HOT SDS/PHENOL RNA PREP

Many protocols for the isolation of total RNA from E. coli exist. Although some of these will provide RNA of sufficient quality for microarray analysis we have settled on the following protocol for the following reasons:

1. Instant lysis of cells using hot SDS quickly inactivates endogenous RNAses. This can be very important due to the extremely short half-life of many E. coli mRNAs and is of particular importance when performing time-course studies.
2. The DNAse treatment of total RNA prior to purification using RNAeasy columns is critical to removing contaminating genomic DNA from RNA samples. Genomic DNA can be used as template by reverse transcriptase.
3. A final purification using a RNA binding resin like that of the RNAeasy column provides very high quality RNA which we and others have found can improve microarray results.

SDS lysis solution:

2% SDS, 16mM EDTA (add 200mM NaCl if cells are grown in low salt media like LB)
Use 0.5 volumes of lysis solution relative to total volume of cell and media.

Protocol

For each sample:

1. Bring 1 volume (based on final volume of cells, media and lysis solution) of acid phenol/chloroform (Ambion) to 65^{o}C.
2. When culture has reached desired O.D., bring 0.5 volume of lysis solution (i.e. 5ml of lysis solution for 10ml of culture) to 100^{o}C in 50ml conical tube (Falcon/Corning) for ~ 5 minutes.
3. Quickly pipette the appropriate volume of cells (typically 10-15ml) directly into boiling lysis solution.
4. Keep at 100^{o}C for 5 min. with periodic mixing.
5. Pour this sample directly into 65^{o}C acid phenol/chloroform seal and parafilm cap (to avoid phenol escaping tube during mixing). Mix well by vortexing at high speed.
6. Keep at 65^{o}C 10 min with periodic vortexing.
7. Spin in samples 15 min at 2500g (Sorval RC3B = 3000 rpm).
8. Carefully transfer aqueous phase (avoiding any of the white interface) to fresh 50ml tube and add an equal volume acid phenol/chloroform
9. Seal cap with parafilm and mix well by vortexing at high speed.
10. Spin at 3000 rpm 15 min. (~2500g)
11. Carefully transfer aqueous phase to fresh 50ml conical and add equal volume of chloroform/isoamyl alcohol.
12. Seal cap and mix well by vortexing.
13. Spin 15 min. at 3000 rpm and remove aqueous phase to 30ml corex tube and add an equal volume of isopropanol. Keep at –20^{o}C at least 2 hrs or overnight.

Next Day:

1. Spin down RNA 20 min. at 15-20,000g (12,500 rpm in SS34 rotor).
2. Pour off supernant and wash pellet with 5ml of ice cold 70% ETOH (prepared with DEPC-H2O)
3. Dry pellet at room temp ~20 min. (note: overdrying can lead to difficulty resuspending pellet)

At this point RNA can either be re-precipitated or purified further using Qiagen RNAeasy columns (we recommend using Qiagen RNAeasy columns).
In either case RNA samples should be treated with RNAse-free DNAse to remove any genomic DNA contamination prior to further purification

DNAse treatment

- Resuspend dried RNA in 450ul DEPC H_2O + 50ul of 10x RQ1 buffer (Promega).
- Add 5ul of RQ1 RNAse-free DNAse (Promega) and incubate 60 min. at 37^{o}C.
- Add 50ul of DNAse stop solution (Promega) (optional: heat inactivate DNAse at 70^{o}C 10 min. We have found that this can sometimes lead to RNA fragmentation. The RNAeasy column should remove DNAse and therefore this step is not required).
    - **ALTERNATIVELY** other RNAse-free DNAse treatments can be used. We have had good success with the Ambion DNA free reagent.
- Proceed to RNAeasy purification or re-precipitation

Qiagen RNAeasy

- Follow the protocol for RNA cleanup provided with the RNAeasy kit

Reprecipitiation

- Phenol/chloroform extract sample 1x using insta-prep tubes (available from 5'- 3'). 250ul RNA sample +250ul acid phenol/chloroform
- Chloroform/Isoamyl alcohol extract samples 1x in same tube
- Remove aqueous phase and precipitate by addition of sodium acetate to 0.3M + 2 volumes 100% ETOH

At this stage you need to determine the quality and concentration of your RNA samples. We typically take A_{260}/A_{280} readings to determine yield and quality and also run 2-3 ug on an agarose gel and look for intact rRNA bands.

If your RNA is at low concentration (<2 mg/ml) it will need to be concentrated. We typically precipitate our RNA samples at this point and store them at –20^{o}C as an ethanol precipitate. This way the required amount of RNA can be spun down when needed.