DNase I footprinting

I. Labeling of probe

- 5 pmol 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 at 37°C for 45 - 60 min. In the meantime, equilibrate Biogel 6 spin column with 50 mM Tris HCl (8.8), 50 mM KCl. Terminate labeling by addition of 1 μl of 500 mM EDTA.
- Pre-spin column at 2,000 rpm for 1 min. Discard liquid. Pre-spin once more at 2,000 rpm for 1 min. Apply labeling reaction, and spin at 2,000 rpm for 4 min.

II. Synthesis of probe by PCR

- Labeled primer #1 x μl (all of eluate from spin column)
- Cold primer #2 10 pmol
- 10 X PCR buffer 5 μl
- (25 mM MgCl2 5 μl)
- Template DNA 10-100 ng plasmid
- 10 mM each dNTP 1 μl
- H2O up to 49.5 μl
- Taq pol 0.5 μl (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 at 4°C (OK for a week or two).

III. DNase I footprinting

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.