

Chapter 3.3

RNA ISOLATION USING PHASE EXTRACTION AND LiCl PRECIPITATION

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The extraction of good quality RNA from plant tissues is a critical step in gene expression studies. We have developed a protocol for RNA isolation from Lotus japonicus roots and leaves that yields RNA that can be successfully used in downstream applications such as reverse transcription-polymerase chain reaction (RT-PCR). The use of an adequate extraction buffer is crucial to obtaining good quality RNA, especially when working with roots. The composition of the extraction buffer has been optimized to avoid contamination with phenolic compounds, which are very abundant in Lotus roots and can inhibit downstream enzymatic reaction such as reverse transcription. In our laboratory, this method yields highly purified RNA suitable for cDNA synthesis and is routinely used for gene expression studies in L. japonicus roots. For Northern blots, dirtier (i.e. phenolic contamination), higher yielding extraction methods might be suitable as well.

PROCEDURE

1. Warm 10 mL of extraction buffer to 65°C in a water bath (do not forget to add β -mercaptoethanol before use).
2. Grind plant tissue in liquid nitrogen and transfer the frozen powder to an eppendorf tube (the volume of powder should not exceed 200 μ L).
3. Add 600 μ L of extraction buffer and 600 μ L of phenol: chloroform: isoamylalcohol (25:24:1 pH 8.0). Mix by inverting the tube and shaking for 5-10 min at 55°C, then separate phases at room temperature by centrifugation 10 min (>10.000 rpm).
4. Collect upper phase and extract again with phenol: chloroform: isoamylalcohol.
5. Take upper phase and add LiCl to a final concentration of 2M. The RNA is precipitated overnight on ice and harvested by centrifugation at max speed 10 min at 4°C.

A.J. Márquez (Editorial Director). 2005. *Lotus japonicus* Handbook. pp. 123-124.
<http://www.springer.com/life+sci/plant+sciences/book/978-1-4020-3734-4>

6. Discard the supernatant, wash the pellet in cold 2M LiCl, spin, and wash again twice in cold 80% ethanol.
7. Dry the pellet (but not over dry) and resuspend it in RNA-Secure-Resuspension Solution (Ambion).
8. Measure the RNA concentration. Ratio A_{260}/A_{280} should be ~2. Optionally check RNA integrity on an agarose formaldehyde gel.
9. If necessary for downstream application perform DNase I treatment 30 min at 37°C (20 U of DNase I per 2 µg of RNA).
10. Inactivate DNaseI by adding 0.2 volumes of DNase Inactivation Reagent (Ambion) for 2 min at room temperature. Centrifuge at 13,200 rpm 1 min and use the supernatant for first strand cDNA synthesis.

EXTRACTION BUFFER

- 2% CTAB (hexadecyltrimethylammonium bromide)
- 2% PVP (polyvinylpyrrolidone 360.000)
- 100 mM Tris-HCl pH 8.0
- 25 mM EDTA
- 2 M NaCl

(Stir with moderate heating, autoclave)

- 2% β-mercaptoethanol (add just before use)