DNase Footprint
(From Kathy Jones)

1) DNA Mix
   ^{32}P labled probe
   10 ul 10% polyvinyl alcohol
   1 ul 10 A $_{260}$ unit/ml poly d(I-C) (from Sigma P9514)
   H_2O to 25 ul
2) Add Z' or TM buffer to a final volume of 50 ul allowing room for the amount of protein extract used.
3) Add protein extract last.
4) incubate on ice 15 min.
5) DNase digestion. (amount of DNase to use must be titrated for best results)
   a) 1 ul of 2.5 mg/ml DNase I is added to 1 ml ice cold water, mix thoroughly by inversion.
   b) Add 100 ul from above to 200 ul ice cold water.
   c) Add 50 ul 10 mM Mg Cl_2, 5mM Ca Cl_2 to the DNA protein mix leave at rooom temp for 1 min.
   d) Add 2.0 ul diluted DNase, mix quickly , incubate at room temp. for 1 min. (If done at 1 min intervals, one can do three reactions at 15-20 second intervals.)
   e) Add 90 ul STOP Mix, vortex, and store on ice.
   f) After all reactions are completed to all;
      Phenol extract.
      Chloroform extract. This may require 1 min microfugation for good seperation.
      Ethanol precipitate.
      70% ethanol wash and vacumn dry.
   g) Add 4-10 ul loading buffer, boil for three minutes and load on sequencing gel.

Footprinting solutions.

	Stock	1 ml
TM buffer		
50mM Tris-HCl pH 7.9	2M	25 ul
12.5mM Mg Cl_2	1M	12.5 ul
1mM EDTA	0.5M	2 ul

1mM DTT	1M	1 ul
20% glycerol	100%	200 ul
0.1M K Cl	1M	100 ul
H_2O	660 ul	

STOP Mix		5 ml
200mM Na Cl	1M	1 ml
20mM EDTA	0.5 M	250 ul
1% SDS	10%	500 ul
250 ug/ml tRNA	5 mg/ml	250 ul