Subject Area(s)
Biology

Associated Unit
Engineering Nature: DNA Visualization and Manipulation

Lesson Title
Restriction Enzymes and DNA Fingerprinting

Header Insert Image 1 here

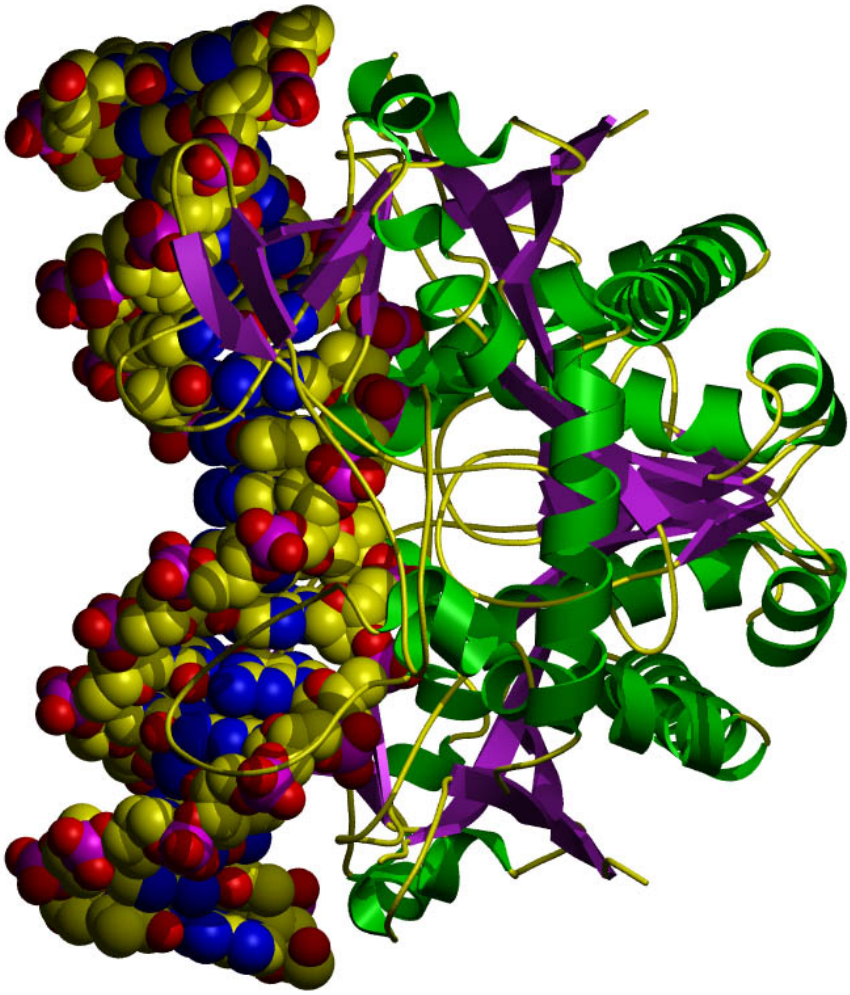

Image 1

ADA Description: Computer generated rendering showing the position of the atoms in a restriction enzyme

Caption: The molecular structure of the Bg II restriction enzyme as reconstructed by computer models

Image file: restriction.jpg

Grade Level 10 (9-12)

Lesson # 2 of 2

Time Required
80 minutes

Summary
The discovery of restriction enzymes and their applications in DNA analysis has proven to be essential for biologists and chemists. This lesson and its associated activity can be used in conjunction with biology lessons on DNA analysis and DNA replication. The lesson focuses on restriction enzymes and their application to DNA analysis and DNA fingerprinting.

Engineering Connection
Microfluidics concepts and devices used to study colloidal particle flow are also employed by biologist to study and filter biomolecules. Gel electrophoresis is one example of engineering applications that are used by biologists to compare fragments of DNA samples.

Engineering Category = #1
Choose the category that best describes this lesson's amount/depth of engineering content:
1. Relating science and/or math concept(s) to engineering
2. Engineering analysis or partial design
3. Engineering design process

Keywords

DNA, electrophoresis, enzymes, genetics, inheritance, pigments, restriction enzyme

Educational Standards
Biology: Texas Essential Knowledge and Skills (112.34. Biology, Beginning with School Year 2010--2011)
(b) (3) Scientific inquiry. Scientific inquiry is the planned and deliberate investigation of the natural world. Scientific methods of investigation are experimental, descriptive, or comparative. The method chosen should be appropriate to the question being asked.
(c) (2) Scientific processes. The student uses scientific methods and equipment during laboratory and field investigations. The student is expected to:

