Experimental Manual in Medical Biochemistry and Molecular Biology

(Second Edition)

Chief Editors Yu Hong Chen Juan

Subeditors Zhang Baifang Du Fen He Chunyan

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Preface

Progress in life science, including medical science, is mainly made by observation and experiments, especially biochemistry and molecular biology experiments. Therefore, it is essential for medical students and life science researchers to master the basic biochemical techniques and methods.

Since publication of the first edition of *Experimental Manual in Medical Biochemistry* in 2008, the pace of biotechnology has been further accelerated, the advances in bioinformatics have generated vast genomics data and subversive biotechnologies such as gene editing have emerged. While the basic biochemistry methods presented in the first edition remain as valuable tools, it is clear that graduate students and researchers need an updated reference book that incorporates classic as well as modern approaches to tackle medicinal research in functional genomics era.

We have significantly revised in the second edition in order to adapt to the recent development of new technology and research strategies in biochemistry and molecular biology. The core chapters of the previous edition were revised to highlight existing strategies and methods for nucleic acid preparation and cloning, gene transfer and expression analysis. All protocols have been evaluated, revised, and sometimes replaced with more efficient or reliable ones. New theory chapters involving the structure and function studies of gene and protein, have been introduced. New technique principles have been added, including DNA methylation technology, epigenetic analysis of chromosomal immunoprecipitation, RNAi, new generation sequencing technology, as well as technologies for protein chemical modification study, spatial structure and protein interaction research.

We thank all colleagues in the department of biochemistry and molecular biology from Wuhan University School of Basic Medical Sciences, and Huazhong University of Science and Technology Tongji Medical College, who have contributed ideas, skills and experience to this manual. We are very grateful to all the experts, especially those who helped with the preparation of the first and second editions, from other universities, who made big contribution to the compiling of various chapters. We specifically would like to thank our lovely graduate students for their contributions in drawing and proofreading. They are Guo Cui, Liu Yu, Shen Wenwen, Liu Tianli, Li Feifei, Zhao Xiaojie, Huang xiaopeng, Zhang Yu, and other students.

This manual is not a complete version. It should be continually modified and updated. We would be very grateful if the users of this manual could bring good feedbacks for the development of this manual.

Yu Hong and Chen Juan

June, 2019

Requirements

Lab safety

1. Eating, drinking, smoking or chewing gum in the laboratory are strictly prohibited.
2. Please wear lab coat and keep your work area clean. In case of spilled reagents, please wipe up as soon as possible. Be aware of objects that can burn or give electrical shocks.
3. Do not turn on an instrument until you have read instructions and consulted the instructors for the use of equipment. If any equipment malfunction is noted, report this immediately to an instructor.
4. Anyone carrying out these protocols will encounter the following hazardous materials: (1) toxic chemicals and carcinogenic or teratogenic reagents, (2) pathogens and infectious biological agents. We emphasize that users must proceed with the prudence and precaution associated with good laboratory practice. Use chemicals with high vapor pressure only in the hood. Handle and dispose of hazardous chemicals properly. Disposal containers are provided.
5. In case of an accident, notify a teacher immediately. For any chemicals splashed into the eye or mouth, or spilled on the skin, please flush immediately with large amounts of cold water using eyewash. For burns, flush with cold water and contact a teacher.

Attendance policy

Students are expected to attend every experiment, and to arrive promptly and well prepared. A student who is absent from a lab without the prior permission of the teacher or does not have documented excuse will receive 0 points on the lab report for that experiment.

Lab reports

Lab reports are due one week after completing the experiment. The reports comprise 1/2 of the practice score; the exam is 1/4, also include quiz in lab and the assessment of lab behavior.

All lab reports must be done on an individual basis. You will be given instructions about the format and the information needed for each experiment. Laboratory reports must include the following: experiment title, date, experiment performed, experimental results (primary data, calculation formulas, tables, graphs or figures, and final results), and discussion.

Content

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Section Ⅰ Theories and Strategies of Biochemistry and Molecular Biology Technology in Medical Research

Chapter 1 Medical Application of Biochemistry and Molecular Biology Technology

1.1 Basic Objectives of Biochemistry and Molecular Biology Technology

Life depends on biochemical reactions. Biochemistry is the subject of studying the chemical substances and vital processes occurring in living organisms. Biochemical research involves the structures, functions and interactions of biological macromolecules, such as proteins, nucleic acids, carbohydrates and lipids, which provide the structure of cells and perform many functions associated with life.

