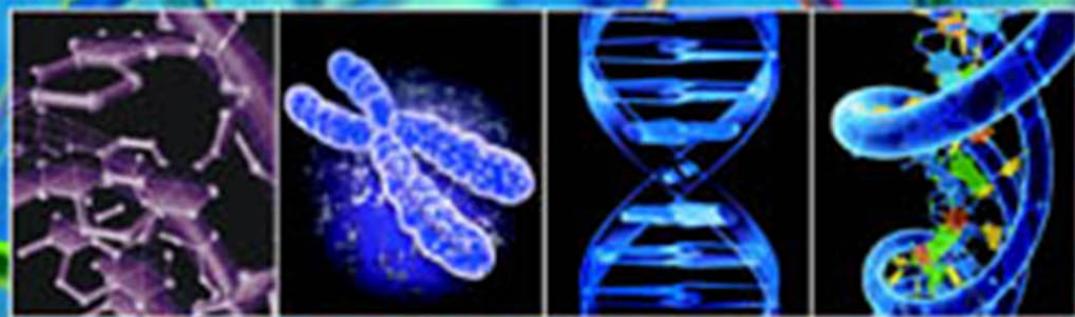


# Recombinant DNA Technology

Sardul Singh Sandhu



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**Prof. Sardul Singh Sandhu**

M.Sc., M. Phill., Ph. D. & D. Sc.

Director

Centre of Scientific Research and Development (CSR  
D)  
Peoples Group, Bhanpur, Bhopal,  
M. P., India



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# Dedication

I dedicate the book “Recombinant DNA Technology” to People’s Group, Bhopal because this group is itself dedicated towards nurturing humanity through health and educational excellence and has treated about 10 lakh patients free of cost in Central India, in 1200 bedded advanced and super speciality hospitals of People’s Group.



Her Excellency President of India Smt. Pratibha Devisingh Patil inaugurated Centre for Scientific Research and Development (C.S.R.D.), People’s Group, Bhopal on 29<sup>th</sup> June 2008. The main aim of this Centre is to excel in the research field of Medical Biotechnology and specially Recombinant DNA Technology, which is the main theme of this book too.



**Left to Right:** Shri Shivraj Singh Chauhan, Chief Minister, M.P., Shri A.S. Sejwal, Health Minister, M.P., Shri Balram Jhakar, Governor, Shri Devisingh Shekhawat, M.P., Her Excellency President of India, Smt. Pratibha Devisingh Patil, Prof. S.S. Sandhu, Director, C.S.R.D., Capt. Ruchi Vijaywargia, CEO, People’s Samachar, Brig. Anil Kohli, President, D.C.I. and Shri. S.N. Vijaywargia, Chairman, People’s Group, Bhopal.

# Foreword

*Recombinant DNA Technology* has appeared at a more appropriate time, for the field is in a period of enormous progress and the prospects for future advances are even greater. It gives me immense pleasure to write the foreword for this book which is primarily meant for the use of undergraduate and postgraduate students pursuing studies in biotechnology and allied fields. It contains the basic information needed to understand this area along with the most current work at the forefront of the field. The author is to be commended for assembling a broad, comprehensive, well-organized overview of this burgeoning field. Although the available literature covers some exercises of one of these disciplines, there has been a conspicuous requirement for a complete book that imparts knowledge on the principles and applications of various techniques of biotechnology. This book follows the revised syllabi of all major universities and covers up-to-date information nearly on all aspects of Recombinant DNA Technology. The book commences with basic and fundamental topics in Recombinant DNA Technology such as the concepts of nucleic acids, genes, restriction enzymes, vectors, cloning, etc, while it culminates with the most sophisticated topics of nucleic acid hybridization, genetic engineering in action, gene expression and transgenic technology. Special attention has been made in defining the fundamental concepts and authentic explanation of various techniques.

