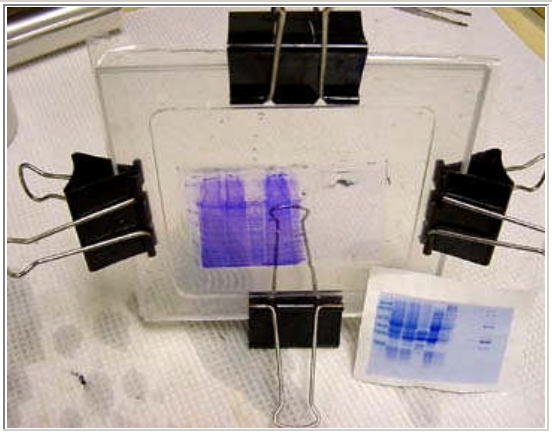
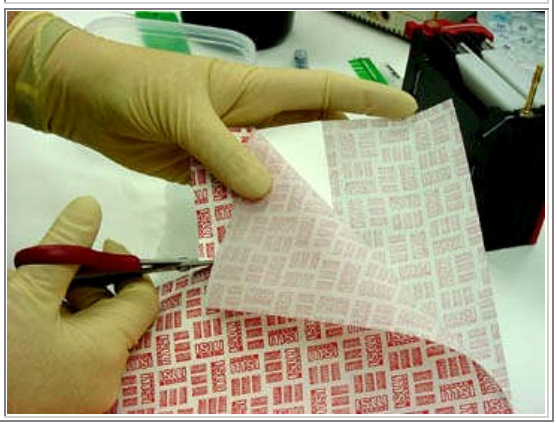
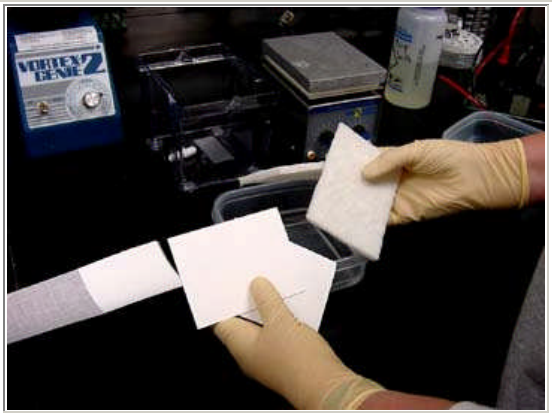
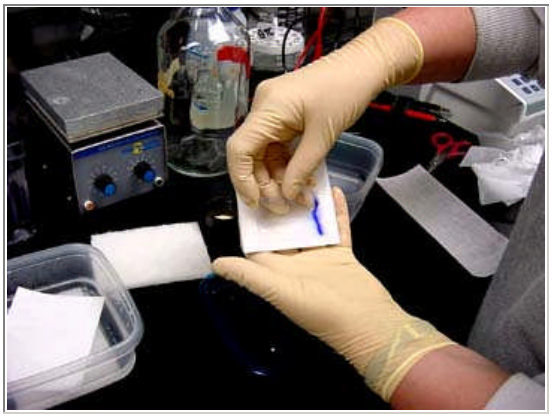
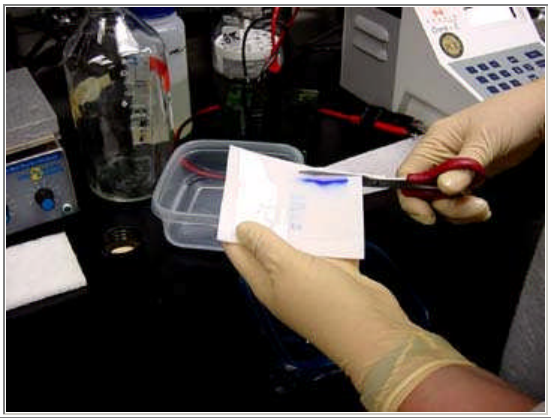
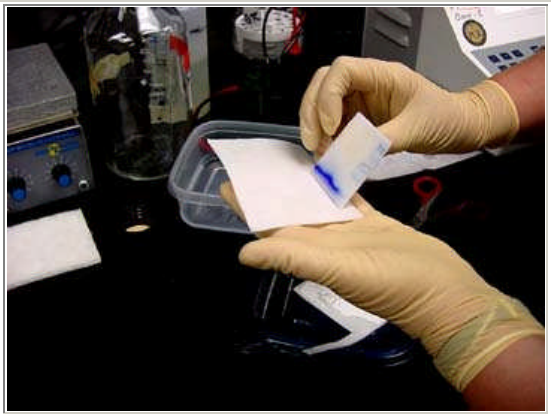
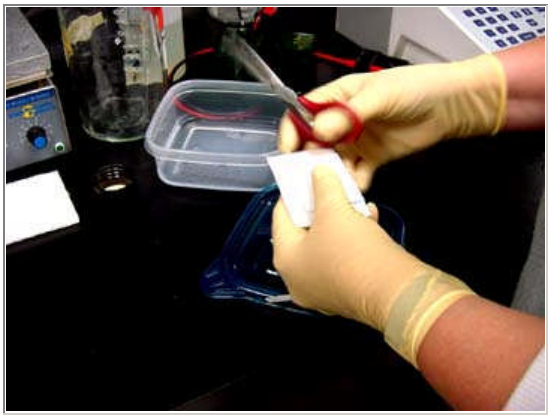
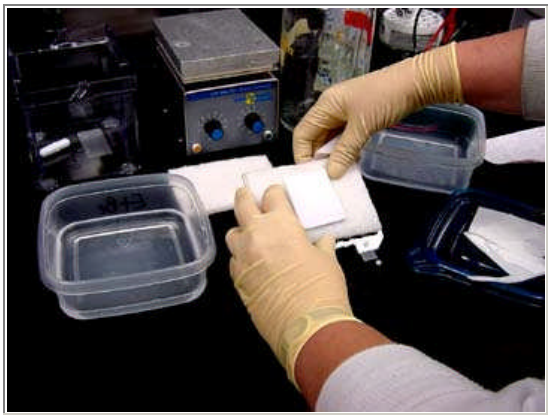
Sandwich the gel between two pieces of wet membrane on the drying rack. Smooth out all bubbles. Apply the frame and clamp. Let dry overnight or as long as it takes. Either method leaves you with a permanent record of your gel. Gels dried on membrane can be quantitated by densitometry.	
BioRad Mini-PROTEAN 3 Cell. More information can be found at www.biorad.com	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.	