The double helix structure model of DNA, proposed by Watson and Crick in 1953, can be considered as the birth of molecular biology. Molecular biology is a branch of biology that concerns the molecular basis of biological processes of replication, transcription and translation of the genetic material. The central dogma of molecular biology is that genetic material which transcribe into RNA and then translate into protein, including emerging novel roles for RNA.

Biochemistry and molecular biology technologies are employed to separate and study the structure and functions of nucleic acids and proteins, to assemble recombinant DNA molecules and produce recombinant proteins, etc. In particular, medical biochemistry and molecular biology technology as an important branch is dedicated to elucidating the structure, function and expression regulation of specific molecules, and the molecular mechanisms of various physiological and pathological conditions, revealing and developing strategies and drugs for the diagnosis and prevention of different diseases.

1.2 Application of Biochemistry and Molecular Biology Technology in Medical Research

Almost all diseases have a biochemical and molecular basis. To a certain extent, all diseases are manifestations of abnormalities in genes, proteins, biochemical reactions, or metabolic processes, each of which can adversely affect one or more critical biochemical functions. New biochemical and molecular genetic tools allow investigators to query and manipulate genomic sequences as well as the entire information of cellular RNA, protein and post-translational modifications of protein at the molecular level.

1.2.1 Gene manipulation

The study of the structure and function of genes is the core of life science at the molecular level and one of the core contents of medical research. It requires many complex experimental techniques, relying on the establishment and development of technical systems. This is not a simple combination of experimental methods, but a combination of theory and technology that complements the molecular biological theory system.

In the early 1970s, techniques for nucleic acid laboratory operations appeared. These techniques, in turn, lead to the construction of DNA molecules consisting of nucleotide sequences from different sources. These innovative products, recombinant DNA molecules, have opened up exciting new pathways in molecular biology and genetics research and have given birth to a new field—recombinant DNA technology. Genetic engineering is the application of this technology in gene manipulation. It is now possible to target a specific region of almost any genome, produce an almost unlimited number of copies, and determine the sequence of its nucleotides. These technological breakthroughs in genetic engineering have a tremendous impact on the life sciences of cells and organisms.

1.2.1.1 Gene diagnosis

Gene diagnosis is the diagnosis of diseases by analyzing the mutation and expression of genes from the level of molecular structure and expression, using the current knowledge of genome and molecular genetic data.

Although much has been understood about human disease from pedigree analysis and study of affected proteins, the specific genetic defect is unknown in many cases. The development of DNA sequencing, recombinant DNA technology, high-throughput DNA microarrays, genomics analysis, and other molecular biology technology have an increasing impact on medical research and clinical medicine. Manipulation of a DNA sequence and the construction of recombinant DNA provide a means of studying how a specific segment of DNA works. Gene mapping localizes specific genes to distinct chromosomes. Gene localization thus can define a map of the human genome. This is already yielding useful information in the definition of human disease. Somatic cell hybridization and in situ hybridization are two techniques used to accomplish this.

The heritable DNA polymorphism can be associated with certain diseases in a kindred and used to search for the specific gene involved. The deletion of a critical piece of DNA, the rearrangement of DNA within a gene, or the insertion or amplification of a piece of DNA within a coding or regulatory region can all cause changes in gene expression resulting in disease.

We can use molecular approaches such as DNA probes to assist in the diagnosis of various biochemical, genetic, immunologic, microbiologic, and other medical conditions-molecular diagnostics.

1.2.1.2 Gene therapy

Gene therapy refers to the introduction of foreign normal genes into target cells to correct or compensate for diseases caused by defects and abnormal genes in order to achieve therapeutic purposes. The way to correct the defect is either to repair the defective gene in situ or to transfer the functional normal gene into a part of the cell genome to replace the defective gene. In a broad sense, gene therapy can also include measures and new technologies to treat certain diseases at the DNA level.

