32P LABELED RIBOPROBES FOR NORTHERNS

1. Add the following in order:

   - H2O 1.75 µl
   - 1 mg/ml BSA 2.5 µl
   - 5 x buffer 5 µl
   - triton X-100 0.25 µl
   - 1M DTT 0.5 µl
   - RNAguard 2 µl
   - 10mM ATP 1 µl
   - 10mM CTP 1 µl
   - 10mM GTP 1 µl
   - 100µM UTP 3 µl
   - DNA 1 µl (1 µg)
   - α32PUTP 10mCi/ml 5 µl
   - T3/T7/SP6 polymerase 1 µl

2. Incubate at 30°C for 3 hours.

3. Dilute into 100 µl by adding:

   - 2 µl RNAGuard
   - 10 µl 10 x DNAase buffer (manufacturer's)
   - 10 µl 100mM DTT
   - 52 µl H2O

   Remove 2 x 1 µl and spot onto DE81 paper for quantification later.

   Add 1 µl DNAase and incubate for 15' at 37°C.

4. Phenol/chloroform extract.

5. Ethanol precipitate by adding 50 µl 7.5 M NH4OAC, 2 µl 50 mg/ml tRNA and 376 µl ethanol.
6. Precipitate and resuspend in 500 μl 50% formamide.

**Quantification.**

Rinse 1 filter:  
5 x 5' in 0.5 M Na₂HPO₄  
2 x 1' in dH₂O  
2 x 1' in 95% ethanol

Dry thoroughly.

Count both filters in aqueous scintillant. Washed filter represents incorporated counts.