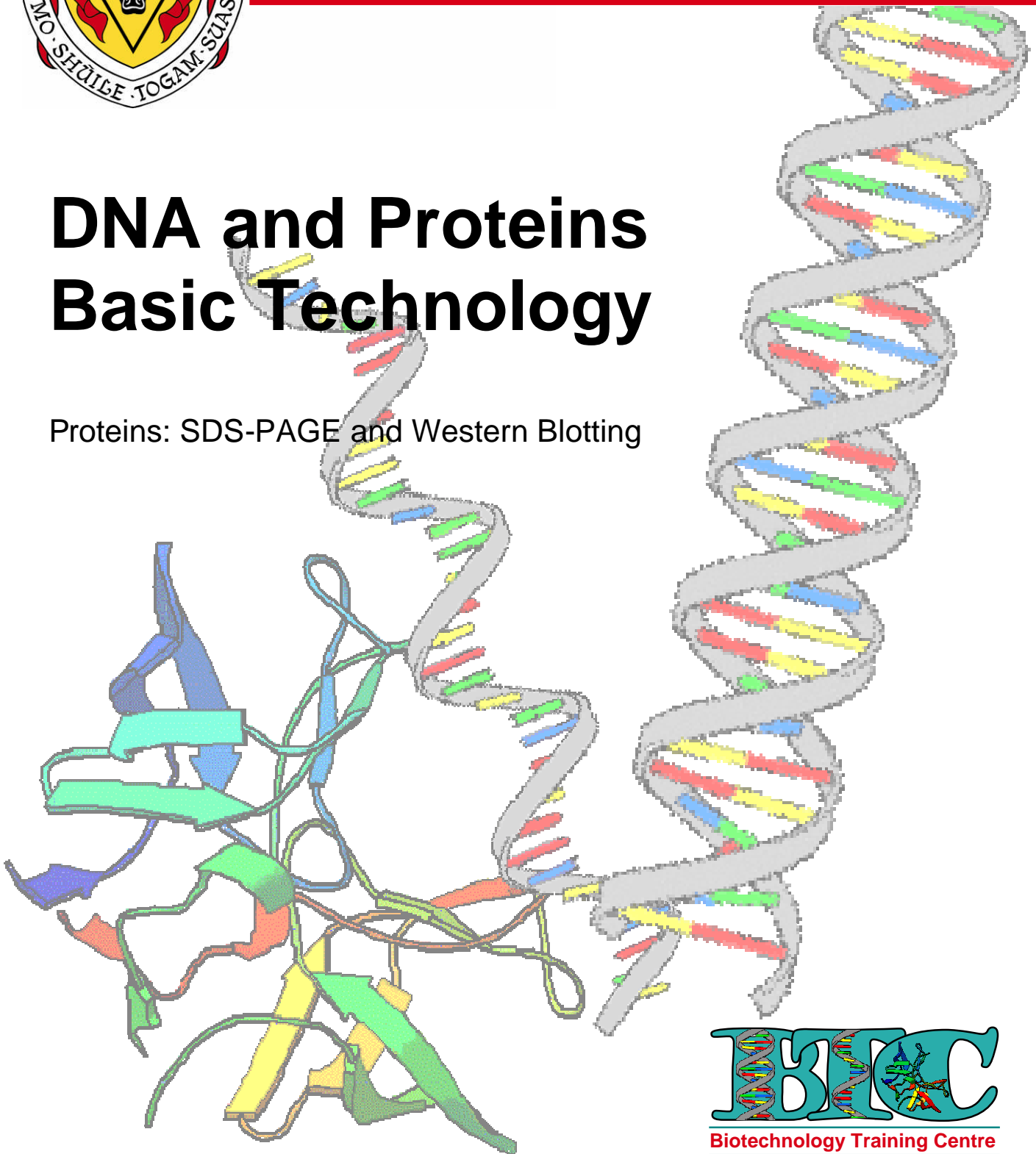




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# DNA and Proteins Basic Technology

