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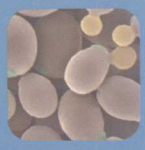


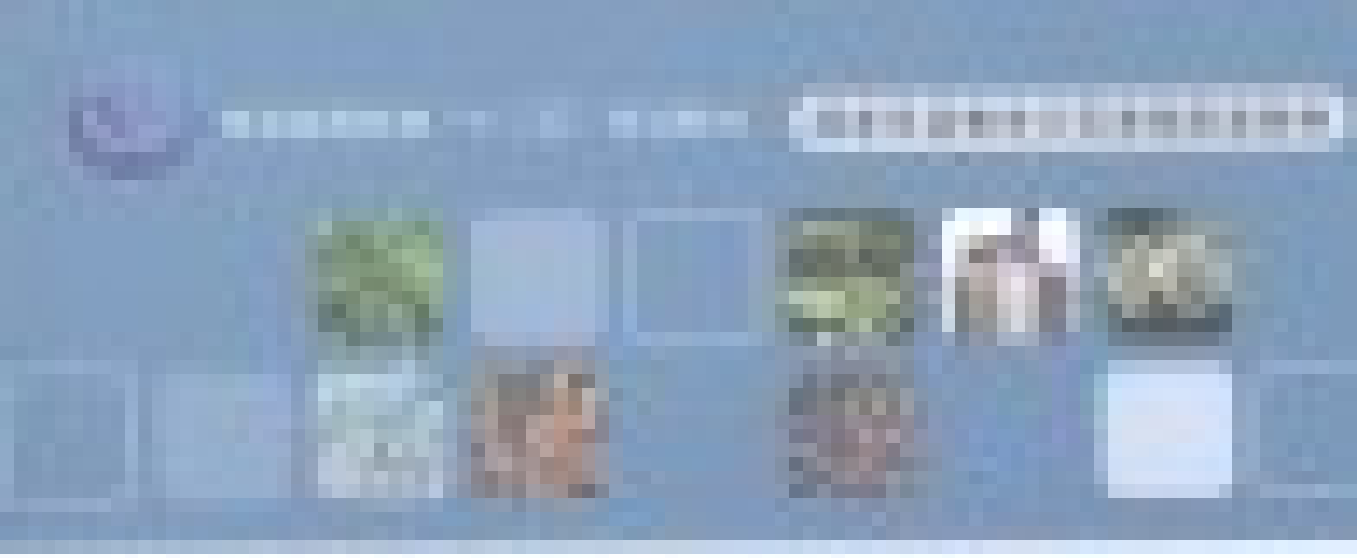

生物技术概论（双语教材）

Essentials of Biotechnology

王 武 主编

（双色版）

科学出版社



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北京

内 容 简 介

本书为国家双语教学示范课程配套教材，全书分为基因克隆、细胞工程、酶技术、工业发酵四个部分，共21章，分别介绍了上述四大生物技术领域的基础知识、技术关键以及发展前沿与动态，并对有关技术的正负面影响提出思辨。附录部分提供了关键词汇的中英文对照；列出了本领域诺贝尔奖获得者名单及其主要贡献；提供了近十年来出版的英文原版专业书目，以利于读者自学、参考。

本书主要供相关专业本科生双语课程使用，也适合作为研究生或高职高专优秀生的选用教材。对于生物技术产业的技术人员，以及准备出国深造的青年学子而言，本书也具有一定的参考价值。

图书在版编目(CIP)数据

生物技术概论＝Essentials of Biotechnology/王武主编. —北京：科学出版社，2012

国家双语教学示范课程配套教材·普通高等教育“十二五”规划教材

ISBN 978-7-03-035239-2

Ⅰ.①生… Ⅱ.①王… Ⅲ.①生物工程-双语教学-高等学校-教材 Ⅳ.①Q81

中国版本图书馆CIP数据核字(2012)第177049号

责任编辑：席 慧/责任校对：刘小梅

责任印制：阎 磊/封面设计：迷底书装

科 学 出 版 社出版

北京东黄城根北街16号

邮政编码：100717

http://www.sciencep.com

北京天时彩色印刷有限公司印刷

科学出版社发行 各地新华书店经销

*

2012年8月第 一 版 开本：787×1092 1/16

2012年8月第一次印刷 印张：22 1/4

字数：513 000

定价：55.00元

（如有印装质量问题，我社负责调换）

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Preface

For years, these authors have been teaching an undergraduate course named "Notes in Biotechnology" in a bilingual English-Chinese format. The National Education Authority has encouraged to build bilingual teaching systems for all undergraduates. This approach will help them improve their comprehension of scientific English, since large amounts of information in the field of biotechnology is constantly emerging and is often expressed in English. The younger Chinese generation has been taking English courses from the Primary Grade 3 through their Ph. D studies, but I have to say that many of them still have difficulty of comprehending academic articles and books with many modern scientific English terms. I'm glad to see that these authors have written this new textbook—*Essentials of Biotechnology* for upgrading their teaching.

