

Denaturing Gels

The formation of single stranded DNA after a double strand break is made can be detected on Southern blots if the DNA from a time course is run on a denaturing gel. Single stranded DNA generally cannot be cut by restriction enzymes and hence runs as longer DNA segments on denaturing gels as more restriction sites become single stranded. A list of some restriction enzymes that are exceptions to this rule and are able to cleave single stranded DNA can be found in the New England Biolabs catalog. When time course DNA is run on native, neutral gels, the single stranded intermediate runs as a heterogeneous smear presumably due to the loss of DNA of varying lengths and due to formation of secondary structures.

Denaturing electrophoresis is carried out according to the procedure of McDonnell et al. (1977) as modified by Sambrook et al. (1989).

1. Melt agarose in 50 mM NaCl, 1 mM EDTA, pour into a gel tray and allow to solidify. Submerge the gel in a gel box in 50 mM NaOH, 1 mM EDTA and allow to equilibrate for 30 minutes or longer. This procedure is used because agarose will decompose if boiled under alkaline conditions. Alternatively melt the agarose in water and cool to 60°. At that temperature the agarose can be adjusted to 50 mM NaOH, 1 mM EDTA by addition of concentrated NaOH and EDTA. Ethidium bromide is omitted because it does not efficiently bind to DNA under these conditions.
2. Prepare the DNA samples by adjusting the solution to 0.3 M sodium acetate and 5 mM EDTA (pH 8.0) followed by addition of 2 volumes of ethanol to precipitate the DNA. EDTA is included to prevent the magnesium from precipitating out under alkaline conditions. After chilling the samples on dry ice (10 min.) and centrifuging in a micro-centrifuge (10 min.), the supernatant is discarded and the pellet is rinsed in 95% or 70% ethanol. As much of the supernatant as possible should be removed before drying the pellet.
Resuspend the pellet in alkaline gel loading buffer (1X buffer: 50 mM NaOH, 1 mM EDTA, 2.5% Ficoll (Type 400) and 0.025% bromocresol green). Size

markers can be denatured by directly adding alkaline gel loading buffer to a small volume of plasmid or phage DNA digested by restriction enzymes.

3. After loading the DNA, a glass plate can be (optionally) placed on the gel to prevent the dye from diffusing from the agarose during the course of the run. This is generally not necessary for short electrophoresis runs. Because of the large currents that can be generated with denaturing gels, gels are usually run slowly at lower voltages.
4. After the DNA has migrated far enough, remove the gel and if desired, stain the gel with ethidium bromide (0.5 ug/ml) in 1x TAE electrophoresis buffer. Two washes of 15 min each works best. The DNA will be faint because the DNA is single-stranded.
5. Soak the gel in 0.25 N HCl for 6-7 minutes with gentle agitation.
6. Rinse the gel with water and soak the gel in 0.5 N NaOH, 1.5 M NaCl for 30 minutes with gentle agitation.
7. Rinse briefly with water and apply the gel to the DNA transfer apparatus. The DNA can be blotted onto either neutral or positively charged nylon membranes using 2x or 10x SSC by any of various methods. We generally employ a capillary transfer table or a vacuum blotter.
8. Southern hybridizations can be carried out according to different protocols. We hybridize according to the protocol of Church and Gilbert (1984). We prepare RNA probes according to Melton et al. (1984) as modified by Promega and random-primed DNA probes according to Feinberg and Vogelstein (1984).

Melton et al Nuc Acids Res. 12:7035 (1984)

Feinberg and Vogelstein Anal. Biochem. 137:266(1984).

Church and Gilbert PNAS 81:1991 (1984).

McDonnel et al J. Mol. Biol. 110:119 (1977)

Sambrook et al. Molecular Cloning, CSH Laboratories, 1989

The most recent version of this protocol may be found at the Haber Lab web page:
<http://www.bio.brandeis.edu/haberlab/jehsite/protocol.html>.