> (B) know that hypotheses are tentative and testable statements that must be capable of being supported or not supported by observational evidence. Hypotheses of durable explanatory power which have been tested over a wide variety of conditions are incorporated into theories;
>
> (C) know scientific theories are based on natural and physical phenomena and are capable of being tested by multiple independent researchers. Unlike hypotheses, scientific theories are well-established and highly-reliable explanations, but they may be subject to change as new areas of science and new technologies are developed;
>
> (D) distinguish between scientific hypotheses and scientific theories;
>
> (E) plan and implement descriptive, comparative, and experimental investigations, including asking questions, formulating testable hypotheses, and selecting equipment and technology;

(c) (10) Science concepts. The student knows that biological systems are composed of multiple levels. The student is expected to:

> (C) analyze the levels of organization in biological systems and relate the levels to each other and to the whole system.

Pre-Requisite Knowledge

Basic human anatomy, high school freshman level biology

Learning Objectives

After this lesson, students should be able to:

- Describe the basic steps involved in DNA fingerprinting
- Know the function of restriction enzymes
- The role of restriction sites along the DNA
- Know that DNA fragments are charged molecules
- The distance traveled by DNA fragments inside the gel tray depends on the length of fragments
- The DNA (genetic code) from related organisms contain similar restriction sites
- Related DNA are cut into similar fragments by the same restrictions enzymes

Introduction / Motivation

Comparison between multiple DNA samples can be performed by a simple technique called restriction fragment length polymorphism (RFLP). This technique is based on several concepts ranging from genetics to physics and engineering. The genetic code contained inside the DNA molecule does not need to be entirely compared between two or more DNA samples but only some portions of it. In the early days of genetics there were no computers and no automated, fast processes for DNA decoding and analysis. Then, how was possible to analyze and compare DNA samples? By analyzing short DNA fragment. Restriction enzymes are a special class of enzymes that can cut the DNA into fragments at specific locations called restriction sites. This a defense mechanism employed by bacteria for protection against viral DNA or genetic code. This concept can also be used to cut DNA into short fragments for analysis.

How can multiple DNA samples be compared? By cutting them with the same restriction enzyme and comparing the fragments. If two or more DNA samples have the same restriction sites then they will be broken into similar fragments. How can someone tell if two DNA fragments are the same? This is accomplished by placing the fragments in a gel tray and applying an electric current along the plate. As the DNA fragments are charged, the positively charged fragments will move toward the cathode and the negatively charged toward the anode. The movement of fragments through the agarose gel solution is determined by their mass—smaller fragments will move further away while longer fragments will move slower.

In the same manner, the pigments that are used to give a certain color to paint will segregate when a drop of water based paint is placed on a filter paper and water is allowed to diffuse through the paper. Different colors that are combinations of multiple pigments will decompose into their respective pigments, thus, allowing for the identification of common pigments.

Lesson Background & Concepts for Teachers

Restriction enzymes attach to DNA and are activated by restriction sequences in the DNA. Once activated, the restriction enzymes will hydrolyze and destroy the bonds between nucleotides. The restriction sequences along the DNA are inherited, thus, people that are related they have similar restriction sequences along their DNA. Cutting DNA samples by the same restriction enzymes and analyzing the resulting DNA fragments by DNA fingerprinting will indicate which DNA samples have similar restriction sequences. During DNA fingerprinting, DNA fragments are placed in agar gel and an electric field is applied along the gel plate. As DNA fragments are electrically charged they will travel through the gel. DNA fragments of same length will travel at the same rate with shorter fragments traveling faster and longer ones slower. DNA samples that

are broken in similar fragments have the same restriction sequences occurring at the same location along the DNA double strand.

Restriction enzymes attach to DNA and cleave it (cut it) randomly or at specific locations. Bacteria are protected from foreign DNA by using its restriction enzymes to destroy the foreign DNA. Restriction enzyme (restriction endonuclease) cuts DNA at specific locations (specific nucleotide sequence) called restriction (recognition) sites. But how is the DNA of the organism (or bacteria) protected against its own restriction enzymes? The DNA is protected by methylases—enzymes that add methyl groups to adenine and cytosine that are part of the recognition sequences (sites) to prevent the restriction enzymes from cleaving the DNA. Restriction enzymes are categorized in:

Type I and III: both restriction and methylation performed by one large enzyme

Type II and III: cleave (cut) DNA at random sites (don't need recognition sequence)

Also, Type II—only restriction, methylase performed by another enzyme.

Examples of restriction enzyme: EcoRI. It cleaves at the GAATTC recognition sequence. The DNA double helix is cut between G and A and two "sticky ends": 5' (AATT) result. Sticky ends can bind to their complement (DNA ligase enzymes fuse two fragments—just the opposite action of restriction enzymes).

