EXPERIMENTAL PROTOCOLS FOR MEDICAL MOLECULAR BIOLOGY IN CHINESE AND ENGLISH

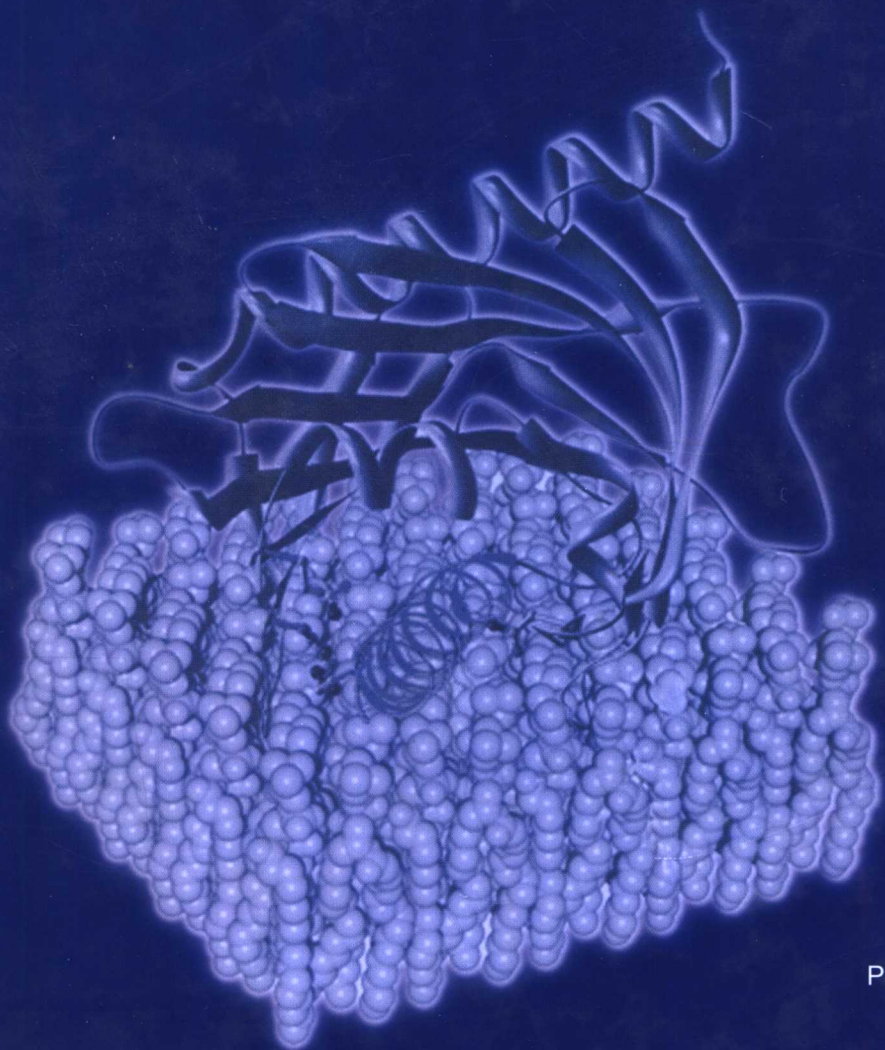
汉英医学分子生物学实验方法

第一版

First Edition

郑维 主编

Edited by Wei Zheng



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Experimental Protocols for Medical Molecular Biology in Chinese and English

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内 容 简 介

本书是一本介绍当今国外常用医学生物学，重点是分子生物学实验室方法的书籍。全书共分十五部分，六十余章。按照实验目的、原理、材料、操作方法及注意事项的结构模式介绍每一实验技术。包括质粒部分，PCR 技术，常用的凝胶电泳，放射性探针的制备，DNA 及 RNA 部分，克隆基因在体外、原核细胞及哺乳动物细胞中的表达，病毒部分，蛋白质检测与分析，真核基因表达调控，组织学，蛋白质组学技术，细胞凋亡，转基因、基因敲除鼠的构建等常用方法；同时附有常用试剂和溶液及常用的生物学网站。采纳的不仅有简便、实用的实验技术，也有经济、高效的试剂盒方法。采用汉英对照，使读者能更方便地理解英语的内容。该书可供从事医学及生物学领域研究的科研人员参考，特别适合准备到国外深造和正在国外从事实验室研究者参考。