The book starts with an introduction to the fundamental concepts of genomics and vectors, restriction enzymes and cloning. The final chapters cover genomic libraries, Polymerase Chain Reaction, blotting techniques, DNA sequencing, protein engineering, transgenic technology and applications of Recombinant DNA Technology. This edition features greatly expanded coverage of the latest innovations in DNA sequencing techniques, therapeutics, vaccines, transgenic plants and animals, the methodology and applications of genetic engineering of plants, and microbial production of therapeutic agents. Updated chapters reflect recent developments in biotechnology and the societal issues related to it, such as cloning, gene therapy, and patenting and releasing genetically engineered organisms. With its broad range of topics, the book is adaptable to different upper-level undergraduate and graduate courses emphasizing particular aspects of modern biotechnology. It includes chapter summaries in the form of a flow chart highlighting key points. The book embraces a glossary defining nearly all the possible terms mentioned in the text. Standard protocols employed in Recombinant DNA Technology are

accurately presented in a user-friendly style at the end. I hope this book will not only appeal to the students of biotechnology but also to those pursuing other interlinked disciplines. This book offers an authoritative, accessible, and engaging introduction to modern genome centered biology from its foremost practitioners. It explores core concepts in Recombinant DNA Technology and Molecular Biology in a contemporary inquiry based context, building its coverage around the most relevant and exciting examples of current research and landmark experiments that redefined our understanding of nucleic acids. As a result, students learn in a compelling way how working scientists make real high impact discoveries. This is an excellent book by any standards and I sincerely hope that students and young researchers make full use of this valuable book.

**PROFESSOR ANTONIO ROSSI**  
*FMRP, University of Sao Paulo,  
Rebeirão Preto, Brazil*

# Preface

Recombinant DNA Technology has been introduced in the curricula of many universities even at Graduation standard. Many books on Recombinant DNA Technology have been published in the past three decades. Some are very expensive others do not contain much details and some topics are superficially explained and are in introductory pattern only, therefore, the students are not getting benefits particularly in developing countries. This book comprises virtually all aspects of Recombinant DNA Technology and consists of eight chapters with details of the areas in the field of Recombinant DNA Technology. Chapter-1: introducing the subject with some milestone inventions in Recombinant DNA Technology. Chapter-2: covers some basic molecular biological studies which provide the general background for understanding the genes. Chapter-3: enumerate the information for isolation, handling and other techniques used while working with DNA. Chapter-4: illustrates the tools used in Recombinant DNA Technology and Chapter-5: provide the detailed account of biology involved in Recombinant DNA Technology like essential enzymes and biological system needed for cloning a gene. Chapter-6: is the detailed account of the strategies used for cloning a gene. Chapter-7: describes in detail how a particular DNA fragment can be chosen from thousands of the cloned DNA sequences and how one can analyze the recombinants. Chapter-8: the practical appliances of Recombinant DNA Technology enumerated the applied part of this technology with references to some universally available examples like gene therapy and gene knock down are also included in this chapter.

At the beginning of all the chapters a flow chart is provided, which illustrates the important points of the chapter, which may provide the essential summaries of each chapter. A glossary of the terms has also been made at the last. The language of the book is also taken care to be understood by the graduate level students. The book may be useful to the readers who are unfamiliar to the subject but have little interest in Recombinant DNA Technology. Low cost protocols for 16 experiments needed for Recombinant DNA Technology are also included in the end so that students could get at least initial exposure to Recombinant DNA Technology at graduate level, and hence they will feel better at master level with gene cloning and other advanced experiments.

I am highly grateful to the following for their support and help in compilation of this book. Dr. Jim Kinghorn and Dr. Shiela E. Unkles (St. Andrews, Fife, Scotland), Prof. G. Venkateshwerlu (Osmania University, Hyderabad),

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Dr. Carlose A. Columbo (IAC, Brasil), Prof Antonio Rossi (USP, Brazil), Prof. G.P. Agarwal, Prof. S.K. Hasija and Prof. R.C. Rajak (R. D. University, Jabalpur), I am also thankful to Mr. Arun Gurtoo, IPS (Rt.) and Ex. Vice-Chancellor, R. D. University, Jabalpur for his kind support.