Proteins: SDS-PAGE and Western Blotting



Revised: April 2006

## Pre-Detection: Electrophoresis and Blotting techniques

Blotting is the method of putting DNA, RNA or protein onto a membrane for further studies and detection. Usually prior to blotting the molecules are separated based on size or mass by electrophoresis. Like total protein electrophoresis, DNA and RNA can be separated by size/mass, but charge is irrelevant (DNA and RNA are negatively charged). In protein electrophoresis for molecular biology, proteins are separated by mass as charge is made negative by the pH of the buffer used. As there are no issues with charge, the only difference with the molecules position in the matrix following electrophoresis is the length of the sequence. Thus it is possible to determine the length of a piece of DNA or RNA or mass of a protein by its position in a gel relative to a standard marker. This type of analysis is important in epidemiology, forensics and in just checking up on your PCR reaction. For proteins many techniques are available to determine function as well as mass.

The first blotting technique described was the Southern blot; published in 1975 by E. M. Southern. He described a technique for detecting specific DNA fragments after electrophoresis so a specific gene could be isolated from a complex DNA mixture. Since then, electrophoresis/blotting has been done with RNA (northern blots) and proteins (western blots). There has been lots of speculation on how you would run an eastern, but no one has solved it to date. [Note that the only technique that earns a capital initial is Southern!] Blots done without electrophoresis are called dot assays or dot blots or slot blots if a linear slot was used – often with filter paper migration of the target towards the probe. All blotting assays are qualitative; they can be made quantitative depending on the fine, painstaking details of specific protocols.

Western blots and protein dot blots are detected using specific antibodies, antibody conjugates and substrates. Western blots differ from DNA/RNA blots in that electrophoresis is used to accomplish the transfer of the proteins from the acrylamide gel to the membrane. For more on Western blots – animated!

## SDS-PAGE Recipes

Acrylamide is a crosslinked polymer gel that enables the use of small amounts of sample, separation of very similar pieces (sequencing gels for example), is optically transparent and is able to take a great deal of heat during the electrophoresis run. Buffers are added to the gels when they are made.

**For BTC workshops resolving GFP, 10% gels are used.**

**A minimum of 10 mLs of resolving gel is needed for 2 0.75mm gels and 15 mLs is needed for 1mm gels. All volumes are in mLs.**



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<b>Resolving Gels</b>					
<b>10%</b>	5 mL	10 mL	15 mL	20 mL	25 mL
H <sub>2</sub> O	1.9	4.0	5.9	7.9	9.9
1.5 M Tris (pH 8.8)	1.3	2.5	3.8	5.0	6.3
30% Acryl/0.8% bis-Acryl	1.7	3.3	5.0	6.7	8.3
10% SDS	0.050	0.100	0.150	0.200	0.250
10% APS	0.050	0.100	0.150	0.200	0.250
TEMED	0.002	0.004	0.006	0.008	0.010
<b>12%</b>	5 mL	10 mL	15 mL	20 mL	25 mL
H <sub>2</sub> O	1.6	3.3	4.9	6.6	8.2
1.5 M Tris (pH 8.8)	1.3	2.5	3.8	5.0	6.3
30% Acryl/0.8% bis-Acryl	2.0	4.0	6.0	8.0	10.0
10% SDS	0.050	0.100	0.150	0.200	0.250
10% APS	0.050	0.100	0.150	0.200	0.250
TEMED	0.002	0.004	0.006	0.008	0.010

<b>Stacking Gels</b>	1 mL	2 mL	3 mL	4 mL	5 mL
H <sub>2</sub> O	0.6.8	1.4	2.1	2.7	3.4
1.0 M Tris (pH 6.8)	0.13	0.25	0.38	0.5	0.63
30% Acryl/0.8% bis-Acryl	0.17	0.33	0.5	0.67	0.83
10% SDS	0.010	0.020	0.030	0.040	0.050
10% APS	0.010	0.020	0.030	0.040	0.050
TEMED	0.001	0.002	0.003	0.004	0.005

### **Buffers**

#### 2x Sample Buffer:

100 mM Tris-Cl, pH 6.8

20% Glycerol

4% SDS

0.2% Bromophenol Blue

200 mM DTT or 10% BME (If using DTT, add from a 1 M stock just before use.)

