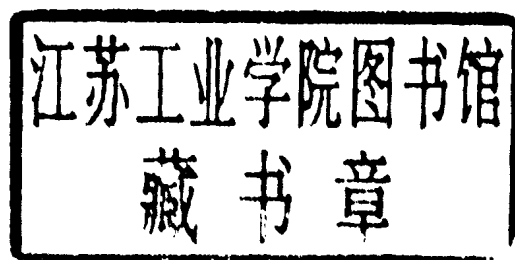




# PRACTICAL PROTOCOLS IN MOLECULAR BIOLOGY

*Editors-in-Chief*

Li Yongming, Zhao Yuqi



Science Press  
Beijing New York

*Responsible Editors:* Zhang Ju, Li Feng

Published by  
Science Press  
16 Dong Huang Cheng Gen North  
Beijing 100717  
China

Science Press New York, Ltd.  
84-04 58th Ave  
Elmhurst, NY 11373  
USA

ISBN 7-03-005077-0/Q • 615  
ISBN 1-880132-14-1

All Right Reserved

© 1995 by Science Press and Science Press New York, Ltd.

No part of the material protected by this copyright notice may be reproduced or utilized any form or by any means, electronic or mechanical, including photocopying, recording, or by any information storage and retrieval system, without written permission from copyright owners.

Printed in China

## 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 numerous 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.

## CONTRIBUTORS

Chen, Dongfen, Ph.D.  
Harvard University Medical School  
Boston, MA, USA

Coats, Stephen R., Ph.D.  
Florida State University  
Tallahassee, FL, USA

Du, Zenzian, Ph.D.  
New England Regional Primate Research  
Center  
Harvard University, Marlborough, MA,  
USA

Epstein, Lloyd M., Ph.D.  
Florida State University  
Tallahassee, FL, USA

Fan, Wufang, D.V.M., Ph.D.  
Human Genome Center  
Lawrence Livermore  
National Lab.  
Livermore, CA, USA

He, Chaoying, M.D.  
Laboratory of Molecular Carcinogenesis  
National Institute of Environmental  
Health Sciences  
Research Triangle Park, NC, USA

Hu, Zhi-Liang, M.S., Ph.D.  
Animal Genome Mapping  
USDA-Meat Animal Research Center  
Cley Center, NE, USA

Jia, Libin, Ph.D.  
National Cancer Institute  
NIH, Bethesda, MD, USA

Jin, Chuanfang, M.D., M.S.  
University of Michigan,  
Ypsilanti, MI, USA

Li, Yi Yang, M.D., Ph.D.  
College of Medicine,  
University of Iowa, Iowa City, IA, USA

Li, Yong Ming, M.D., Ph.D.  
The Picower Institute for Medical  
Research,  
Manhasset, NY, USA

Liang, Peng, Ph.D.  
Dana-Farber Cancer Institute  
Harvard University Medical School  
Boston, MA, USA

Liu, Qiang, M.D./Ph.D. Candidate  
University of Illinois at Champaign-  
Urbana,  
Urbana, IL, USA

Lu, Yang, Ph.D.  
GeneTherapy Inc.  
Rockville, MD, USA

Marilyn Ma Bui, M.D.,  
Ph.D. Candidate  
College of Medicine,  
University of Florida,  
Gainesville, FL, USA

Rong, Xianghong, M.D.  
National Institute of Environmental  
Health Sciences  
Research Triangle Park, NC, USA

She, Jin-Xiong, Ph.D.  
College of Medicine,  
University of Florida,  
Gainesville, FL, USA

Shen, Wenyang, Ph.D.  
Whitehead Institute,  
MIT,  
Cambridge, MA, USA

Tao, Tao, M.D., Ph.D.  
Case Western Reserve University,  
Cleveland Heights, OH, USA

Wang, Ming Jing, M.D., Ph.D.  
Center for Molecular Genetics  
UCSA School of Medicine  
La Jolla, CA, USA

