Acrylamide Gels for Resolving Small (25bp-700bp) DNA fragments

Procedure:

1. Assemble gel apparatus (Stockinger lab has two different apparatus, a mini gel from Idea Scientific and a big gel apparatus from Hoefer; for more specific information see also the directions from Idea Scientific or Amersham/Hoefer).

After each use the glass plates should be washed using soap, hot/warm water and a soft sponge. Rinse the plates first with hot/warm water and then tap dH_2O. Place the plates into the slotted plate holder to drip/air dry and for storage. Similarly all combs, spacers, cams, clamps, etc should have been washed, or the very least, rinsed of all residue after each use.

Locate a clean & level working surface (your bench). Lay one plate onto the bench. Place a spacer on each side. (Use the SpacerMate to help keep the spacers on the edge of you need to.) Lay the second plate on top of the spacers. Pick the plate sandwich up and place a clamp on each side. (Loosely tighten one screw on each clamp in order to help position things.) Place the gel sandwich so that it stands vertical on either your bench or into the gel casting cradle (without the gasket), loosen the screw on each side so that the spacer drops flush with the surface of the bench top (or gel cradle). Tighten all the screws along both clamps firmly but not overly. Insert the gasket, thin, gray-side up into the cradle (remove the gel first) and place the gel sandwich onto the gasket. Insert the cams into the holes on the sides and turn until they lock vertically (120^o to 150^o and the glass edge will appear to darken as it seals against the plate).

2. Prepare the appropriate acrylamide concentration depending on the size of DNA fragments that you want to resolve. See Table 5-6 in Sambrook and Russell, Molecular Cloning 3rd ed VIII, p5.42 for different acrylamide concentrations and the effective range of DNA fragment sizes separated. Add the APS and dissolve. Then add the TEMED immediately prior to pouring the gel.

Stock Solution	Vol. for one 50ml thick gel:	
	5% Gel	12% Gel
ddH_2O	31.67ml	20.0ml
10X TBE	5.0ml	5.0ml
80% Glycerol	5.0ml	5.0ml
30% Acrylamide	8.33ml	20.0ml
APS	32mg	32mg
TEMED	25µl	25µl

3. Insert the selected comb into the gel assembly apparatus. Do this so that the one end of the comb is all the way in and the other is just barely in; i.e., the comb sits at a diagonal.

4. Obtain a syringe that will hold the volume of gel solution to be poured. Fit the syringe with a needle that will fit in between the glass plate sandwich (Use 18g1-1/2 needle for 1.5mM spacer thickness gels; 22g1-1/2 for 0.75mM spacer thickness). Draw the gel solution up into the syringe. This will take a little bit of effort. Tip the gel and gently syringe in the gel solution in between glass plates allowing it to run down the lower side of the gel (be careful not to chip the glass plates with the metal part of the syringe). Ideally this should be done such that no air bubbles are introduced into the gel solution. If the gel polymerizes before you get it all out of the syringe try chilling the gel solution on ice next time before adding the Temed; this will slow down the polymerization.

5. Let the gel polymerize ~40min +.

Note: You may leave this overnight; simply cover the top portion of the gel with saran wrap so that the gel does not dry out.

6. Remove comb by pulling it out using one motion.

7. Add 1X gel running buffer to the wells. Wash the wells by pipetting the buffer up and down.

8. Load samples into wells
9. Use an appropriate sized marker (pTZ19U cut with *Hpa*II is a great marker).
10. Place gel assembly to lower reservoir chamber.
11. Attach upper reservoir chamber.
12. Add 1X TBE buffer to the chambers.
13. The large 15cm gels can be run gel at ~70V 14-16h; or at ~125-150V for 2-4 h to resolve 25-700bp fragments.

Stock Solutions:

30% Acrylamide (100ml) (Note that this recipe differs slightly from that in Ausubel but this is what I have always used as 30%.)

(a)	Acrylamide	29g
(b)	Bis-acrylamide	1g
(c)	ddH_2O to	100ml
(d)	Filter through 0.45μM filter	

Add a small quantity of BIO-RAD AG 501-X8 (D) ion exchange resin; i.e., enough to cover bottle bottom.

Store in brown bottle @ 4°C.

Acrylamide hydrolyzes to acrylic acid and ammonia. Normally it should be made and used with in one month. However the ion exchange resin absorbs free radicals and this allows much longer storage. I have stored mine for 4 months plus this way.

10% Ammonium persulfate (APS)

(a)	APS	100mg
(b)	ddH_2O	1ml
(c)	Store at –20°C	

APS does not last very long at 4°C. If you make up a large volume (e.g., 10ml) of stock solution, then aliquot it out into 1.0ml volumes; freeze all but your working stock at –80°C and store the working stock at -20°C. Alternatively, simply go directly to the APS stock in the RT desiccator, scoop out a tiny portion of APS and drop this directly into the gel solution. This is not a very accurate way to do things; however APS merely serves as a catalyst for the polymerization of acrylamide and so its exact concentration in the reaction is not critical (unless you add way too much and your gel polymerizes before you get it out of the syringe.)

10X TBE

	1L	2L	4L
Tris Base	108g	216g	432g
Boric Acid	55g	110g	220g
EDTA	7.45g	14.9g	29.2g
ddH_2O to volume	1L	2L	4L
Filter through 0.45μM			
Store RT			

Gel loading buffer dye (good for just about all applications)

	50ml	5X Conc
80% Glycerol	47ml	75%
Bromophenol Blue	125mg	0.25%
Xylene Cyanol	125mg	0.25%
1M Tris (pH.7.4)	0.5ml	10mM
5M NaCl	0.1ml	10mM
0.5M EDTA	1.0ml	10mM
10% SDS	0.5ml	0.1%

The general rule of thumb is to use 1μL of gel loading buffer dye per 5μL of sample.

Acrylamide Elution Buffer (for crush and soak method of DNA band extraction from acrylamide gels)

Stock Solution	Volume	Final Concentration
NH_4Ac (10.0M)	5.0ml	0.5M
MgAc (1.0M)	1.0ml	10mM
EDTA (0.5M)	0.2ml	1mM
10% SDS	1.0ml	0.1%
ddH_2O	92.8ml	

Sterile filter (NH_4Ac is volatilized by autoclaving)