

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.

Restriction Enzyme Cleavage Of Dna Lab Answers

Ying Liu



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Addison-Wesley Biology Addison Wesley,1996-04 , *Principles of Genetics* D. Peter Snustad,Michael J. Simmons,2015-10-26 *Principles of Genetics* is one of the most popular texts in use for the introductory course It opens a window on the rapidly advancing science of genetics by showing exactly how genetics is done Throughout the authors incorporate a human emphasis and highlight the role of geneticists to keep students interested and motivated The seventh edition has been completely updated to reflect the latest developments in the field of genetics *Principles of Genetics* continues to educate today s students for tomorrows science by focusing on features that aid in content comprehension and application This text is an unbound three hole punched version *Genetics Abstracts* ,1990 Cumulated Index Medicus ,1999 Virology Abstracts ,1980-07 INIS Atomindex ,1988 *Cleavage of Mouse DNA by a Restriction Enzyme as a Clue to the Arrangement of Genes* ,1974 *Single Molecule Study of DNA Configuration on Restriction Enzyme Cleavage Rate Using Optical Tweezers* Svenja-Marei Kalisch,Heinz Helmers,Gijs Wuite,2006 Restriction Endonucleases Alfred Pingoud,2004-02-19 Restriction enzymes are highly specific nucleases which occur ubiquitously among prokaryotic organisms where they serve to protect bacterial cells against foreign DNA Many different types of restriction enzymes are known among them multi subunit enzymes which depend on ATP or GTP hydrolysis for target site location The best known representatives the orthodox type II restriction endonucleases are homodimers which recognize palindromic sequences 4 to 8 base pairs in length and cleave the DNA within or immediately adjacent to the recognition site In addition to their important biological role up to 10 % of the genomes of prokaryotic organisms code for restriction modification systems they are among the most important enzymes used for the analysis and recombination of DNA In addition they are model systems for the study of protein nucleic acids interactions and because of their ubiquitous occurrence also for the understanding of the mechanisms of evolution The present book deals with all aspects of restriction endonucleases including nomenclature diversity evolution genetics structure and function mechanism of target site location and DNA recognition enzymology protein design and provides a description of the history of the discovery of and the research on restriction enzymes

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