Biotechnology and Bioinformatics Summer Camp 2010

Acrylamide Gels

Acrylamide gels give better resolution of nucleic acids than agarose gels. They are thinner and give better sensitivity to bands where we have less material. Generally you stain these after running them – you can use either ethidium bromide or SYBR Green.

Overview:

Prepare the vertical gel sandwich.
Prepare the acrylamide solution.
Pour the gel.
Slot the gel into the upper buffer chamber and seal with agarose.
Add buffer, check for leaks.
Aliquot out samples and mix with loading dye.
Load samples into gel wells.
Load size standards into edge wells.
Electrophorese
Stain
Document bands on GelDoc station
Use GelDoc software to analyze the size and intensity of the gel bands.

1. Acrylamide Gels: we are going to pour 8% acrylamide gels, having 18 wells. You will load 3ul of your sample + 1ul of 4X loading dye per well of your ISSR samples. You will electrophorese at 120V. Because we are not adding a denaturant, the DNA will stay double stranded.
    a. Prepare the apparatus: take two glass plates, 3 spacers, one comb and 9 clips.
        i. Make a sandwich of the plates, with the spacers along 2 edges and the bottom, put the comb on top. Clip the spacers into place, 3 per side and bottom, none on top.
        ii. Seal along the side edges and bottom, where the spacers are (not on the top) with melted agarose (1% in water is fine).
    b. Prepare the gel solution. We have a 30% stock of acrylamide, and will run this in 1X TBE. Each gel sandwich requires about 8ml, so if you make 10ml you will have plenty. In an 25 ml flask add:
        i. 10ml (8%) = x ml (30%) so use 2.6 ml of acrylamide stock
        ii. 1 ml of 10X TBE stock
        iii. Add 6.4 ml of water, swirl to mix
        iv. Add 6ul per ml of 10% Ammonium persulfate (APS) = 60ul
        v. Add 0.5ul per ml of TEMED = 5ul
        vi. Swirl quickly and thoroughly to mix
        vii. Pipette the solution using a green pump and a Pasteur pipette, down one side of the gel (just inside a side spacer). Avoid air bubbles. Fill to the top, then push down the comb so the tops of the teeth are in contact with the solution– you want as little contact with the air as possible.
        viii. You will be able to tell when polymerization has occurred by the difference in the diffraction of light around the wells of the comb.
    c. A test for complete polymerization: pull some of the solution up into the Pasteur pipette, let it sit – when it is polymerized your gel should be as well.

   d. Remove the comb and bottom spacer, leave the side spacers in place even though you remove the clips.
   e. Slide the gel into the gel box. Clamp in place with two clips, one on each side, where the top chamber extends down.
   f. Seal around the edges of the entry slot with melted 1% agarose.
   g. Add 1X TBE buffer (you will need ~600 ml per gel rig, half in the top and half in the bottom).
   h. Load samples (the wells will hold ~12 ul if well-formed). Don't forget to reserve the end lanes for size standards, and to load them last.
   i. Attach the electrodes (red at the bottom), set on constant voltage at 120V, electrophorese for 2 hours
   j. Stain with SYBR Green dye (in 50 ml of water put 5ul of dye) for 5 minutes
   k. Rinse in ddH2O for 15 seconds.
   l. Place on transilluminator in Gel Doc.
   m. Take picture
2. Sample prep: remove 3 ul of each sample, add 1ul of loading dye, pipette into the wells. This can be done in tubes but is usually easier on a piece of Parafilm, since it will bead up there. For the acrylamide gel you may want to heat denature the size standard for 1 minute at 95C in a temperature block (then quick-spin in a microfuge) in order to have single-stranded product to compare to your unknown.

Materials needed
Acrylamide gel rig: 2 glass plates, 3 spacers, 1 combs,9 clips, gel tanks (top and bottom), electrodes
Agarose for sealing

15 ml Falcon tubes to mix gel components
5 ml serological pipette
10 ml serological pipette
10X TBE
Molecular Biology Grade water
30% acrylamide stock
TEMED
10% APS

4X loading dye
DNA ladder for sizing

SYBR Green

Gel Doc station