PRIMER EXTENSION ANALYSIS OF RNA

Materials:

2x hybridization buffer: 1.25 M KCl
10 mM Tris pH 7.9
1 mM EDTA

50 fmole of kinased primer per reaction

Primer extension buffer: 10 mM $MgCl_2$
5 mM DTT
20 mM Tris pH 8.3 at 37°C
100 µl/ml actinomycin D
0.3 m each dXTP

Procedure:

1. Resuspend RNA in: 8 µl TE
   2 µl 2x hybridization buffer
   1 µl primer
2. Incubate at 57°C for 1 hour.
3. Add 23 µl primer extension buffer
   10 units AMV reverse transcriptase
4. Incubate at 37°C for 30 min.
5. Add 300 µl EtOH, ppt. speed vac.
6. Wash with 70% EtOH.; speed vac.
7. Resuspend in 4 µl formamide dyes
   2 µl 0.1 N NaOH
8. Heat to 95°C for 5 min.
9. Load onto denaturing PAGE.

Reference:

Dignam et al. 1983. Nuc. Acids Res. 11, 1475-1489.
Dynan and Tijian. 1985 Nature 316, 774-778.