Teacher Prep & Follow-up

STUDENT LAB BRIEFING

To get the most out of the laboratory, we suggest that you prepare your class prior to coming to the DNA Learning Center (DNALC). Make sure your students have a good understanding of the structure and function of DNA. They should also read the enclosed **Carolina Tips** article, "DNA Restriction Analysis." After reading this article, students should be able to discuss:

- Discovery of restriction enzymes and their physiological role in bacteria.
- Use of electrophoresis and agarose to separate DNA fragments.
- Use of restriction enzymes in genetic engineering.

You may also wish to have the students read over the enclosed lab protocol.

AT THE DNA LEARNING CENTER

Before starting the experiment, the instructor will briefly review the purpose of the laboratory and the uses of DNA restriction analysis. Students will be introduced to the lab equipment and given an opportunity to practice using micropipettes. The lab protocol will be discussed step by step. Students will have a 30-to-40-minute period during the electrophoresis step to eat their lunch/snack if they wish. Teachers must supervise students in the lunchroom, or students may choose to eat in the school bus, if available. Students will not be permitted to eat in the laboratory or in the hallways of the DNALC.

RESULTS AND DISCUSSION

In addition to the questions in the lab protocol, you can discuss the ethical ramifications of DNA fingerprinting and genetic screening, the Human Genome Project, and genetic engineering. There are often articles in journals on teaching bioethics, for example "Genetic Engineering—A Lesson on Bioethics for the Classroom" by Kerri Armstrong and Kurt Weber (*The American Biology Teacher*, May 1991, Volume 53(5)). There is also a very good section on societal issues in *A Sourcebook of Biotechnology Activities* from the National Association of Biology Teachers and the North Carolina Biotechnology Center.

Answers to student questions are in bold.

1. Why is water added to tube "–" in Part 1, Step 2?

   Water is used in place of a restriction enzyme to serve as a control. This enables students to observe DNA in the uncut state. Furthermore, the water ensures that the reaction volume is 10 µl, bringing the salts in the 2X buffer to the proper 1X working concentration.

2. What is the function of restriction buffer?

   Restriction buffer provides the proper conditions of salt and pH for optimal activity of the restriction enzymes.

3. What are the two functions of loading dye?

   Sucrose in the loading dye increases the density of the DNA mix so it sinks to the bottom of the well when loaded. The bromophenol blue and xylene cyanol migrate through the gel visibly to show how far the invisible DNA has migrated and indicate when to turn off the power.

4. What is the effect of run time on the observed pattern of restriction fragments? How would the gel concentration affect the observed pattern?

   The bands spread out more the longer the gel is electrophoresed. An increase in the concentration of agarose in the gel would result in a "tighter" gel matrix, which would more effectively separate smaller DNA fragments. A lower concentration of agarose would separate larger fragments of DNA.

5. Examine the photograph of your stained gel. Compare your gel with the ideal gel shown below, and try to account for the fragments of *Lambda* DNA in each lane. How can you account for differences in separation and band intensity between your gel and the ideal gel?

   Bands on the ideal gel are probably more spread out because the gel was electrophoresed for a longer period of time. Band intensity depends upon the mass of DNA; the greater the mass, the more intensely stained the band.

6. What is the identity of the unknown restriction enzyme?

   The number and spacing of DNA bands in the "?" gel lane should precisely match that in either the *Eco*RI or *Hin*dIII gel lanes.