

DNase I footprinting

I. Labeling of probe

- 5 pmoles of primer #1
- 1.5 μ l of 10 X T4 PNK buffer
- 1 μ l of 32 P ATP (ICN, 7,000 Ci/mmol, 167 μ Ci/ μ l)
- H₂O to 14 μ l
- 1 μ l 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 μ l 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 μ l (all of eluate from spin column)
- cold primer #2 10 p moles
- 10 X PCR buffer 5 μ l
- (25 mM MgCl₂ 5 μ l)
- template DNA 10-100 ng plasmid
- 10 mM ea dNTP 1 μ l
- H₂O up to 49.5 μ l
- Taq pol 0.5 μ l 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 μ l 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 μ g/ μ l 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 μ g/ μ l carrier DNA.

Reaction

- probe 0.5 - 1.0 μ l
- 5X FP buffer 4 μ l

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

mix well gently.
Add DNA binding protein

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