

Preparation of RNA and RT-PCR

Caution: all procedures for RNA experiments must be carried out on ice or in cold-room.

1. Prepare RNA-extracting buffer containing 2 M guanidinium thiocyanate, 12.5 mM sodium citrate, pH 7.0, 50 mM β -mercaptoethanol, 0.25% *N*-lauroylsarcosine, 0.1 M sodium acetate, and 50% phenol, or use TRIzol (GIBCO-BRL) for RNA extraction.
2. For RNA prep from tissue homogenates, use 4 ml of RNA-extracting buffer for 100 ug tissue; for culture cells, add 10 ml to 100 mm-dishes to lyse the cells.
3. Add 2 ml of chloroform to cell lysate, mix vigorously by vortex for 15 sec.
4. Store on ice for 10 min.
5. Spin at $8000 \times g$ at 4°C for 20 min
6. Save the aqueous phase, mixed with an equal volume of cold isopropanol.
7. Store on ice for 10 min.
8. Spin at $8000 \times g$ at 4°C for 20 min
9. Wash pellet with 1 ml of 75% ethanol.
10. Dissolve the pellet in 50ul DEPC-treated dH_2O (dH_2O is pretreated with 0.1% DEPC overnight and autoclaved).
11. Store RNA prep on -70°C . It could be good for RT-PCR at least three months and freeze-thaw for 3-5 times.
12. Mix 1-10 ug of total RNA (according to the abundance of target gene) with 1ul 0.5 ug/ul oligodT in 10 ul dH_2O .
13. Heat at 65°C for 5 min in a preheated heat block, cool rapidly on ice.
14. Spin briefly.
15. Add: 2 ul 5x First Strand buffer (GIBCO-BRL)
 - 1 ul 2.5 mM dNTP
 - 1 ul RNasin (PROMEGA, 40u/ul)
 - 1 ul 0.1 M DTT
 - 1 ul SuperScript RT (GIBCO-BRL, 100 u/ul)

- Sometimes you could add 1 ul ^{32}P -dCTP in reaction to label cDNA to monitor the RT reaction.
16. Add dH₂O to adjust reaction volume to 20 ul.
 17. Spin briefly.
 18. Place at 37°C water bath for 60 min.
 19. Take 1ul of reaction mixture for following PCR reaction.
 20. Set up PCR reaction (50 ul):
 - 1 ul RT mixture
 - 5 ul 10x PCR buffer
 - 1 ul 2.5 mM dNTP
 - 1 ul upstream primer (5 pmol/ul)
 - 1 ul downstream primer (5 pmol/ul)
 - 1 ul Taq DNA polymerase (2.5 u/ul)
 - 40 ul dH₂O
 21. Mix well and spin briefly.
 22. Run on PCR cycles.
 23. Load 5 ul of PCR mixture onto 1% agarose gel to check the result.