Abstract

This book describes medical biological techniques most frequently used in laboratories abroad. The book, consisting of fifteen parts and more than sixty chapters, mainly describes the current protocols for cellular and molecular biology, including plasmids, PCR, gel electrophoresis, preparation of radiolabeled probes, DNA and RNA, expression of cloned genes in E. Coli, and mammalian cells, detection and analysis of proteins, regulation of expression of eukaryotic genes, histology, techniques for proteomics, detection of apoptosis, generation of transgenic and knockout mice as well as commonly used reagents and solutions and websites in biology as appendixes. The people who participated in writing this book are postdoctoral fellows, research associates, technicians, and assistant professors in the USA, and they have extensive laboratory experiences. This well – organized book contains the most advanced progress in laboratory techniques and, therefore, is highly practicable. The methodologies adapted in this book not only include concise and practicable experimental techniques but also some economic, highly efficient, and commonly used protocols of commercial kits. As this book is written in English with a Chinese contrast, its unique novelty and practicality would benefit the readers in their understanding of the English version. It is very suitable for those who are engaged in research in the medical or biological fields, especially those who want to go abroad to enhance their training or are doing laboratory research abroad.

序　言

随着医学生物学知识和技术的迅速成长和不断更新，对于国内的科研工作者及学生，他们均有了解当今细胞和分子技术的需求。一天，在美国伊利诺伊州芝加哥大学工作的年轻科研专家郑维，产生了编写一本汉英文常用实验方法书籍的想法。中国科研者在了解这些方法的同时，亦可学习专业的英语单词。然后，他成功地召集了一批在美国各自领域工作的年轻的专家级中国科研者，由他们组成的编委及编者来撰写这本很好的专业书，几乎囊括了所有基础的细胞和分子学方法。

郑维在中南大学湘雅医学院（前湖南长沙的湖南医科大学）获得博士学位。怀着对分子和细胞生物学的浓厚兴趣以及对科学的热衷，他于 2001 年来到美国。起初，他在西北大学，后到芝加哥大学做博士后。

这本书与其他书籍有两点不同，第一，此书大多为在美国工作的中国博士后所撰写，他们非常熟悉实验方法，编写此书时他们结合了当今的方法及切身体会和经验，而不是单纯地将一些英语课本翻译成中文；第二，本书采用汉英文形式，研究者使用这本书的时候，能快捷地学习一些专业的英语术语。我坚信，对于国内的生物医学研究者来说，郑维及同仁们编写的这本书将是一本非常有实用价值的工具书。

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Foreword

With the rapid growth in the knowledge and technology of medical biology, there are increasing requirements for scientists, students, and researchers in China to learn modern cellular and molecular technology. One day, a young scientist Wei Zheng at the University of Chicago, Illinois, USA, had an idea that there should be a textbook on commonly used experimental protocols written in both Chinese and English; therefore, it would be easier for Chinese researchers to follow the protocols and to learn the professional English vocabulary. He then successfully organized an editorial board consisting of a group of young Chinese scientists in the USA, who are experts in their own fields, to write this excellent textbook which covers almost all basic cellular and molecular protocols. Wei did his Ph. D. studies at Xiangya Medical College of Central South University (formerly Hunan Medical University), Changsha, Hunan. He was an outstanding student with a profound interest in molecular and cell biology. Wei's intense commitment to science actually led him to come to the USA in 2001, initially to Northwestern University and then University of Chicago as a postdoctoral fellow.

Two things make a difference in this book from other books. First, this book is written by the Chinese postdoctoral fellows, research associates, technicians, and assistant professors in the USA who are working in the specific fields, and are very familiar with the experiments. Therefore, this book combines the existing protocols with their own experiences, and is not just a Chinese translation of some English textbooks. Second, as I mentioned earlier, this textbook is written in both Chinese and English, allowing Chinese researchers to quickly learn professional English terms when they utilize the book. I believe that this special textbook written by Wei and his colleagues provides researchers in China with a very practical tool for their biomedical studies.

