RNA isolation protocols

I obtained these protocols from Shu-Hsing Wu

I. TRIzol Method

This is a modification of the procedure originally described by Chomczynski P and Sacchi N. 1987. Signal-Step Method of RNA Isolation by Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction. *Analytical Biochemistry* 162: 156-159

Materials and Reagents

TRIzol Reagent (Commercially available from many venders, components see below)
0.8 M sodium citrate / 1.2 M NaCl
Isopropanol (2-Propanol)
Chloroform
DEPC-Water
75% ethanol prepared with DEPC-Water
RNase Inhibitor (e.g., aseERASE TM BIO 101 Cat. 2601-104)
50 mL sterile plastic screw-cap centrifuge tubes

1. Grind 1g tissue in liquid nitrogen in a mortar and pestle.
2. Transfer powdered tissue to a 50 mL sterile plastic screw-cap centrifuge tube containing 15 ml TRIzol reagent. Incubate samples at room temperature or at 60°C for 5 min.
3. Homogenize tissue with homogenizer for 15 seconds. Repeat once.
4. Centrifuge samples at 12,000 x g at 4°C for 10 min.
5. Transfer supernatant into new sterile 50 mL sterile plastic screw-cap centrifuge tube. Discard pellet.
6. Add 3 mL chloroform to each tube in hood. Shake tubes vigorously with vortex for 15 sec.
7. Let tubes sit at room temp 2-3 min. Centrifuge tubes at 10,000 x g at 4°C for 15 min.
8. Carefully pipet aqueous phase into a clean screw-cap centrifuge tube; discard interphase and lower phase into waste.
9. Precipitate RNA by adding isopropanol and 0.8 M sodium citrate/1.2 M NaCl, half volume of the aqueous phase each. Cover tube and mix by gentle inversion. Let sit at room temperature for 10 min.
10. Centrifuge tubes at 10,000 x g at 4°C for 10 min. Discard supernatant.
11. Wash pellet with 20 ml of 75% ethanol. Vortex briefly.
12. Centrifuge at 10,000 x g at 4°C for 10 min. Discard supernatant; briefly dry pellet on kimwipe.
13. Add 100-250 μL DEPC-Water, to pellet. Resuspend RNA by pipetting up and down a few times.
14. Add 1 μL RNase inhibitor aseERASE to a 250 μL RNA sample. If having problems resuspending the RNA pellet, we suggest incubation at 55 - 60°C for 10 min.
15. Transfer sample to microcentrifuge tube at room temperature.
16. Spin samples at high speed in microcentrifuge tube for 5 min at room temperature (to pellet the material that would not resuspend).
17. Transfer RNA solution (supernatant) to a new tube. Determine RNA concentration and quality by spectrophotometry.

Note: For optimal spectrophotometric measurements, RNA aliquots should be diluted with water or buffer with a basic pH. Water with pH< 7.5 falsely decreases the 260/280 ratio.

TRIzol Reagent (Commercially available from many venders)

Home-made recipe for 1 L:

Reagents	Final Concentration
Phenol in saturated buffer 380 mL	38%
Guanidine thiocyanate 118.16 g	0.8 M
Ammonium thiocyanate 76.12 g	0.4 M
Sodium acetate, pH 5.0 33.4 mL of 3 M stock	0.1 M
Glycerol 50 mL	5%
DEPC-Water Adjust the final volume to 1 L	

II. Pine Tree Method

This method was originally described by Chang S., Puryear J., Cairney J. (1993) A Simple and Efficient Method for Isolating RNA from Pine Trees. *Plant Molecular Biology Reporter* 11: 113-116.

Materials and Reagents*

♠**Extraction buffer** (For RNA extraction Pine Tree Method)

	For 100ml solution:
2% CTAB (hexadecyltrimethylammonium bromide)	2g CTAB
2% PVP (polyvinylpyrrolidone K 30)	2g PVP
100 mM Tris-HCl pH 8.0	10ml 1M Tris
25 mM EDTA	5ml 0.5M EDTA
2.0 M NaCl	40ml 5M NaCl
0.5 g/L spermidine	1ml stock (100x)
	Add DEPC-H_2O to 100ml

Mix well (may need overnight stirring) and autoclave

2% beta-mercaptoethanol **(add just before use)**

♠Chloroform:isoamyl alcohol (24:1)

♠10 M Lithium chloride

1. Warm 5 mL extraction buffer to 65°C in a water bath, quickly add 1g ground tissue and mix by inverting the tube and vortexing.
2. Extract two times with an equal volume of chloroform:isoamyl alcohol, separating phases at room temperature by centrifugation for 10 min at 12,000 x g. Centrifuge longer if phases are not well separated.
3. Add 1/4 volume 10 M LiCl to the supernatant and mix. The RNA is precipitated overnight at 4°C and harvested by centrifugation at 12,000 x g for 20 min. (Shorter precipitations time may also be used with lower yield: 1hr precip. will obtain ~30% yield)
4. Optional: Wash pellet with 20 ml of 75% ethanol. Vortex briefly. Centrifuge at 10,000 x g at 4°C for 10 min. Discard supernatant; briefly dry pellet on kimwipe.
5. Dissolve pellet into 100-250 μL DEPC-H_2O and proceed with polyA RNA selection directly.