Seed RNA Extraction Protocol

Required Solutions and Preparation

Extraction Buffer (EB)
1M Tris-HCl pH9.0
1% SDS
autoclave

PCI
Phenol: Chloroform: Isoamyl alcohol (25:24:1)
The 24:1 chloroform: isoamyl alcohol can be prepared in advance and stored. PCI should be prepared fresh by mixing 1 part of TE-saturated phenol to 1 part of the 24:1 chloroform: Isoamyl alcohol solution. This can be stored up to 1 week at room temp in the dark.

Also prepare:

- 3M NaOAc pH5.6 (autoclaved)
- 4M LiCl (autoclaved)
- 2M LiCl (autoclaved)
- 100% and 70% EtOH
- mortar and pestles
- 13 eppen tubes/sample
- ddH_2O (autoclaved)

Protocol

1. After harvesting, siliques should be immediately frozen in liquid N_2. Samples are stored at -80°C until the extraction
2. Place all solutions, eppen etc on ice to cool. Set centrifuge to 4°C. All steps are performed on ice and all centrifuge steps are at 4°C and 10,000 rpm
3. All supernatant removal should also be carried out with a fine pippette. We use either a 200 µl yellow pippette tip attached to a pasteur pippette for this, or a fine pasteur pippette made using a

bunsen burner

4. Add 300µl of EB and 300 µl of PCI to an eppen tube on ice
5. Cool the mortar & pestle with liquid N_2, add 10 seed pods (or 0.05g of 8 DAF siliques) per sample and homogenise *very well*, adding N_2 as necessary to keep sample frozen
6. Once fully ground, use small spoon to transfer sample to the eppen tube with the buffer
7. vortex for 3 min and divide the sample into 2 eppen tubes
8. Centrifuge for 1 min and collect sup into new eppen
9. Add an equal volume of PCI and vortex
10. Centrifuge for 5 min and collect sup into new eppen
11. Centrifuge again for 5 min and collect sup into new eppen
12. Add 0.1 vol 3M NaOAc and 3 vol 100% EtOH, invert 4 times to mix
13. Place at -80°C (dry ice) for 10 min
14. Centrifuge for 5 min, discard sup and vacuum dry pellet
15. Dissolve pellet with 100µl ddH_2O (may take time)
16. Centrifuge for 10 min and collect sup into new eppen
17. Add equal volume of 4M LiCl, invert 4 times to mix
18. Place on ice on 4°C cold room overnight or -80°C (dry ice) for 1 hr

NEXT DAY

19. Centrifuge for 15 min, discard sup. Small pellet should be visible, be careful as it moves easily
20. Add 1 mL of 2M LiCl and carefully invert once to mix
21. Centrifuge for 2 min and discard sup
22. Add 1 mL of 70% EtOH and invert once to wash
23. Centrifuge for 2 min, discard sup and vacuum dry
24. Dissolve pellet in 5 to 10µl ddH_2O
25. Combine the 2 solutions/sample into one eppen
26. Centrifuge for 5 min and collect sup into new tube
27. Store at -30°C or lower
28. Expected RNA amounts: 4DAF, 1-2µg; 6DAF, 2-3µg; 8DAF, 6-8µg; 10DAF, 8-10µg; 12DAF, 6-8µg; 14DAF, 3-4µg

Reference

Adapted from:

Naito S., Hirai M.Y., Chino M. and Komeda Y. (1994). Expression of a soybean (*Glycine max* [L.] Merr.) seed storage protein gene in transgenic *Arabidopsis thaliana* and its response to nutritional stress and to abscisic acid mutations. *Plant Physiol.* **104:** 497-503.