

## Background

Bacteria have circular **plasmids** that are usually several thousand base pairs in length. Plasmids are used in recombinant DNA technology to transfer genes from one organism to another. A plasmid will have an origin of replication site and may also contain genes for antibiotic resistance.

Recombinant DNA contains DNA from more than one organism. For example, the gene for human insulin can be inserted into bacteria with the help of a plasmid. The bacteria will then secrete human insulin that can be harvested economically and with great purity. The millions of diabetics throughout the world can then use the insulin.

You have been provided with a paper plasmid and a piece of cellular DNA. The DNA contains a gene called Green Fluorescent Protein (GFP) and a gene for resistance to the antibiotic ampicillin. If these genes are inserted into the plasmid, then the bacteria that have the plasmid will:

1. Be resistant to the antibiotic ampicillin.
2. Be able to glow IF the bacteria have been fed arabinose sugar. Arabinose sugar activates the GFP gene, which causes the bacteria to glow.

How are these genes inserted into the bacterial plasmid to begin with? These genes were cut from DNA and inserted into the plasmid. Your task today is to model this process by:

1. Finding the best restriction enzyme to cut out the GFP and antibiotic resistance (shaded on the cellular DNA).
2. Cutting the plasmid so the gene of interest can be inserted into it.

## Part A: Modeling Recombinant DNA Technology

1. Study your plasmid DNA. Notice there are sequences that give antibiotic resistance to bacteria from antibiotics. There is also an origin of replication site. If any of these sequences are cut, the ability of the bacteria to reproduce or to have antibiotic resistance will be destroyed.

2. Construct your plasmid by cutting the strips of the plasmid DNA along the lines. Tape the ends together to form a circular plasmid. Tape them together in the same order they are on the paper. For example, the bottom of strip number 1 would be taped to the top of strip number 2.

### Sequence

Ampicillin

Kanomycin

Tetracycline

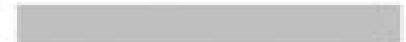
### Shading on Plasmid



3. Construct your strip of cellular DNA by cutting out the strips and taping them together in a linear piece. Again, be sure to tape them in the order they are on the sheet.

Origin of Replication

Origin of Replication



4. Run each restriction enzyme card along the plasmid. Mark any matching restriction sites you find using a different color for each enzyme. The restriction enzymes may cut once, more than once, or not at all. Complete the table in analysis question #1 as you work.

5. Run each restriction enzyme card along the cellular DNA. Mark any matching restriction sites you find using a different color for each enzyme. The restriction enzymes may cut once, more than once, or not at all. Complete the table in analysis question #1 as you work.

6. Now you must decide which restriction enzyme to use in your pretend transformation. Here are the requirements:

- Use a restriction enzyme that can cut out the gene of interest (shaded on the cellular DNA).
- You want to cut as close to the gene of interest on the cellular DNA as you can so that little extraneous DNA will be included.
- You must also choose a restriction enzyme that can cut the plasmid only once so the gene can be inserted into it.

# Dna Technology Section Study Guide Answers

**Susan McMurry**



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