

MODULE 2: LECTURE 1

ENZYMES IN GENETIC ENGINEERING: RESTRICTION NUCLEASES: EXO & ENDO NUCLEASES

2-1.1 Introduction:

A **restriction enzyme** is a nuclease enzyme that cleaves DNA sequence at a random or specific recognition sites known as restriction sites. In bacteria, restriction enzymes form a combined system (restriction + modification system) with modification enzymes that methylate the bacterial DNA. Methylation of bacterial DNA at the recognition sequence typically protects the own DNA of the bacteria from being cleaved by restriction enzyme.

There are two different kinds of restriction enzymes:

- (1) **Exonucleases** catalyses hydrolysis of terminal nucleotides from the end of DNA or RNA molecule either 5' to 3' direction or 3' to 5' direction. Example: exonuclease I, exonuclease II etc.
- (2) **Endonucleases** can recognize specific base sequence (restriction site) within DNA or RNA molecule and cleave internal phosphodiester bonds within a DNA molecule. Example: EcoRI, Hind III, BamHI etc.

2-1.2 History:

In 1970 the first restriction endonuclease enzyme *HindII* was isolated. For the subsequent discovery and characterization of numerous restriction endonucleases, in 1978 Daniel Nathans, Werner Arber, and Hamilton O. Smith awarded for Nobel Prize for Physiology or Medicine. Since then, restriction enzymes have been used as an essential tool in recombinant DNA technology.

Module 2 Lecture 1 Enzymes In Genetic Engineering

**João Manuel R. S. Tavares, Nilanjan
Dey, Amit Joshi**



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Enzymes represent one of the most fascinating and meaningful entities in the biological world. In the fields of genetic engineering and recombinant DNA technology, enzymes serve as vital tools. Enzymes acting as biological catalysts have been known to all of us since our primary education. Nonetheless, learning about enzymes at the graduate level can be difficult by a lack of comprehension of the principles and a misunderstanding of the terminology employed in enzymology. This book has been written to augment the understanding of enzymology among graduate and postgraduate students. The author of this book has extensive experience teaching enzymology and has proven and admired competence in the field. Balanced text, well-illustrated concepts, and an organized stepwise ascent of the topics are key features of this book. Besides fundamentals, this book also covers some of the emerging areas of enzymology, such as enzyme immobilization, reversible covalent modification, and proteolytic activation. This book not only provides information for enzymologists but also does so in a manner that will also aid non-enzymologists in making proper use of these biocatalysts in their research. This book consists of a detailed discussion of key concepts of enzymology: enzyme kinetics, modes of enzyme regulation, isozymes, enzyme immobilization, DNA cloning, and genome editing. Difficult concepts have been simplified with analogies and examples. Furthermore, this book's material aligns with the majority of university curriculum and exams, both domestically and internationally.

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