DNase I footprinting

I. Labeling of probe

- 5 pmoles of primer #1
- 1.5 µl of 10 X T4 PNK buffer
- 1 µl of 32P ATP (ICN, 7,000 Ci/mmol, 167 µCi/µl)
- H2O to 14 µl
- 1 µl of T4 PNK
- incubate @ 37 °C 45 - 60 min. In the mean time, equilibrate Biogel 6 spin column with 50 mM Tris HCl (8.8), 50 mM KCl.
  Terminate labeling by addition of 1 µl 500 mM EDTA.
  Pre-spin column @ 2,000 rpm 1 min. Discard liquid. Pre-spin once more @ 2,000 rpm 1 min. Apply labeling reaction, and spin @ 2,000 rpm 4 min.

II. Synthesis of probe by PCR

- labeled primer #1 x µl (all of eluate from spin column)
- cold primer #2 10 p moles
- 10 X PCR buffer 5 µl
- (25 mM MgCl2 5 µl)
- template DNA 10-100 ng plasmid
- 10 mM ea dNTP 1 µl
- H2O up to 49.5 µl
- Taq pol 0.5 µl an example of amplification condition (hsp 70 promoter, p29-p58)
  95 °C 2 min.-(95 °C 20 sec.-55 °C 30 sec.-72 °C 30 sec) x 30 cycles-72 °C 5 min.

  Purify PCR product through 6 % PAGE in 0.5 X TBE.
  Identify product by exposure to X-Ray film (~1 min.). There will be three bands. From top, they are the ssDNA product, the dsDNA product, and the probe. Cut out dsDNA DNA band, crush and soak overnight in 0.1 % SDS, 0.5 M ammonium acetate, 10 mM magnesium acetate. Alternatively, recover DNA by DEAE paper. Extract DNA with phenol-chloroform, and recover by EtOH ppt.

  Dissolve DNA in 100 µl of TE. Store @ 4 °C (OK for a week or two).

III. DNase I foot printing

Solutions:

- 5X FP buffer: 75 mM Tris-HCl (pH 7.4), 250 mM KCl, 0.5 mM EGTA, 25 % glycerol, 2.5 mM DTT.
- competitor mix: 0.5 µg/µl fragmented E. coli DNA, 20 mg/ml BSA
- DNase I stop buffer: 20 mM Tris-HCl pH8.0, 20 mM EDTA, 0.8 % SDS, 0.1 µg/µl carrier DNA.
  Reaction
- probe 0.5 - 1.0 µl
- 5X FP buffer 4 µl

- competitor mix 1 µl
- H2O to bring reaction to 20 µl (including protein)

mix well gently.
Add DNA binding protein

- Incubate @ 26 °C 30 min. Add 2 µl of 10 u/ml DNase I (Boehringer, diluted in 50 mM MgCl2, 10 mM CaCl2).
- Incubate @ room temp. for 1 min. Add 60 µl of DNase I stop buffer. Add 2 µl of 10mg/ml Proteinase K, and incubate overnight @ 37 °C. Add 20 µl of 10 M ammonium acetate and 250 µl of 100 % EtOH. Recover DNA and air dry. Count by Geiger, and dissolve in appropriate vol. of sequencing loading buffer (dissolve so that cpm/µl will be equal among samples). Run samples on 6-8 % sequencing gel.