# DNA Technology – Mapping a plasmid

A first step in working with DNA is mapping the DNA molecule. One way to do this is to use restriction enzymes (restriction endonucleases) that are naturally found in bacteria to cut the DNA molecule into fragments, and then perform a gel electrophoresis on the treated DNA. The fragments of DNA can be separated according to their molecular weight as they travel through the gel. The results of the sorted DNA fragments are used to construct a map of the DNA molecule.

There are many different restriction enzymes, each of which recognizes one specific nucleotide sequence. Many restriction enzymes work by finding palindrome sections of DNA (regions where the order of nucleotides at one end is the reverse of the sequence at the opposite end). If a DNA molecule is treated with a number of restriction enzymes, the combination of fragments produced can provide information for making a gene map. Any DNA can be mapped, from plasmids to human DNA. This process of cutting the DNA into fragments is also known as **digesting** the DNA. In real life, bacteria use their restriction endonucleases to literally digest foreign DNA that they encounter in their environment.

Restriction enzymes are also used to make DNA fingerprints, which allows us to identify the source of an unknown sample of DNA. The technique of DNA fingerprinting is important in forensic laboratories where it is used to provide evidence in criminal and paternity cases. DNA fingerprinting can also be used to determine prenatal conditions and diseases that are based on genetic predispositions of the parents. Because each of us has unique DNA, DNA fingerprinting provides positive identification with great accuracy, in contrast to more conventional identification methods, such as blood-typing, which could (and can) only be used to exclude a suspect.

How do restriction enzymes work?
Restriction enzymes catalyze the cutting of both strands of a DNA molecule at very specific DNA base sequences, called recognition sites. Recognition sites are typically 4 - 8 DNA base pairs long. There are over 100 different restriction enzymes, each of which cuts at its specific recognition site(s). A restriction enzyme cuts tiny sticky ends of DNA that will match and attach to sticky ends of any other DNA that has been cut with the same enzyme. DNA ligase joins the matching sticky ends of the DNA pieces from different sources that have been cut by the same restriction enzyme. This is used extensively in the field of DNA technology to produce substances such as insulin and interferon, and to splice genes that alter a cell or organism from its original DNA for some benefit. For example, in agriculture we have use gene splicing to delay the ripening process of tomatoes, to make more nutritious corn, to make rice that contains carotenes and to produce plants with natural pesticides.
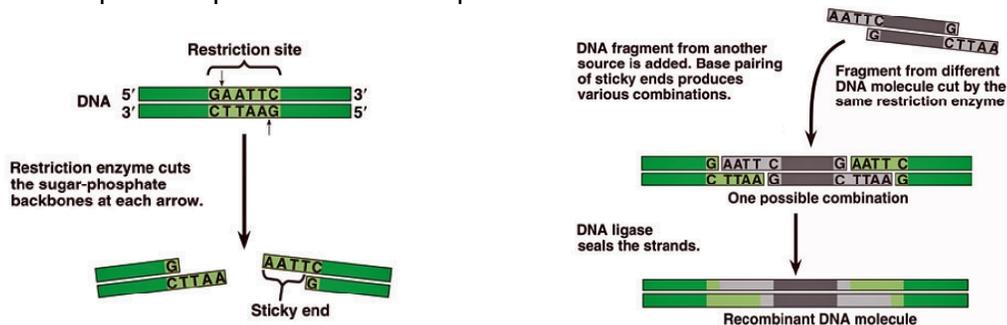
In general, the longer a DNA molecule, the greater the chance that it will have a specific recognition site. The average human chromosome contains over 100 million base pairs, so that a restriction enzyme such as Eco R1 might cut a human chromosome into 750,000 fragments. The differences in DNA base sequences for each of us means that no 2 humans will have exactly the same pattern of recognition sites for restriction enzymes, so that each human's DNA will fragment differently.

