Name: _____ TOC#

Manipulating and Analyzing DNA

Restriction Enzymes, Recombinant DNA, and Gel Electrophoresis

Introduction:

Today you will examine three extremely important aspects of biotechnology; restriction enzymes, recombinant DNA and gel electrophoresis. You will use two different websites to understand both topics. By the end of today you should be able answer the flooring questions:

- What are restriction enzymes? How and why are they used in biotechnology?
- How do restriction enzymes play a role in recombinant DNA?
- How does gel electrophoresis work and how are restriction enzymes important?

Restriction Enzymes Background:

Biotechnology is the manipulation of the biological capacity of cells and their components. For thousands of years people have used biotechnology by using yeast to make flour into bread and grapes into wine. Today, we are using biotechnology to study the basic processes of life, diagnose illnesses, and develop new treatments for diseases. Biotechnology also provides the means to analyze similarities and differences in DNA between different species (interspecific variation) and between individuals of the same species (intraspecific variation). Since genes and DNA change as a species evolves, interspecific analysis of DNA provides a picture of evolutionary relationships between different species.

Some of the tools of biotechnology are the natural components of cells. Restriction enzymes are made by bacteria to protect themselves from viruses. They inactivate the viral DNA by cutting it in specific places. DNA ligase is an enzyme that exists in all cells and is responsible for joining together strands of DNA. Scientists use restriction enzymes to cut DNA at specific sequences called recognition sites. They then rejoin the cut strands with DNA ligase to make new combinations of genes.

1. From reading the paragraphs above; what is a restriction enzyme? Why are they used?

Restriction Enzyme Activity:

Go to the following website: http://www.dnalc.org/resources/animations/restriction.html. Before you start the animation, read the paragraph above the animation box. As you click through the animation, be sure to read everything. These questions are in order.

1. First of all, a restriction enzyme is an enzyme. Enzymes are made of _____ and

   _____ chemical reactions.

2. Where were restriction enzymes originally found? What did they do?

3. Restriction enzymes are _____, for example EcoR1, binds to _____

4. These sequences are called _____. What does it mean to say that DNA restriction enzymes produce "sticky ends?" Explain.

5. What does DNA ligase do? Explain.

Recombinant DNA Background:

Scientists use restriction enzymes to create recombinant DNA. Recombinant DNA sequences typically contain genes (composed of DNA) from two or more organisms. Recombinant DNA can be inserted into plants, animals, or bacteria in order to great transgenic species, or species that express foreign DNA. For example, the human insulin gene can be inserted into bacteria, which will then produce human insulin.

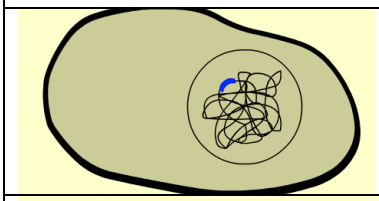
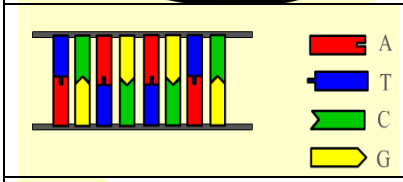
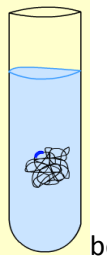
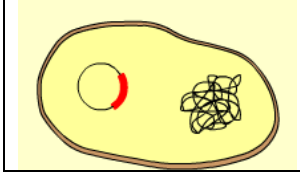
1. From the paragraph above; why do scientists create recombinant DNA?