How biotechnology should be presented in this new edition is a crucial task that the authors have to face. With the ever changing and increasingly blurred boundaries across Biology, Biochemistry, Microbiology, Physics, Chemistry, Genetics, Logics, and Information Technology, etc., biotechnology can no longer be regarded as a clearly defined subject. Since it is an enormously complicated and rapidly growing field, I understand the comprehensive approach is to collect and compile the knowledge referring to important discoveries, key theories, broadly applied techniques and classical examples of application. Four parts, including *Gene Cloning*, *Cell Engineering*, *Enzyme Technology*, and *Industrial Fermentation*, are considered as the most significant domains to be presented in this textbook, and each part is divided into several chapters which are subsequently composed of many sections. I appreciate that the authors have made every effort to limit the content to a reasonable length, in spite of the massive information that must be included in a modern, up-to-date text in biotechnology. Therefore, some of the topics are discussed briefly, yet the major significance and the main points are essentially kept. At the beginning of each Chapter, there is an abstract written in Chinese to help students catch the main idea at the first glance. Beyond the main text, an index of term translations, a list of Nobel Laureates in the related field, and the suggested further readings are provided in the Appendix section.

I believe that writing this textbook, especially in English, was no doubt a time consuming and energy demanding task. The authors must have carried a heavy burden of responsibility. Fortunately they have completed this work. I have no doubt that "Essentials of Biotechnology" will be of great benefit to biotechnology teachers and students especially those who wish to further studies abroad or pursue careers in bio-industry.

Lun Shiyi
Professor
Academician of the Chinese Academy of Engineering
June,2012

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Part I Gene Cloning

Chapter 1 Introduction to Gene Cloning

自 1970 年，科学家已实现了对生物体内的基因组进行切分，提取出含有基因的片段，将供体和载体片段连接成重组 DNA 分子，最终转化入合适的宿主细胞，表达出一种新的遗传性状。所谓的基因克隆是生物技术领域最具创新性的工作，借此，人类有可能培育出全新设计的植物和动物，并根据需要调整它们的遗传特性。这一技术也使得人类可以透彻地研究遗传物质的本质和功能，鉴别染色体中某个基因的位点。基因克隆技术为现代社会带来了各种全新的生物产品和克隆品种，但是对那些因不良因子或危险性遗传特性导入生物体而产生的负面效应，人类还必须保持高度的警惕。

1.1 Brief Overview

Gene cloning, as one of the most innovative technologies, allows scientists to discover the sophisticated essence of life and to manipulate genes in life. For a long time, **biologists** had been seeking to understand how **genetic factors** are organized and how they work in living organisms. Several generations of biologists had contributed their understanding to life issues, especially since the behavior of genes was revealed by molecular geneticists. The efforts they made led to an almost incredible technology—gene cloning, which arose by the early 1970s. **Mendel**, for example, having counted the **offspring** of pea plants, suggested that there exist some **hereditary factors** (later called **genes**) by inferring from the traits and numbers of **progeny** of the parent plants crossed. Later, people took almost a hundred years to know in detail the real genetic elements and how they are expressed. Now, based upon the powerful means of gene cloning, one could easily extract the **genetic materials**, cut the essential fragments from a genome, manipulate ***in vitro*** the genes, and **transform** the cloned piece into living cells to get totally novel **recombinants**.

Genes are at the very heart of life, in terms of information technology (IT), they are the master program of life. Collectively, genes constitute the **life's blueprint** and determine all the properties and capabilities of an organism. In biological terms, this master program is called the **hereditary substance**, the **chromosomes**. Constituted by chains of so called DNA molecules (in some cases RNA molecules), chromosomes carry

the genes with "**code words**" or instructions of the master program.

There is an identical set of this master program in every cell of an individual species. For example a corn plant has about a billion cells, each with a set of this master program. In different sections of the plants different parts of the program are active, giving rise to differentiated tissues such as leaves, seeds and root. The living organism is like a huge computer network, much more complicated than any man-made one. Still, science has a very incomplete understanding on how this master programs is able to make bio-system cooperated in a very harmoniously and effectively coordinated way.

Although the knowledge about the master program in some **prokaryotes** are well known, but in some **eukaryotic genome** only 1～3 percent of it are carrying "code words". The existing purpose and function of the remaining 97～99 percent are very little known. But this doesn't obstruct the progress on gene cloning, since the general roles of the DNA, RNA and protein molecules are gradually revealed in detail by molecular biologists.