What comes after the restriction enzymes?
Using Restriction enzymes to cut DNA into fragments is just the first step. The DNA fragments must then be separated from each other to determine the pattern of cuts and the relative sizes of the DNA fragments. **Gel electrophoresis** is a procedure used to separate and analyze the DNA fragments generated by restriction enzymes. Gel electrophoresis separates charged molecules based on their molecular weight. The materials used in gel electrophoresis consist of a chamber containing a buffer solution which has positive and negative electrodes at either end; and a "gel" which consists of pores that act like a microscopic sieve. An electric current is used to "drive" molecules that are placed in wells made in the gel from the negative electrode of the gel electrophoresis chamber toward the positive electrode. The rate at which molecules move through the gel is relative to their molecular weight. As the molecules are separated they appear as distinct bands on the gel. DNA fragments have a strong negative charge in neutral pH so they are well suited for the technique of gel electrophoresis. For DNA mapping, more than one restriction enzyme will be used, and distance a band travels in the gel for the different enzymes is used to construct the map.
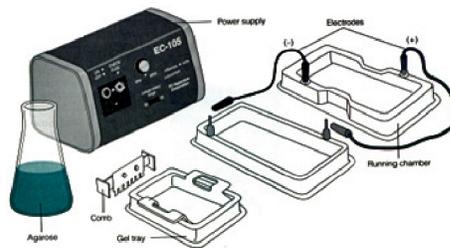
Gel Electrophoresis Chamber

Procedure
Although we will not be doing a gel electrophoresis, data from a gel digest of a *Bacillus anthrax* plasmid is provided so you can do a DNA map. The *Bacillus anthrax* plasmid is 4000bp (4Kb) long. Note the origin position as well as the reference molecular weight markers on the gel. Two restriction enzymes, A and B, were used to obtain two individual digests, A and B. They were combined to produce the third digest.
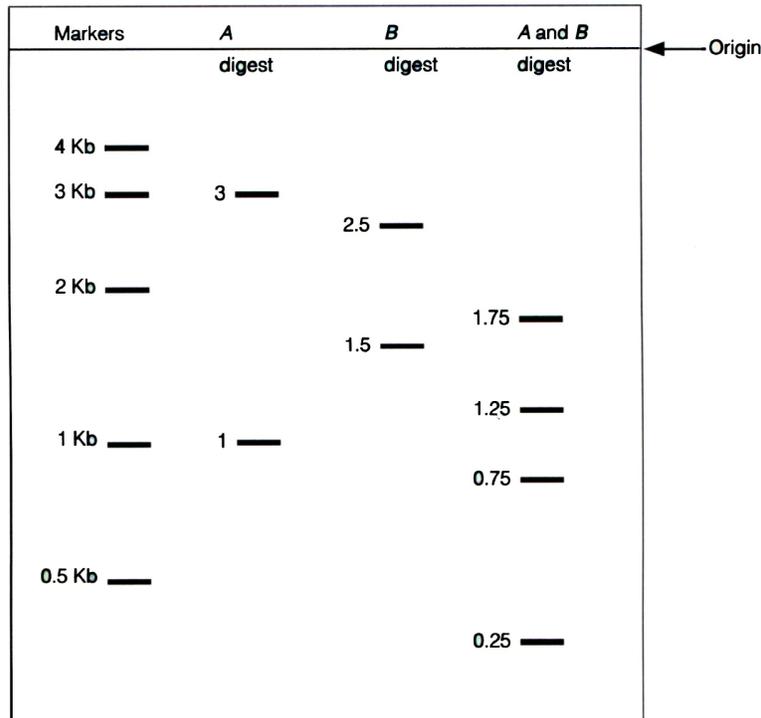
The restriction enzyme fragment pattern for the digest of *Bacillus anthrax* plasmid

Determining the Number of Fragments
How many fragments were produced by enzyme A? _____
How many fragments were produced by enzyme B? _____
How many fragments were produced by the combined digest (A and B)? _____

Fragment Size
Fragment size is relative to molecular weight, and must be determined by comparing the fragment distance to the molecular weight markers. The fragment size has been provided on the gel pattern for this exercise.

