Paper Plasmid Digest

Bacteria DNA is not in the form of chromosomes the way our DNA is. Bacterial DNA is circular. Bacteria have only one primary piece of DNA, but they also have small supplementary pieces of DNA that they pick up from their surroundings. These small pieces of circular DNA found in bacteria are called **plasmids**. Plasmids are used in many biotechnology applications because they are pieces of DNA that are easily transferred from one organism to another. They can be cut apart and genes can be inserted in them to make transgenic organisms, or organisms with a gene from another species.

**Restriction enzymes** are enzymes that cut DNA at specific sequences. Most restriction enzymes cut crooked, meaning they leave a single-stranded overhang off one end of the DNA. This overhang is called a “sticky end” because it will hydrogen bond with another overhang if they are complementary. Some restriction enzymes cut straight across. These produce what we call “blunt ends”.

**Procedure:**

1. Locate the four strips of base codes. On each strip, the two rows of letters indicate the nucleotide bases; the small circles between the bases indicate the weak hydrogen bonds.
2. Use your scissors to carefully cut along the SOLID lines.
3. Glue the four numbered strips together by gluing together the matching symbols (& with &, @ with @, etc.). It should create a circular plasmid. Make sure the letters are on the outside of the circle.
4. Scan along your plasmid to find the restriction sites for EITHER HindIII OR BstEII (your teacher will tell you which one to do).
5. Use your restriction enzyme (your scissors) to cut at the appropriate restriction site listed below. There will be multiple restriction sites for your enzyme on the plasmid.

**BstEII Restriction Enzyme:**

5’ – G G T T A C C – 3’ 

3’ – C C A A T G G – 5’

**HindIII Restriction Enzyme:**

5’ – A A G C T T – 3’ 

3’ – T T C G A A – 5’

**Electrophoresis** is a biotechnology technique that separates molecules (usually DNA or protein fragments) on the basis of size and charge in a gel matrix (agarose gel). This allows you to visualize differences between physical pieces of DNA depending on how they are “cut” by restriction enzymes.

Next week we will do electrophoresis with real DNA that we cut with real restriction enzymes. Today, we will visualize the process of electrophoresis using our paper models of plasmid DNA that we cut with scissor restriction enzymes.

In electrophoresis, an electric current is run through a gel with wells loaded with DNA fragments. These are the same gels we used when we pipetted. DNA is negatively charged, so when a current is applied, the DNA pieces start moving toward the positive charge. Small pieces of DNA move the fastest through the gel, and large pieces move slower. So in a set period of time, small pieces move farther in the gel than large pieces. Separate your DNA pieces from smallest to largest, and count the number of base pairs on each piece. Record the number of base pairs for each piece of DNA here:

On the board is a drawing of an electrophoresis gel with base pair numbers listed on the side. Each group gets one well of the gel. Go to the board, claim your gel, and tape your DNA pieces at the appropriate distance from the well based on the base pair length of each piece of DNA. Compare your restriction digest to that of the other groups.

**Analysis Questions:**

1. How can you tell which groups had the same restriction enzyme as your group?

1. How would restriction enzymes be used to insert a gene from one organism into another organism?
2. What would the electrophoresis gel look like if we cut the same piece of DNA with BOTH restriction enzymes?