Wang, Qing Feng, M.D., M.S.  
North Shore University Hospital-Cornell  
University,  
Mahasset, NY, USA

Wang, Xuhong, M.A.  
Amgen Center,  
Thousand Oaks, CA, USA

Wu, Jiarui, M.S., Ph.D. Candidate  
Institute of Cell Biology,  
Swiss Federal Institute of Technology,  
Zurich, Switzerland

Xu, Yang, M.D., Ph.D.  
Liver Center  
Albert Einstein College of Medicine  
Bronx, NY, USA

Xu, Anlong, Ph.D.  
Alliance Pharmaceutical Corp.  
San Diego, CA, USA

Xue, Bao Guo, Ph.D.  
Harvard University  
Medical School  
Boston, MA, USA

Yang, Bin, M.D., Ph.D.  
University of Iowa  
Iowa City, IW, USA

Yang, Jinghua, Ph.D.  
Department of Molecular and Cellular  
Biology  
Harvard University Medical School  
Boston, MA, USA

Yu, Xiaobo, Ph.D.  
Kea College of New Jersey  
Union, NJ, USA

Zhang, Yi, M.S., Ph.D. Candidate  
Florida State University,  
Tallahassee, FL, USA

Zhang, Liping, M.D., M.S.  
College of Medicine,  
University of Florida,  
Gainesville, FL, USA

Zhang, Liang, M.D., Ph.D.  
University of Chicago,  
Chicago, IL, USA

Zhao, Yuqi, M.S., Ph.D.  
Northwestern University Medical School  
The Children's Memorial Hospital  
Chicago, IL, USA



# Table of Contents

---

Foreword .....	i
Contributors .....	iii
 <b>PART ONE</b>	
<b>DEOXYRIBONUCLEIC ACID (DNA) .....</b>	<b>1</b>
Section 1. Electrophoresis .....	3
1.1 Agarose Gel Electrophoresis .....	3
1.2 Pulse-field Gel Electrophoresis (PFGE) .....	5
1.3 Polyacrylamide Gel Electrophoresis (PAGE) .....	8
1.4 Denaturing Gradient Gel Electrophoresis (DGGE) .....	10
1.5 Two-Dimensional Agarose Gel Electrophoresis .....	13
I. Neutral/Neutral 2-D Gel .....	14
II. Neutral/Alkaline 2-D Gel .....	15
Section 2. Quantitation of DNA .....	18
Section 3. Vectors .....	20
3.1 Plasmid Vectors .....	20
3.2 Yeast Vectors .....	21
3.2.1 Fission Yeast Vectors .....	21
3.2.2 Yeast Artificial Chromosome (YAC) Vector .....	25
3.3 Retroviral Vectors and Gene Transfer .....	27
Introduction of Retrovectors .....	27
Section 4. DNA Cloning .....	35
4.1 Vectors Selection .....	35
4.1.1 Plasmid for General Purposes .....	35
4.1.2 Plasmid for Special Purposes .....	35
4.2 TA Cloning of PCR Products .....	36
4.3 Construction of Genomic DNA Libraries .....	38
4.4 Screening of DNA Libraries .....	43
I. Standardized Hybridization for Screening Bacterial Colonies .....	44
II. Plating Phage for Plaques .....	44
III. <i>In Situ</i> Hybridization of Bacteriophage Plaques .....	45
4.5 cDNA Hybridization Selection .....	46
4.6 Rapid Clone Screening Using Polymerase Chain Reaction .....	55
Section 5. DNA Transformation and Transfection .....	57

