01-11-08

RNA Isolation (using Promega SV Total RNA Purification kit, revised protocol by Y.S.)

1. Grow O/N culture in 1 ml HH at 37°C.
2. Spin down cells and wash 2X with 1ml HN no Mg and resuspend in 1ml same media.
3. Inoculate 1:50 in 5ml of appropriate medium (HL or HH). Grow 4h in 37°C shaker.
4. Spin down cells in an RNAse free microcentrifuge tube and remove supernatant.
5. Store pellet at -80°C until future use.
6. Resuspend pellet in 100ul TE buffer containing ~0.4 mg/ml lysozyme (freshly prepared) and incubate at RT for 6 min.
7. Add 75ul RNA lysis buffer (RLA, in 4°C) and mix by inversion several times.
8. Add 350ul RNA dilution buffer (RDA) and mix by inversion several times.
9. Add 180 ul absolute ethanol (for RNA, in -20°C, mix by inversion several times.
10. Assemble spin column by inserting column into collection tube. DO NOT TOUCH THE BOTTOM OF THE SPIN COLUMN.
11. Pour solution into spin column assembly.
12. Spin at 12,000xg for one minute. (Spin an additional minute if solution doesn't completely pass through.) Discard flow through.
13. Add 600 ul RNA wash buffer (RWA) to column.
14. Spin at 12,000xg for one minute. (Spin an additional minute if solution doesn't completely pass through.) Discard flow through.
15. Add 250ul RNA wash buffer (RWA) to column.
16. Spin at 12,000xg for two minutes. Discard flow through.
17. Transfer column to elution tube.
18. Add 100ul RNAse free H_2O to spin column.
19. Spin at 12,000xg for one minute. (Spin an additional minute if solution doesn't completely pass through.) Discard column.
20. Add 10ul of 10x DNAseI buffer to RNA solution (flow through).
21. Add 5ul of DNAse I. Mix by pipetting.
22. Incubate in dry water bath at 37°C for one hour.

Phenol Extraction/Ethanol Precipitation of RNA

1. Add 100ul Phenol:Chloroform:IsoAmylAlcohol solution to RNA solution. Vortex 30 seconds. Spin at 12,000xg for one minute.
2. Transfer upper level to RNAse-free tube. Add 100ul Chloroform:IsoAmylAlcohol (24:1). Vortex 30 seconds. Spin at 12,000xg for one minute.
3. Transfer upper level to RNAse-free tube.
4. Add 1/10 volume of 3M ammonium acetate (pH 5.5) and 3x volume of cold absolute ethanol to precipitate RNA. Invert 20-30 times to mix and place at -20°C for 15 min.
5. Spin at 12,000xg at 4°C for 10 min.
6. Rinse pellet with 100 ul cold 70% ethanol. Spin at 12,000xg for one minute. Remove supernatant.
7. Air dry at 37°C for 5 minutes and resuspend pellet in 50 ul RNAse-free distilled H_2O.
8. Check RNA concentration on spectrophotometer. From exponential phase cultures, you should obtain 50-60 ug RNA.