#### Running buffer:

25 mM Tris

250 mM glycine

0.1 % SDS

Prepare a 5X running buffer by dissolving 15.1 g of Tris base and 94 g of glycine in 900 mL of dd H<sub>2</sub>O. Add 50 mL of 10% SDS and adjust to 1000 mL with H<sub>2</sub>O.

Recipes as described in Sambrook and Russell. Molecular Cloning: A Laboratory Manual. 3rd Ed. 2001. Cold Spring Harbor Laboratory Press. pA8.43

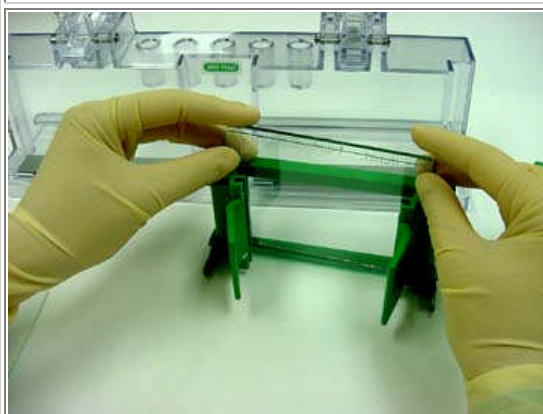
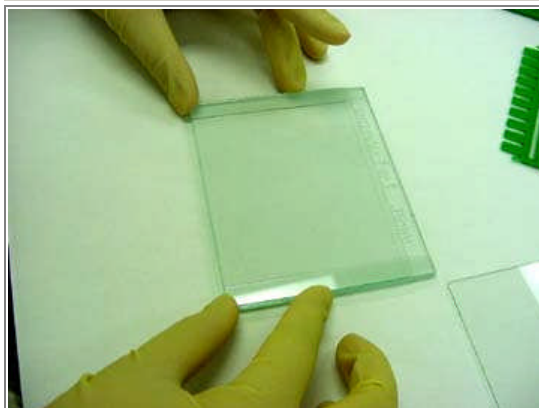
## SDS-PAGE (with the BioRad Mini-PROTEAN 3 Cell)

The protocol below illustrates SDS-Polyacrylamide Gel Electrophoresis (PAGE) using the BTC equipment (BioRad). Specifics of the protocol for SDS-PAGE will change based on the directions for your apparatus.



Clean the plates REALLY well (squeaky clean!) with ethanol or Windex.

Stack the plates on top of each other, (thin one on top). Make sure the bottom of the plates is aligned.



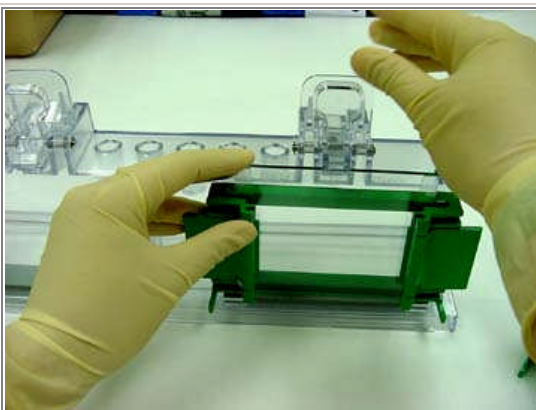
Place the plates into the casting frame keeping the short plate facing front. Ensure both plates are flush on a level surface.



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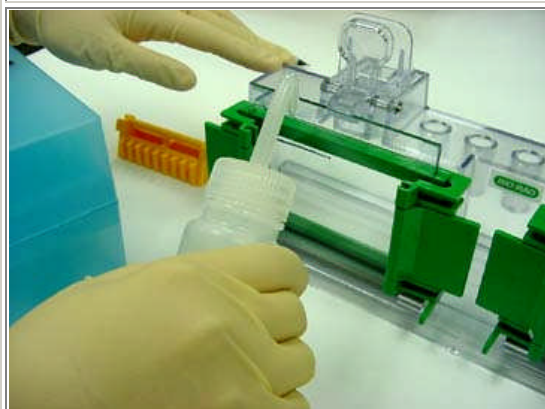
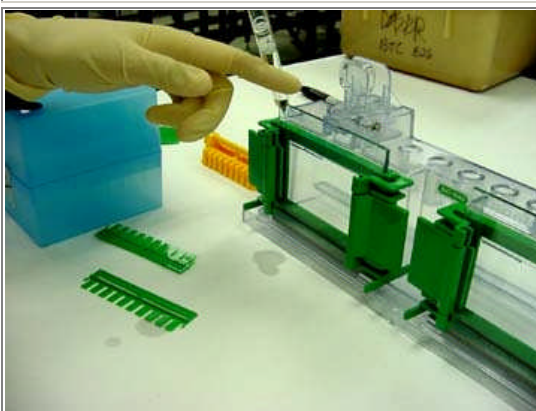