Just as any IT expert knows that adding just one "code Syllable" (binary code) may be disastrous to a computer program, so too it is well known in genetics that changing even a little code word in the master program could mean a big difference between being healthy and carrying deadly hereditary diseases. Insertion of genes, as done by genetic **engineers**, does not add just one syllable, but many thousands of code syllables. The results that come from the gene cloning are not always predictable, which should remind scientists of their responsibility for the whole biological society.

"**Clone**", a term derived from an ancient Greek word "Klon", means the germ line from asexual propagation, for example in the case of grape **vegetative propagation**. Later, cloning was also defined as changing an organism's DNA to make it more beneficial. In this way, the history of cloning can be traced back to very early times when people started to perform **selective breeding**. Breeders have found ways to alter or change the genes of animals and plants to their advantage for thousands of years.

But the practical technology of gene cloning came much later. Gene cloning (also called **gene manipulation** or genetic engineering) means the molecular manipulation of genetic materials *in vitro*. In many cases, genes mostly from often totally unrelated species are recombined and inserted into another genetic "master program" by certain fascinating means. The essence of gene cloning can be considered as a series of four key steps: Generating donor DNA fragments; Joining the DNA fragments to a **vector** or **carrier molecule**; Introducing the cloned molecule into a host cell; Selecting required **clones**(recombinants). The noun "clone" comes from the **colonies** of identical host cells produced during amplification of the cloned fragments. Gene cloning is sometimes referred to as "molecular cloning" to distinguish the process from the cloning of whole organisms.

Genes from species e. g. fish, corn, bacteria and viruses have been inserted into other species in genetic engineering projects and the cloned bacterial cells producing

human hormone are not surprising to us now.

1.2 Historical Events

The Tab. 1. 1 lists out some of the notable events which strongly propelled the progress of gene cloning. Most contributors won the **Nobel Prices** for their beautiful works done during the last century. The British microbiologist **F. Griffith** became the first person to find the genetic material extracted from the dead bacterial cells in 1928, which could be transformed into living cells and express the related **hereditary feature. G. W. Beadle & E. L. Tatum** declared the "One Gene One Enzyme" theory to illustrate the relationship between the genetic unit and enzyme protein molecule during 1940. **Chase & Hershey** worked on virus genetics in the **Lab. of Cold Spring Harbor**, in 1951, they finally proved that **phage** DNA performed the genetic role. The discovery of the structure of **Double Helix DNA** by **J. Watson** and **F. Crick** in 1953 provided the stimulus for the development of genetics at the molecular level. The following few years saw a period of intense activities and excitements as the main features of genes and their expression were determined. In 1966, a stage was set for the appearance of the new genetics, based upon the establishment of the complete set of the **Genetic Code**.

Tab. 1. 1 Some of the notable events

Year	Contributors	Events
1928	F. Griffith	DNA transformation to bacteria
1940	G. W. Beetle & E. L. Tatum	One gene one enzyme
1949	E. Chargaff	Analysis of 4 nucleotide bases
1951	H. Chase	DNA is the major gene carrier
1953	J. Watson & F. Crick	DNA double helix structure
1959	A. Kornberg	DNA replication
1960	J. Monod	Lac-operon
1964	M. W. Nirenberg & P. Leder	Genetic triplet codon
1970	H. O. Smith	DNA restriction enzyme
1972	P. Berg & B. Cohen	DNA cloned into *E. coli*
1975	H. M. Temin	Revetro-transcriptase
1977	F. Sanger & W. Gilbert	DNA sequencing
1986	T. R. Cech	Self splicing of intron, indicated existence of ribozyme

In 1967 an enzyme named **DNA ligase** was isolated, which catalyzes the joining of the **complementary ends** of two DNA pieces together (some time also works for the **blunt ends** of two DNA pieces in lower efficacy), a prerequisite for the construction of recombinant molecules, and can be regarded as a sort of **molecular glue**. In 1970, H. O.

Smith declared the discovery of the first **DNA Restriction Endonuclease**, a major milestone in the development of genetic engineering. Restriction enzymes are essentially molecular scissors that cut DNA sequence at precisely defined sites. Such enzymes can be used to produce DNA fragments that are suitable for joining to other fragments with the same cutting. Thus, by 1970, the basic tools required for the construction of **recombinant DNA** were available. Right away, P. Berg & B. Cohen eagerly performed the very primitive cloning works. The first recombinant DNA molecules were generated at **Stanford University** in 1972, utilizing the cleavage properties of restriction enzymes (scissors) and the ability of DNA ligase (glue) to join DNA fragments together. When the cloned **antibiotics resistant markers** were expressed in the *E. coli* host cells, the importance of these first **tentative experiments** can hardly be overestimated. The **methodology** was extended in 1973 by joining DNA fragments to the **plasmid** which is an **extra-chromosomal genetic element** isolated from the **bacterium**. These recombinant molecules behaved as either the vectors or the **replicons**; that is, they could help the cloned fragments **transform** and **replicate** in *E. coli* host cells. Thus, by creating recombinant molecules *in vitro*, and placing the recombinant molecules in a bacterial cell where it could be replicated *in vivo*, the cloned DNA fragments could be isolated from bacterial colonies that form clones (colonies derived from a single cell, in which all cells are identical) when grown on **agar plates**. This development marked the emergence of the technology that became known as gene cloning (Fig. 1.1).

