**Restriction Enzymes/Gel Electrophoresis Reading**

**Restriction Enzymes**

The discovery of restriction enzymes (RE's) made genetic engineering possible.  RE's first made it possible to work with small, defined pieces of DNA.  Before RE's were discovered, a scientist might be able to tell that a chromosome contained a gene of interest to him.  He might be able to purify the protein or use genetic analysis to tell what other genes were close to "his" gene, but he could not physically locate the gene on the chromosome nor manipulate it.  The scientist could purify the chromosome but then he had a huge piece of DNA containing thousands of genes.  Before the discovery of RE’s the only way to break the DNA into smaller segments was to physically shear it.  But these fragments would be random and not reproducible.  RE's, for the first time, allowed scientists to cut DNA into defined pieces reproducibly, and these pieces could be put back together in new ways for analysis and protein engineering.  Now scientists could study small specific regions of chromosomes.

So, what are restriction enzymes?  Restriction enzymes, or restriction endonucleases, are proteins that recognize and bind to specific DNA sequences and cut the DNA at or near the recognition site.  Restriction enzymes were originally discovered through their ability to break down, or "restrict" foreign DNA.  Naturally RE’s are found in bacterial cells where they protect the cell by acting as DNA scissors, cutting "foreign" DNA into pieces so that it cannot function.

The RE's used in laboratories generally recognize specific DNA sequences of 4 or 6 base pairs.  These recognition sites (restriction sites) are symmetrical or **palindromic. They are the same when read left to right on both strands of DNA. Remember DNA is complementary. A’s pair with T’s and G’s pair with C’s. When reading DNA you ready from the 5’ end to the 3’ end (left to right).**  Below are some examples of restriction enzymes and their recognition sequences, with arrows indicating cut sites. You can see that both sequences on each of the two strands of DNA are the same.

 

BstEII 5’ GGTNACC 3’ HindIII 5’ AAGCTT 3’

3’ CCANTGG 5’ 3’ TTCGAA 5’

 

 

BamHI 5’ GGATCC 3’ SmaI 5’ CCCGGG 3’

3’ CCTAGG 5’ 3’ GGGCCC 5’

 

Notice that some of the RE's (BstEII, HindIII, and BamHI) introduce two staggered cuts in the DNA (producing single-stranded tails or "sticky" ends), while others (SmaI) cut each strand at the same place (producing "blunt" ends).

G AATTC CCC GGG

CTTAA G GGG CCC

sticky ends        blunt  ends

DNA fragments can be put back together (ligated) using the enzyme DNA ligase.  As you might expect any blunt ended-DNA can be ligated but sticky ended-DNA fragments are more selective.  The sticky ends (or tails) must be complementary for ligation to occur. For example the sticky end above that is a single strand of DNA – “AGCT” must be ligated to another sticky end with the complementary bases that would allow the A’s to from a bond with T’s, the C’s with G’s, the G’s with C’s and the T’s with A’s.

**Gel Electrophoresis**

Electrophoresis is a technique used to separate and sometimes purify macromolecules - especially proteins and nucleic acids (such as DNA and RNA) - that differ in size, charge or conformation. As such, it is one of the most widely-used techniques in biochemistry and molecular biology.

When charged molecules are placed in an electric field, they migrate toward either the positive or negative pole according to their charge. In contrast to proteins, which can have either a net positive or net negative charge, **nucleic acids have a consistent negative charge imparted by their phosphate backbone, and migrate toward the anode (the positive electrode).**

Proteins and nucleic acids are electrophoresed within a matrix or "gel". Most commonly, the gel is cast in the shape of a thin slab, with wells for loading the sample. The gel is immersed within an electrophoresis buffer that provides ions to carry a current and some type of buffer to maintain the pH at a relatively constant value.

The gel itself is composed of either agarose or polyacrylamide, each of which have attributes suitable to particular tasks:

**Agarose** is a polysaccharide extracted from seaweed. It is typically used at concentrations of 0.5 to 2%. The higher the agarose concentration the "stiffer" the gel. Agarose gels are extremely easy to prepare: you simply mix agarose powder with buffer solution, melt it by heating, and pour the gel. It is also non-toxic. The buffer solution maintains the proper pH and salt concentration with 0.75% to 2.0% agarose added to form the gel. The agarose forms a porous lattice in the buffer solution and the DNA must slip through the holes in the [lattice](http://faculty.plattsburgh.edu/donald.slish/glossary.html#lattice) in order to move toward the positive pole. This slows the molecule down. Larger molecules will be slowed down more than smaller molecules, since the smaller molecules can fit through the holes easier. As a result, a mixture of large and small fragments of DNA that has been run through an agarose gel will be separated by size.

**Agarose gels have a large range of separation, but relatively low resolving power.** By varying the concentration of agarose, fragments of DNA from about 200 to 50,000 base pairs (bp) can be separated using standard electrophoretic techniques.

**Polyacrylamide** is a cross-linked polymer of acrylamide. The length of the polymer chains is dictated by the concentration of acrylamide used, which is typically between 3.5 and 20%. Polyacrylamide gels are significantly more annoying to prepare than agarose gels. Because oxygen inhibits the polymerization process, they must be poured between glass plates (or cylinders). 

***Acrylamide is a potent neurotoxin and should be handled with care!*** Therefore we will **not** be using this type of gel.

**Polyacrylamide gels have a rather small range of separation, but very high resolving power.** In the case of DNA, polyacrylamide is used for separating fragments of less than about 500 bp. However, under appropriate conditions, fragments of DNA differing is length by a single base pair are easily resolved. In contrast to agarose, polyacrylamide gels are used extensively for separating and characterizing mixtures of proteins. We will **NOT** be using polyacrylamide gels but rather agarose gels.

The agarose gels are visualized on an ultraviolet (U.V.) trans-illuminator by staining the DNA with a fluorescent dye (ethidium bromide). The DNA molecular weight marker is a set of DNA fragments of known molecular sizes that are used as a standard to determine the sizes of your unknown fragments.

The higher the voltage, the faster the electrophoresis time.

**Use online resources to complete the following questions.**

For a visual on **RESTRICTION ENZYMES (endonuclease)** we will be using this website: [http://highered.mcgrawhill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120078/bio37.swf::Restriction%20Endonucleases](http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120078/bio37.swf::Restriction%20Endonucleases)

For a visual on **GEL ELECTROPHORESIS** we will be using this website: <http://www.dnai.org/b/index.html>

-**GEL ELECTROPHORESIS**: Click on “**Techniques**”, then click on “**Sorting & Sequencing**” tab at the top**.** Go through the 2D animations of **gel electrophoresis** and cycle sequencing. We will do the electrophoresis in lab.

1. Describe what restriction enzymes do.

2. Why are restriction enzymes important (name a scientific application)?

3. What is agarose?

4. How does gel electrophoresis work?

6. What are some practical applications for gel electrophoresis (why is it useful)?