Gel Electrophoresis=Pre-Lab

*Read and annotate (comment/question, etc)

Name _____

Date _____ Period ____

Background Information

Restriction Enzyme	Organism
Bgl I	*Bacillus globigii*
Bam HI	*Bacillus amyloliquefaciens H*
Eco RI	*Escherichia coli, strain RY 13*
Eco RII	*Escherichia coli, strain R 245*
Hae III	*Haemophilus aegyptius*
Hind III	*Haemophilus influenzae* R_d

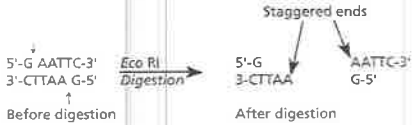
Figure 1

The discovery of restriction enzymes began a new era of molecular genetics. These enzymes cut DNA in a highly specific and reproducible way. This, in turn, made molecular cloning, DNA mapping, sequencing and various genome projects possible.

Restriction enzymes are endonucleases that catalyze cleavage of phosphodiester bonds within both strands of DNA. They require Mg^{+2} for activity and generate a 5 prime (5') phosphate and a 3 prime (3') hydroxyl group at the point of cleavage. The distinguishing feature of restriction enzymes is that they only cut DNA at very specific base sequences. Restriction enzymes are produced by many different species of bacteria (including blue-green algae). Over 2,000 restriction enzymes have been discovered and characterized. More recently, intron-coded yeast mitochondrial endonucleases have been discovered that also cut DNA. The recognition sequences for these enzymes yield very few cuts in DNA and promise to be important new biological reagents for DNA analysis.

Restriction enzymes are named according to the organism from which they are isolated. This is done by using the first letter of the genus followed by the first two letters of the species. Only certain strains or sub-strains of a particular species may produce restriction enzymes. The type of strain or substrain sometimes follows the species designation in the name. Finally, a Roman numeral is always used to designate one out of possibly several different restriction enzymes produced by the same organism.

A restriction enzyme requires a specific double-stranded recognition sequence of nucleotide bases to cut DNA. Recognition sites are usually 4 to 8 base pairs in length. Cleavage occurs within or near specific enzyme recognition sites. The cleavage positions are indicated by arrows. Recognition sites are frequently symmetrical, i.e., both DNA strands in the site have the same base sequence when read 5' to 3'. Such sequences are called palindromes. Consider the recognition site and cleavage pattern of *Eco* RI as an example.



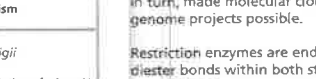
As shown above, *Eco* RI causes staggered cleavage of its site. The resulting ends of the DNA fragments are also called "sticky" or "cohesive" ends.

This is because the single-stranded regions of the ends are complementary.

Some restriction enzymes, such as *Hae*III, introduce cuts that are opposite each other. This type of cleavage generates "blunt" ends.

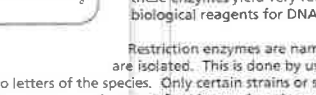
Background Information

The recognition sites of some restriction enzymes contain variable base positions. For example, *Ava* I recognizes:



Keep in mind that A pairs with T and G pairs with C. Consequently, there are four possible sequences *Ava* I recognizes. Recognition sites of this type are called degenerate.

There are certain recognition sites that are separated by a certain number of totally variable bases. For example, *Bgl* I recognizes:



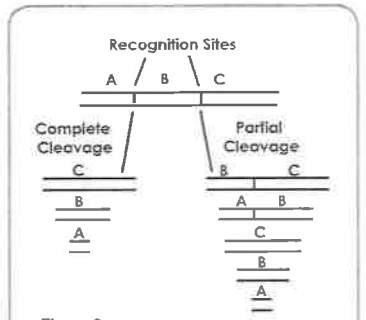


Figure 2:
DNA fragments obtained from complete digestion (left) and partial digestions (right).

There are 625 possible sequences *Bgl* I can cleave. The only bases the enzyme truly "recognizes" are the six G-C base pairs at the ends, which forms a palindrome. In the case of *Bgl* I, these true recognition bases must always be separated by 5 base pairs of DNA, otherwise the enzyme cannot properly interact with the DNA and cleave it. Recognition sites like that of *Bgl* I are called hyphenated sites.

In general, the longer the DNA molecule, the greater the probability that a given restriction enzyme recognition site will occur. The probability of DNA digestion is directly proportional to the size of the enzyme recognition palindrome. Thus, an enzyme that recognizes four nucleotides will cut DNA on average once every 256 base pairs, while an enzyme that recognizes five base pairs will cut DNA once every 1024 base pairs. Human chromosomal DNA contains 3 billion base pairs and has a large number of restriction enzyme recognition sites. Plasmid DNAs usually contain only a few thousand base pairs and contains fewer restriction enzyme sites.