Since September 1990, the world's first attempt to treat adenosine deaminase deficiency-related severe combined immunodeficiency (ADA-SCID) with gene therapy has achieved gratifying results, gene therapy has made some progress in a variety of diseases, including genetic diseases, tumors, infectious diseases and so on. Because gene therapy is a new method which is different from any other therapies in the past, it is still in the early stage of development, and it is mainly aimed at diseases which have no other effective therapies in clinical routine treatment. Therefore, it will be a long time before gene therapy can be used as a routine therapy for diseases.

1.2.2 RNA study

RNA, like DNA, plays an equally important role in life activities. In most organisms, RNA is the transcription product of DNA, which participates in the replication and expression of genetic information. RNA is much smaller than DNA, but its species, size and structure are much more complex than DNA, which is closely related to its functional diversity. RNA transcript can be divided into two major categories: mRNAs encoded for proteins; the other RNAs that does not encode protein products, i. e., non-coding RNAs, including tRNA, rRNA, etc. , and especially newly identified small interfering RNA (siRNA), microRNAs (miRNAs) and long non-coding RNAs (lncRNA) that have been found to play important roles in gene expression regulation in recent years, and are closely related to the occurrence and development of many diseases, such as tumors and diabetes mellitus.

1. 2. 2. 1 MicroRNA microarray

A microRNA (abbreviated miRNA) is a class of small non-coding RNA molecule (containing about 22 nucleotides) found in plants, animals and some viruses, that functions in RNA silencing and post-transcriptional regulation of gene expression. The miRNA is partially or completely complementary to the 3′ untranslated region (3′-UTR) of the target mRNA, thereby inhibiting transcription or causing degradation of the target mRNA. Thousands of miRNAs are found in many mammalian cells, which appeared to target about 60% of genes. Many miRNAs are evolutionarily conserved, which implies their important biological functions and the relationship with disease.

High-throughput quantification of miRNAs can be carried out by microarray/chip. Be hybridized onto microarrays, slides or chips with probes to hundreds or thousands of miRNA targets, relative levels of miRNAs can be determined in different samples. By simultaneously analyzing expression level of disease-associated miRNAs and mRNAs in chip system, researchers can detect changes of miRNAs in the sample and analyze the regulatory activity of these miRNAs on target mRNAs. Some miRNA molecules have been found to be biomarkers for the early tumorigenesis, which is helpful for the early diagnosis of cancer.

1. 2. 2. 2 The application of siRNA

The mechanism of RNA interference (RNAi) is that siRNA uses the principle of base pairing to control the stability of mRNA molecules containing sequences complementary to them. This discovery has made us a big step forward in understanding eukaryotic gene expression regulation and gene function. In addition, the strong binding of siRNA to target genes allows for their powerful ability in down-regulation of gene expression, making siRNA-based anti-disease treatment possible. Since the first discovery of RNAi in 1998, it has only been two decades, but the treatments developed based on this mechanism have entered the clinical trial stage, and many biotechnology companies specializing in the development of RNAi treatment technologies.

1. 2. 2. 3 LncRNA microarray

Long non-coding RNAs (lncRNAs) are a class of RNAs longer than 200 nucleotides, which cannot be translated into protein. They are found to be located between coding genes or within introns. LncRNAs regulate gene expression by several different mechanisms, including gene transcription regulation, post-transcriptional regulation and epigenetic regulation.

Recognition that lncRNAs function in various aspects of cell biology has focused increasing attention on their potential to contribute towards medical research. Although many studies have implicated that aberrant expression of lncRNAs is related to a variety of human diseases, especially cancer and neurological disease (e. g. , Alzheimer's disease), there is poor understanding of their role in causing disease. Recently, a number of studies examining single nucleotide polymorphisms (SNPs) associated with disease states have been mapped to lncRNAs. Many SNPs associated with certain diseases are found within non-coding regions and the complex networks of non-coding transcription within these regions make it particularly difficult to elucidate the functional effects of polymorphisms.

With lncRNA chip, researchers can detect the expression changes of lncRNAs associated with specific biological processes or diseases, rapidly in a high throughput manner. This will contribute to the subsequent lncRNAs functional studies and/or biomarker screening.

1.2.2.4 CircRNA study

Circular RNA (circRNA) is a special kind of non-coding RNA molecule. Different from traditional linear RNA (containing 5′ and 3′ ends), circRNA molecule has a closed ring structure and is not affected by RNA exonuclease. It is more stable and difficult to degrade. CircRNA has tissue specificity and its expression in diseases is different from that in normal cases. It can be used as a biomarker for disease diagnosis.

