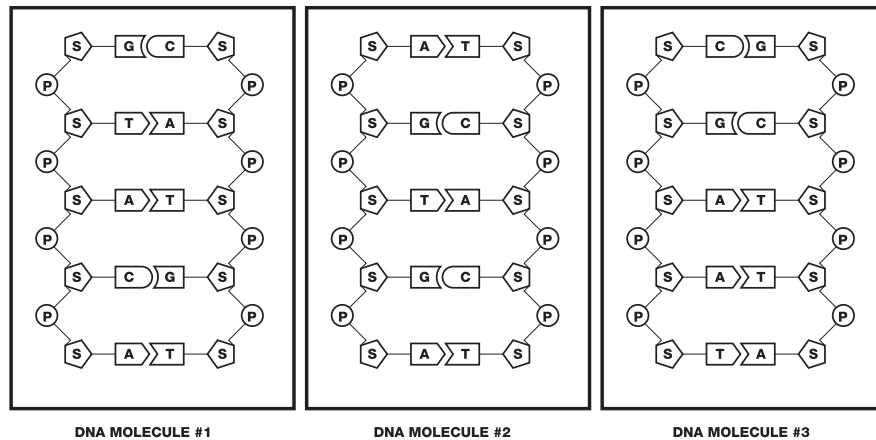


Appendix B

Prelab Activity 1 A Review of Restriction Enzymes

DNA consists of a series of nitrogenous base molecules held together by weak hydrogen bonds. These base pairs are in turn bonded to a sugar-phosphate backbone. The four different nitrogenous bases are **adenine**, **thymine**, **guanine** and **cytosine**. (**A**, **T**, **G**, and **C**: Remember the base-pairing rule is A-T and G-C). Refer to the figures below to review the structure of a DNA molecule.

Fig. 1. The Structure of DNA



If a **segment** of DNA is diagrammed without the sugars and phosphates, the base-pair sequence might appear as:

Read to the right----> A C T C C G T A G A A T T C.....>

<....T G A G G C A T C T T A A G <----Read to the left

Look at the linear sequence of bases (As, Ts, etc.) on each of the strands:

1. Describe any pattern you might see in the upper sequence of bases.
2. Compare the bases in the upper portion of the molecule to those in the lower portion. Describe any relationship you can see.
3. Now look at the upper sequence of bases and compare it to the lower. Do you notice any grouping of bases that when read toward the right on the upper strand and read to the left on the bottom strand are exactly the same?

You may have discovered that the sequence of base pairs is seemingly random and that the two strands are complementary to each other: **A**s are paired with **T**s and **C**s are paired with **G**s. You may have also noticed that a portion of the top strand **GAATTC** (read toward the right), has a counterpart in the lower strand, **CTTAAG** (read toward the left). Example sequences are:

GAATTC
CTTAAG

AAGCTT
TTCGAA

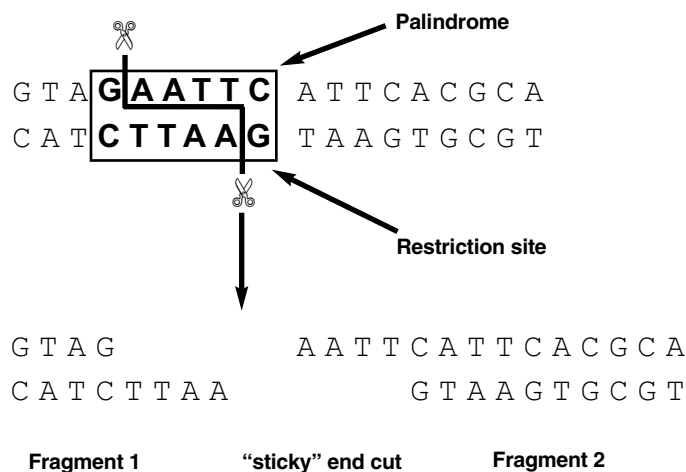
CTGCAG
GACGTC

When such a sequence is looked at together with its complementary sequence the group reads the same in both directions. These sequences, called palindromes, are fairly common along the DNA molecule.

