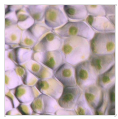

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Hornwort RNA extraction

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ABSTRACT

The same protocol used for DNA extraction is used for RNA extraction with the addition of an overnight RNA precipitation step with LiCl.

MATERIALS

NAME	CATALOG #	VENDOR
Polyvinylpyrrolidone	PVP40	Sigma – Aldrich
Chloroform	319988	Sigma

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

SAFETY WARNINGS

All solutions used during this protocol were prepared using DEPC water!!

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. Mix by inversion and incubate in 60 ^{o}C in a water bath for 30 min (or incubate on ice for 15 min).

3 Remove from heat, and let cool to room temperature for 4 to 6 min.

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

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

6 Transfer aqueous phase to a new 30 ml centrifuge tube. 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 6 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. Dry pellet.

10 Dissolve in 400 µl TE and add 100ul 8M LiCl. Incubate o/n at 4 ^{O}C.

11 Spin 10 min to pellet RNA . Remove supernatant (contains DNA).

12 Dissolve pellet with 400ul DEPC dH2O. Add 40ul NaoAc (pH 4.5) and 1ml 95% EtOH and freeze at -80 oC for 10 min. (optional: wash pellet with 95% EtOH).

13 Spin for 10 min at 13K and resuspend pellet in 40ul DEPC dH_2O.

14 If necessary (pellet not dissolved): incubate at 60 ^{O}C for 10 min and then on ice for 30 min. Pellet debris by spinning tube for 10 min at 13K. Transfer supernatant (RNA) to a new tube.Extraction buffer:100mM Tris-HCL pH 8 (2M) 25ml.