INTRODUCTION

When biologists clone a gene in order to produce human insulin, they create a recombinant plasmid that has the human insulin gene. To do so, they use restriction enzymes to create DNA fragments that contain the plasmid components and then use DNA ligase to join those fragments together. (*DNA ligase* is an enzyme that catalyzes the joining of the DNA fragments.) As part of the gene cloning process, biologists have to *verify* that they have created the recombinant plasmid they need—that is, the one with the gene of interest as well as all the necessary components for the protein of interest to be made. In this chapter, you will continue to work with the tools of genetic engineering as you verify that you have the recombinant plasmid you need to produce the red fluorescent protein.

CHAPTER 4A GOALS

By the end of this chapter, you will be able to do the following:

- Describe why it is important to verify products created in the genetic engineering process
- Predict the relative speed of DNA restriction fragments and plasmids through a gel during gel electrophoresis
- Separate and identify DNA restriction fragments and plasmids using gel electrophoresis

WHAT DO YOU ALREADY KNOW?

Discuss the following questions with your partner and write your ideas in your notebook. Be prepared to discuss your responses with the class. Don't worry if you don't know all the answers. Discussing these questions will help you think about what you already know about gel electrophoresis and verification in the lab.

1. Why do DNA restriction fragments and plasmids separate when analyzed by gel electrophoresis?
2. Why is it important to identify and verify a recombinant plasmid?

VERIFICATION

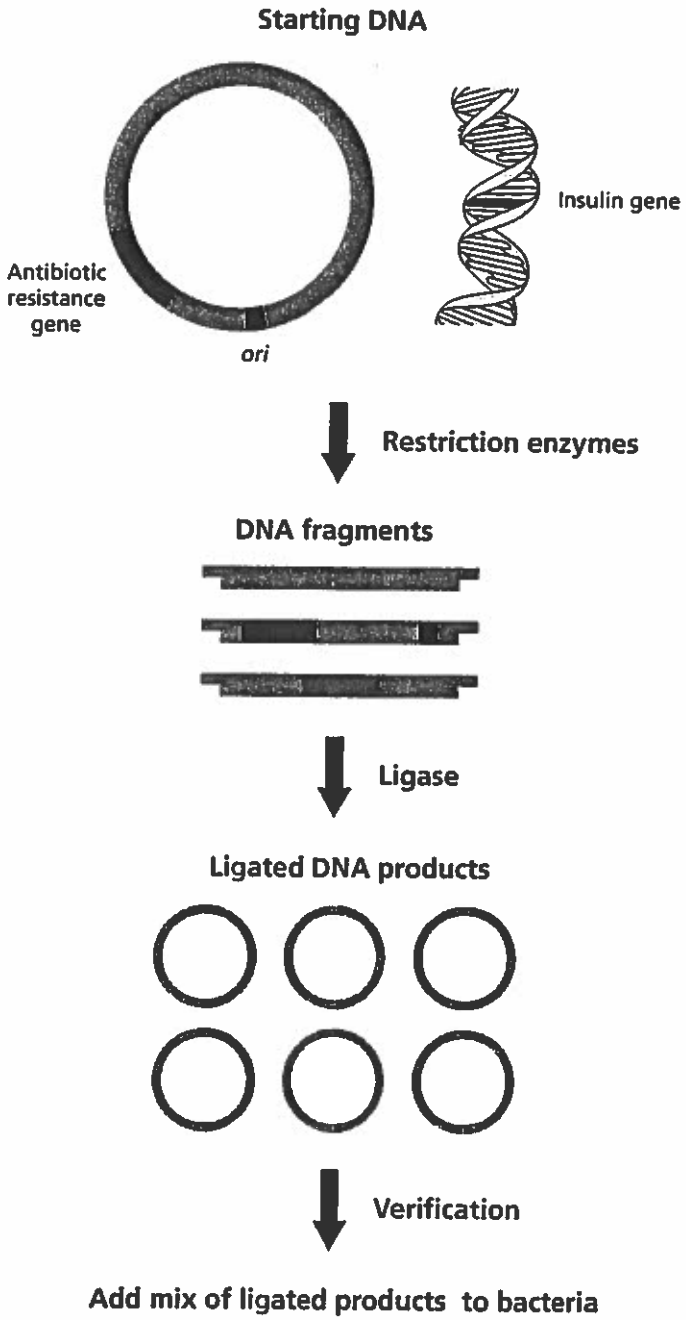
It is important to verify work in the lab—there are many sources of potential error in any procedure, including the procedures used in cloning a gene. In gene cloning, there is also the problem that some procedures are not selective. For example, when a DNA ligase is used to *ligate*—bind together—DNA fragments, many different combinations result from the *ligation* process. Unless you verify your work, you do not know if you have made the recombinant plasmid that is needed.

HOW TO VERIFY THE RECOMBINANT PLASMID

Figure 4A.1 shows how to verify your results when making a recombinant plasmid. You verify that the restriction digest and ligation procedures worked by comparing the products of both procedures with each other and with what you started with.

