Analysis of Precut DNA

Restriction Analysis — Links to Biotechnology

- Hundreds of restriction enzymes are now known, and they have provided the catalyst for the molecular biology revolution in the last part of the twentieth century.

- The restriction enzymes studied in this investigation are EcoRI, PstI, and HindIII.

- In this investigation, the enzymes have been used to digest bacteriophage lambda DNA.

Background

-One of the basic tools of modern biotechnology is DNA splicing: cutting DNA and linking it to other DNA molecules. The basic concept behind DNA splicing is to remove a functional DNA fragment — let’s say a gene — from one organism and to combine it with the DNA of another organism in order to study how the gene works. The desired result of gene splicing is for the recipient organism to carry out the genetic instructions provided by its newly acquired gene. For example, certain plants can be given the genes for resistance to pests or disease, and in a few cases to date, functional genes have been given to people with nonfunctional genes, such as those who have a genetic disease like cystic fibrosis.

Restriction Enzymes

The ability to cut and paste, or cleave and ligate, a functional piece of DNA predictably and precisely is what enables biotechnologists to recombine DNA molecules. This is termed recombinant DNA technology. The first step in DNA splicing is to locate a specific gene of interest on a chromosome. A restriction enzyme is then used to cut out the targeted gene from the rest of the chromosome. This same enzyme is also used to cut the DNA of the recipient into which the fragment will be inserted.

Restriction enzymes are biomolecules that cut DNA at specific sites. Restriction enzymes, also known as endonucleases, recognize specific sequences of DNA base pairs and cut, or chemically separate, DNA at that specific arrangement of base pairs. They were first identified in and isolated from bacteria that use them as a natural defense mechanism to cut up the invading DNA of bacteriophages — viruses that infect bacteria. Any foreign DNA encountering a restriction enzyme will be digested, or cut into many fragments, and rendered ineffective. These enzymes in bacteria make up the first biological immune system. There are thousands of restriction enzymes and each is named after the bacterium from which it is isolated. For example:

EcoRI = The first restriction enzyme isolated from Escherichia coli bacteria

HindIII = The third restriction enzyme isolated from Haemophilus influenzae bacteria
PstI = The first restriction enzyme isolated from *Providencia stuartii* bacteria

Each restriction enzyme recognizes a specific nucleotide sequence in the DNA, called a restriction site, and cuts the DNA molecule at only that specific sequence. Many restriction enzymes leave a short length of unpaired bases, called a “sticky” end, at the DNA site where they cut, whereas other restriction enzymes make a cut across both strands creating double stranded DNA fragments with “blunt” ends. In general, restriction sites are palindromic, meaning they read the same sequence of bases forwards and backwards on the opposite DNA strand.

**Lambda Phage DNA**

Lambda DNA comes from a bacterial virus, or bacteriophage, which attacks bacteria by inserting its nucleic acid into the host bacterial cell. Lambda is a lytic bacteriophage, or phage, that replicates rapidly inside host cells until the cells burst and release more phages to carry out the same infection process in other bacterial host cells. Bacteriophage lambda is harmless to man and other eukaryotic organisms, and therefore makes an excellent source of DNA for experimental study.

In this investigation, students observe the effects of three restriction enzymes on lambda genomic DNA. Since the lambda genome is significantly large, with approximately 48,000 base pairs, each restriction enzyme will cut the DNA several times and generate restriction fragments of different sizes. In this kit, three separate samples of lambda DNA have been precut using the three different restriction enzymes, and one sample remains undigested. Each sample produces DNA fragments whose size can be estimated when run on an agarose gel using electrophoresis.
**Electrophoretic Analysis of Restriction Fragments**

The three-dimensional structure or shape of a restriction enzyme allows it to fit perfectly in the groove formed by the two strands of a DNA molecule. When attached to the DNA, the enzyme slides along the double helix until it recognizes a specific sequence of base pairs which signals the enzyme to stop sliding. The enzyme then chemically separates, or cuts, the DNA molecule at that site — called a restriction site. In this way, a restriction enzyme acts like molecular scissors, making cuts at the specific sequence of base pairs that it recognizes.

If a specific restriction site occurs in more than one location on a DNA molecule, a restriction enzyme will make a cut at each of those sites, resulting in multiple fragments of DNA. Therefore, if a given piece of linear DNA is cut with a restriction enzyme whose specific recognition sequence is found at five different locations on the DNA molecule, the result will be six fragments of different lengths. The length of each fragment will depend upon the location of restriction sites on the DNA molecule.

A DNA fragment that has been cut with restriction enzymes can be separated using a process known as **agarose gel electrophoresis**. The term electrophoresis means to *carry with electricity*. Agarose gel electrophoresis separates DNA fragments by size. DNA fragments are loaded into an agarose gel slab, which is placed into a chamber filled with a conductive buffer solution. A direct current is passed between wire electrodes at each end of the chamber. Since DNA fragments are negatively charged, they will be drawn toward the positive pole (anode) when placed in an electric field. The matrix of the agarose gel acts as a molecular sieve, or a matrix of holes, through which smaller DNA fragments can move more easily than larger ones. Therefore, the rate at which a DNA fragment migrates through the gel is inversely proportional to its size in base pairs. Over a period of time smaller DNA fragments will travel farther than larger ones. Fragments of the same size stay together and migrate in single bands of DNA. These bands will be seen in the gel after the DNA is stained.

An analogous situation is one where all the desks and chairs in the classroom have been randomly pushed together. An individual student can wind his/her way through the maze quickly and with little difficulty, whereas a string of four students would require more time and have difficulty working their way through the maze.

**Visualizing DNA Restriction Fragments**

DNA is colorless so DNA fragments in the gel cannot be seen during electrophoresis. A blue loading dye, containing two blue dyes, is added to the DNA solution. The loading dye does not stain the DNA but make it easier to load the gels and monitor the progress of the DNA electrophoresis. The dye fronts migrate toward the positive end of the gel, just like the DNA fragments. The “faster” dye co-migrates with DNA fragments of approximately 500 bp, while the “slower” dye co-migrates with DNA fragments approximately 5 kb in size. Staining the DNA pinpoints its location on the gel. When the gel is immersed in Fast Blast DNA stain (diluted to 1x for overnight staining or 100x for quick staining), the stain molecules attach to the DNA molecules trapped in the agarose gel. When the bands are visible, your students can compare the DNA restriction patterns of the different samples of DNA.
The DNA pattern that will be obtained by your students following electrophoresis of DNA samples that have been digested using three different restriction digestion enzymes is shown in Figure 1. By convention, the lanes are numbered from the top left. Notice that each restriction enzyme produces a unique banding pattern in each lane. The relative size of fragments contained in each band can be determined by measuring how far each band has traveled from its origin. Since the fragment sizes are known for the HindIII digest, this sample will function as a DNA standard or marker.