.doi.org/10.17504/protocols.io.bud2ns8e

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ABSTRACT

The purpose of this protocol is to extract high-molecular-weight genomic DNA from gametophyte tissue of the moss *Physcomitrella patens*, which contains lots of phenolic compounds. This protocol was modified from a CTAB method for DNA isolation from moss protonemal tissue (Ano et al., 2012). gDNA isolated using this modified CTAB protocol was pure, comparable to gDNA quality extracted from commercial extraction kits. Besides, the yield (69 µg/g FW) was relatively high. The high-quality gDNA is useful for PCR and Southern blotting for the detection of genome editing.

ATTACHMENTS

da28bjkff.pdf

DOI

dx.doi.org/10.17504/protocols.io.bud2ns8e

PROTOCOL CITATION

Lei Zhu 2021. High-Molecular-Weight gDNA extraction protocol from moss gametophytes. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bud2ns8e

KEYWORDS

high-molecular-weight, genomic DNA extraction, Physcomitrella patens

CREATED

Apr 21, 2021

LAST MODIFIED

May 02, 2021

OWNERSHIP HISTORY

Apr 21, 2021 Urmilas

Apr 27, 2021 Lei Zhu University of California, Riverside

PROTOCOL INTEGER ID

49306

GUIDELINES

This protocol was designed for the isolation of high quality genomic DNA from moss gametophyte tissue. It might be useful for the extraction of gDNA from other plant species or tissues.

MATERIALS TEXT

Reagents:

- 2x CTAB buffer: 2% CTAB (Hexadecetyltrimethylamonium Bromide), [M]**1.4 Molarity (M)** NaCl, [M]**100 Milimolar (mM)** Tris-HCl pH 8.0, [M]**20 Milimolar (mM)** EDTA.

OPTIONAL: 1% PVP-40 can be added to 2x CTAB buffer for removing phenolic compounds in DNA extraction.

- 10% CTAB: 10% CTAB, [M]**0.7 Molarity (M)** NaCl.
- Chloroform:isoamyl alcohol (24:1)
- Tris-saturated Phenol (pH > 7.5)
- Phenol:chloroform (25:24)
- 100% Ethanol
- 70% Ethanol
- [M]**10 mg/ml** RNase A
- [M]**3 Molarity (M)** Sodium Acetate (NaOAc, pH 5.2)
- TE: [M]**10 Milimolar (mM)** Tris-HCl, 1mM EDTA, pH 8.0

Materials:

3-week-old moss gametophytes, **2 g** - **5 g** of fresh weight (FW).

Equipment:

- Mortar and pestle
- Liquid nitrogen
- **50 mL** conical tube (sterile, DNase free)
- Eppendorf tubes, **1.5 mL** (sterile, DNase free)
- Desktop centrifuge for **50 mL** conical tubes
- Microcentrifuge
- Water bath
- Incubator

SAFETY WARNINGS

1. Be careful when handling liquid nitrogen. Avoid splash.
2. Wear thermal gloves and safety glasses when grinding tissue.

BEFORE STARTING

1. Prepare reagents as listed.
2. Fill liquid nitrogen in a small thermal tank.
3. Pre-chill mortar and pestle with liquid nitrogen.
4. Pre-heat 2x CTAB buffer at **65 °C** .
5. Set water bath to **65 °C** .
6. Set incubator to **37 °C** .

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Harvest Plant Materials

1 Harvest 3-week-old moss gametophyte tissue.

2 Carefully remove rhizoids from leafy gametophytes.

> IMPORTANT: Rhizoids mixed in the green leafy tissue will cause pigment co-precipitated with DNA. Be careful to remove rhizoids from leafy gametophytes.

3 Pat dry leafy gametophytes with clean towel papers.

4 Weigh **2 g** – **5 g** fresh weight of leafy gametophytes.

5 Grind leafy gametophytes to fine powder in liquid nitrogen using the mortar and pestle.

> IMPORTANT: Grinding moss leafy gametophytes to fine powder is very important. If the powder is coarse, the efficiency of extraction will be much lower than expected.

6 Transfer the ground powder to a **50 mL** conical tube.

7 Put the conical tube in a **-80 °C** freezer with cap open until all liquid nitrogen evaporate.

Crude Extraction in CTAB Buffer 2h 35m

8 

Add **10 mL** pre-heated 2x CTAB buffer to the **50 mL** conical tube that contains ground gametophyte powder.

9 

Close the cap and shake the tube vigorously to mix CTAB buffer and gametophyte powder.

10  45m

Incubate the conical tube in **65 °C** water bath for **00:45:00** .

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11  10m

Shake the tube to mix buffer and gametophyte powder every **00:10:00** .

12 Take the conical tube out of the water bath and cool down to **Room temperature** .

13 

Add **10 mL** chloroform: isoamyl alcohol to the conical tube. Mix well.

14 Then put the tube to a rotator for **00:20:00** at **Room temperature** . 20m

15  5m

Centrifuge at **1000 x g** for **00:05:00** at **Room temperature** to separate phases.

16 

Transfer the upper supernatant to a new 50 mL conical tube. Add **1 mL** 10% CTAB and **10 mL** chloroform: isoamyl alcohol to the new tube. Shake well.

17  5m

Incubate the conical tube at **Room temperature** for **00:05:00** .

18  5m

Centrifuge the conical tube at **1000 x g** for **00:05:00** to separate phases.

19 

Transfer the upper aqueous supernatant to a new conical tube. Add **10 mL** isopropanol and mix well.