Plasmids and many viral DNAs are circular and double-stranded. If circular DNA contains one recognition site for a restriction enzyme, when cleaved, it will form a linear molecule. By contrast, if a linear DNA molecule that contains a single recognition site is cleaved once, it will generate two fragments. The size of the fragments produced depends on how far the restriction enzyme sites are from each other. If a DNA molecule contains several recognition sites for a restriction enzyme, it is possible that under certain experimental conditions not all sites are cleaved. Incompletely cleaved fragments of DNA are called partial digestion products. These partials can arise if low amounts of enzyme are used or the reaction is stopped after a short time. Reactions containing "partials" usually contain molecules that have not been completely cleaved.

Background Information

Depending on the distances between recognition sites, digestion of DNA by a restriction enzyme will produce DNA fragments of varying lengths. In order to analyze such a mixture of DNA fragments, scientists use a technique called agarose gel electrophoresis.

Agarose gel electrophoresis separates DNA fragments according to size (see figure). First, DNA molecules are added into depressions (or "wells") within a gel, and then an electrical current is passed through the gel. Because the sugar-phosphate backbone of DNA has a strong negative charge, the current drives the restriction fragments through the gel towards the positive electrode.

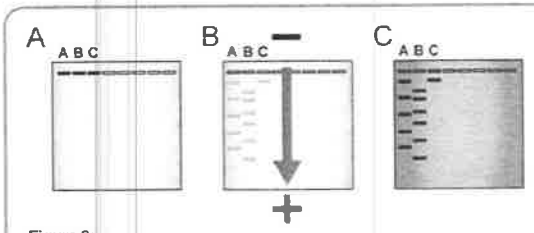


Figure 3:
Overview of Agarose Gel Electrophoresis

At first glance, an agarose gel appears to be a solid at room temperature, but on the molecular level, the gel contains small channels through which the DNA can pass. Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. After the current is stopped, the bands can be visualized using a stain that sticks to DNA.

While electrophoresis is a powerful separation technique, it is not without its technical limitations. Most significantly, if two different fragments share a similar size, they will migrate together through the gel and may appear as a single band. In addition, if digestion results in a broad distribution of DNA sizes, the fragments may stain as a smear. Lastly, DNA with a streamlined secondary structure (such as supercoiled DNA) can pass through the gel more quickly than similarly-sized linear DNA, which prevents an accurate comparison of size.

In this experiment, we will be analyzing the products of a restriction digest of linear DNA isolated from a virus that infects *E.coli*, known as bacteriophage Lambda. Lambda DNA, which contains approximately 49 kilobases (49,000 base pairs), is cut by the restriction enzymes *Eco* RI and *Hind* III five and seven times, respectively. Using agarose gel electrophoresis, we will estimate the lengths of these fragments, and then determine which enzyme was used to digest the DNA.

Background Information

Lambda DNA is used in this experiment that is isolated as a linear molecule from the *E.coli* bacteriophage lambda. It contains approximately 49,000 base pairs and has 5 recognition sites for *Eco* RI, and 7 for *Hind* III. The smaller fragments generated by a restriction enzyme, such as those generated by *Hind* III digestion of Lambda DNA, may not be visible after separation on agarose gel electrophoresis. Smaller fragments will be first to run off the gel during electrophoresis. Since there is less mass in the bands containing smaller fragments, they stain with less intensity and may be less detectable. Stoichiometric cleavage of a pure sample of DNA results in equal amounts of fragments.

Quick Reference:

Lambda DNA cut with *Hind* III using a standard curve will be plotted on semi-log graph paper. The following are the sizes - length is expressed in base pairs.

23130	9416	6557	4361
2322	2027	564	

The discovery of restriction enzymes in the late 1960s had an enormous impact on molecular biology. Due to the exact specificity of restriction enzymes, and the specific cleavage patterns generated when various cut DNA fragments run on gels, restriction enzymes also enables for mapping of DNA. A common application of this is restriction fragment length polymorphism (RFLP), used for e.g. paternity testing. In RFLP, specific human genomic DNA-areas are cut by several restriction enzymes, and the fragments are subjected to electrophoresis. The generated fragment pattern is unique for a given individual, but shares certain similarities with patterns generated by related individuals.

Lambda DNA is used in this experiment is isolated as a linear molecule from the *E. coli* bacteriophage lambda. It contains approximately 49,000 base pairs and has 5 recognition sites for *Eco* RI, and 7 for *Hind* III. The smaller fragments generated by a restriction enzyme, such as those generated by *Hind* III digestion of Lambda DNA, may not be visible after separation on agarose gel electrophoresis. Smaller fragments will be first to run off the gel during electrophoresis. Since there is less mass in the bands containing smaller fragments, they stain with less intensity and may be less detectable. Stoichiometric cleavage of a pure sample of DNA results in equal amounts of fragments.

The Lambda DNA cut with *Hind* III will be served as a marker, providing a set of RFLPs of known sizes (standard). Variations in cleavage patterns obtained from Lambda DNA digested with different restriction enzymes will be analyzed, along with the "uncut" lambda DNA.