Lock the pressure cams to secure the glass plates. Make sure the rubber pad is seated correctly and its surface is clean and smooth. Secure the plates in the casting stand by engaging the spring loaded lever.



Insert the comb into the space between the thick and the thin plate, then using a marker make a line a few millimeters under the comb indicating the maximum gel level.

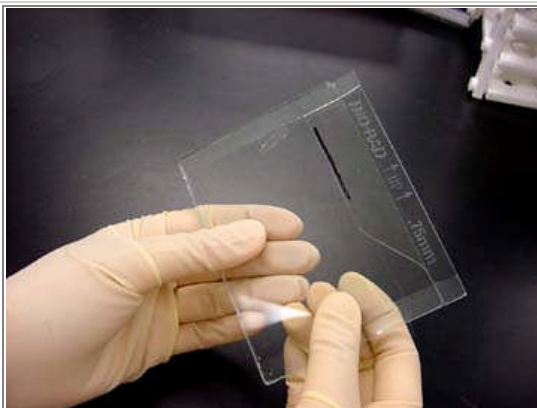
Make the gel solution according to the recipe for the % and volume needed. Do not add TEMED until you are really ready to cast the gel. Remove the comb and using the pipette insert the gel in between the glass plates. Stop when gel level reaches the marker line.



The gel needs to set for 30 min or so. To prevent the gel from drying out, add a layer of water or butanol on top of the gel.

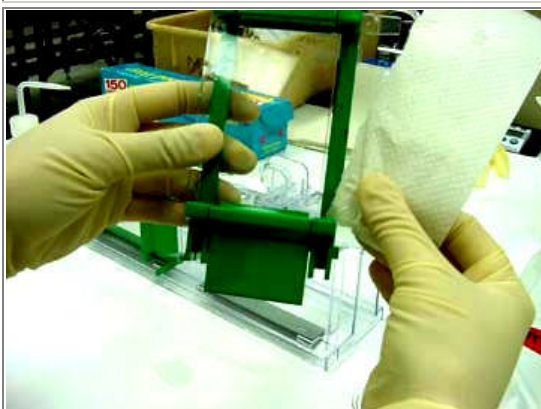


You should be able to see the set gel as a line of change in the refraction between the water and gel layers. Once the gel has set, remove plates from the casting frame. Wash with water to remove any unlinked acrylamide.



Add water to the space and wrap the plates in two layers of saran wrap. Proceed to pouring the stacking gel or place plates at 4°C until use.

To dispose of extra gel, use a spatula to scrap off beaker into the biohazard waste bin.



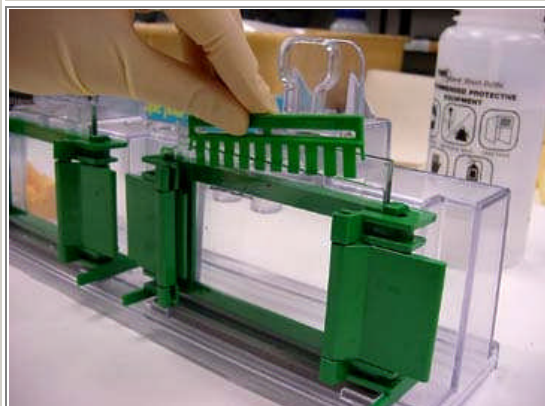
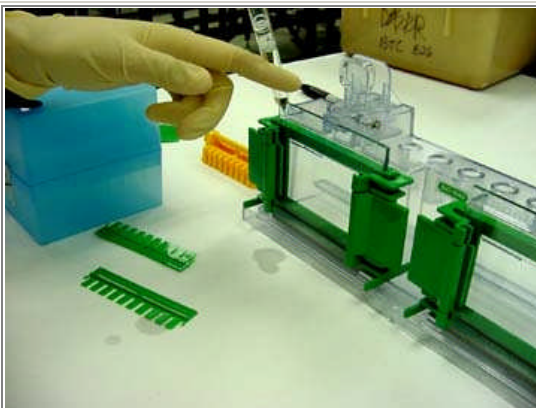
Clean the top part of the space between the plates. Prepare the stacking gel.