20 Incubate the conical tube **On ice** for **00:10:00** . 10m

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21 5m

Centrifuge the conical tube at **1000 x g** for **00:05:00** to precipitate the pellet.

22 Decant the supernatant. Air dry the pellet.

23 Dissolve the pellet in **4 mL** TE buffer.

Tap the pellet gently until it dissolves completely.

24 5m

Centrifuge the tube at **1000 x g** for **00:05:00** .

25

Transfer the supernatant to a new **50 mL** tube. Discard pellets.

26 Add **400 µl** **3 Molarity (M)** sodium acetate and **8 mL** 100% pre-chilled ethanol to the new tube. Mix well.

27 20m

Incubate the tube **On ice** for **00:20:00** .

28 5m

Centrifuge the tube at **1000 x g** for **00:05:00** to precipitate the pellet.

29

Rinse the pellet with 70% ethanol. Centrifuge the tube and discard the supernatant.

30 Air dry the pellet. Dissolve the pellet in **700 µl** TE. Transfer DNA in TE to a new **1.5 mL** Eppendorf tube.

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Tap the tube gently. Wait until the pellet is completely dissolved. DNA can be stored at this step for overnight at **4 °C** .

31 

Add RNase A into DNA solution at a final concentration of **50 µg/ml** . Mix well.

32 

30m

Incubate the Eppendorf tube at **37 °C** for **00:30:00** .

Removal of protein 11m

33 

Add **700 µl** Tris-saturated Phenol. Mix thoroughly.

34 

5m

Centrifuge at **16.000 rcf** for **00:05:00** at **Room temperature** to separate phases.

35 Transfer the upper phase to a new **1.5 mL** Eppendorf tube. Leave white stuff in the middle layer between two phases. Discard the middle and lower phases.

36 Repeat steps 33-35 to extract DNA in phenol one more time.

37 

After transferring the upper supernatant to a new tube, add an equal volume of phenol: chloroform to the new tube. Mix thoroughly.

38 

3m

Centrifuge the Eppendorf tube at **16.000 rcf** for **00:03:00** at **Room temperature** to separate phases.

39 

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Transfer the upper phase to a new Eppendorf tube. Discard the pellet.

40 Add **700 µl** chloroform: isoamyl alcohol to the new tube. Mix thoroughly.

41  3m

Centrifuge the tube at **16.000 rcf** for **00:03:00** at **Room temperature** to separate phases.

42 Transfer the aqueous supernatant to a new **1.5 mL** Eppendorf tube.

43 Repeat steps 40-42 once to remove the phenol residue.

Precipitation of Genomic DNA 20m

44 

Add 1/10 volume of **3 Molarity (M)** sodium acetate and 2x volume of pre-chilled 100% ethanol to the supernatant. Mix well.

45  10m

Incubate the tube **On ice** for **00:10:00** .

46  5m

Centrifuge the tube at **16.000 rcf** for **00:05:00** to collet the pellet. Discard the supernatant.

47 Add **1 mL** 70% ethanol to wash the pellet. Finger tap the tube to mix.

48  5m

Centrifuge the tube at **16.000 rcf** for **00:05:00** . Discard the ethanol.

Pellet may not be attached tightly to the bottom of the Eppendorf tube. Be careful to pour off the supernatant.

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49 Air-dry the pellet by leaving the Eppendorf tube in the fume hood. Wait until ethanol residue evaporated, but not over-dry the pellet.

50 Dissolve the pellet in **100 µl** TE. Tap to dissolve genomic DNA.

51 Store extracted genomic DNA in the fridge until use.

Additional Notes:

1. The entire procedure takes about 4 hours.
2. The yield and quality of gDNA from four preps are listed below. FW, fresh weight.

Table 1. The yield and the quality of genomic DNA.

A	B	C	D	E
Gametophyte Tissue	Extraction Method	Yield (µg/g FW)	OD 260/280	OD 260/230
WT	This method	69.21± 8.43	1.95 ± 0.03	2.21 ± 0.12
WT	NucleoSpin PlantDNA Extraction Kit	5.81± 0.70	1.99 ± 0.02	2.20 ± 0.05

The yield and OD number were calculated from 4 extractions. WT, wild type.

3.

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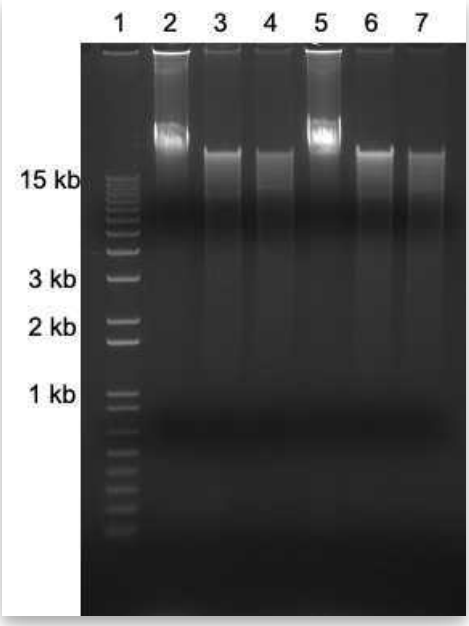

Figure 1. The integrity of the extracted DNA. Genomic DNA isolated from WT (lane 2) and the mutant (lane 5) were in high quality. WT DNA digested with BsaI (lane 3) and ScaI (lane 4), together with mutant DNA digested with BsaI (lane 6) and ScaI (lane 7), suggesting that the isolated genomic DNA were free of endonuclease inhibitors. Lane 1, Invitrogen 1 kb plus DNA ladder. WT, wild type.

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