^{32}P LABELED RIBOPROBES FOR NORTHERNS

1. Add the following in order:

H_2O	1.75 µl
1 mgml^{-1} 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)
α^{32}PUTP 10mCiml^{-1}	5 µl
T3/T7/SP6 polymerase	1µl

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 H_2O

   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 NH_4OAC, 2 µl 50 mgml^{-1} 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 Na_2HPO_4
2 x 1′ in dH_2O
2 x 1′ in 95% ethanol

Dry thoroughly.

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