Analyzing the Data

Experiment 7 introduces you to restriction digestion and gel analysis.

**Your analysis of this experiment should include:**

- a discussion of how fragment sizes are distributed across a gel
- a discussion of how fragment size and migration distance affect accuracy of size estimation
- a discussion of the difference between cut and uncut plasmid

You are also asked to make a simple map of the plasmid pRHR30. pRHR30 is a recombinant plasmid comprised of a pUC9 vector and an insert into the EcoRI site. When you calculate a restriction fragment size, you are, in effect, calculating the distance between the two cut sites that generated the fragment. In order to calculate the distance between the sites of different enzyme sites, it is necessary to perform a series of double digests using two different restriction enzymes. However, in this experiment, the digestions are simplified because you already know the location of many sites. You have the map of pUC9 and the sites on the vector will anchor one of the two sites on each fragment. Given the precision of your gels, the four restriction sites in pUC9 will appear to be located in the same spot. In the example below, a fragment with *PstI, BamHI,* and *HindIII* sites is inserted into the *EcoRI* site of pUC9. A gel of the plasmid shows the fragment sizes of each digestion. The map is easily constructed by plotting the distance of each site from the anchor site on the vector. The direction of the site is clear (in this case clockwise) because if the sites were plotted in a counterclockwise direction, they would map within pUC9.

![Diagram of pUC9 and gel analysis](image-url)
In experiment 8 you were asked to prepare a composite gel consisting of 0.5%, 1.0%, and 1.5% agarose slabs and run 1kb+ and λ HindIII molecular weight standards on each. You were also asked to use both the orange and blue tracking dyes. Once you have measured the bands on each slab, the data should be plotted on a single graph that shows three curves. The 1kb+ and λ HindIII molecular weight standards from each slab should be plotted on a single line with the data points for each clearly identified.

**Your analysis of this experiment should include:**

- a discussion of how agarose concentration affects migration rate
- a discussion of why you might consider using different agarose concentrations
- a comparison of the two tracking dyes and why you might use one or the other in an experiment.

**Writing the Report**

Although these two experiments were presented as distinct, stand-alone assignments, they are indeed related to each other. Your challenge in writing this report is to weave them together into a single unified project. Although you did the experiments in the order #7 then #8, you do not necessarily have to follow this order when you describe them in your paper. You may present them in any order that makes sense to you.

The format of the paper follows that of a standard journal article and is clearly laid out in the lab manual and on the course web site. Please read the instructions carefully and be sure to ask questions if you are unsure.

**Required data elements:**

- **Experiment 7~**
  - Gel Red photograph of the gel
  - cell phone photograph of gel
  - table of migration distances and molecular weights for standards
  - graph of the standard curve
  - table of migration distances of unknown bands and estimation of molecular weights
  - final plasmid map

- **Experiment 8~**
  - Gel Red photograph of the gel
  - table of migration distances and molecular weights for standards
  - graph of the three standard curves
**Points to Remember:**

**Methods and Materials** are GENERIC. Do not refer to specific cells or DNA’s. Those are part of experimental design and belong in Results. In Results you describe the experiment.

For example:

**How does one saw a piece of wood:** “The wood to be cut was measured and marked with a pencil to indicate where the cut was to be made. A wood saw was held at a 45° angle against the edge of the wood and repeatedly moved back and forth until the cut was complete.”

**How does one nail two pieces of wood together:** “Wood glue was spread on each piece of wood. The two pieces of wood were braced together and a nail was held vertically against the two pieces. The nail was hit repeatedly with a hammer until the head of the nail was flush with the surface. This process was repeated until both pieces were fastened together firmly.”

These descriptions are GENERIC. You are not explaining why you are cutting the wood or nailing it together. You can use the same descriptions to explain how to build a table, a bookcase, a house, or anything else made of wood.

In the Results section you explain what you want to build (i.e. this is the experiment) and how you intend to use the hammer and saw to accomplish this goal. You do not have to explain how you hammer and nail wood. That was described in the Methods and Materials.

**Avoid trivial details:**

*The tube was tapped several times to mix the contents.*  
*The tube was briefly centrifuged to bring the contents to the bottom.*  
*The reagent was carefully added to the tube.*

**Do not present a diary of your afternoon in the lab:**

*While the reaction mix was incubating, we poured a gel.*

The **Discussion** must reflect the goals laid out in the **Introduction.** If you said in the Introduction that you are studying several aspects of restriction digestion and electrophoresis, then you must explain how your results reflect the various aspects of restriction digestion and electrophoresis that you described in the Introduction.
Tables and Figures:

In a journal article, there are only tables and figures. Please label your data elements as such. Do not use labels such as “Gel #1” or “Graph #3.”

All figures and tables must be consecutively numbered, in the order in which you discuss them in the paper. Do not refer to all figures from each experiment as Fig 1-1, 1-2, 2-1, etc.

Any figure or table that you include in your paper must be specifically mentioned and referred to in the text of the paper.

I am not into busy work so I don’t mind if you and your lab partner have the same figures and tables, but:

MAKE SURE THAT THE WRITING OF THE PAPER IS CLEARLY INDEPENDENT!!

Format:

Please type your paper single-spaced, with broad margins.

I am not impressed with papers that are formatted to look like journal articles. If you turn in such a paper, do so only because you enjoy the challenge. It will make no difference to your grade.

Your report should have a cover page with both your name and your lab partner’s name in the upper right hand corner. Please include your lab section.

Do not hand in your paper in a binder. The paper should simply be stapled in the corner.

Title of Your Report