To make a map you must determine the relative positions of the restriction sites on the plasmid. It helps to know that the double digest is an extension of the two single digests. The bands you can see in the "A" digest are essentially then subjected to the "B" enzyme digest. Smaller band numbers seen in the double digest can be combined to add up to the "size" of the bands in the A digest. In a similar fashion, the smaller bands of the double digest can be combined to add up to the "size" of the bands in the B digest.

Starting the Map
Determine which two bands in the double digest make up the small A fragment. Mark the bands on the fragment line below, and indicate the size of each. These two bands represent where the B restriction site is on the fragment.

Small A   _____

Determine which two bands in the double digest make up the large A fragment. Mark the bands on the fragment line below, and indicate the size of each. These two bands represent where the B restriction site is on the fragment.

Large A   _____

Determine which two bands in the double digest make up the small B fragment. Mark the bands on the fragment line below, and indicate the size of each. These two bands represent where the A restriction site is on the fragment.

Small B   _____

Determine which two bands in the double digest make up the large B fragment. Mark the bands on the fragment line below, and indicate the size of each. These two bands represent where the A restriction site is on the fragment.
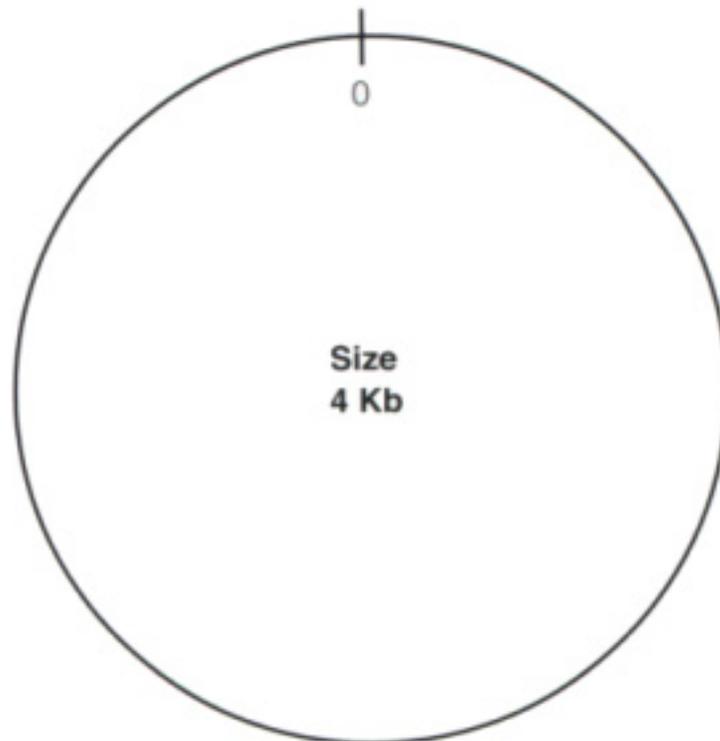
Large B   _____

Superimposing fragments to map the plasmid.
To determine the relative position of the restriction sites on the plasmid, you need to align each of the fragments from the single digests to create one longer fragment, and superimpose one on the other. The alignment of the two large fragments from the plasmid digest is shown below.

1.   Combined A and B Large Fragments
        Large A fragment
        A_____A (3)
        B   1.25 | 1.75   B

        Large B fragment
                                   B_____B (2.5)
                                   A   1.75 | 0.75   A

        Large A and B superimposed
        A   B   A   B
        _____|_____|_____
                 1.25 | 1.75 | 0.75

2.   Do an alignment below for the combined A and B small fragments
        Small A fragment

        Small B fragment

        Small A and B superimposed

3.   Align your two superimposed fragments (the superimposed large and the superimposed small fragments) and look for a repeat.

You can now make a linear map of the plasmid.

The data from your linear map can be redrawn to form the circular plasmid with its restriction sites identified. You can use any point as your start point (0) since the plasmid is circular. This information is useful when we use plasmids to carry desired DNA into a host cell.

0

**Size
4 Kb**