PRACTICAL PROTOCOLS IN MOLECULAR BIOLOGY

Editors-in-Chief
Li Yongming, Zhao Yuqi

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About the editors:

Dr. Yong Ming Li was born in China, where he received his bachelor of traditional Chinese medicine from the Liaoning College of Traditional Chinese Medicine. After a few years of clinical practice, he came to the United States to pursue graduate study. He received his M. S. in physiology from the Illinois State University and his Ph. D. in immunology from the University of Illinois at Urbana—Champaign. Subsequently, he was appointed to positions of Staff Investigator and Research Scientist at the Picower Institute for Medical Research, Long Island, New York. His current research interest focuses on molecular medicine. Dr. Li has received the Doolen Scholar Award for the Study of Aging and the Research Award from American Diabetes Association. Editing of this book represents his efforts in promoting the development of molecular medicine in China.

Dr. Yuqi Zhao, born in Qingdao, China, has received his B. S. degree from Shandong College of Oceanography, China, and M. S. and Ph. D. degrees from the Oregon State University, the United States. His post—doctoral training was at the College of Physicians and Surgeons of Columbia University, New York, where he was also a junior faculty for two years. Dr. Zhao is currently an assistant professor at the Northwestern University Medical School and director of Molecular Diagnostics Laboratory at the Children's Memorial Hospital, Chicago. Dr. Zhao is a molecular biologist. He has published papers numerously in various scientific journals. In addition to co—editing this book, he has also edited two other books and is serving as a member of editorial board for three scientific journals in the United States.

Foreword

Molecular biology is one of the most rapidly evolving scientific fields and it is instrumental to changes in many other areas of life sciences. Its impact on our lives is not only due to evolutionary discoveries such as DNA double helix, genetic codes, reverse transcriptase, and polymerase chain reaction, but also to broad applications of the techniques related to them. From cloning the gene of Huntington's disease to DNA fingerprinting for forensic identification, the techniques in molecular biology are essential in solving many problems that have puzzled scientists for decades. It is not an overstatement that development of new molecular biology techniques plays a vital role in advancing our understanding of life.

Practical Protocols in Molecular Biology was initiated through Association of the Chinese Professionals in the United States and is contributed mainly by overseas Chinese scientists and bench researchers. The goal of this book is to promote modern biotechnology development in China by introducing the State-of-Art methods of molecular biology. It is worthwhile mentioning that the book itself is a product of the modern technology of computer networking. The initial organization, protocol selection, preliminary manuscript review, and editorial discussions of this book were all conducted through the Internet and Bitnet electronic mail network. Although the contributors communicate often from all parts of the world, most of us, if not all, have not yet met in person.

This book intends to be a concise and comprehensive bench-top reference book for beginners and experienced investigators in laboratory research, and it is also essential for biotechnology industry. It contains selected, current, practical, and commonly used protocols in molecular biology and related fields. Each protocol is written in the simplest possible format. We strongly recommend, however, that readers should first gain a necessary background in molecular biology before using these protocols. The two well-known laboratory manuals, *Current Protocols in Molecular Biology* (Ausubel et al., 1989, commonly referred as the "Red book") and *Molecular Cloning* (Sambrook et al., 1989, commonly referred as the "Maniatis book"), are good resources for rationale and technical details.

One strength of this book is that many of the included protocols have been personally tested or used routinely by the contributing authors. These protocols have been repeatedly modified or optimized from the original protocols, and therefore are very reliable and reproducible. Also the protocols which have been selected have proved to be more simple and straightforward than many similar protocols described elsewhere. Some original protocols developed by the contributing authors or affiliated laboratories also add a unique aspect to this book. These protocols range from the development of new methods for improving current laboratory operation to the cutting-edge new approaches in gene cloning such as mRNA differential display, a method that has gained wide application within the past years. In addition, many protocols that require the use of commercial kits are also

mRNA differential display, a method that has gained wide application within the past years.In addition, many protocols that require the use of commercial kits are also introduced in this manual. For obvious reasons, using commercially available kits is a common practice in many laboratories. The selection of these products, in certain cases, only reflects the contributor's personal preference. Readers can often find substitute products at their own convenience. We want to ensure you that this book neither receives any support from any of the companies listed nor intends to advertise any commercial products.

We have made every effort to maintain consistency throughout the book. Each section intends to be independent of other parts, so readers do not have to cross-reference different protocols. Because of this feature, some methods may overlap with others. However, this should not influence the effectiveness of each individual protocol.

You find that *Practical Protocols in Molecular Biology is* easy to read and applicable to your specific needs. However, due to the diverse background of the different contributing authors, equipment or specific experiments described may have been designed explicitly for their own purpose. Therefore, you may have to modify some of these protocols to fit your own applications accordingly.

We have urged each contributing author to cite original references whenever it is possible. However, it is sometimes difficult to trace a protocol to its original developer. Therefore, we may have unintentionally missed mentioning someone who definitely deserves the credit. We would be delighted to add their names, once identified, to the list of future editions. We also welcome suggestions, comments or submission of new protocols from colleagues and readers. Your effort is critical in improving the quality of this book for future editions.

Finally, we would like to express our gratitude to Science Press, New York, Ltd and Science Press, Beijing, China for their help in publishing this book, and specifically to Mr. Ju Zhang and Mr. Weilun Tan for their enthusiasm and excellent technical support in this endeavor. Our gratitude extends to Roche Diagnostics System, Inc. They kindly permit and provided the picture used for the cover of this book. We thank Dr. Ray Wu for writing a foreword for this book. Dr. Wu is a pioneer in molecular biology, who has developed numerous molecular biology methods including the first method for sequencing DNA. Our thanks also go to Drs. Zhi-Liang Hu and Baoguo Xue, who served as a computer networking coordinators to make our communication among the authors easier and spontaneous. Drs. Xiaowu Qu, Wuhang Fan, Libin Jia and David Palmer have provided their help in editing this book that we appreciate greatly. We are especially indebted to Betty Nhan, Annie Tan, and many people who reviewed this manuscript and made invaluable suggestions.

