

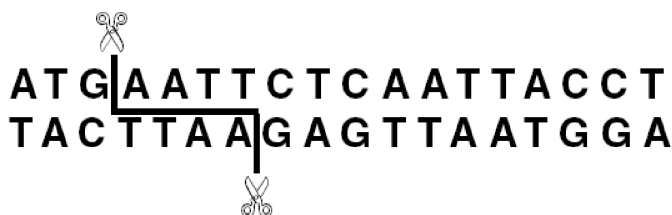
Prelab-Restriction Digestion of DNA Samples

How can we detect differences in base sequences?

At first sight, your task might seem rather difficult. You need to determine if the **linear** base pair **sequence** in the DNA samples is identical or not! An understanding of some historically important discoveries in **recombinant DNA technology** might help you to develop a plan.

In 1968, Dr. Werner Arber at the University of Basel, Switzerland and Dr. Hamilton Smith at the Johns Hopkins University, Baltimore, discovered a group of enzymes in bacteria, which when added to **any** DNA will result in the breakage [**hydrolysis**] of the sugar-phosphate bond between certain specific nucleotide bases [**recognition sites**]. This causes the double strand of DNA to break along the recognition site and the DNA molecule becomes fractured into two pieces. These molecular scissors or "cutting" enzymes are **restriction endonucleases**.

Two common restriction enzymes (endonucleases) are EcoRI and PstI which will be provided to you in this lab procedure. To better understand how EcoRI and PstI may help you in performing your DNA fingerprinting experiment, first you must understand and visualize the nature of the "cutting" effect of a restriction endonuclease on DNA:



The line through the base pairs represents the sites where bonds will break if the restriction endonuclease EcoRI recognizes the site **GAATTC**. The following analysis questions refer to how a piece of DNA would be affected if a restriction endonuclease were to "cut" the DNA molecule in the manner shown above.

1. How many **pieces** of DNA would result from this cut? _____
2. Write the **base sequence** of the DNA fragments on both the left and right side of the "cut".

Left:

Right:

3. What differences are there in the two pieces?

4. DNA fragment size can be expressed as the number of base pairs in the fragment. Indicate the size of the fragments [mention any discrepancy you may detect].

- a) The smaller fragment is _____ base pairs (bp).
- b) What is the length of the longer fragment? _____

5. Consider the two samples of DNA shown below - single strands are shown for simplicity:

Sample #1

CAGTGATCTCGAATTCGCTAGTAACGTT

Sample #2

TCAATGAATTCCTGGAATCAGCAAATGCA

If both samples are treated with the restriction enzyme EcoRI [recognition sequence **GAATTC**] then indicate the number of fragments and the size of each fragment from each sample of DNA.

Sample # 1

of fragments: _____

Sample # 2

of fragments: _____

List fragment size in order: largest —> smallest

Sample # 1

Sample # 2

