GEL ELECTROPHORESIS CONCEPT QUESTIONS

1. What is a marker or ladder? Why is this considered a standard in this lab? *A marker or ladder is a set of DNA fragments and the base pair length of each fragment is known. It is considered a standard because it can be used as a tool from which to measure the lengths of your unknown DNA fragments.*

2. Why is a ladder or standard necessary part of this lab? Why does each lab team need to run their own ladder or standard? *A ladder or standard is necessary to in order determine the length of DNA fragments as measured in base pairs. Each student's gel may run for a different time length, run at slightly different voltages or the agarose gel may be at a slightly different concentration, all of which affect the spacing of the DNA fragments and hence need to be compared to a standard treated in exactly the same manner.*

3. How does the size of the DNA fragment affect its movement or migration through the agarose gel during electrophoresis? *Larger size fragments move more slowly through the gel, while smaller fragments move more rapidly.*

4. Name three components found in the sample loading buffer. What is the purpose of each of these components?

   Buffer – provide the necessary salt concentration to stabilize DNA.

   Glycerol – weigh down the sample, helps hold sample in loading wells and gel

   Tracking dyes – Bromophenol blue and Xylene cyanol, provide visual feedback on where the DNA samples are located as the gel runs.

5. Predict what would happen if you forgot to add the sample loading buffer? *The samples would float out of the wells into the running buffer, it would make loading gels visually difficult and there would be no way to see if the DNA molecules were traveling in the gel.*

6. Why do you add 1X TAE buffer to the gel box? *To make an electric connection between the electrode strips in the gel box.*

7. What would happen if you added water instead of the 1X TAE buffer and ran the gel with the water? *There would be no electrical connection made in the gel box and therefore no current, hence no movement of DNA.*

8. Why is uncut DNA included as a part of this lab? *As a control.*

9. How can you tell that the restriction enzyme digestion has occurred? *You should see more than one DNA fragment or band in the lane containing the digested sample.*

10. What is the purpose of placing the gel in Carolina Blu –2 (a stain) after the electrophoresis? *It stains the DNA. The Carolina Blu stain is positively charged and adheres to the negatively charged pieces of DNA.*

11. Predict, in your journal, what the gel will look like after it has been electrophoresed and stained with Carolina Blu-2. *The lane with the standard or ladder should have many fragment lengths or bands, the uncut DNA lane would contain only one fragment that traveled the least, the unknowns would have a varied amount of fragments and banding patterns and these may or may not match others on your gel.*