	Gather filter paper and fiber pads (2 of each). Soak in transfer buffer.
If using PVDF membrane, wet in methanol then transfer buffer. For nitrocellulose, wet in transfer buffer only. [For BTC workshops, use PVDF membrane.]	
	Place the membrane on a filter pad. Apply the gel to the wet membrane.
Trim the membrane and filter pad to the size of the gel.	

	Apply the other filter pad to the other side of the gel.
Trim the filter pad to the size of the gel/membrane/filter already cut.	
	Place the gel/membrane sandwich to the fiber pads.
Assemble the sandwich in the gel holder. [For the BTC equipment, the gel side goes to the black side of the holder.]	

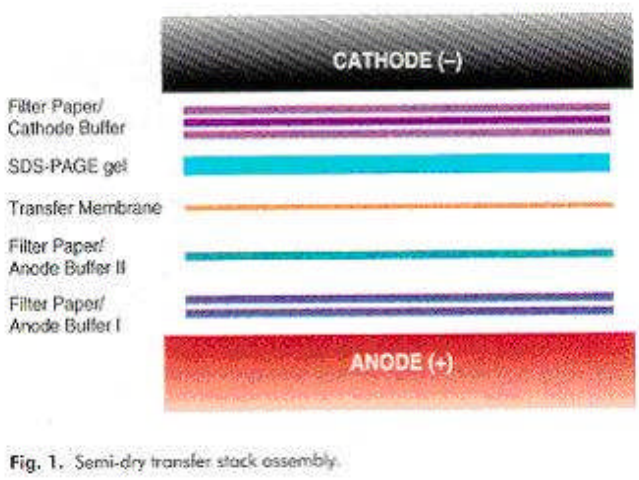
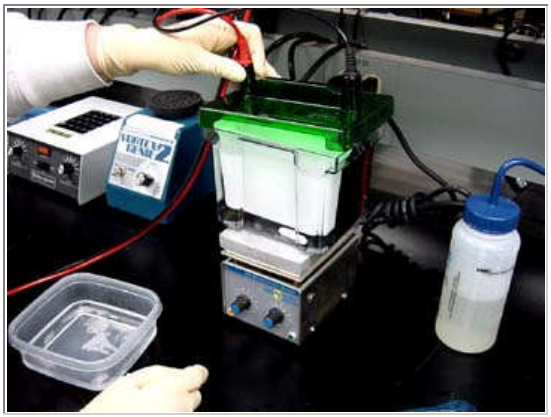
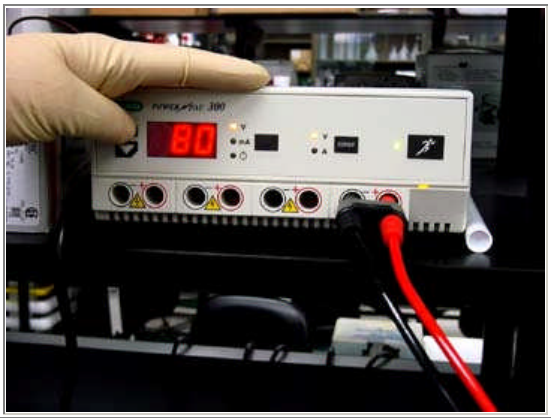