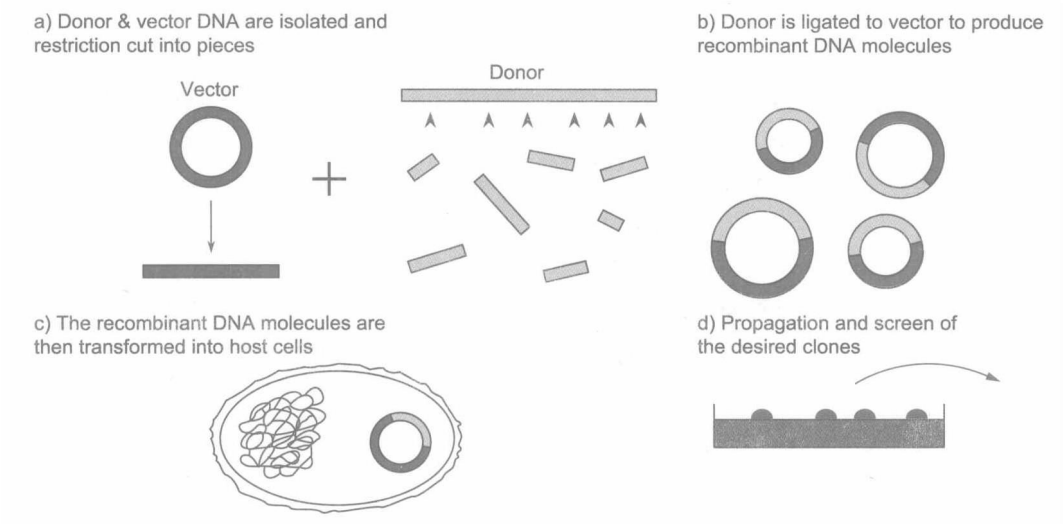

Fig. 1.1 Sketch map of gene cloning

The application of this new technology spread very quickly, and a sense of urgency and excitement prevails in the genetics research community. Now scientists could alter DNA sequence and join different DNA molecules from any organisms together to form a

completely different organism. This was dampened somewhat by the realization that the new technology could give rise to potentially harmful organisms exhibiting undesirable characteristics. It is to the credit of the biological community that measures were adopted to regulate the use of gene manipulation and that progress in contentious areas was limited until more information became available regarding the possible consequences of the inadvertent release of organisms containing recombinant DNA. However, within 40 years, the gene cloning has entered a booming period. More and more **genetically modified organisms (GMOs)**, for example the GMO crop plants, have been produced particularly for commercial benefits; the debate has been re-opened about the safety of these organisms and the consequences of releasing GMOs into the **fragile environment**.

1.3 Significance and Alertness

Theoretically, genetic engineering has advanced the understanding of many aspects of genetic function and genomic organization in prokaryotes. It also helps distinguish between **exons**, the coding section, and **introns**, the non coding parts within the eukaryotic genes. It provides powerful means for searching in detail on the genomes of any living organism, if there is a need.

Practically, through gene cloning techniques, enzymes, vitamins, antibodies, vaccines, even synthesizing **human insulin, human growth hormone, alpha interferon, hepatitis B vaccine**, and other medically useful substances can be produced simply from bacterial cells. Genetic engineering also enables us to perform gene therapy for curing the crippling hereditary diseases like **haemophilia, phenylketonuria** etc. Based on this technique, some of the hereditary diseases can possibly be corrected by replacing "bad" genes with "normal" ones.

A number of valuable genes such as ***Nif* gene (Nitrogen fixation gene)** were finally introduced into other bacteria cells. The plants originally without the function of **nitrogen fixation** may be genetically adjusted to fix nitrogen in order to improve the **soil fertility** and crop production. The **transgenic agro-plants and livestock** are now really the highlight in the area of life science and technology.

In 1980, the "new" microorganisms created by recombinant DNA research were deemed patentable, and in 1986 the **U. S. Department of Agriculture** approved the sale of the first living genetically altered organism—a virus, used as a **pseudo rabies vaccine**, in this viral genome a single gene has been destroyed on purpose. Since then thousands of patents involving genetically altered microorganisms and plants have been released. Nevertheless, special concern has been focused on such achievements for fear that it might result in the introduction of unfavorable and possibly **dangerous traits** into microorganisms, plants and animals that were previously free of "**negative" traits**—e. g. , resistance to antibiotics, production of toxins, or a tendency to cause diseases.

Summary