

Ram Balak Mahto
Guest faculty
Zoology department
v.s.j college Rajnagar Madhubani
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FOOTPRINTING-P1

DNase I footprinting is used to precisely localise the position that a DNA binding protein, *e.g.* a transcription factor, binds to a DNA fragment. A DNA fragment of a few hundred bp is labeled at one end and then incubated with the proteins suspected to bind. After a limited digestion with DNase I, the reaction is quenched, DNA is precipitated and analysed on a denaturing polyacrylamide gel. This protocol uses ³²P-radioactively labeled DNA.

Materials and Reagents

1. Oligonucleotides (usually 20-30 mer) to amplify a suitable fragment (100-400 bp) encompassing the region to be tested for protein binding ability
2. Plasmid DNA carrying the cloned required region to use as template for the PCR amplification
3. [-³²P] ATP (3,000 Ci/mmol, 30 μCi = 3 μl/labeling) (*e.g.* NEN, catalog number: BLU502A)
4. Polynucleotide kinase (PNK) (*e.g.* Biolabs, catalog number: M0201)
5. Agarose
6. Purified protein (or enriched crude bacterial extracts see below)
7. DNase I (*e.g.* Sigma-Aldrich, catalog number: D5025)
8. Phenol
9. Chloroform
10. Herring sperm DNA (*e.g.* Sigma-Aldrich, catalog number: D6898; Roche, catalog number: 223 646)
11. BSA (*e.g.* Biolabs, catalog number: B9001)
12. Acrylamide
13. Urea
14. DTTP (*e.g.* Biolabs, catalog number: N0447)
15. Taq polymerase (5 units/μl) (*e.g.* Biolabs, catalog number: M0267)

16. DNA Marker (*e.g.* 100 bp ladder, Biolabs, catalog number: N3231)
17. Antarctic alkaline phosphatase (Biolabs, catalog number: M0296)
18. MspI (Biolabs, catalog number: N3032)
19. 40% Acrylamide stock (19:1 acrylamide: bis acrylamide) (*e.g.* Euromedex, catalog number: EU0076-C)
20. TBE buffer
21. Binding buffer (see Recipes)
22. DNase I dilution buffer (see Recipes)
23. DNase I stop buffer (see Recipes)
24. DNase I stock (see Recipes)
25. Loading formamide dyes (see Recipes)
26. Denaturing Sequencing gel (6% acrylamide) (see Recipes)
27. Hepes-Glutamate (see Recipes)

Equipment

1. Suitable space for working with ³²P radioactivity
2. Image quantification apparatus (*e.g.* Typhoon GE Healthcare Life Sciences; X-ray film and developing materials)
3. PCR machine
4. Small horizontal agarose gel apparatus
5. Transilluminator (preferably 365 nM)
6. Apparatus for running a 30 cm sequencing gel (*e.g.* Model S2 Vertical sequencing apparatus, now sold by Biometra)
7. Power supply capable of producing 2,000 volts and 60 watts)
8. Geiger counter to monitor for radioactivity and any contamination.
9. Heating block at 90 °C
10. Gel drying apparatus