5.1 Transformation of Plasmid DNA into Bacterial Cells . . . . .	57
5.1.1 Transformation of <i>E. coli</i> by TSS Buffer . . . . .	57
5.1.2 Transformation of <i>E. coli</i> by Rapid Freezing . . . . .	57
5.1.3 Preparation and Transformation of Competent <i>E. coli</i> Using Calcium Chloride . . . . .	58
5.1.4 Transformation of Bacterium, <i>Pseudomonas Syringae</i> . . . . .	60
5.2 Yeast DNA Transformation . . . . .	61
5.2.1 Spheroplast-based Protocol for <i>S. Pombe</i> Transformation . . . . .	61
5.2.2 Lithium-Acetate Based Protocol for Yeast Transformation . . . . .	63
5.3 DNA Transfection of Mammalian Cells . . . . .	64
5.3.1 Transfection of Mammalian Cells with Calcium Phosphate . . . . .	64
5.3.2 DEAE-Dextran Transfection Method . . . . .	66
5.3.3 Cationic Liposome Mediated Transfection . . . . .	68
5.3.4 Liposome-Mediated Transfection . . . . .	69
Support Protocol: Glycerol Treatment for Transfection . . . . .	70
Support Protocol: Hirt Extraction of Episomal DNA . . . . .	71
5.4 Retrovirus-Mediated Gene Transfer . . . . .	72
Section 6. Gene Expression and Regulation . . . . .	75
6.1 Expression of Recombinant Proteins in <i>Escherichia Coli</i> . . . . .	75
6.2 Expression of Proteins with Baculovirus in Insect Cells . . . . .	78
6.3 Expression of Exogenous DNA in <i>Xenopus</i> Oocytes and Embryos . . . . .	84
6.4 Transient Expression in Mammalian Cells with Vaccinia VTF7-3 . . . . .	90
6.5 Antisense Techniques . . . . .	91
6.5.1 Oligodeoxynucleotides -- Synthetic DNA/RNA . . . . .	91
6.5.2 Antisense RNA: RNA Transcripts from Vectors/cDNA Clones . . . . .	95
6.5.3 Methods for Transduction of Antisense RNA or DNA into Target Cell . . . . .	96
6.5.4 Assay for Antisense DNA or RNA Functions . . . . .	97
Section 7. DNA Extraction . . . . .	102
7.1 Plasmid DNA Extraction . . . . .	102
7.1.1 Mini-scale Preparation of Bacterial Plasmid DNA . . . . .	102
I. A Boiling Method . . . . .	102
II. An Alkaline Lysis Method . . . . .	103
III. Mini-Preparation of Plasmid DNA for Sequencing . . . . .	104
IV. Mini-Preparation of Single-stranded Plasmid DNA . . . . .	105
V. Quick Mini-Preparation of Plasmid DNA . . . . .	106
VI. Mini-Preparation of Plasmid DNA without Phenol Extraction . . . . .	107
VII. Mini-Preparation of Plasmid DNA from <i>Pseudomonas syringae</i> . . . . .	108
VIII. Mini-Preparation of Plasmid DNA by Commercial Kits . . . . .	109
(I) Magic Mini-preps DNA Purification System . . . . .	110
(II) Two-Minutes Mini-Prep by INSTA-MINI-PREP Kit . . . . .	111
7.1.2 Large-scale Preparation of Bacterial Plasmid DNA . . . . .	112
I. Large-preparation of Plasmid DNA by Alkaline Lysis . . . . .	112
II. Large-prep of Plasmid DNA by PEG Precipitation . . . . .	113
III. Large-scale Preparation of Single-stranded Plasmid DNA . . . . .	115
IV. Large-prep of Plasmid DNA by Cesium Chloride Centrifugation . . . . .	116

