

Yeast RNA Extraction

(submitted by Martin Kos, Tollervey lab)

Small scale version of a protocol described by Tollervey and Mattaj. (1987).
EMBO J. 6/2:469-76.

1. Spin up to 10 O.D. of yeast culture and transfer into an Eppendorf tube
2. Wash with 1 ml water and pellet the cells
3. Add 100 μ l GTC/phenol mix (separate solutions in original protocol)
100 μ l glass/zirconia-silica beads (glass beads in the original protocol)
Vortex for 5 minutes on a multitube vortexer at 4°C room.
4. Spin 1 s ! (to get all the liquid from the lid down)
Add 700 μ l GTC/phenol mix
Vortex and incubate at 65°C for 5 min.
5. Spin 1 s !
Add 350 μ l Chloroform
120 μ l 3M NaOAc (pH 5.2)
Vortex for 20 s
Spin 5 min, room temperature
6. Transfer 550 μ l of the upper phase into a new tube
add 500 μ l phenol/chloroform
Vortex 20 s and spin 5 min, RT
7. Transfer 500 μ l upper phase into a new tube
and extract with 500 μ l chloroform.
8. Transfer 450 μ l of the upper phase
add 1 ml 100% Ethanol (-20°C)
Incubate on ice for 10 minutes.
9. Spin 10 min, 20000g, 4°C.
10. Wash pellet with 70% EtOH
11. Resuspend in 50 μ l water.

This works very well for ribosomal RNA. It can be scaled up for larger volumes. If there is too much protein in the interphase after step 6 then the phenol/chloroform extraction should be repeated. If the RNA is used only for Northern, then step 7 can be skipped.

For Northern blot load ~1 μ l per lane for ribosomal RNA, or more for mRNA.

GTC mix 100g Guanidine Thiocyanate (Sigma G9277)
 100 ml H₂O
 10.6 ml 1M Tris-HCl pH 8.0
 4.24 ml 0.5M EDTA pH 8.0

Dissolve 10 min at 60 – 70 °C
Add 2.1 ml β-mercaptoethanol
 21.2 ml 20% sarkosyl

GTC/Phenol mix 1:1 with phenol for RNA