STOP Sol^n 230mg AmAc
1µl 100mM EDTA
10µl β-Mercap
+~790 dH2O.
KMnO4 31mg/ml

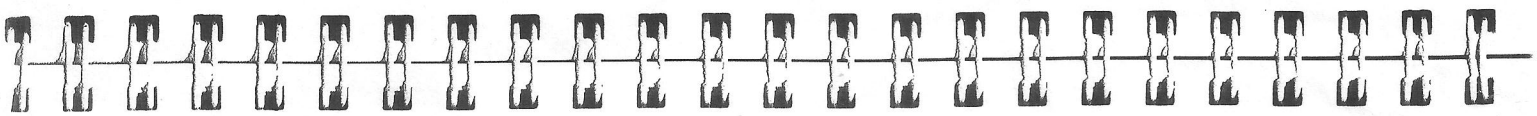
Protocol 6. **Potassium permanganate footprinting.** N. Savery, T. Belyaeva and S. Busby

Reagents

Absolute ethanol⚠
10% Acetic acid/10% methanol⚠
10% Ammonium persulfate (freshly made)
5× Binding buffer (25% glycerol, 0.5 M NaCl, 25 mM $MgCl_2$, 0.5 mM EDTA, 5 mM DTT, 0.25 mg/ml bovine serum albumin,100 mM Tris-HCl pH 8.0)
10× Buffer for *Taq* DNA polymerase, usually supplied with enzyme (typical composition 500 mM KCl, 15 mM $MgCl_2$, [illegible] mg/ml gelatin, 0.5% Tween 20, 100 mM Tris-HCl pH 8.3)
D[illegible]aturing acrylamide mix⚠ [6% acrylamide (acrylamide: bisacrylamide ratio 19:1), 7 M urea, 1× TBE buffer]
Deoxyribonucleotide stock containing each dNTP at a final concentration of 2.5 mM
DNA sample (fragment or plasmid)
70% Ethanol⚠
Gel loading buffer [40% deionized formamide⚠ (see *Protocol 4*, Note 1), 5 M urea, 5 mM NaOH, 1 mM EDTA, 0.025% Bromophenol blue, 0.025% xylene cyanol]
20 mg/ml Glycogen
Protein samples
3 M sodium acetate pH 7.0
Sterile distilled water
Stop solution (3 M ammonium acetate, 0.1 mM EDTA, 1.5 M 2-mercaptoethanol)
Taq DNA polymerase
TEMED
TBE buffer (89 mM Tris-borate, 89 mM boric acid, 2 mM EDTA)
10× TBE (107.8 g Tris base, 55 g boric acid, 7.44 g EDTA) make up to liter with deionized water, and store at room temperature

Equipment

Autoradiography equipment (X-ray film, cassettes and developing facilities) or phosphorimager.
Controlled temperature block
Gel dryer
Microcentrifuge and microcentrifuge tubes
Perspex radiation shield
Pipette plus tips
Thermal cycler for PCR



200 mM $KMnO_4$ (freshly prepared)
Mineral oil
O[illegible]nucleotide primer end labeled with ^{32}P ⚠
P[illegible]ol/chloroform/isoamyl alcohol (25:24:1 by vol. saturated with 0.1 M Tris-HCl pH 8.0)
Sequencing gel apparatus (power supply, electrophoresis tank, glass plates, spacers and combs)
Vacuum desiccator
3MM Whatman paper

Procedure

1 Mix the DNA (4–10 nM) ① with the proteins to be footprinted, in a total volume of 20 µl of 1× binding buffer. ②

2 Incubate the mixture at 37°C for 30 min. ③

3 Add 1 µl of 200 mM $KMnO_4$ solution to the sample. Mix by stirring gently with the pipette tip for 15 sec. ④

4 Incubate the mixture at 37°C for 4 min.

5 Add 50 µl of stop solution and mix well. [1]

6 Add 130 µl of TE buffer and extract with 200 µl of phenol/chloroform/isoamyl alcohol. Transfer the aqueous phase to a fresh microcentrifuge tube.

7 Precipitate the DNA by adding 1 µl of 20 mg/ml glycogen and 400 µl of cold absolute ethanol. Mix, and incubate at −70°C for 15 min. [2]

8 Centrifuge for 15 min in a microcentrifuge at 4°C, and discard the supernatant.

PTO

Notes

Time required: 1 day

Footprinting reactions (12 samples) – approximately 8 h.
Electrophoresis, fixing and drying gel – approximately 3 h.
Autoradiography – overnight or longer.

① The DNA sample may be either a purified fragment or an intact plasmid. Nicks in the DNA will cause a high background and care should be taken to avoid their introduction during sample preparation.

② Some experimentation may be necessary in order to find the optimal conditions.

③ The exact duration of this incubation is not critical, but should be sufficient for the protein–DNA complex to form. The temperature may also be altered.

④ $KMnO_4$ is present in excess in this reaction.

⑤ Primers should be designed to anneal 60–70 bp from the area of interest and should be 15–20 bases in length to ensure specificity. End label 50 pmol of primer using T4 polynucleotide kinase and [γ-^{32}P ATP] then dilute to 1 µM.

- 70%. EtOH wash.
- 1M Piperidine @ 90°C 30 min
- See G4 ladder protocol

N.B. FIXER FOR SEQUENCING GECS 10%. Methanol 10%. Acetic acid