TRANSLATION PROTOCOL

A typical 10(l reaction contains:

- 35mM Tris-acetate, pH 8.0
- 190mM K (glutamate)
- 30mM NH4OAc
- 2mM DTT
- 12mM Mg (Ac)2
- 40(M 19 aa's - met
- 2mM ATP
- 5mM each CTP, UTP, and GTP
- 20mM phosphoenol pyruvate
- 1mg/ml E. coli tRNA
- 35mg/ml PEG 8000
- 20(g/mL folinic acid
- 2mM IPTG
- 0.5(g DNA template
- 1(l 35S Met
- 3(l bacterial lysate

This is incubated at 37(C for 45 min. for coupled T1 andT2

Note: Each batch of S-30 must be optimized for Mg (OAc)2 and K(glutamate)concentration.