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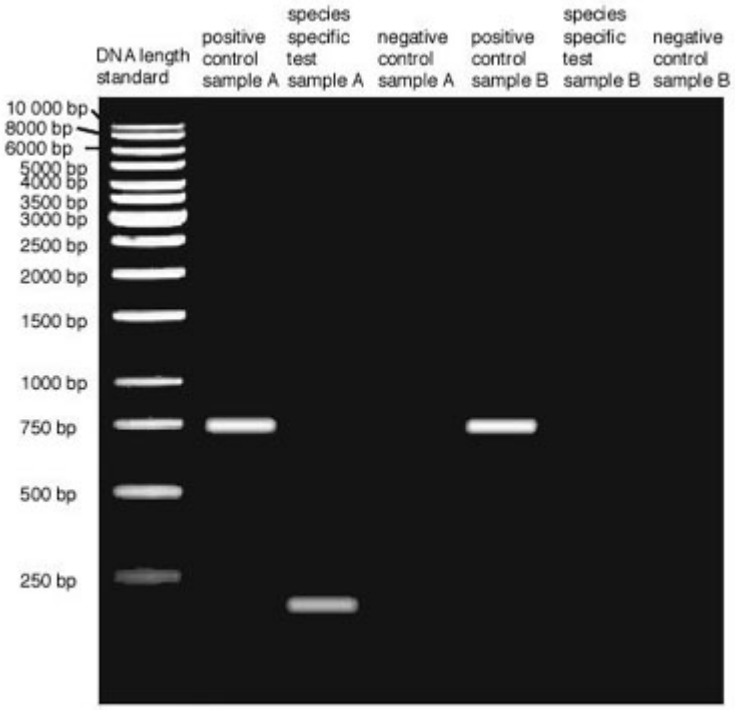

Figure 1

ADA Description: Picture showing horizontal bars on a black background

Caption: Figure 1. Example of gel electrophoresis indicating the length of DNA fragments (number of base pairs). Notice that shorter fragments have traveled longer through the agarose gel

Image file: gel.jpg

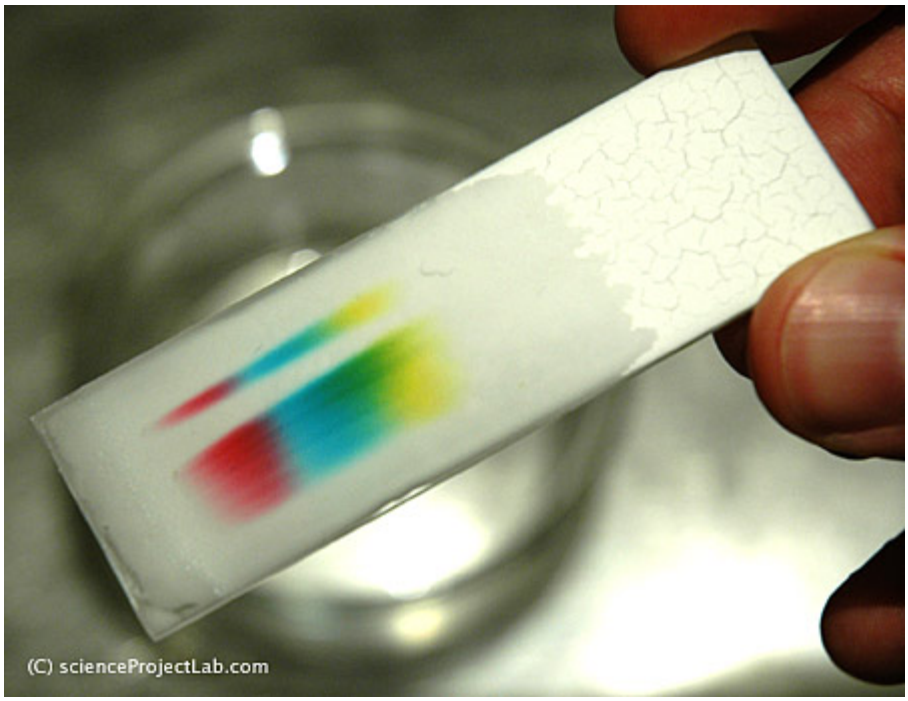

Figure 2

ADA Description: The image shows a filter paper with four colors

Caption: Figure 2. Example of paper chromatography in which different pigments have been differentiated

Image file: chromato.jpg

Associated Activities

DNA Forensics and Color Pigments

Lesson Closure

Assessment

See the assessment worksheet attached to the associated activity.

Lesson Extension Activities

Additional Multimedia Support

References

Attachments

Other

Redirect URL

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