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Last but not the least; I am truly grateful to my wife Mrs. Ranjit Kaur Sandhu, daughter Ishwinder Kaur Sandhu, son Repudaman Singh Sandhu and my Parents for their cooperation and moral support, and I am thankful to all those who helped me directly or indirectly.

PROF. SARDUL SINGH SANDHU

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# About the Book

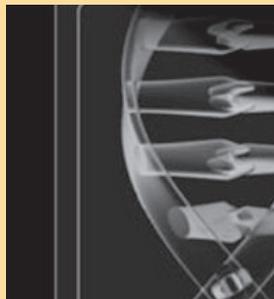
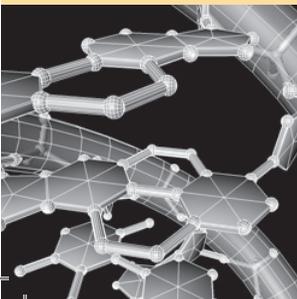
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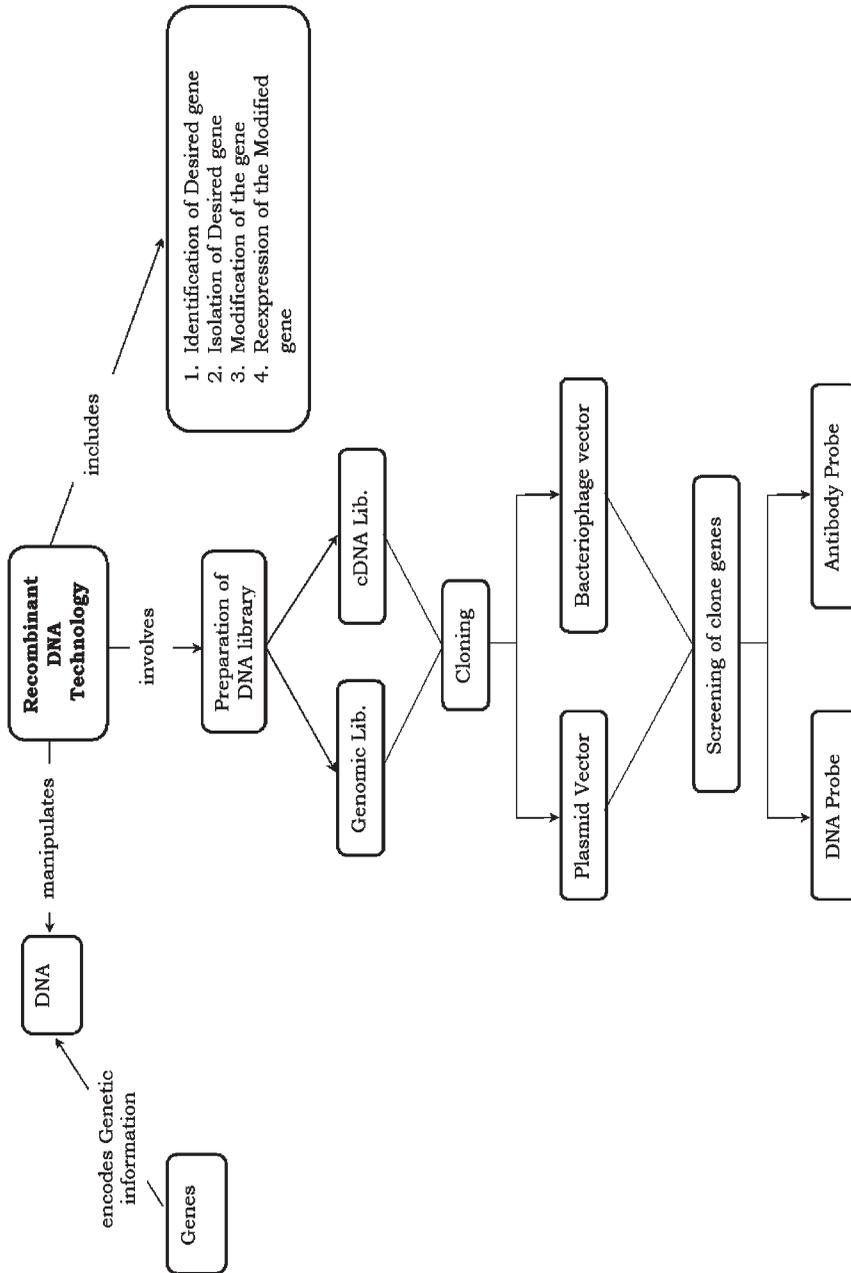
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# Introduction



