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Hornwort DNA extraction

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ABSTRACT

The gametophytic tissue of hornworts is rich in polysaccharides (Renzaglia et al., 2009) and it also seems to be rich in polyphenolics. Both compounds pose a problem for DNA.

A modified CTAB protocol was found to be optimal for hornwort genomic DNA extraction (adapted from Porebski et al. 1997). This protocol uses polyvinyl pyrrolidone to remove polyphenolics and contains an extra ethanol precipitation step with a relatively high NaCl concentration compared standard DNA extraction protocols. At NaCl concentrations higher than 0.5 M,polysaccharides remain in solution and they do not co-precipitate with DNA.

MATERIALS TEXT

Extraction buffer:

100mM Tris-HCL pH 8 (2M)	25ml
1.4M NaCl (5M)	140ml
20mM EDTA pH 8 (0.5M)	20ml
2% CTAB	10gr
0.3% β-mercaptoethanol	150µl/50ml

1 Grind 0.5-2 g of tissue using mortar and pestle in the presence of liquid nitrogen until finely ground. Transfer frozen ground tissue to a 30 ml tube.

2 Add 10 ml of 60 ^{o}C extraction buffer and 100 mg PVP-40/g tissue (5µl of RNAse A (100mg/ml)). Mix by inversion and incubate in a water bath in 60 ^{o}C for 30 min.

3 Add 12 ml of chloroform:IAA (24:1) and mix by inversion to form an emulsion.

4 After mixing thoroughly, spin at 10,000 rpm for 10 min at RT.

5 Transfer aqueous phase to a new 30 ml centrifuge tube.

6 Repeat chloroform:IAA extraction to remove cloudiness (PVP) in aqueous phase. Spin at 7,000 rpm for 10 min at RT.

7 Add ½ volume of 5M NaCl to the final aqueous solution recovered. Mix well. Add two volumes of cold (-20 ^{O}C) 95% ethanol. Mix by inversion. Place in freezer (-20 ^{O}C) for 10 min to accentuate precipitation. Spin at 13,000 rpm for 15 min.

8 (optional) Resuspend in 2 ml of TE and repeat step 7.

9 Pour off supernatant and wash pellet with cold (0 to 4 ^{O}C) 70% v/v ethanol. Spin at 13,000 rpm for 5 min, pour off supernatant and dry pellet for 5 min.

10 Dissolve in 50 µl TE (preferably o/n at 4 ^{O}C). Transfer to a 1.5 ml tube.