1.2.3 Protein study

Proteins perform most processes in cells: they catalyze metabolic reactions, use nucleotide hydrolysis to do mechanical work, and serve as the major structural elements of the cell. The great variety of protein structures and functions has stimulated the development of a multitude of techniques to study them. Protein biochemistry continues to be an essential part of modern biological research.

In order to study protein function in vitro, one must isolate a single type of protein from thousands of other proteins present in a cell. Besides some technologies for studying protein properties, structure and function, purification of protein is a basic step for characterization and study of protein. Depending on the specific properties of proteins, a series of processes and methods are used for purifying proteins. Thanks to recombinant DNA technology to produce a large amount of a given protein, protein purification has become much easier. Proteins produced in abundance by genetic engineering and protein engineering can be used for medical research, diagnostic testing, therapy (e.g., insulin, growth hormone, and tissue plasminogen activator) and vaccines preparation (e.g., hepatitis B).

The occurrence and development of diseases are related to the changes of some proteins. Protein microarray can simultaneously detect the content of all proteins in biological samples that may be related to certain diseases or environmental factors. It is also important to monitor the process of diseases or to predict and judge the effect of treatment.

If we use these proteins to construct chips to screen many candidate chemical drugs and directly screen out the chemical drugs that interact with target proteins, the development of drugs will be greatly promoted. Protein microarray is helpful to understand the interaction between drugs and their effector proteins. It can also link the action of chemical drugs with diseases, whether drugs have toxic and side effects, and determine the therapeutic effect of drugs. It can provide experimental basis for guiding clinical medication.

1.2.4 Omics

With the progress of scientific research, it is found that only one direction (genome, proteome, etc.) cannot explain all biomedical problems. Scientists propose to study the structure of human tissues and cells, the interaction between genes, proteins and other molecules from a holistic point of view, and reflect the function and metabolism of human tissues and organs through holistic analysis. Omics is the science of studying all the components of a molecule such as DNA, RNA, protein, metabolite or other molecule in a cell, tissue or the whole organism. It mainly includes genomics, proteiomics, transcriptomics and metabolomics, which have been applied to identify causal targetable pathways for disease development. These methods are appealing as these analytes are closer to phenotype development. Benefiting from the rapid development of sequencing, analytical techniques the improvement of bioinformatics platforms, omics has been widely used in medical research and had a remarkable achievement.

Genomics refers to the science of genome mapping, nucleotide sequence analysis, gene mapping and gene function analysis of all genes. The aim is to elucidate the structure, composition of genome, regulation of gene expression, the relationship between structure and function, the interaction between genes and genes, and to reveal the relationship between genes and diseases.

Transcriptomics, the transcriptome as the research object, studies all the RNA molecules in cells, and understands the temporal-spatial relationship of all transcripts produced by genes and its biological significance. In a narrow sense, transcriptomics usually refers to the study of mRNA, which is the bridge and link between genome structure and function.

Proteomics essentially refers to studying the characteristics of proteins on a large scale, including protein expression level, post-translational modification, protein-protein interaction and so on, so as to obtain a comprehensive understanding of cell metabolism, disease occurrence and other processes at the protein level.

Metabolomics is a newly developed discipline and an important part of systems biology. The concept of metabolomics comes from the metabolome. The metabolome is the complete complement of metabolites (small molecules involved in metabolism) present in a cell or an organism. Metabolomics is the in-depth study of their structures, functions, and changes in various metabolic states qualitatively and quantitatively. Unlike genomics research, genetic scores are predictive of possible changes, metabolic traits and metabolic phenotypes represent changes that are currently taking place. Therefore, metabolomics research can explore the underlying cause with a unique perspective. With the development of metabolomics detection technology, the role of metabolomics in drug discovery has become increasingly prominent. Furthermore, because metabolomics can be used for disease diagnosis and monitoring, it will undoubtedly play an important role in precision medicine. For example, given that energy metabolism abnormalities are one of the typical features of malignant tumors, the use of metabolomics methods to identify and quantify cancer metabolites may promote the development of anti-tumor drugs for energy metabolism and offer better individualized medical treatment options.

In summary, omics characterization is essential to complement genetic and biomarker studies to understand disease mechanism and therapeutics targeting.