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前　言

在湖南医科大学获外科学博士学位后，我到美国西北大学医学院实验室做博士后研究，目前在著名的芝加哥大学医学院做博士后。不同实验室的培训，使我掌握了许多常用的实验室技术，以及目前发展起来的新的、热门的研究方法和技术。我逐渐萌发了将国外常用实验方法以中英文对照的形式介绍给国内研究人员的想法。通过与中国协和医科大学出版社联系，得到了他们的大力支持，在此实表感谢！这亦更坚定了我完成此书写作的信心与决心。故此，我邀请了一批在美国实验室工作的专业人士，协作一起完成了此书的写作。历时约一年，我们基本完成了英文实验方法的收集和整理，并加入自己切身的实验体会和经验，然后翻译成中文。

由于编写本书的初衷是为从事医学生物学研究的科技工作者介绍先进、常用的实验方法，力求实用，因此基础理论与基础知识介绍不多，如实验目的及实验原理只是简略说明，重点是具体实验操作步骤及注意事项。使读者按介绍的方法进行实验，能获得较满意的结果。全书共分十五部分，约六十余章。重点介绍分子生物学方面的实验方法。包括质粒部分、PCR 技术、常用的凝胶电泳、放射性探针的制备、DNA 及 RNA 部分、克隆基因在体外和原核细胞及哺乳动物细胞中的表达、病毒部分、蛋白质检测与分析、真核基因表达调控、组织学、蛋白质组学技术、细胞凋亡、转基因和基因敲除鼠的构建等常用方法，并附有常用试剂和溶液以及常用生物学网站。实验室技术特别是分子生物学技术的不断扩充和突飞猛进，导致当今科学的革命，也随之带来了它的商业化。特别在美国，各种特殊用途的成套试剂盒大量出现，使许多实验方法更简便可靠，效率增高，而且在一定程度上减少了误差率。但考虑到国内实际情况，我们力求介绍一些经济而高效并得到广泛使用的试剂盒方法。

本书内容采用汉英文对照，使其更富新颖性和实用性，读者亦能方便地理解英语的内容。当今实验室技术不断更新，新的名词不断涌现，单词及术语的翻译我们力争准确、贴切。本书充分反映了当今国外实验室技术的最新进展，资料新颖详实，结构层次清晰，具有很高的实用价值。可供医学及生物学研究领域的科研人员参考，特别适合准备到国外深造和正在国外从事实验室研究者参考。

在本书编写过程中，得到了美国芝加哥大学医学院章坚助理教授的极大关心和支持，并在百忙之中亲为作序。原在湖南医科大学卫生部英语培训中心具多年英语教学经验、现在美国发展的郭惠老师对本书的汉英文翻译，提供了极大的帮助。德国希尔敦市 Qiagen 公司的 Karin Schulz 和 Christian Starke，美国威斯康星州麦迪逊市普洛麦格公司的 Linda K. Cripps 对本书引用该公司试剂盒给予了大力支持，对此一并表示衷心的感谢！科学的发展日新月异，新的方法不断涌现，已有的技术亦适应不断变化的现实而得到改进。由于我们知识水平的限制以及编写时间的仓促，本书的错误、遗漏在所难免，恳请读者不吝赐教，以便将来再版时参考校正。但愿本书的问世，能为我国科研事业的提高和发展，起到铺路石和阶梯的作用。

郑　维

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Preface

After receiving my M. D. degree in general surgery at Hunan Medical University, I have spent my post-doctoral training in several laboratories at the Northwestern University, and I am now working at the University of Chicago as a research associate. The extensive training received from different laboratories has allowed me to become familiar with many commonly used laboratory techniques as well as newly developed modern and state-of-the-art technology. Because of my own experience, I would like to provide the most frequently used laboratory methodologies in both Chinese and English languages to domestic researchers. Here, I want to express my sincere appreciation to the Peking Union Medical College Press who has strongly and kindly supported this idea. Their support has also strengthened my confidence in writing this book, together with a group of young Chinese scientists in the USA. With great effort on everyone's part, we completed the entire writing process in English, and the translation into Chinese within approximately a year. Of note, unlike others this book combines both existing methodology in the literature and our own experiences.