Restriction Enzymes — Molecular Scissors

Viruses called bacteriophages frequently infect bacteria. These viruses inject their own DNA into bacteria and force the bacteria to multiply the DNA. Bacteria have evolved restriction enzymes, to cut up and destroy the invading viral DNA. Bacteria prevent destruction of their own DNA by modifying certain DNA bases within the specific enzyme recognition sequence, which allows them to protect their own DNA while cutting up viral DNA. This could be considered a very primitive immune system. Restriction enzymes search the viral DNA for specific palindromic sequences of base pairs, such as **GAATTC**, and cut the DNA at these sites. The actual sequence of DNA is called a **restriction site**. Some restriction enzymes may leave a short length of unpaired nucleotide bases, called a “sticky” end, at the DNA site where they cut, whereas other restriction enzymes make a cut across both strands at the same place creating double stranded DNA fragments with “blunt” ends.

Look at the DNA sequence below:



A restriction enzyme cut the DNA between the **G** and the **A** in a **GAATTC** palindrome.

- How many bases are still paired to the left of the “cut”?
- How many bases are still paired to the right of the “cut”?
- Counting the number of paired bases, is the right fragment the same size as the left fragment?
- How could you describe the size of each fragment in terms of the number of base pairs in the fragment?

An important feature of restriction enzymes is that each enzyme only recognizes a specific palindrome and cuts the DNA only at that specific sequence of bases. A palindromic sequence can be repeated a number of times on a strand of DNA, and the specific restriction enzyme **will cut all those palindromes**, no matter what species the DNA comes from.

8. If the **GAATTC** palindrome is repeated four times on the same piece of linear DNA, and the restriction enzyme that recognizes that base sequence is present and digests the DNA, how many DNA fragments will be produced?

9. If the **GAATTC** palindrome repeats are randomly found along the DNA strand, then what can you say about the sizes of the fragments that will be produced when the DNA is digested with a restriction enzyme that recognizes that sequence?

The table below shows palindromic sequences that are recognized by the enzymes that are used to digest the DNA you will be analyzing in this activity.

Palindromic sequence	Name of restriction enzyme that recognizes the palindrome
GAATTC CTTAAG	<i>EcoRI</i>
AAGCTT TTCGAA	<i>HindIII</i>
CTGCAG GACGTC	<i>PstI</i>

Below is the summary of what we have learned so far:

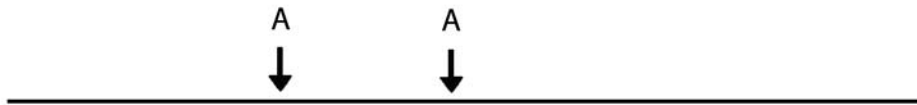
- A sequence on one strand of DNA and its complementary sequence on the other strand can form a palindrome i.e.,

GAATTC
CTTAAG
- Palindromes can be detected by restriction enzymes
- Restriction enzymes cut the palindromes at restriction sites
- Restriction enzymes recognize specific palindromes
- Cutting DNA at restriction sites will produce DNA fragments
- Fragment size can be described by the number of base pairs a fragment contains

Applying What You Have Learned

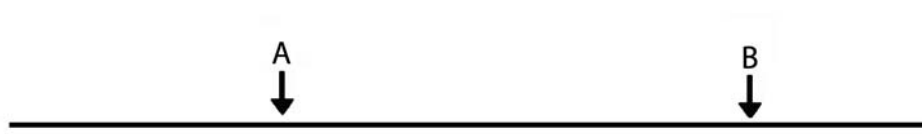
A linear DNA molecule is represented below. The DNA is represented by one line, although in actuality, DNA has two strands.

1. If the DNA molecule has two restriction sites, for restriction enzyme A, how many fragments would be produced if the DNA is cut by that enzyme?



2. Number each fragment.
3. Which fragment is the largest?
4. Which fragment is the smallest?

5. Draw a DNA molecule that has five randomly spaced restriction sites for a specific palindrome. How many fragments would be produced if each site were cut by that specific restriction enzyme?
6. Label each fragment.
7. Rank them in order of size from largest to smallest.



In this diagram A and B are different palindrome sequences on a DNA strand. Only the restriction enzyme that recognizes site B is present.

8. Explain why only two fragments would be produced.