Gel Electrophoresis of Nucleic Acids

- Always wear gloves; ethidium bromide is a powerful mutagen!
- For a 1.5% gel in the small gel box, weigh out 0.45 g agarose into a 250 ml flask.
- Add 30 ml 0.5X TBE buffer, and plug the flask opening with 2 crumpled Kimwipes.
- Microwave for 30 s, or until agarose dissolves.
- Carefully remove flask (it's very hot) and cool to about 50ºC.
- In the meantime, tape the open ends of the gel tray with lab tape and insert the desired comb(s) to get the size and number of wells you want.
- Add 1.5 µl ethidium bromide to the molten agarose solution and swirl to mix.
- Pour agarose solution into the gel tray. Gently tilt the gel tray back and forth to distribute the gel evenly.
- Let stand 20 min or until gel solidifies, and remove the comb(s).
- Add 0.5X TBE to the gel box (use the same batch as you used for making the gel). You can re-use the same TBE for several days. Add enough to cover the entire gel to a depth of 1 mm.
- On a piece of parafilm, mix together loading dye and your samples/ladders. Pipet out 1 µl drops of loading dye onto the parafilm, one for each sample. Then add 4 µl ladder or sample to each drop and pipet to mix. Load 4 µl of the mixture into the gel well. These amounts can vary depending on your well size.
- Don't forget to note which samples are in which wells.
- Put the lid on the gel box, making sure the nucleic acids (which are negatively charged) will move in the correct direction (toward the positive, red terminal). Typically, we run the gel for 25 min at 95V.
- When the gel is done, slide it out of the gel tray onto the UV table. Be sure you are wearing goggles and gloves!
- Turn on the UV lamp and use the Polaroid camera to take a photo of the gel.
- Note the electrophoresis conditions on the gel photo and tape it in your notebook with the date and well labels.