Denaturing Urea PAGE - Small Gel

1. Prepare denaturing polyacrylamide gel solution. Use Gibco/BRL apparatus.

	7.2 %	9.6 %	12 %
10X TBE	2.5 mls	2.5 mls	2.5 mls
Urea (ultrapure)	10.5 g	10.5 g	10.5 g
40% Acrylamide	4.5 mls	6 mls	7.5 mls
ddH_2O	10.5 mls	9 mls	7.5 mls
Total Volume	25 mls	25 mls	25 mls

   Use 40% acrylamide stock for DNA/RNA gels. Do not use 30% stock for SDS-PAGE gels. Mix in 50 ml beaker. Heat gently in microwave (~ 15 seconds) to dissolve urea. Syringe filter (0.45 µm) gel mix into sidearm flask and degas for 5-10 minutes.

   • If urea is not ultrapure grade, deionize as follows: Add 0.05 g AG501-X8(D) resin. Mix at room temperature for 5 minutes. Spin in IEC for 5 minutes at 2000 rpm.

2. Pour gel. Add 25 µl TEMED and 50 µl 25% APS. Pour gel to ~ 0.5 cm from top. Insert clean, dry comb at an angle to prevent trapping of bubbles. Push all the way down, but don't trap any bubbles.

3. Pre-electrophorese gel. Use 1 X TBE in upper and lower reservoirs. Remove comb. Run at 20 watts for 15 minutes. Warmth plus urea denatures nucleic acids.

4. Denature samples. To RNA, add an equal volume of sample buffer (100 µl formamide + 1 µl 0.5 M EDTA, pH 8 + 1 µl 100X BPB). Heat for 1 minute at ~95°C (tubes in steam over boiling water in bath). Chill on ice. Spin briefly. Thaw frozen RNA samples just before denaturation and loading.

5. Load samples. Blow out wells with syringe and needle. A load volume of 3-5 µl will give tight bands. Up to ~15 µl can be loaded.

6. Run gel. Use 10 watts (~35°C). Up to 15 watts (~50°C) is okay, but may distort bands.

Percent	Bromophenol Blue (BPB)	Xylene cyanol FF (XC)
4%	30 nt	155 nt
6%	25 nt	110 nt
8%	20 nt	75 nt
10%	10 nt	55 nt

   • For RNase protection (30-50 nt): run 12% gel and stop when BPB is 1-2 cm from bottom.

   • For 5.8S and 5S (~150 nt): run 12% gel for 3.5-4 hours. BPB will run off at ~1.5 hours.

7. Ethidium bromide staining. Gently agitate for 10 minutes in 10X (5 µg/ml) EtBr in ddH_2O.

8. Fix gel. Cut gel down to sample lanes + 1-2 lanes each side. Fix gel in 3 X 5 minute washes of 10% MeOH, 10% AcCOOH (dilute 5X destain 1 in 5). Use ~10 X gel volume for each wash.

9. Fluorography with En^{3}Hance (for ^{3}H and ^{14}C):
   A. Immerse in minimum amount of En^{3}Hance. Swirl gently for 30 minutes in covered glass dish.
   B. Precipitate En^{3}Hance with slow stream of dH_2O (house distilled) for 15 minutes.
   C. If gel is 12% or more, soak in 2% glycerol for 5 mintues before drying.

10. Dry gel. Place gel on plastic wrap, front side down. Place wet 3MM paper on gel. Turn over and dry at 50°C. Gel should dry in less than 1 hour.