Figure 4A.1: Verification method when making a recombinant plasmid

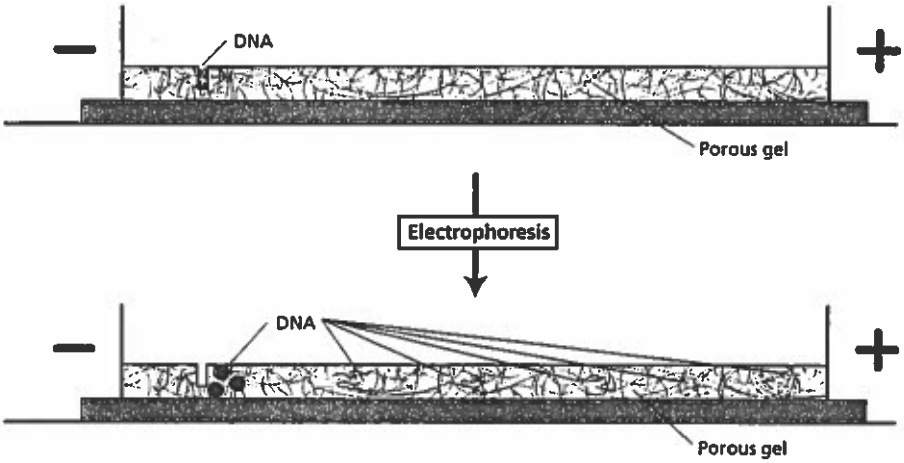
Verify that the restriction digest and ligation procedure was successful by comparing the products of the restriction digest, the products of the ligation procedure, and the starting materials



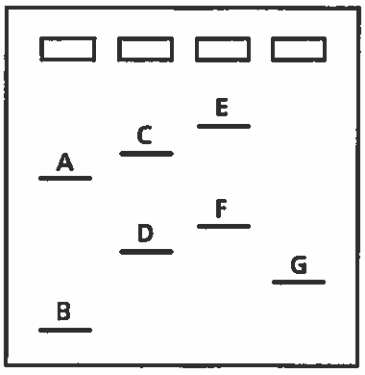
GEL ELECTROPHORESIS

Gel electrophoresis is used extensively in DNA verification and purification. In order to identify or purify DNA restriction fragments, it is necessary to separate the various-sized DNA molecules. Gel electrophoresis separates biomolecules primarily according to their molecular size, which for DNA is measured by the number of base pairs. The backbone of a DNA molecule, because of its phosphate groups, is negatively charged and therefore will move away from the negative (black) electrode and toward the positive (red) electrode. Because it is easier for small DNA molecules to move through the agarose matrix, they will migrate faster than larger DNA molecules. See **Figure 4A.2.**

Figure 4A.2: Separation of DNA by size using gel electrophoresis



CONSIDER: After different DNA fragments and plasmids have been separated by gel electrophoresis, the gel is stained to show bands that indicate the location of each kind of fragment and plasmid. The drawing of a stained gel below shows a series of bands that have been labeled with letters. The locations of the wells are also shown. What is the order of the fragments, from smallest to largest?



PLASMID CONFIGURATIONS

While short, linear pieces of DNA move as expected when run on gel electrophoresis, the movement of plasmids is not as straightforward. This is because a plasmid can exist in different configurations that move at different rates through the gel. There are three *plasmid configurations*:

- The most common plasmid configuration is *supercoiled*. You can visualize this configuration by thinking of a circular piece of plastic tubing that is twisted. This twisting or supercoiling results in a very compact molecule, one that will move through the gel very quickly for its size. This configuration is only seen in plasmids that have been replicated in bacteria because supercoiling of a plasmid requires an enzyme that is found in the bacterial cell. It is the default natural plasmid configuration found in bacteria.
- The second plasmid configuration is a *nicked circle*. You can visualize this configuration as a large floppy circle. This plasmid has a break in one of the covalent bonds located in its sugar-phosphate backbone along one of the two nucleotide strands. This circular plasmid configuration will not move through the agarose gel as easily as the supercoiled configuration. Although it is the same size, in terms of base pairs, it will be located closer to the well than the supercoiled form.
- The third plasmid configuration is a *multimer*. You can visualize this configuration by thinking of two or more plasmids that are connected like links in a chain. This configuration is only seen in plasmids that have been replicated in bacteria, because multimers form when plasmids are replicated so fast that they end up linked together. If two plasmids are linked, the multimer will be twice as large as a single plasmid and will migrate very slowly through the gel. In fact, it will move slower than the nicked circle.

The possible plasmid configurations are shown in **Figure 4A.3**.

Figure 4A.3: Plasmid configurations

Supercoiled Nicked circle Multimer

CONSIDER: If you used gel electrophoresis to separate the same plasmid that has all three configurations, the supercoiled plasmid would move the fastest, while the multimer would move the slowest. Why do the different plasmid configurations move the way they do through the gel? Explain in words or a drawing.

DID YOU KNOW?

History of Genetic Engineering

Genetic engineering is not a new phenomenon—it has been done for centuries in plant and animal breeding. Throughout history, humans have used selective breeding to produce organisms with desirable traits. The science of agriculture began with the selection of wild grasses and subsequent breeding to form the precursors of modern staples such as wheat, rice, and maize. In *selective breeding*, two members of the same species are paired as breeding partners in order to encourage desirable characteristics in the offspring. For example, cows that have been observed producing large volumes of milk may be bred to pass that trait on to future generations.

Selective breeding is one way for humans to nurture desirable traits in plants and animals, but it is much older and less predictable than genetic modification. Genetic modification is more precise than classical breeding, and, in many cases, it is much quicker. Our current understanding of genetics and heredity allows for the manipulation of genes and the development of new combinations of traits and new varieties of organisms. Actually going into a cell and changing its genome by inserting or removing DNA is a very new technology. But keep in mind that genetic modification isn't some "unnatural" process to create monsters! Genetic modification and traditional selective breeding are limited by the same constraints, and natural-occurring mutations may (but not always) have a negative outcome as well.