ACRYLAMIDE GEL ELECTROPHORESIS

Acrylamide gel electrophoresis is the method of choice for separating nucleic acids less than 1000 bp in length. The gel can be run native (TBE) or denaturing (TBE + 8.3 M urea).

Materials:

-Sugden apparatus
-2 three finger clamps
-8 bulldog clamps
-3 spacers and 1 comb (1.5 mm)
-2 glass plates (fitted to Sugden apparatus)
-lubriseal in a 10 ml plastic syringe
-two rubber 'ears'
-vacuum dessicator
-power supply and leads
-ethidium bromide (Sigma)
-10X TBE
    108 g TRIS BASE
    55 g Boric Acid
    9.3 g Na_2EDTA
    dH_2O to 1 liter
-30% acrylamide solution: 29 g acrylamide/1 g bis acrylamide (Biorad)
-TEMED (BIORAD)
-Ammonium Persulfate (Biorad)
-Urea ultrapure (Schwartz and Mann)
-DNA buffer
    10 mM TRIS pH 7.4
    5 mM NaCl
    1 mM EDTA
-50% glycerol dyes (xylene cyanol Bromophenol blue)
-Formamide (Fluka)

Procedure:

1. Clean glass plates with ethanol followed by ddH_2O. Dry with Kimwipes.

2. The spacers will be placed along the two sides and the bottom of the smaller glass plate. To insure a watertight seal the spacers are greased with lubriseal. The excess grease is removed from the inside edge of the spacers with a kimwipe and the spacers are positioned on the glass plate. Often leaks occur at the junctions between the spacers, so it is best to put an extra dab of lubriseal between the spacer junctions.

3. The larger glass plate is placed on top of the smaller one. The two plates are clamped together, and the grease seal is checked for visible defects. Two clamps are put on either side while four clamps are put on the bottom. The gel form can stand vertically on the bottom clamps.

4. It requires 50 mls of solution to fill the gel form. For native gels 5 mls of 10X TBE is combined with the appropriate volume of acrylamide solution. dH_2O is added to a final volume of 45 mls followed by the addition of 50 µl TEMED. The polymerization reaction is initiated by the addition of 5 mls of a 1% ammonium persulfate solution. The final solution is mixed well, degassed in the vacuum dessicator and pipetted into the gel form. (After the addition of the ammonium persulfate it takes 5 minutes for the acrylamide to polymerize). The comb is inserted at an angle to ensure that bubbles do not form under the teeth.

5. Acrylamide-urea gels are made in the same manner except that 25 g of urea is placed in the tube before the aqueous additions. To increase the rate at which the urea dissolves it is best to gently heat the tube under hot tap water. Be sure that the solution has cooled back to room temperature before the addition of TEMED and ammonium persulfate, as the polymerization reaction proceeds much faster at elevated temperatures.

6. The gel is allowed to cure at least 2 hours before running. After this period the comb is removed by a gentle side by side motion. Rinse out each well vigorously with dd H_2O from a squeeze bottle.

7. Greased rubber "ears" are placed at either end on top of the smaller glass plate. This forms a notch which matches the notch in the upper reservoir tank of the sugden apparatus. Lubriseal is applied around the notch to ensure a watertight seal between the gel form and the upper reservoir of the sugden apparatus.

8. The clamps on the bottom of the gel form are removed along with the bottom spacer. One clamp is left on either side and the gel form is placed in the sugden apparatus. A three finger clamp is placed on either side of the apparatus to hold the gel form in place.

9. 1000 ml's of TBE are needed for the reservoirs. After the two tank are filled use a crooked pasteur pipette to remove bubbles at the bottom of the gel form.

10. Clean the wells with a pasteur pipette. With a urea gel, it is especially critical to rinse out the wells just before the samples are applied, as the urea leaches into the sample wells.

11. Samples are loaded with a micropipette. for aqueous gels the samples are usually in DNA buffer (xylene cyanol - Bromophenol blue), but any low salt buffer will do. Acrylamide urea gel samples are in 90% formamide - 5% glycerol-dyes 0.5 X TBE. The samples are heated to 90°C and rapidly cooled just before application to the gel.

12. Gels are run at from 25 mAmp to 45 mAmps. On a 5% acrylamide gel xylene cyanol runs at about 350 bp and bromophenol blue runs at about 75 bp.

13. At the end of electrophoresis the gel form is removed from the apparatus, and the plates are cleaned of lubriseal with a paper towel soaked in ethanol. The spacers are removed and the glass plates are pried apart with a spatula.

Comments:

1. Acrylamide Gels

a) The percentage of acrylamide may be varied from 4-10%. 4% gels are best from fragments 400-1000 bp in length, 5% is a good standard matrix for 4 base recognition restriction digests, and 8-10% gels are good for fragments <250 bp.

b) The well size is rather critical to resolution. I find 1 cm wells convenient while wells less than 0.5 cm wide give variable results.

c) Gels are stained with ethidium bromide by immersing the gel and glass plate into a TBE + 0.5 µg/ml ethidium bromide solution for 15 minutes. The gel is transferred onto a black plexiglass background for photography.

d) To dry gels, the gel and glass plate are immersed in 1000 ml of 5% glycerol for 30 minutes with occasional agitation. This step is particularly important for acrylamide urea gels. The gel is transferred onto wet Biorad drying paper, covered with saran wrap, and placed on the gel dryer. TBE gels take 2-3 hours to dry, and acrylamide- urea gels take 3-6 hours to dry. The longer time corresponding to high percentage gels.

2. Preparative Gels

a) Preparative gels differ from analytical gels only in the width of the wells. For a 100 µg sample usually a 2.5 cm well is used.

b) To visualize the bands the gel is transferred onto a saran wrap covered fluorescent PEI plate. The gel is illuminated with a UV light source (short wave if possible, long wave alone doesn't work) DNA absorbs the fluorescence of the PEI plate, and is seen as a dark band on a bright background.

Maniatis, T. et al., Biochemistry 14: 3787-3794.