RNA extraction method*

0.8g of tissue

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Before you do, check the needed items (next page)

1. Add 8ml TLE, 800μl 10% SDS and 2.7ml of phenol to a 30ml Corex tube. Put on ice.

2. Chill mortar and pestle with liquid nitrogen. Grind 0.8g tissue to fine powder and add to previous Corex tube with scratch rods.

Place a plastic weigh boat floating on liquid nitrogen and sweep the powder into the boat.

3. Put in Fisher tissue homogenizer and mangle for ca. 2 mins.

Put other samples on ice and also all following steps.

4. Add 2.7ml chloroform and homogenize for another 2 mins.

5. Cover tube w/ parafilm and centrifuge at 12,000g, 4°C for 20mins.

6. Transfer upper layer to clean tube and add 5ml of phenol/chloroform (1:1). Centrifuge at 12,000g, 4°C for 20mins.

7. Repeat step 6.

8. Transfer upper layer to clean tube and add 5ml of chloroform and centrifuge as above.

9. Transfer upper layer to a very clean 15ml Corex tube and add 1/3 volume 8M LiCl. Leave at cold room overnight.

Alternatively you can continue to next step immediately if you see lots of precipitation.

10. Centrifuge at 14,000g for 20mins, 4°C. Do a quick 70%EtOH wash.

11. Dry the pellet and redisolve in 330μl of DEPC-H_2O, stay on ice; transfer to a clean 1.7ml eppendorf. Add 110μl of 8M LiCl. Vortex and sit on ice for >2hrs.

If the pellet is very difficult to resuspend, add double amount of water and LiCl.

12. Centrifuge at 14,000g, 20mins, 4°C. Remove supernatant, wash with 70% EtOH briefly, dry and resuspend in 125μl DEPC-H_2O. Add 12.5μl 3M NaOAc and 355μl 95% EtOH. Vortex and sit for >2hrs at -20°C.

13. Centrifuge at 14,000g, 20mins, 4°C. Remove supernatant, wash w/ 70% EtOH briefly, dry and resuspend in 50μl DEPC-H_2O. Take OD reading and/or run on formaldehyde gel w/ standards.

*This method is based on the one described by Michael Frohlich 3/99, which was modified from Steve Jacobsen's and Neil Olszewski's methods.

The items you need for clean and prepared:

Chloroform washed 30ml (4/sample) and 15ml (1/sample) COREX tubes
Mortar and pestle
New or chloroform washed scratch rods
DEPC treated water
TLE (0.2M Tris, 0.1M LiCl, 5mM EDTA, pH8.2) (RNase-free)
10% SDS (RNase-free)
8M LiCl (RNase-free)
Phenol (~8ml/sample); chloroform (13ml/sample)
70% EtOH (RNase-free)
95% EtOH (RNase-free)
3M NaOAc (RNase-free)
Liquid Nitrogen