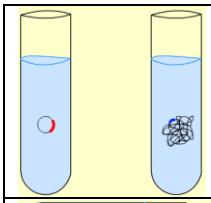
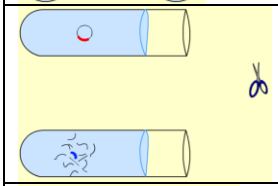
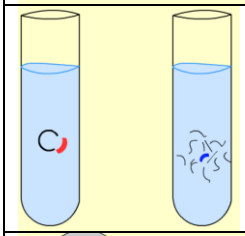
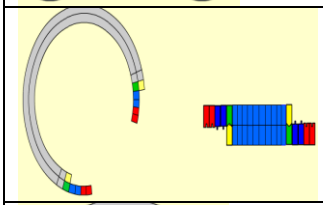
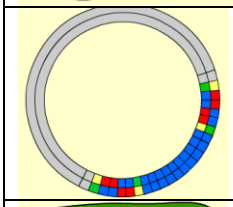
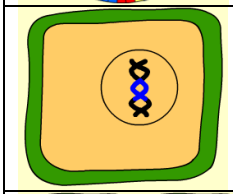
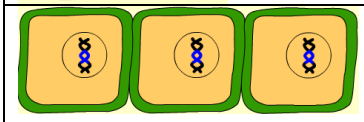
Recombinant DNA Activity:

Go to the following link: http://www.hhmi.org/biointeractive/dna/DNAi_genetic_eng.html

1. Fill in the blanks from the paragraph:
   A new gene can be _____ into a _____ called a _____. This is done by _____ with a _____, which allows a _____. The ends of the new piece of DNA are _____ by an enzyme called _____. The genetically engineered bacteria will now _____ _____.

Go to the following link: http://www.bioteach.ubc.ca/TeachingResources/Applications/GMOpkgJKloseGLampard2.swf. This animation will show you the steps of creating recombinant DNA and making a transgenic plant. Answer the following questions.

Picture	Text
	
	
 both screens	• •
	

	
	
	
	
	
	
	
Summarize the entire process of creating recombinant DNA and a transgenic plant (you may use pictures and words)	

Gel Electrophoresis Background

In any species genome, there is also a lot of "junk DNA." This does not perform any known function and, therefore, is free to vary at random. Intraspecific analysis of DNA (sometimes called "DNA fingerprinting") provides an accurate means of comparison between individuals of the same species. It is used extensively in the field of forensic science.

Scientists identify differences in DNA sequences by measuring the length and number of fragments created by digestion with restriction enzymes. A technique called gel electrophoresis is used to separate fragments according to length. DNA fragments, cut with specific restriction enzymes, are placed on one end of the specially prepared block of agarose called a gel. The gel is like a sponge with small holes in it. AN electric current is applied across the agarose which causes the strands to migrate through the gel. (Since DNA molecules are negatively charged, they migrate towards the positively charged electrode.) The larger fragments move more slowly and are found nearer the point of origin, while the smaller fragments are found further from the origin (closer to the positive electrode). Scientists then use a special stain to make the DNA fragments visible as bands. By counting the number of bands the researchers can tell how many fragments exist. By observing the distance each fragment has migrated, they determine how big each fragment is.

Gel Electrophoresis

Go to the follow website: http://learn.genetics.utah.edu/content/labs/gel/

Click through the online activity, and read the information to answer the questions below. These questions go in order of the activity.

1. By what factor does gel electrophoresis sort DNA?

2. What other types of molecules can gel electrophoresis be used to sort?

3. The gel is the _____ that _____ DNA strands. It's like a sponge made of Jello with many small _____ in it.

4. Where do we put DNA in the gel?

5. ANALYSIS: What do you think you must do to the DNA before you place it in the gel?

6. By adding an _____, we can make the DNA move.

7. Is the DNA at the positive or negative side of the gel? Which way will the DNA move?

8. Which strands will move the farthest, why?

9. We can't see a single strand of stained DNA, but we can see _____.

10. What causes the bands in the gel?

In the following questions, follow along completing the steps of running a gel, and answer these questions.

11. Make the gel in step 1. What are the ingredients of agarose gel?

12. What does the liquid buffer do?

13. As the gel cools, _____ will form in it.

14. What is the comb for?

15. Why do we use the DNA Size Standard?

16. In the last step, the stain we use on the DNA is a chemical that _____ to DNA and shows up under _____ light.

17. Although we can't see _____ DNA strands, we can see large groups of stained DNA strands. These groups will show up as bands in the gel.

18. How many base pairs long was each of the three bands of DNA that showed up?

19. ANALYSIS: explain, in detail, why we use gel electrophoresis.