

Lab Report 1

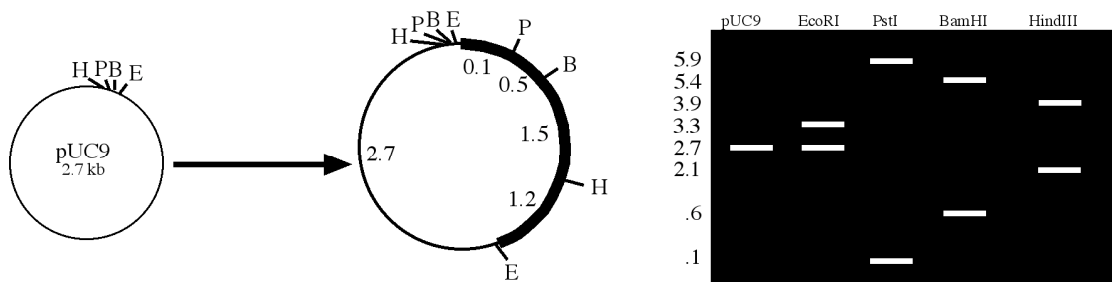
Experiments 1 and 2

Analyzing the Data

Experiment 1 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
- how fragment size and migration distance affect accuracy of size estimation
- 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 *Pst*I, *Bam*HI, and *Hind*III sites is inserted into the *Eco*RI 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 in pUC9.



In experiment 2 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 in a single unified project. Although you did the experiments in the order #1 then #2, 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 are:

Experiment 1~

- photograph of the 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 2 ~

- photograph of the gel
- table of migration distances and molecular weights for standards
- graph of the three standard curves

Points to Remember

- 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.
- In a journal article, there are only tables and figures. Please label your data elements as such. Do not have a label such as "Gel #1" or "Graph #3."
- Any figure or table that you include in your paper must be specifically mentioned and referred to in the text of the paper.
- 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.
- 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 in 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.

| |
|---------------------------------------|
| Your name Lab Partner Section # |
| Title of Your Report |

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