PREPARATION OF S-30

*Before beginning preparation, make sure all buffers have been cooled to 4(C. It is also useful to prepare dialysis tubing prior to starting this prep. All steps should be performed at 4(C, except where noted.

DAY 1:

Inoculate bacterial strain mre 600 (RNase -) into 2x10ml of growth media and grow overnight to saturation.

Note: Since no antibiotic can be used, it is useful to monitor for contamination with a negative control (no bacteria).

DAY 2: (12 to 16 hours work)

1. Inoculate each 10 mL saturated culture into 1L of growth media.
2. Monitor growth at 30 min. intervals by measuring OD650nm.
3. When OD650=0.8, chill each flask rapidly in ice (use ice-filled sink).
4. Harvest cells in Beckman centrifuge at 4500 rpm (3500x g) for 15 minutes.
5. Resuspend cells in 400ml wash buffer. Pellet cells by centrifugation at 4500 rpm for 20 minutes, then repeat wash once again. After second wash, scrape off any dark material on surface of pellet.
6. Resuspend cell THOROUGHLY in cold lysis buffer at 0.25g/ml (expect 10-20g of cells). Pass through 22 gauge needle to ensure complete resuspension.
7. Pass suspension through French pressure cell at 6000 psi. Immediately add DTT to 1mM.
8. Centrifuge lysate twice at 30 000x g (18 000 rpm in Ti 50.2 for 30 minutes at 4(C. Transfer supernatant to a clean fresh tube.
9. Measure protein concentration for 2 (l sample by BCA assay. Adjust to 10-15(g/(l with lysis buffer if necessary.

   Note: Expect to dilute approx. 2X.
10. Add 1ml for pre-incubation mix for each 6.5ml of S-30 lysate.

    Note: Prepare mix fresh immediately before use, and make absolutely no more than required due to extreme expense of PEP. Incubate with shaking at 25(C for 1 hour.
11. Add micrococcal nuclease to 25U/ml S-30 plus Ca (acetate)2 to 1mM final concentration. Incubate 30 minutes at 37(C.
12. Add EGTA to a final concentration of 4mM to inactivate micrococcal nuclease.
13. Transfer to 2x15cm lengths for dialysis tubing (molecular weight cutoff of 6000-8000da).
    Note: Preparation of tubing
    - boil 10 min. in2% NaHCO3/1mM EDTA
    - boil 10 min. in 1mM EDTA
    - rinse thoroughly and soak in ddH2O for more than 1 hour at 4(C.

Dialyze four times against 2L for dialysis buffer for 1 hour at 4(C.

14. Snap freeze in liquid nitrogen in 0.5-1.0ml aliquots and store at -80(C.

Note: Total volume of lysate is approx. 80ml