# RNA Isolation from *Arabidopsis* Pollen Grains

Yongxian Lu

Carnegie Institution for Science, Stanford University, Stanford, CA, USA

**[Abstract]** This purpose of this experiment is to isolate high quality RNA from pollen grains, which lays the foundation for further studies, like gene expression analysis and cDNA cloning. We describe a simple and robust method to isolate RNA from Arabidopsis pollen grains.

## Materials and Reagents

1. Mannitol (Thermo Fisher Scientific/ VWR International)
2. TRIzol reagent (Life Technologies, Invitrogen™)
3. Chloroform (Thermo Fisher Scientific)
4. 75% ethanol
5. Liquid nitrogen
6. RNase-free water
7. Isopropyl alcohol

## Equipment

1. Ceramic mortar and pestles
2. Nanodrop (Thermo Fisher Scientific)
3. Centrifuges (Eppendorf)
4. Vortexer (VWR International)
5. Fume hood
6. 500 ml flask
7. 50 ml falcon tubes
8. 100 µm nylon mesh

## Procedure

A. Pollen collection:

To collect mature pollen grains, stage 13 flowers (Sanders *et al*., 1999) should be used.

1. Collect flowers and put into a 500 ml flask.
2. Add 300 ml ice-cold 0.3 M mannitol.

3. Hand-shake the flask vigorously for 2 min.
4. Filter the pollen suspension through 100 µm nylon mesh.
5. Collect pollen by centrifugation using 50 ml falcon tubes. (450 *x g*, 5 min, 4 °C). Repeat this step until all the pollen suspension is finished.
6. Transfer pollen pellet into a 1.5 ml centrifuge tube. You can stop here by storing pollen at -80 °C, or proceed to the RNA isolation steps.

B. Homogenization:

7. Put into liquid $N_2$.
8. Homogenize pollen with mortar and pestles. Try to be as quick as possible at this step.
9. Add TRIzol reagent (1 ml reagent/ 50-100 mg tissue, the sample volume should not exceed 10% of the volume of TRIzol used for homogenization, as suggested by the TRIzol protocol provided by the manufacturer).

C. Phase separation:

10. Incubate the homogenized samples for 5 min at RT to permit the complete dissociation of nucleoprotein complexes.
11. Add 0.2 ml of chloroform per 1 ml of TRIzol reagent under fume hood. Cap sample tubes securely.
12. Shake tubes vigorously by hand for 15 sec and incubate them at RT for 2 to 3 min.
13. Centrifuge the samples at no more than 12,000 *x g* for 15 min at 4 °C.
14. Transfer the upper aqueous phase to a fresh tube.

D. RNA precipitation:

15. Precipitate the RNA from the aqueous phase by mixing with isopropyl alcohol (Use 0.5 ml of isopropyl alcohol per 1 ml of TRIzol reagent used for the initial homogenization).
16. Incubate samples at RT for 10 min.
17. Centrifuge at no more than 12,000 *x g* for 10 min at 4 °C.
18. Discard the supernatant.

E. RNA wash:

19. Wash the RNA pellet with 75% ethanol (use at least 1 ml of 75% ethanol per 1 ml of TRIzol reagent used for the initial homogenization).
20. Mix the sample by vortexing.
21. Centrifuge at no more than 7,500 *x g* for 5 min at 4 °C.
22. Discard the supernatant. Now you get the RNA pellet at the tube bottom.

F. Dissolving RNA:

23. Briefly dry the RNA pellet on bench at RT (10-20 min).
24. Dissolve RNA in RNase-free water by passing the solution in a few times through a pipette tip.
25. Incubate at 55 to 60 °C for 10 min. Tap the tube several times during the incubation.
26. Use Nanodrop to test the quantity and quality of the RNA.
27. Store the RNA sample in -80 °C for future use.

## References


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