RNA Extraction from RNase-Rich Senescing Leaf Samples

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[Abstract] Isolation of intact, full-length high quality RNAs is essential for RNA sequencing, reverse transcription PCR analysis of gene expression as well as RNA gel blot analysis. This simple yet easy protocol is developed to meet this need; in addition to regular samples, this protocol is especially good for isolating RNAs from RNase-rich samples such as senescing leaves and ripening fruits (from which RNAs isolated using standard method are generally degraded to certain degree). The total RNA yield varies from 900 μg total RNA/g non-senescing leaves to 200 μg total RNA/g senescent leaves.

Materials and Reagents

1. Guanidinium thiocyanate (Thermo Fisher Scientific, catalog number: BP221)
2. NaCitrate
3. N-Lauroylsarcosine (Sigma-Aldrich, catalog number: L9150)
4. β-mercaptoethanol (β-ME)
5. Glacial HAc
6. NaOH
7. Phenol (J.T.Baker®, catalog number: 2859)
8. Ethylenediaminetetraacetic acid (EDTA)
9. Sodium dodecyl sulfate (SDS) (Sigma-Aldrich, catalog number: L5750)

10. Tris (hydroxymethyl) aminomethane
11. Chloroform
12. Isopropanol
13. Diethylpyrocarbonate (DEPC)
14. Ethanol
15. Extraction buffer (EB) (see Recipes)
16. 2 M NaAcetate (pH 4.0) (see Recipes)
17. 2 M NaAcetate (pH 5.0) (DEPC treated) (see Recipes)
18. H_2O-saturated phenol (no buffer required) (see Recipes)
19. Citrate-EDTA-SDS solution (CES) (see Recipes)
20. Tris-EDTA-SDS solution (TES) (see Recipes)
21. 100 ml 0.75 M NaCitrate (see Recipes)
22. 60 ml 10% Sarkosyl (see Recipes)

Equipment

1. Vortex Mixer
2. Beckman high speed centrifuge with JS13.1 rotor or the like
3. 15-ml Corning centrifuge tube

Procedure

1. 0.2-0.5 g sample tissue ground in liquid N_2 (better grinding ensures a higher RNA yield).
2. Add to 15-ml Corning centrifuge tube containing 5 ml EB (2.5 ml EB if sample is less than 0.2 g), vortex 2 min at room temperature.
3. Add 0.5 ml 2 M NaAcetate (pH 4.0) to the tube, vortex 1 min at room temperature.
4. Add 5 ml H_2O-saturated phenol, vortex 1 min at room temperature.

5. Add 1 ml chloroform, vortex 1 min at room temperature.
6. Spin 10 min at 10,000 *x g*, 4 °C (8,000 rpm, JS13.1 rotor), transfer aqueous phase to a new 15-ml Corning centrifuge tube.
7. Add equal vol. isopropanol to the new tube, screw cap and mix by inverting several times, keep the tube at -20 °C for >1 h.
8. Spin 10 min at 3,000 *x g*, 4 °C (4,500 rpm JS13.1), discard supernatant.
9. Add 2 ml CES or TES to redissolve pellet.
10. Add 2 ml chloroform, vortex 1 min at room temperature.
11. Spin 10 min at 3,000 *x g*, 4 °C, transfer aqueous to new tube.
12. Add 1/10 vol. 2 M NaAcetate (pH 5.0), equal vol. isopropanol, pellet by spinning 10 min at 3,000 *x g*, 4 °C.
13. Wash the pellet with 75% and 100% ethanol, respectively, air dry the pellet, and dissolve the pellet with 50-200 µl DEPC-treated CES or TES (for RT PCR or other analyses involving enzymes, DEPC-treated CE or TE without SDS should be used). The total RNA yield from senescent leaves is low, so a reduced final volume is suggested.

Recipes

1. EB

	in 100 ml	in 300 ml
4 M Guanidinium thiocyanate (MW 118.2)	47.28 g	141.84 g
25 mM NaCitrate (pH 7.0) (MW 294.10)	3.33 ml of 0.75 M	10 ml of 0.75 M
0.5% Sarkosyl (N-Lauroylsarcosine, 293.4)	5 ml of 10% Soln	15 ml of 10%
0.1 M β-ME (in 5 ml EB, add 35 µl conc)	53.8 ml H_2O	

2. 2 M NaAcetate (pH 4.0) (MW 82.03)

   11.49 ml glacial HAc (17.4 M)

   70 ml H_2O

Titrate w/ NaOH to pH 4.0 (this pH value is essential)

ddH_2O to 100 ml

3. 2 M NaAcetate (pH 5.0) (MW 82.03) (DEPC treated)

   11.49 ml glacial HAc (17.4 M)

   70 ml H_2O

   Titrate w/ NaOH to pH 5.0

   ddH_2O to 100 ml

4. H_2O-saturated phenol (no buffer required)

5. Citrate-EDTA-SDS solution (CES) for 100 ml

   10 mM NaCitrate(pH 7.0) 1.333 ml 0.75 M NaCitrate (pH 7.0)

   (DEPC) 1 mM EDTA (pH 8.0) 200 µl 0.5 M EDTA

   0.5% SDS 2 ml 25% SDS

6. Tris-EDTA-SDS solution (TES)

   10 mM Tris (pH 7.5)

   1 mM EDTA (pH 8.0)

   0.5% SDS [DEPC]

7. 100 ml 0.75 M NaCitrate

   15.761 g Citric acid (MW 210.14)

   80 ml ddH_2O

   Titrate w/ NaOH to pH 7.0

   ddH_2O to 100 ml

8. 60 ml 10% Sarkosyl

   6 g N-lauroylsarcosine (MW 293.4)

   60 ml ddH_2O

   65 °C stir

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References

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