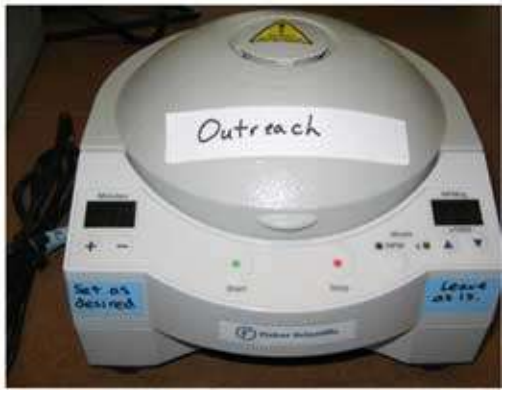
EQUIPMENT GLOSSARY AND INSTRUCTIONS

1.5 ml microtube: larger of the two tube sizes supplied. (aka microfuge tube)



Centrifuge

a. Plug centrifuge into its power supply
b. Plug power supply in to wall outlet. Everything should be on (there is no on/off switch)
c. Load samples and ensure they are properly balanced (equal volumes loaded directly opposite each other). Please supervise students when using the centrifuge to ensure they balance samples properly.
d. Press inner lid on (one of the centrifuges has an inner lid while the other is missing it) and close centrifuge cover.
e. RPM display should read "13". Leave as is for all uses except for cell pelleting during transformation protocol, use up and down arrows to adjust the speed (rpm).
f. Mode should have RPM lit, the mode lights (rpm and g) are faint on one centrifuge model.
g. Set minutes appropriately.
h. Press Start symbol when ready. It should beep when done and the lid will then unlock.

Floater: for holding tubes in a water bath

Gel Equipment: see gel instructions below

PCR machine:

- Plug in and toggle the switch at the back if the machine doesn't turn on.

Put samples in smaller holes, close lid and lock (switch to tube symbol with round lid)

Display should read 'Main-menu Outreach'

To select the Outreach program hit 'Enter', will see a menu with 'Outreach' selected

Hit Enter again to start the program, display will say ‘testing program’ before starting to cycle.
If the initial display does not read ‘Main-menu’, press ‘Exit’ until it does and follow instructions as above.

PCR microtube: very small tubes

Pipettes:

Three sizes supplied; 1-10ul, 2-20ul, 20-200ul
There are two buttons on the top, one for dispensing liquid and one for ejecting the tip
Set the volume on the pipette using the dial at the top. (Turn slowly, DO NOT ROTATE past the maximum volume!)
Ensure that a new tip is placed on the pipette for each solution used
The top button has two stops. To draw up liquid, press down to the first stop, put tip in solution and release slowly. To dispense liquid, press down to second stop.

Toothpicks 

Gel inside ziplock with cover in place

Closeup of pink bands (evidence of insert segment)

UV Transilluminator:

Plug in.
Place gel on transilluminator in plastic bag and place clear shield over it to block the UV rays.
Turn on the transilluminator

Vortex:

Plug in.
Speed can stay at max (7-8) for all uses. It can be use on "touch" which goes when a sample is pressed into it, or "ON" where it operates continuously.

Gel Electrophoresis

If you attempt to run the power supply when it is not connected to a gel box with liquid in it, the supply will shut itself off. This is a safety feature of the equipment. Connect the leads to the gel boxes before turning on the power.

When washing up the gel boxes, please be careful not to damage the platinum wire located inside the box near the corner of the box. These are very expensive to repair.

Gels are best viewed on the same day you run the experiment. The bands will diffuse if they are left overnight. The gels can be made up ahead of time and it takes about an hour to make 2 - 3. Simply store your gel in plastic wrap in a fridge overnight.

NB: Earlier versions of instructions for the Biotechnology Kits suggested a very high voltage for running high speed gels. This setting has been lowered and we now recommend you use 240V - 250V.

Preparing the Agarose Gel:

1. Prepare the gel mold. See directions for Low and High speed gel set up below.
2. Take 10 ml of 10X TBE and add 90 ml of tap water, making 1X TBE.
3. Open the packet of agarose and put into the microwaveable container.
4. Pour in 80 ml of the 1X TBE and swirl to mix.
5. Microwave on high for approx. 30 seconds. Swirl again. Careful the solution will be hot. Microwave another 30 seconds and keep an eye out as it can boil over. The solution should be clear and just on the point of boiling when all the agarose is dissolved.
6. **Put on gloves** and add 8 ul of Ethidium Bromide (orange solution provided in microfuge tube) and allow to cool for a couple of minutes before pouring into the gel box (make sure you have place a comb in the mold).

Running a Low Speed Gel (run time ~30-40mins)

1. Turn the gel mold sideways in the gel box so the open ends are sealed. Alternatively, tape can be placed across the open ends but be careful to press hard to create a tight seal to the plastic. Place on a level surface.
2. Place a comb in with the flat side of the comb facing the direction of migration (i.e. towards the positive red electrode) and make sure it does not touch the bottom, although it should be close.
3. Prepare gel as above. When gel has solidified place it in gel box with the end with the wells (comb) towards the negative electrode.
4. Cover with approx. 1 L of **1X TBE** (diluted from the 10X TBE concentrate supplied) in the gel box and remove the comb.
5. Power settings for a Low Speed Gel should be **150V** and run time is around 30 - 40 min. After students load their samples, be sure to use one well for the DNA marker.

<u>For the new blue low speed gel box:</u>

1. Put the gel mold into box with open ends lengthwise (see pictures on website)
2. Slide two gel forms in together on either side of the mold, is put in separately they will be uneven and won't form a good seal.
3. Put in the comb and pour gel as above.

Running and Viewing a Gel

1. Connect the leads from the gel to the power supply (black to black etc). Plug in power supply using cord from centrifuge power supply, turn on power switch (only on model labeled VWR, other model has no ON switch). This is the large

button with horizontal line.

2. Set the voltage for high or low speed system. For the EC105 model you will have to select the ‘high’ or ‘low’ voltage range and use the dial to set voltage.
3. Make sure all selections are set to Volts.
4. Press RUN to start VWR model, the EC105 will automatically run once connected to gel.
5. Once the gel has finished, put it into a sealed Ziploc bag for ease of viewing in the transilluminator. Return the gel with the kit as it is a hazardous waste.
6. Rinse the gel apparatus well in running tap water being careful not to damage the platinum wire running along one corner on the inside of the gel box.