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Part One

DEOXYRIBONUCLEIC ACID (DNA)

Section 1. Electrophoresis

1.1 Agarose Gel Electrophoresis

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Electrophoresis is now the most popular technique used for the separation and purification of DNA fragments. When loaded on a piece of "gel", namely a porous supporting media containing electrolyte, and made subject to static electric field, DNA molecules migrate toward the anode because they carry negatively charged phosphate residues along the side of their backbone. As the ratio between the driven force from the electric field and the resistance from the gel goes down when the length of DNA goes up, the DNA fragments of different length will be rendered different mobility, leading to the separation according to the size of the molecules. Such a process may be monitored by "running" tracking dyes or molecular weight markers together with the samples. The molecular weight markers also provide a measure for the determination of the size of the DNA fragments.

According to the materials used to make the gel, gel electrophoresis may be divided into two subcategories: agarose electrophoresis and polyacrylamide electrophoresis. By contrast, agarose gel is poorer in separation resolution, but superior in separation range. Generally speaking, it may be applied to DNA fragments with size ranging from 0.2 to 50 kb. The following is a detailed protocol for the preparation of agarose gel and the implementation of agarose gel electrophoresis in DNA fragments separation.

Applications

To separate, identify, and purify DNA fragments ranging from 0.2-50 kilobases.

Protocol

I. Preparation of the gel

1. Weigh proper amount of agarose (e.g., 1.0 g agarose in 100 ml TAE buffer makes 1% agarose) and dissolve in TAE buffer by heating it in a microwave.
2. Pour the agarose slowly (avoid bubbles) onto a gel bed with the comb inserted. Use the plastic transfer pipette to remove the bubble if there is any. Currently, many varieties of gel beds are available commercially and those can be used according to the instruction. However, the "traditional", as well as economical and simple gel bed can be made by sealing a proper sized plastic or a glass with the masking tape. Make sure the seal is tight to prevent agarose from leaking, and this can be achieved by running your finger along the edge of the gel bed several times.
3. Let gel polymerize for about 20 to 60 min depending upon the size of the gel.
4. While the agarose gel is polymerizing, prepare the DNA sample and mix proper amount of DNA with the loading buffer containing the dye such as bromophenol blue.
5. Remove the comb after the agarose gel has polymerized and place the gel bed onto the electrophoresis tank with the wells near the cathode (black terminal). Fill the tank with proper amount of TAE buffer. Usually the buffer is about 1 cm above the gel. Add ethidium bromide (final concentration is 1 μg/ml) to the TAE buffer and mix it well. Alternatively, ethidium bromide can be added to the agarose gel. This can be done by boiling the agarose in the

Microwave and cooling to about 50°C and then adding ethidium bromide.

II. Loading DNA samples and gel running

6. Hold the pipette perpendicular to the well and add DNA sample slowly with the pipette tip just beneath the opening of the well.
7. After all the samples have been loaded to the wells, connect the gel tank with the power supply properly (black to cathode [−] and red to the anode [+]). Set the voltage and time before turning on the power supply.
8. Make sure the leads have been properly connected by watching the platinum wire at the black terminal near the wells. Bubbles should be slowly rising if the leads have a good connection.
9. Allow electrophoresis to progress for appropriate time. The timing of electrophoresis depends on the length of the gel and the amount of voltage applied. The longer the gel and the lower the voltage, the longer time is needed. However, high voltages are significantly less effective at resolving large DNA fragments.

III. Gel photography

DNA fragments separated on the agarose gel after electrophoresis can be visualized and photographed under the UV light. Many kinds of cameras are available commercially and they can be easily manipulated following the instructions. In principle, the aperture and the speed are the two key factors for taking a good picture. For example, to increase exposure, i.e. to make image more intense, a longer exposure time is required and this can be achieved by slowing the shutter speed, or increasing the aperture. In contrast, to decrease exposure, i.e. to make pictures darker, a shorter exposure time or smaller lens opening is required.

Materials

Reagents: agarose, TAE (Tris-Acetate, EDTA) [concentrated stock solution 50X: Tris base, 242 g; glacial acetic acid, 57.1 ml; 0.5 M EDTA (pH 8.0), 100 ml; add to 600 ml dH_2O, stir vigorously, bring to 1000 ml with dH_2O].

Ethidium bromide stock (10 mg/ml): add 1 g of ethidium bromide to 100 ml of H_2O, stir with a magnetic stirrer for several hours and transfer to a dark bottle and store at 4°C (Ethidium bromide is a powerful mutagen. Wear gloves and a mask when weighing it out. In case of contact, immediately flush with copious amounts of water);

TBE [5x stock solution (1 liter): 54 g Tris base, 27.5 g boric acid, 20 ml 0.5 M EDTA, pH8.0].

10 x loading buffer: 0.25% bromophenol blue, 0.25% xylene cyanol FF, 15% Ficoll in water.

Equipment: beaker, graduated cylinder, stir bar, microwave, Pan balance, gel bed, comb, electrophoresis tank, and power supply.

Notes

1. Both TAE and TBE are common buffer used. TBE has relative higher buffering capacity than TAE.
2. The loading dye bromophenol blue migrates with DNA of about 0.5 kb and provides an index of the mobility of the fastest fragments.
3. The migration of the DNA depends on the following factors: