

TRIS-LiCl RNA extraction procedure for Grape

Adapted by Tattersall et al (2005) Am J Enol Vitic 56:400-406 from: Wang SX, Hunter W, Plant A. 2000. "Isolation and Purification of Functional Total RNA from Woody branches and Needles of Sitka and White Spruce" Biotechniques 28:292-296.

All solutions treated with 0.1% DEPC (vol/vol) except those with TRIS.

All glass baked. Reusable plastic-ware treated with 5% bleach, rinsed in depc-water and autoclaved.

Solutions needed:

Homogenization Buffer

Final Volume: 500 ml

Amount (g)	Compound	mol/L	g/L
12.11	200 mM Tris base-pH with HCl to 8.5) or 50 ml of 2M solution	0.200	24.22
7.5	1.5% (wt/vol) lithium dodecylsulfate		15
6.358	300 mM LiCl	0.300	12.717
1.861	10 mM sodium EDTA	0.010	3.72
5	1% (wt/vol) sodium deoxycholate (AKA cholic acid)		10g
5 ml	1% (vol/vol) NP-40 (AKA tergitol NP-40)		10 ml

Autoclave the buffer.

Remove 50 ml aliquot for each 3-4 g sample to be processed.

Immediately before use add for ea 50 ml: (amounts of first three may be doubled)

150 ml

double

The 150 ml double will do six 25 ml extractions. For 12, double these amounts.

ie. ATA (0.2532 g), DTT (0.9252 g), thiourea (0.228 g), and PVPP (6.0 g)

0.0211	1 mM aurintricarboxylic acid (0.0422g)	0.001	0.422	0.1266 g
0.0771	10 mM dithiothreitol (AKA DTT) (0.1542g)	0.010	1.5425	0.4626 g
0.019	5 mM thiourea (0.038)	0.005	0.3806	0.114 g
1	2% (wt/vol) PVPP		20 g	

TE Buffer

Final volume: 250 ml

Amount (g)	Compound	mol/L	g/L
0.394	10 mM Tris (pH with HCl) (pH 7.5)	0.01	1.576
0.093	1 mM EDTA	0.001	

Other solutions/chemicals

3.3 M sodium acetate

Ethanol (absolute)

Isopropanol

10 M LiCl

5 M Potassium acetate (not pH adjusted)

DEPC treated H₂O

Procedure:

Day 1

1. Grind 3 g leaf tissue (or 8-9 g berry) in liquid nitrogen cooled mortar. Grind to fine powder. Put tissue powder in 50 ml falcon tube. **Separated 1.5 grams tissue for the extraction into a 50 ml Falcon tube**
2. **Slowly** add five volumes (50 ml) Homogenization buffer to tissue in tube. Invert and vortex till fully mixed. **Added 25 mls of homogenization buffer to each tube with a 25 ml pipette**
3. Transfer homogenate to 250 ml BEAKER and allow to freeze slowly at -80 for at least 2 hours. (freeze at -20°C for one hour, then transfer to -80) (may leave at -80 overnight or longer). **Mixed in the Falcon tube by vortex and shaking, then placed directly into the -80 freezer**

Day 2

4. Transfer to a 37°C water bath until thawed. **Approx 15 minutes**
5. Transfer to two 35 or 50 ml Oakridge tubes. Balance. **Used a 30 ml ungraduated tube for each sample and balanced with Wang buffer.**
6. Centrifuge $5000\times g$ 20 min. at 4°C (5750 rpm; 13.1 rotor in Beckman JT2/21ME). Transfer supernatant to clean tubes (35 ml conical is good), (if needed, filtering it through 1 or 2 layers of miracloth that has been autoclaved.) **May pour off supernatant and do not need miracloth if the pellet is firm.**
7. Mix supernatant with 1/30 volume 3.3 M sodium acetate (0.106 M final conc.)
 $21\text{ mls}/30 = 0.7\text{ mls sodium acetate}$
8. Add 100% EtOH until 10% (vol/vol) (= 1/9 volume). Invert to mix.
 $21\text{ mls}/9 = 2.3\text{ mls ETOH}$
9. Incubate on ice 10 min.
10. Centrifuge at $5000\times g$ 20 min. 4°C . Transfer supernatant to clean 50 ml polypropylene tubes.
Must use a pipette to transfer. Take care to leave all of the polysaccharide pellet in the tube. Measure the volume transferred.
11. Add 1/9 volume 3.3 M sodium acetate (0.33 M final conc)
 $22\text{ mls}/9 = 2.44\text{ mls sodium acetate}$
12. Add isopropanol to conc. of 33% (vol/vol) (= 1/2 volume)
 $22\text{ mls}/2 = 11\text{ mls isopropanol}$
13. Incubate -20°C 2 h (may leave overnight)

Day 3

14. Centrifuge 5000x g 30 min. 4°C
15. Discard supernatant, re-suspend pellet in 3 ml TE Buffer (prechilled)
16. Incubate on ice 30 min.
17. Centrifuge 5000x g 30 min. 4°C. Transfer supernatant to clean 14 ml tubes. **Use a pipette to measure the volume.** May recombine samples at this point, in which case, double the volumes for succeeding steps—doubled amounts are in parentheses.
18. Retain supernatant, mix with 1/4 volume 10 M LiCl (2.5 M final conc.)
3 mls/4 = 0.75 mls LiCl
19. Incubate overnight **on ice** in cold room or refrigerator

Day4

20. Centrifuge 10000x g 30 min. 4°C (8000 rpm; 13.1 rotor)
21. Discard supernatant, re-suspend pellet in 1.5 ml **pre-chilled** TE buffer.
(vortex and pipette) (3 ml)
Supernatant may be poured off. Use a pipettor to resuspend the pellet.
22. Add 1.5 volumes 5 M potassium acetate (not pH adjusted) and vortex to mix.
1.5 mls X 1.5 = 2.25 mls potassium acetate
23. Incubate 3 h on ice.
24. Centrifuge 10000x g 30 min. 4°C
25. Re-suspend pellet in 1 ml **pre-chilled** TE buffer (can incubate on ice 1 h) (2 ml)
26. Add 1ml Phenol:chloroform:isoamyl (25:24:1), vortex. Spin 10,000xg 15 min. 15°C.
Remove upper phase to new tubes. If upper phase very small, back extract.
Need at least 0.8 ml. If less, add another ml of TE and vortex and spin again.
Follow with an equal volume of chloroform:isoamyl (24:1), vortex, spin as above.
Remove upper phase to new tubes.
27. Mix with 1/9 volume 3.3 M sodium acetate and 2 volumes EtOH
1000µl/9 = 111µl sodium acetate
1 ml X 2 = 2 ml EtOH
28. Incubate -20°C for a minimum of 2 hours (can leave overnight)

Day5

29. Centrifuge 10000x g 30 min. 4°C. Pellet is RNA
30. Wash pellet with 500 µl (1 ml) absolute EtOH (do not re-suspend). **Use chilled EtOH.**
Keep pellets on ice.
31. Centrifuge 10000x g 10 min. **If pellet does not dislodge, this step is not necessary.**
32. Remove EtOH. Air dry pellet (about an hour) and re-suspend in 500 µl (1 ml) DEPC treated H₂O. Cover tubes with tissue to keep dust out while drying. Pellet will be hard to see.
Remove excess EtOH from above the pellet with a pipettor as the pellets are drying.
33. Place in 65°C water bath for 8 minutes if needed to re-suspend pellet completely.
Transfer to Eppendorf. **Probably will resuspend without this step.**

Steps 1-2 take	1-2 hrs	Day1	Day1	Day1
Step 3	2hrs +			
Steps 4-12	2 hrs	Day2		Day2
Step 13	2 hrs +			
Steps 14-18	2-2.5 hrs	Day3		
Step 19	O/N			
Steps 20-28	7 hrs	Day4	Day2	Day3
Step 29	2 hrs +			
Steps 30-34	2 hrs	Day5		Day4