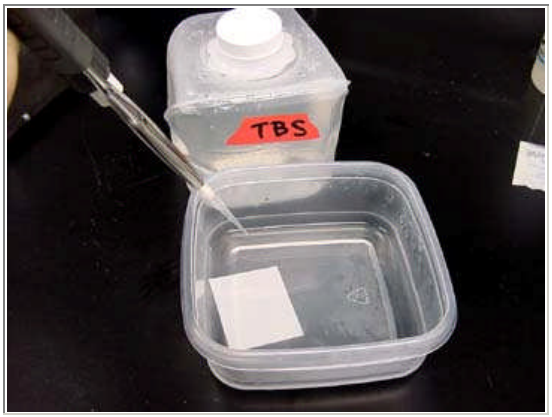
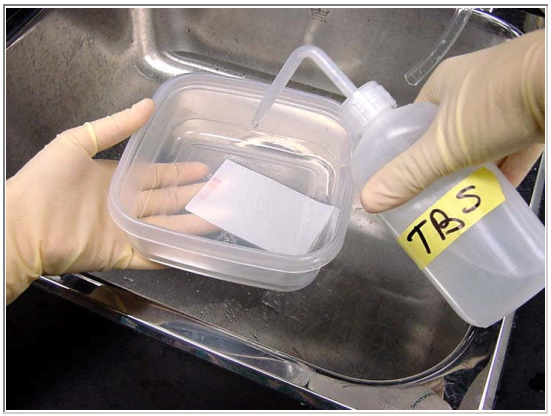
Fig. 1. Semi-dry transfer stack assembly

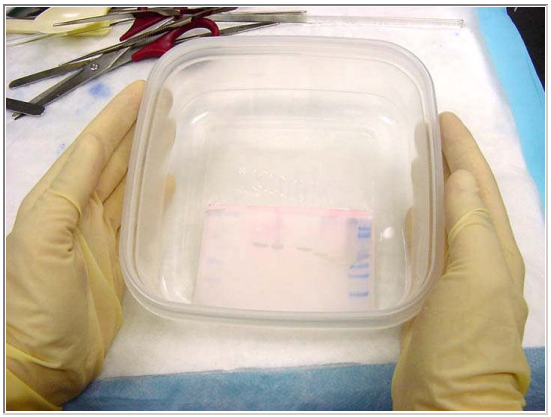
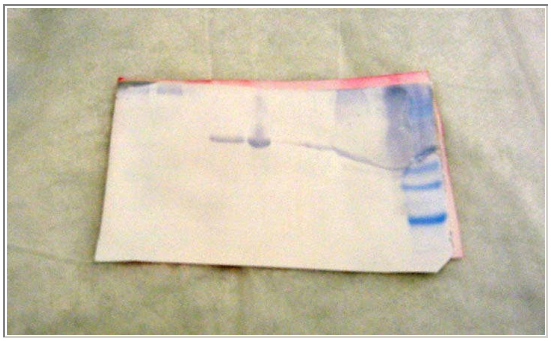
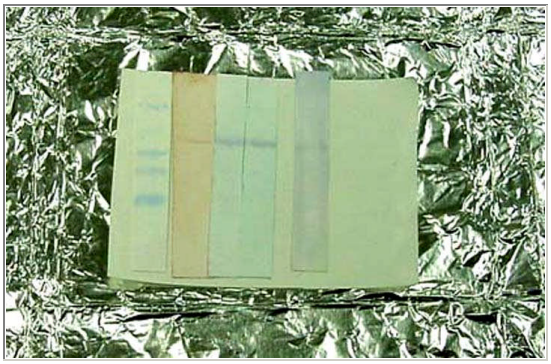
The final gel/membrane sandwich should look like this.

Assemble the sandwich holders into the electrode (black to black).

Place the electrode into the gel box.

	Insert an ice block.
Fill the chamber with cold transfer buffer.	
	Add a magnetic stir bar to the chamber and place the apparatus on a magnetic stirrer. Put the top on the chamber.
Transfer the proteins from the gel to the membrane by applying current. [For BTC workshops, 1 hour at 80 volts.]	

	After transfer is complete remove the membrane from the sandwich and place into block solution and incubate with moderate shaking for 1 hour at RT. [For BTC workshops, 1% skim milk powder in TBS.]
Add primary antibody at the appropriate dilution to the block solution and incubate with mixing for 1 hour.	
	After washing the gel 3-4 times with TBS, add the secondary antibody at the appropriate dilution and incubate with mixing for 1 hour.
Wash the blot with TBS 3-4 times.	

