DNase I Footprinting

A method for determining the location of a protein binding site, DNase I Footprinting Analysis involves endonuclease treatment of an end labeled DNA fragment bound to a protein. Limited digestion yields fragments terminating everywhere except in the footprint region, which is protected from digestion.

1. Preparation of DNA substrate:

DNA to be analyzed must be cloned in such a way as to present a restriction site for an enzyme leaving a 5' overhang (3' recessed OH) 50-150 bp from the putative binding site. This site is labeled by "filling in" the recessed site with 32P-dNTP's using DNA polymerase. The probe is then cut from the remaining plasmid by a second restriction enzyme, 150 bases on the opposite side of the binding site. This releases a 200-300 bp probe, labeled at one end (see figure below).

Digest 5-10 picomoles of plasmid (10-20µg of a 3000bp construct) with an enzyme which will leave a recessed 3' end 50-150bp from the binding site.

Ethanol precipitate the DNA, and wash 1X with 70% Ethanol.

Add 50 µl of 1X Klenow buffer containing 50 µCi of each 32P dNTP. Add 10 units of Klenow fragment in < 2 µl, and incubate 30 minutes at 25°C.

Klenow buffer: 50mM Tris HCl, pH 7.6
12mM MgCl
1mM DTT
50 µg/ml BSA

Chase reaction with 5 µl of 2mM each dCTP, dGTP, dTTP, dATP, to ensure complete polymerization.

Ethanol precipitate twice with 0.1 vol 3M Sodium Acetate and 3 volumes of Ethanol, or purify with a spin column or glass powder elution to remove unincorporated label.

Cut with a restriction enzyme to release a 200-300 bp end labeled probe.

Run probe on a 1.5-2% agarose gel and recover fragment by electroelution. Further purification may be necessary to ensure consistent results. Ion exchange mini columns provide good results.

If volume is >50µl, ethanol precipitate the probe, and reconstitute in 50 µl TE buffer.

2. Bind Protein to DNA Probe:
Mix 105 cpm of probe with 200 µl of DNase Footprinting Buffer:

- 10mM Tris HCl, pH 7.6
- 4mM MgCl2
- 1mM CaCl2
- 150mM KCl
- 2mM DTT
- 100 µg/ml BSA
- 2mg/ml calf thymus DNA
- pH 7.6 (adjust buffer if necessary)

3. Add 20 µl protein sample. A series of dilutions covering 4-5 orders of magnitude will allow calculation of the binding affinity.

4. Prepare a blank tube with 20 µl of Assay buffer.

5. Incubate 30-45 minutes, at equilibration temperature.

6. The optimum time and temperature must be determined for each DNA/probe combination.

7. DNase I Treatment:

   DNase treatment proceeds for only 2 minutes, so stop solution and a dry ice Ethanol bath must be prepared before beginning the treatment.

Stop Solution: 6.5 ml Ethanol
50 µl 1mg/ml tRNA
0.5 ml Ammonium Acetate saturated solution.

Cool Stop Solution to -70°C prior to use.

Prepare DNase I solution:

The amount of DNase I required will vary depending upon the purity, age and storage conditions used for the enzyme. Start with 0.1 mg/ml DNase I and adjust to get an average of 1 nick per DNA molecule.

Dissolve DNase I in Assay/Equilibration buffer without BSA or calf thymus DNA.

To each 200 µl sample of protein/DNA, add 5 µl DNase I solution.

Reproducible pipetting is essential at this stage if different DNA:protein ratios are to be compared.

Incubate at Equilibration temperature exactly 2 minutes, then add 800 µl Stop Solution @-70°C.

Precipitate DNA at -70°C for 30 minutes. Wash pellet with 70% Ethanol twice, and remove all supernatant. Air dry or speedvac 10 minutes.

Redissolve pellet in 6 µl of loading buffer, and run on a standard denaturing PAGE gel.
Preparing the DNA substrate for DNase footprinting analysis. A circular construct containing the protein binding site is linearized with a restriction endonuclease, yielding two free ends, which are both labeled. One end is then cut away in a second round of restriction digestion, leaving an end labeled probe which carries the binding site.

Interpreting the results of DNase Footprinting
The figure below shows an electrophoresis gel of idealized results. Bands correspond to DNase I cleavage sites. As the amount of protein present increases, the footprint area is progressively protected from cleavage. The concentration of protein required to give 50% protection can be mathematically related to the equilibrium constant for protein binding. See CPMB Section 12.4 for a complete discussion of this method of analysis.

An electrophoresis gel showing successive DNase footprinting reactions conducted with increasing titrations of binding protein.

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