

Lab 11: Restriction Enzyme Cleavage of DNA and Electrophoresis

Overview:

Molecular biologists use many techniques to analyze DNA. In this lab, you will utilize several of these techniques to study the DNA of a lambda bacteriophage. Restriction enzyme digests of phage *lambda* DNA will be used to demonstrate techniques for separating and identifying DNA fragments using gel electrophoresis.

Objectives

1. Demonstrate how restriction enzymes are used in genetic engineering.
2. Use electrophoresis to separate DNA fragments
3. Determine unknown DNA fragment sizes when given fragments of known size.

Introduction

Restriction endonucleases are essential tools in recombinant DNA methodology. Several hundred endonucleases have been isolated from a variety of prokaryotic organisms. In nature, an endonuclease is a prokaryotic cell's defense against foreign DNA inserted by a virus. The endonuclease cuts DNA at palindromes sites. Restriction endonucleases such as EcoRI and HaeII are named according to a specific system of nomenclature. The letters refer to the organism from which the enzyme was first isolated. The first letter of the name stands for the genus name of the organism. The next two letters represent the second word of the species name. The fourth letter (if there is one) represents the strain of the organism. Roman numerals indicate whether or not the particular enzyme was the first isolated, second, or so on.

Examples: EcoRI	E = genus <i>Escherichia</i>	HaeII	H = genus <i>Haemophilus</i>
	co = species <i>epithet coli</i>		ae = species <i>aegyptius</i>
	R = strain RY13		II = second endonuclease
	I = first endonuclease isolated		isolated from organism

These restriction endonucleases recognize specific DNA sequences in double stranded DNA (usually a 4-6 base pair sequence of nucleotides) and digest the DNA at these sites. The result is the production of fragments of DNA of various lengths corresponding to the distance between identical DNA sequences within the chromosome. Some restriction enzymes cut cleanly through the DNA helix at the same position on both strands to produce fragments with blunt ends. Other endonucleases cleave each strand off-center at specific nucleotides to produce fragments with 'over hangs' or **sticky ends**. By using the same restriction enzyme to cut DNA from two different organisms, complementary sticky ends will be produced and can realign in a 'template-compliment' manner allowing DNA from two sources to be **recombined**.

By taking DNA fragments and systematically reinserting the fragments into an organism with minimal genetic material, it is possible to determine the function of particular gene sequences. In this way, the genome or chromosomal character of the organism can be dissected, rearranged, and tested for function and organization. Further, when a gene sequence of interest has been identified, it is possible to remove it from a chromosome and insert it into a plasmid that is capable of generating many copies of itself and the foreign gene sequence. This process is known as **gene cloning**. Finally, the sequence can be transferred to other systems to produce a desired effect as done during the pGLO transformation lab. Another well-known example of genetic engineering is the cloning of the human insulin gene. The gene was removed from human chromosome number 11 using restriction endonucleases and was inserted into an *E. coli* plasmid at a site that was cut with the same restriction enzyme as was used to cut out (excise) the human insulin gene. Restriction endonucleases and gene cloning have made possible the large-scale production of human insulin in the laboratory.

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