	Add developer.
Rock the blot by hand or on a platform rocker, watching for the developing lines of protein.	
	When development is complete, wash the blot with water and allow to dry.
To store blots, keep them in the dark to prevent fading.	
Credits: Models, reagents, text and layout - Summer volunteers from St. Mary's High School, Calgary, AB - With thanks and hope you had fun!; Wendy Hutchins - photos	

Detection by HRP-conjugates using KLP Chemiluminesence kit.

Reagent preparation

Detector Block Solution – to be prepared fresh daily

1. Based on the total desired 1X Detector Block volume, weigh out 0.2% - 1% w/v Detector Block Powder. For detection with AP and CDP-Star, use 0.2% and for detection with HRP and LumiGLO, use 1%. (.2-1g of Detector Block Powder per 100 mL of 1X Solution)

2. Place the Detector Block Powder in a flat-bottom, screw cap container and add molecular biology grade water to a volume equivalent to 4/5 of the total desired 1X Detector Block volume. Shake the container vigorously until the powder is fully solubilized.

3. Dilute the solution with 1:5 v/v 5X Detector Block Solution. Ex., for 50 mL of 1X Detector Block:

Detector Block Powder		0.1 g (AP/CDP-Star detection only)
	or	0.5 g (HRP/LumiGLO detection only)
Molecular Biology Grade H_20		40 mL
5X Detector Block Solution		10 mL

Biotin Wash Solution (HRP and LumiGLO detection only)

1. Dilute 1 part 10X Biotin Wash to 9 parts molecular biology grade water. Mix well.

The above reagent is used for biotinylated DNA probes used in Southern blotting protocols. For Western blots, you can use TBS or PBS or these plus Tween 20 detergent. [Also for Western blots, blocking with milk powder is effective for this reagent.]

LumiGLO – to be prepared fresh before its use (HRP and LumiGLO detection only)

1. Mix together 1 part LumiGLO A to 1 part LumiGLO B.

Protocol

Detection with HRP-SA and LumiGLO

Or any other HRP-conjugate

Steps

1. Prepare enough 1X Detector Block blocking/diluent solution for the block step and for the HRP-SA conjugate dilution.

2. Incubate Southern blot with 1X Detector Block Solution for 30 minutes in a tray approximately the same size as the blot.

Southern, Northern or Western

3. Dilute HRP-SA conjugate at 1:500 in fresh 1X Detector Block (60 µL conjugate + 30 mL blocking solution). Mix well.

If you use a measured amount of block, you can add your 1° detection to the block solution.

Conjugate dilutions are supplied by the manufacturer and can vary from this.

4. Pour off the blocking/diluent solution (from step 2) from the membrane and add the diluted HRP-SA solution. Incubate for 30 minutes **with rocking.**

5. Transfer membrane to a clean container. Wash the membrane in 1X Biotin Wash Solution. Perform 3 washes for 5 minutes each.

The reaction will proceed faster if your last wash is TBS alone. The Biotin wash contains detergent that can sometimes interfere with the enzyme/substrate reaction.

6. Prepare enough LumiGLO Chemiluminescent Substrate to completely immerse the membrane by mixing equal volumes of Solutions A and B.

7. Incubate membrane for 1 minute in LumiGLO Chemiluminescent Substrate. Blot membrane on filter paper to remove excess substrate. Place membrane in a hyb bag or between sheet protectors and expose to X-ray film for an initial exposure of 10 minutes. Adjust exposure time for optimal signal-to-noise ratio.

8. Develop film either manually or by using a mechanical processor.

NOTES ON...LumiGLO Chemiluminescent Substrate

- LumiGLO can be used with nitrocellulose, nylon, and PVDF membranes.
- The LumiGLO reaction does not need to be protected from light.
- For maximum signal, expose membrane to film immediately after incubation with LumiGLO. The reaction and film exposure are performed at room temperature.
- For most applications, exposures of one hour or less produce sufficient sensitivity.
- LumiGLO is an extremely sensitive substrate. Insufficient washing of membranes or contamination of substrate with HRP will result in non-specific background. Following incubation with HRP-SA, it is important to transfer membranes to a clean container and wash thoroughly to remove excess enzyme and prevent background problems. Always use a clean container for the substrate incubation.
- Do not allow LumiGLO to contact film. LumiGLO solution will case dark spots to appear on the film.

A special thank you is given to KPL and Mandel Scientific, the Canadian supplier of KPL kits, for the kind donation of some of the kits in use and for the exceptional pricing offered on others. Over the past years, several workshops and courses, and a group of high school coop students have benefited from their generosity.

Notes: