2.3 Load the GFP samples in the center wells and put spaces in between other groups's samples if possible (15ul per lane). There are only 2 total protein standard samples for each gel and they are loaded into the leftmost and rightmost lanes (7.5 per). **Remember to make a legend of the gel lanes so you can identify which group is responsible for which lane.*

2.4 Run the gel at __ volts for __ hours. During this time become familiar with the iBlot tool and prepare the reagents (namely Tri-Glys transfer buffer) after reading page 3.

2.5 Shut off the power and recycle the SDS using a funnel.

2.6 See the section regarding how to remove and handle the gel. Then clean the gel box using soapy water and then DI water before letting it dry.

2.7 Ask your professor as to whether your group is western blotting via iBlot (directions 3a) or via capillary model (directions 3b).

Western Blot 3(a) iBlot tool-

Materials:

- Gloves.
- Copper Anode (bottom layer) which will include the transfer membrane. **Keep this attached to its plastic bottom**
- Polyacrylamide gel with GFP and protein standard.
- Piece of filter paper. **soaked in transfer buffer**
- Novex Tris-Gly transfer buffer.
- Copper Cathode (top layer).
- iBlot sponge with metal clip.

3.1(a) Turn on the iBlot tool and change the settings to program 4, while you read the section on how to remove and handle polyacrylamide gels.

3.2(a) Place the copper Anode layer with its plastic container in the setup first. Then place the nitrocellulose transfer membrane on top, then your gel. The next layer of this Western Blot sandwich is the filter paper, which must be soaked in Tris-Glycene transfer buffer. The last step is to place the copper Cathode layer on top and fit the sponge into the lid of the iBlot. The Sponge's metal clip should be in the top right corner when the lid is open*.*The iBlot manual has pictures, ask for it.**

3.3(a) Close the lid. If you have a closed circuit you will see a red light turn on next to the start/stop button. This means you are good to go, press start. ** If you do not see the red light, it is likely that your Western Blot sandwich does not have a closed circuit. First take off the top layer and use a 10ml pipet to wet the filter paper and gel before trying again.* **If you do see a red light but the machine beeps angrily at your group then you have a short circuit. Turn the IBlot off then on and open the iBlot and make sure the anode/cathode layers are NOT touching before trying again.***

3.4(a) While the iBlot is running, read ahead and prepare the reagents needed to either block and probe immediately after transferring or to preserve the gel in saran wrap (stored at 4 deg Celsuis) to be blocked and probed later. This depends on how much time you have left. **If you are storing the gel, be sure to tape your gel legend to the saran wrap so each group knows where their protein is on the nitrocellulose transfer membrane. **

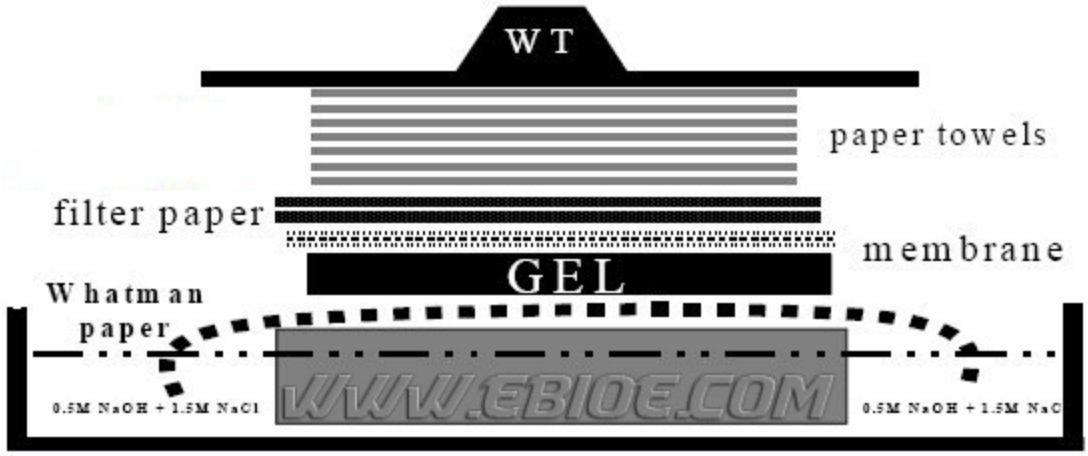
Western Blot 3(b) Capillary model- Rather than drawing out the proteins using an electric (and thus faster) current, the capillary method uses paper towels (and thus cheaper) to draw the transfer buffer through the gel. Larger pieces will take longer than smaller pieces to cross the membrane. As such, some of the heavier protein fragments from your Kaleidoscope protein standard may not be present on your nitrocellulose transfer membrane.

<u>Materials:</u>

- Gloves.
- Horizontal gel box. **the very ones used for DNA electrophoresis**
- One microfuge tube rack (your weight).
- 5 paper towels (one will be the wick, four will be regular paper towels, all will assist in capillary action).
- Two pieces of filter paper.
- Four strips of parafilm **These will frame your transfer membrane**
- One nitrocellulose transfer membrane.
- Polyacrylamide gel
- Novex Tris-Gly transfer buffer.

  3.1(b) Take your 5 paper towels and cut a middle-fingers length off of all of the simultaneously. Put the smaller pieces aside to be used in the paper towels layer (as seen in the picture below). Take one of the larger pieces and fold the edges so that it drapes over the gel box stage. **make sure that your <u>wick</u> does not touch the far sides of the gel box**. Take what is leftover, fold the larger pieces in half, and pile them with the rest of the paper towel layer.

  3.2(b) Put on gloves and read the section on how to remove and handle Polyacrylamide gels. Otherwise begin to layer your materials as seen in the picture below. There are special instructions for the transfer membrane.** the transfer buffer is added LAST**



3.3(b) In the filter paper box there will be three different types of paper. One blue which we don't need, and two white. The shinier white piece is your transfer membrane, place it atop your gel and frame its edges using parafilm.**otherwise your paper towel layer will touch the wick, causing the capillary action to bypass your gel and the protein will not leave for the transfer membrane*.* The other white piece is the filter paper, take two and stack them atop the transfer membrane.

3.4(b) After weighing down your paper towel layer with a microfuge tube rack, two people will use 10mL pipets to simultaneously add a total of 50mL (transfer buffer) to each side of the gel box. Add additional mL every half hour.

3.5(b) the experiment runtime is__hour(s). Afterwards, immediately mark which side of the transfer membrane faced the gel during the blotting process. Clean the rig, and prepare to either store the transfer membrane or probe with antibodies depending on the remaining time.

How to remove and handle Polyacrylamide gels-

1. Wearing gloves, use a metal utensil (i.e. scalpel) to pry the gel out of its casing. Start with the corners and then the sides.
2. While the gel is still attached to the bottom half of the casing, use your utensil to cut off the wells to make transport easier.
3. Wet the tips of your gloves with transfer buffer and remove slowly. Polyacrylamide gels are prone to tearing. You can use your utensil to lift the gel in one hand and peel with the other hand.