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Pour the stacking gel.



Insert the comb and allow the gel to set.

Remove the comb carefully.



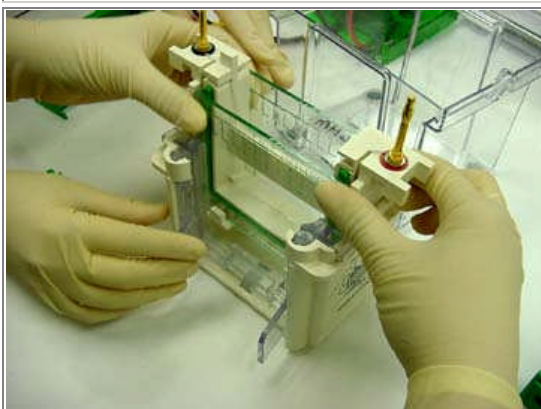
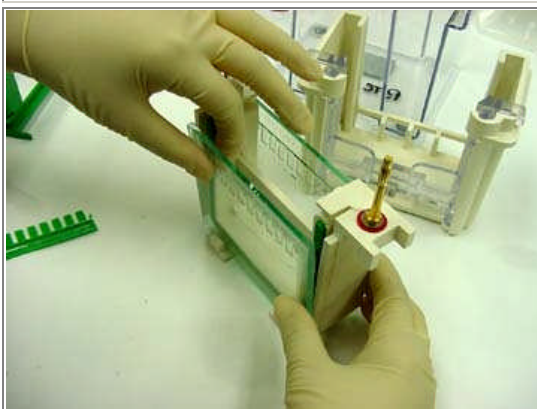
Wash the wells with distilled water really well at least 3 times to make sure there are no bits of acrylamide floating in the wells.

Drain the wells really well between washes by blotting on tissue paper.



Using filter paper wicks, ensure the wells are really free of bits of acrylamide if present.

Mount gels with short plate to the center to create the electrode well.



Assemble gel apparatus according to manufacture's protocol.

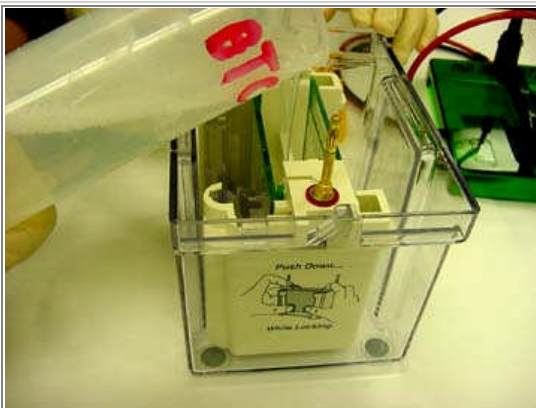




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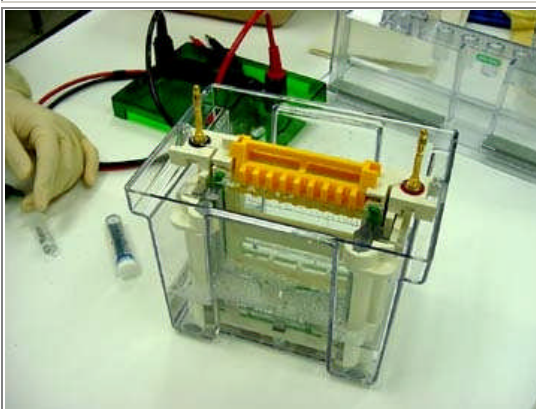


Pour running buffer into center or top well.



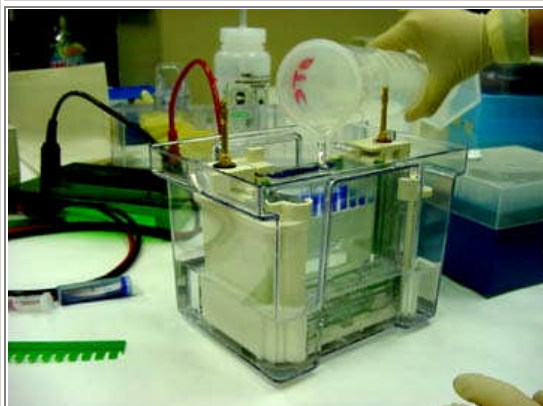
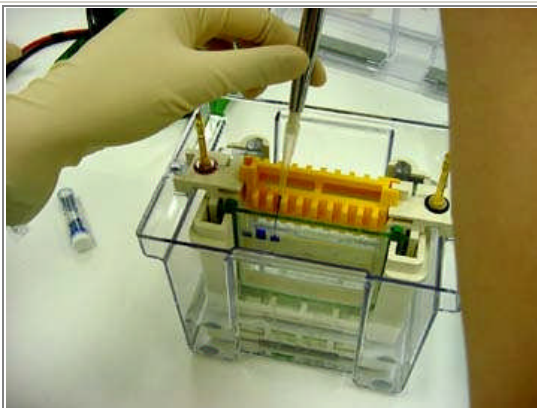
Pour running buffer into the outer or bottom well.

Ready to load.



Add equal volumes of the sample and 2X loading dye. After preparing samples, place them in boiling water for 3 min. Remove and keep on ice or at  $-20^{\circ}\text{C}$  until loading the gel.

Load the samples into the wells using a gel loading tip. Remove the casting guide.



Remove the loading guide. Top up center well with running buffer if required.

Put on the top and connect electrodes.



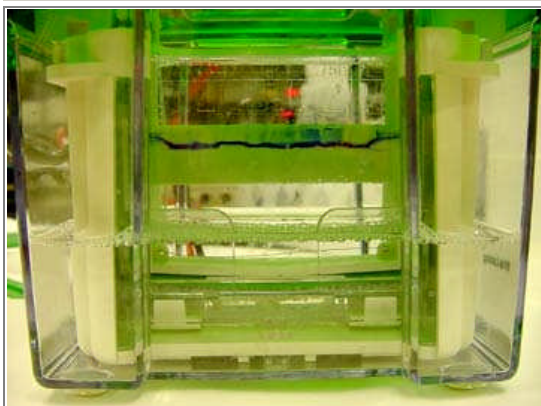
Plug in electrodes to the powerpack, ensuring red to red and black to black.



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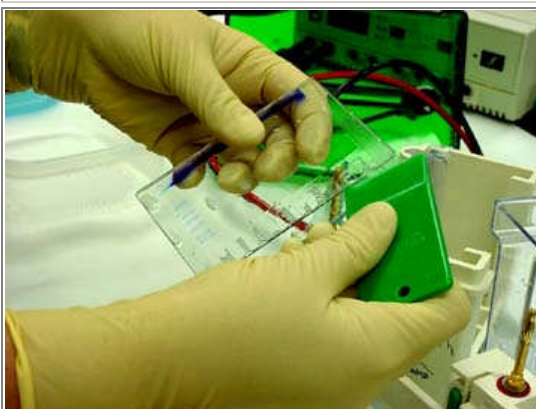


Start the current. Run until the samples completely enter the stacking gel. As the gels run, the apparatus heats up and the center well buffer level can fall or leak. Be prepared to check that a) the gel is still running and b) top up the center well if needed.



When the blue dye front reaches the resolving gel, you can increase the current. Run until the dye front reaches the bottom of the gel plates. Turn off the current.

Disassemble the apparatus and pry the plates apart. The gel should stick to one plate.



Remove the stacking gel from the resolving gel and discard. Identify the order of your lanes by nicking the gel in a corner.



Flip the gel into  
Coomassie Blue stain for protein  
staining, fixative for silver staining  
or  
into transfer buffer for [western  
transfer](#).



Coomassie Blue Staining:  
Put the gel and stain on a platform-  
rocker for 30 min.

Pour off the stain and rinse the gels  
with water or previously used  
destain.



Add fresh destain to the gels.

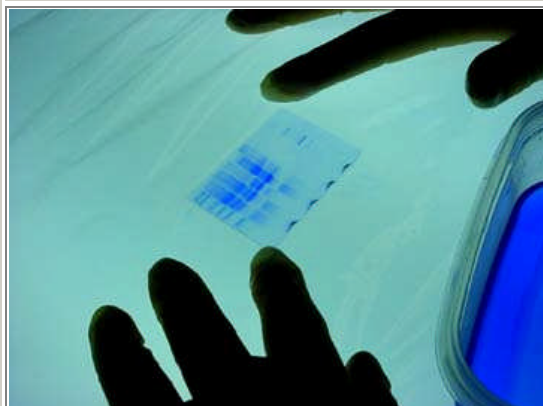




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During destaining, a kimwipe or kleenex can be used to absorb the dye. Put back on the platform-rocker and destain until background is clear.



Check the gel to make sure if it's sufficiently destained.

Mount the gel onto a piece of blotting paper.



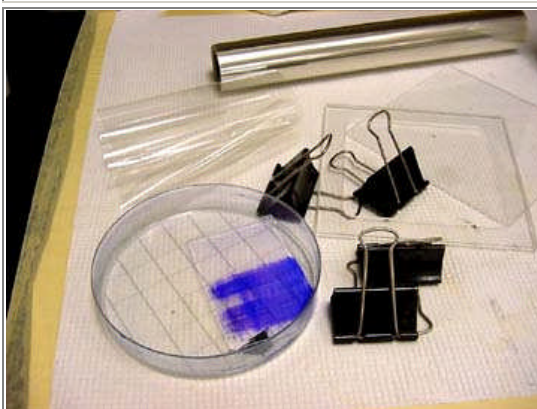
Cover the gel with plastic wrap.

Place the gel, blotting paper side down, in a vacuum blotter.



Dry under vacuum with 80°C heat for 1 hr.

Alternately, gels can be dried in a frame sandwiched between pieces of dialysis membrane.  
Hint: Soaking your gel in destain plus 30% glycerol may prevent cracking during drying.



Wet the dialysis membrane.

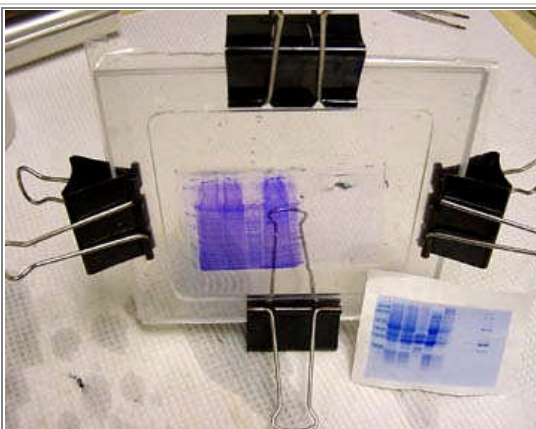


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Sandwich the gel between two pieces of wet membrane on the drying rack. Smooth out all bubbles. Apply the frame and clamp. Let dry overnight or as long as it takes.

Either method leaves you with a permanent record of your gel. Gels dried on membrane can be quantitated by densitometry.



BioRad Mini-PROTEAN 3 Cell. More information can be found at [www.biorad.com](http://www.biorad.com)

Credits: Models, reagents, text and layout - Jennifer Sparling, Lesley Ing, and Vicky Lau (2002 summer volunteers from St. Mary's High School, Calgary, AB); Wendy Hutchins, Drew MacCannell - photos

## Western transfer of proteins to membrane using MiniTransblot (BioRad)



When the SDS-PAGE is done, remove the gel from the apparatus and pry the plates apart. Remove the stacking gel and discard. Flip the gel into transfer buffer.

Cut a piece of membrane larger than the gel. Do not touch the membrane with bare fingers.