The major purpose of this book is to introduce modern and commonly used methodologies to those who are performing biomedical research. Therefore, this book is mainly focused upon experimental procedures and helpful issues rather than basic theories and knowledge such as experimental purpose and principle. The readers should be able to perform the experiments following the protocols described and obtain ideal results. This book, consisting of fifteen parts and more than sixty chapters, mainly describes the current protocols of cellular and molecular biology, including plasmids, PCR, gel electrophoresis, preparation of radiolabeled probes, DNA and RNA, expression of cloned genes in *E Coli*, and mammalian cells, detection and analysis of proteins, regulation of expression of eukaryotic genes, histology, techniques for proteomics, detection of apoptosis, and generation of transgenic and knockout mice, as well as commonly used reagents and solutions and websites in biology as appendixes. Rapid growth in experimental technology, especially molecular biology techniques, has not only resulted in revolutions in biomedical science, but has also led to the generation of many commercial products in biological research. In the USA and western countries, many specialized and commercially available kits make experiments rather easy, reliable, and highly efficient. Also, to some extent, these kits can reduce experimental errors. However, in combination with domestic situations, we tried to introduce some economic, highly efficient, and commonly used protocols of commercial kits. As this book is written in English with Chinese contrast, its unique novelty and practicality would benefit the readers in their understanding of the English version. At present, more and more new cellular and molecular techniques are available, and more new professional words and terms are springing up, thus we have tried our best to translate these words and terms into Chinese more precisely. This well-organized book contains the most advanced progress in laboratory techniques, and therefore, is highly practicable. It is very suitable for those who are engaged in research in the medical or biological fields, especially those who want to go abroad to enhance their training or are doing laboratory research abroad.

During the process of preparing this book, we obtained expert assistance from Jian Zhang, an assistant professor at the University of Chicago, Chicago, Illinois, USA, who personally wrote a foreword for our book. Hui Guo, who has had many years of English teaching experience and once worked in the Center for English Training of the Ministry of Public Health in Hunan Medical University, provided great help in the English – Chinese translation of the book. Karin Schulz and Christian Starke from Qiagen Inc., Hilden, Germany and Linda Cripps from Promega Inc., Madison, Wisconsin, USA offered us assistance in the citation of their company's kits. We

sincerely thank all for their valuable assistance and support.

With the rapid development in science and rapid growth in new methodology, the existing technology is being modified and improved day to day. Due to the limitations of our knowledge and relatively short period of time in preparing and writing this book, there might be some errors or missing information. We welcome the readers to point out those errors so that we may correct them in further publications. With hope, the publication of this book can provide some paths or steps for the improvement and development of our country's scientific research.

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目　录

Contents

1. 质 粒

1.1 用碱裂解并 SDS 法制备小量质粒 DNA

【目的】 用碱裂解并 SDS 法从小量（1～2ml）培养的细菌制备质粒 DNA。

【原理】 碱裂解法是纯化质粒前最常使用的裂解细菌的方法之一。产生碱裂解产物包括四个基本步骤。

1. 悬浮 将收获的细菌悬浮在含有 RNA 酶 A 的 Tris-Cl/EDTA 缓冲液中。

2. 裂解 细菌被氢氧化钠/SDS 裂解。十二烷基硫酸钠（SDS）溶解细胞壁的磷脂和蛋白成分，导致细胞内容物的裂解和释放。氢氧化钠使染色体和质粒 DNA 以及蛋白质变性。RNA 酶 A 使得裂解期间释放出来的细胞内 RNA 被消化。

3. 中和 裂解物被酸性的醋酸钾中和。高盐浓度的溶液使十二烷基硫酸钾沉淀，加上变性的蛋白质、染色体 DNA 和细胞残骸共同沉淀在不溶性的含盐去污剂的复合物中。可复性和以共价键结合关闭的质粒 DNA 通过复性保留在溶液中。

4. 裂解物的澄清 高速离心清除沉淀的残骸，得到清亮的裂解液。

【材料】

1. 试剂和溶液

（1）碱性裂解溶液Ⅰ

50mmol/L 葡萄糖

25mmol/L Tris-Cl（pH 8.0）

10mmol/L EDTA（pH 8.0）

（2）碱性裂解溶液Ⅱ

0.2mmol/L 氢氧化钠

1% SDS

（3）碱性裂解溶液Ⅲ（100 ml）

5mol/L 醋酸钾	60 ml
冰醋酸	11.5 ml
水	28.5 ml

（4）质粒选择性抗生素

（5）乙醇

（6）含 20μg/ml RNA 酶 A 的 TE 液（pH 8.0）

10mmol/L Tris-Cl（pH 8.0）

1mmol/L EDTA（pH 8.0）

20μg/ml RNA 酶 A