

# Polysome Preparation Protocol

## 1 Gradient preparation

1.1) Prepare 10%, 20%, 30%, 40%, 50% sucrose solutions in the following buffer:

Tris-HCl pH 8 20mM  
KCl 140mM  
MgCl<sub>2</sub> 5mM  
DTT 0.5mM  
Cycloheximide 0.1mg/ml  
Heparin 0.5mg/ml

1.2) Underlay 2.2ml of each 10%, 20% 30% 40% 50% sucrose, starting with the 10%, to make 11ml gradients. Store overnight at 4° to allow equilibration.

## 2. Polysome extraction

Routinely, extracts are made from 500ml of culture and split to 6 gradients. The volumes in the following protocol are for ~80ml culture (1 gradient).

2.1) Grow cells to OD<sub>600</sub> 0.4-0.6. in YPD

2.2) Add cycloheximide to a final conc. of 0.1mg/ml, cool immediately and spin down the cells 6K rpm/4min/4°.

We noticed that extended incubation on ice prior to centrifugation lead to increase in the 80S fraction.

2.3) 2 x Resuspend cells in 2.5ml of fresh lysis buffer and spin as above.

Lysis buffer is:

20 mM Tris-Cl pH 8.0,  
140 mM KCl,  
1.5 mM MgCl<sub>2</sub>,  
0.5 mM DTT,  
1% Triton X-100,

0.1mg/ml cycloheximide,  
1mg/ml Heparin

2.4) Resuspend in 0.7ml lysis buffer, transfer to corex tubes and add 2/3 vol. chilled glass beads (0.45-0.55 mm). 4 x vortex hard 20 sec and cool on ice 100 sec.

2.5) Spin 4.7K rpm (2600g)/5min/4°. Transfer supernatant (~500ul) to 1.6ml tube.

2.6) Spin 9.5K rpm/5 min/ 4°. Transfer to new 1.6 ml tube.

2.7) Bring to final volume of 1 ml and load ~0.8 ml per gradient.

2.8) Spin 35K/ 160min/ 4° in SW41 rotor.

### **3. Fractions collection**

Gradients are fractionated using ISCO collection system with the following setting:

Pump speed 0.75ml/min  
Fraction time 1.2min/fraction  
Chart speed 60cm/hr.

These settings result 14 fractions with volume of ~0.9ml. Sensitivity of the OD254 recorder is set to 1 or 2, depending on the desired resolution. Collect fractions into tubes containing 2ml 8M guanidinium-HCl (final Guanidinium conc is 5.5M).

### **4. RNA extraction for microarray analysis**

To obtain sufficient amounts of mRNA for the microarray analysis, identical fractions from at least 3 gradients were pooled together. Indicated volumes are for material from 3 gradients. 75% of the resulting RNA was used for labeling.

4.1) Pool similar fraction and precipitate by adding equal volume of 100% Ethanol and incubation overnight/ -20°.

4.2) Spin 10K/20 min/ 4° using SS34 rotor. Wash with 1ml 85% Ethanol and spin as above.

4.3) Resuspend in 400ul TE pH8, transfer to 1.6 ml tube and precipitate again by adding 0.1 vol. 3M NaAcetate pH5.3 and 2.5 vol. 100% Ethanol.

4.4) Resuspend in 650ul DDW and remove any residual proteins by extracting once with Tris buffered Phenol:Chloroform. Take 500ul of the aqueous phase to a new tube.

4.5) Bring to 1ml with DDW and add LiCl (1.5M final conc.) to remove any residual Heparin. Incubate overnight in -20°. Spin down, wash with 75% Ethanol, air dry and resuspend in 155ul.

4.6) To remove the LiCl, precipitate again with 0.1 vol. 3M Na Acetate pH5.3 and 3 vol. 100% Ethanol, wash with 75% Ethanol and air dry.

4.7) Resuspend in 1mM Tris pH 7.4 (20ul for material from 3 gradients, usually 10-15ul from this are used for labeling).