# Recombinant DNA Technology in a Nutshell



# 1

## Introduction

The “Recombinant DNA Technology” generally known as genetic engineering is the ability to alter the genetic make-up of an organism. Advancement in the technology takes place in relatively privacy of academic research laboratories and the secrecy of the corporate world. By the time new inventions reach to the public, they are usually already being applied, and become old. Hence, we have to educate our students at basic level so that they handle these technologies immediately in graduate and postgraduate levels. Above all, if we are poor in technology the credit of biodiversity of a nation will be taken by the developed countries.

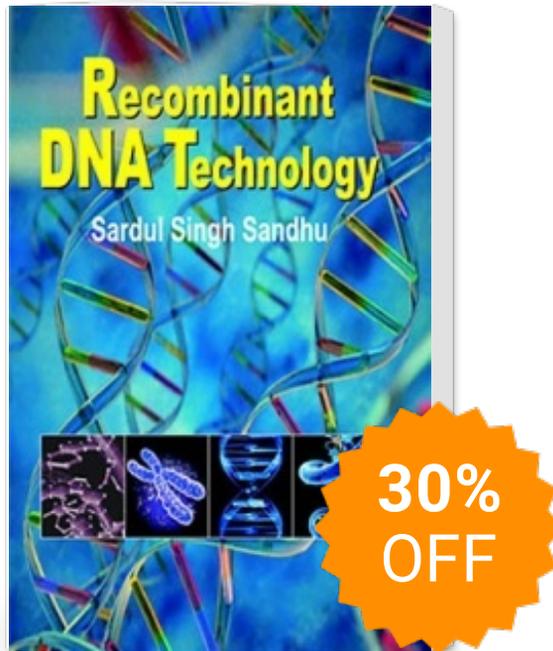
The progress in any scientific world of Recombinant DNA Technology is dependent on the availability of the methodology and that extends the range and sophistication of experiments which may be performed in genetic engineering. Over the last 30 years or so this has been demonstrated in spectacular fashion by the emergence of Recombinant DNA Technology. This field has grown rapidly to the point where, in many laboratories around the world, it is now a routine work to isolate a specific DNA fragment from the genome of an organism, determine its base sequence, and assess different functions. The technology is readily accessible by individual scientists, without the need of large-scale equipment or resources outside the scope of a reasonably good research laboratory is particularly striking in this respect.

The term “Recombinant DNA Technology” is often thought to be rather emotive or even trivial, yet it is probably the label that most scientists would recognize. However, there are several other terms which may be used to describe the technology, which include genetic engineering, gene manipulation, gene cloning, genetic modification and the new genetics. The legal definitions used in administering regulatory mechanism of Recombinant DNA Technology in all the countries where this science is practiced are coined and practiced for Intellectual Property Rights (IPR) too. Although many diverse and meticulous techniques are involved, the basic principles of Recombinant DNA Technology are reasonably simple.

The premise on which the technology is based is that a genetic information, encoded by DNA and arranged in the form of genes singly or in cluster, is a resource which can be manipulated in various ways to achieve certain goals in both pure and applied biological sciences.

In the present book we will observe the main aim, traits, principles, techniques and applications of Recombinant DNA Technology. In the last few chapters

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