7.1.3 Yeast Plasmid DNA Isolation . . . . .	118
I. Mini-prep of Yeast Plasmid DNA . . . . .	118
II. Large-prep of Yeast Plasmid DNA . . . . .	119
III. Isolation of YAC DNA . . . . .	120
7.2 Genomic or Other DNA Extraction . . . . .	121
7.2.1 Extraction of Genomic DNA from Mammalian Cells . . . . .	121
7.2.2 Isolation of Eukaryotic DNA . . . . .	123
7.2.3 Isolation of Genomic DNA from Plants . . . . .	126
7.2.4 Plant DNA Extraction with CTAB . . . . .	128
7.2.5 Isolation of DNA Fragments from Agarose Gel . . . . .	130
7.2.6 Elution of DNA Fragment from Agarose Gel . . . . .	131
 Section 8. Enzymatic Modification of DNA . . . . .	133
8.1 DNA Polymerases and Polymerization . . . . .	133
8.2 Generation of Unidirectional Deletion with Exonuclease III . . . . .	134
8.3 Dephosphorylation of DNA with Phosphatase . . . . .	136
8.4 Phosphorylation of DNA with Polynucleotide Kinase . . . . .	137
8.5 Preparation of RNA-free DNA by Treating with RNase A . . . . .	138
8.6 DNA Ligation . . . . .	139
8.7 Labeling DNA Probe with <sup>32</sup> P or Biotin . . . . .	141
 Section 9. DNA Detection and Mapping . . . . .	143
9.1 Southern Hybridization . . . . .	143
9.2 A Quick Southern Hybridization Method . . . . .	149
9.3 DNA Restriction Fragment Length Polymorphism (RFLP)	
Analysis . . . . .	150
9.4 Analysis of Microsatellite Polymorphism Using the Polymerase Chain Reaction . . . . .	154
I. Radioactive Method . . . . .	154
II. Non-radioactive Method . . . . .	155
 Section 10. DNA Sequencing . . . . .	157
10.1 Double/Single- Stranded DNA Sequencing Using Klenow Enzyme . . . . .	157
10.2 RNA and cDNA Sequencing with <i>Taq</i> DNA Polymerase . . . . .	160
 Section 11. Mutagenesis . . . . .	163
11.1 Generation of Unidirectional DNA Deletion Mutants . . . . .	163
11.2 Oligonucleotide-Mediated Site-Directed Mutagenesis of Cloned DNA . . . . .	170
Protocol 1: How to Make Helper Phage R408 . . . . .	172
Protocol 2: How to Titer Phage Stock . . . . .	172
Protocol 3: Quick Mini-Plasmid Preparation for Sequencing . . . . .	173
 <b>PART TWO</b>	
<b>RIBONUCLEIC ACID (RNA) . . . . .</b>	<b>175</b>
 Section 1. RNA and Gel Electrophoresis . . . . .	177

1.1 RNA and RNase-Free Environment	177
1.2 RNA Gel Electrophoresis	178
Section 2. Isolation of RNA	181
2.1 Single Step RNA Isolation	181
2.2 Single Step Isolation of RNA with TRI REAGENT	182
2.3 Isolation RNA from <i>E. coli</i>	183
2.4 RNA Isolation from Animal Tissues by CsCl Method	184
2.5 Isolation of Poly (A) <sup>+</sup> RNA with Oligo (dT) Cellulose	185
2.6 Prepare RNA Transcripts from RNA Expression Vectors	186
Section 3. RNA Synthesis and Processing	190
3.1 Chemical Synthesis of RNA	190
3.2 RNA Labeling	192
3.3 RNA Sequencing	194
3.4 Site Specific RNA Cleavage by Catalytic RNA	196
3.5 Reverse Transcription Polymerase Chain Reaction (RT-PCR)	198
3.6 Competitive RT-PCR	200
3.7 One Step RT-PCR	203
3.8 Messenger RNA Differential Display	204
3.9 Nuclear Run-Off Transcription Assay	209
3.10 RNA splicing <i>in vitro</i>	212
Section 4. RNA Detection and Mapping	214
4.1 Northern Hybridization	214
4.2 Northern Hybridization: a Quick Method	216
4.3 <i>In Situ</i> Hybridization	218
4.4 RNase A/T1 Protection and RNase H Focusing	225
4.5 Fe(II)-EDTA Protection Assay	229
4.6 Primer Extension for Mapping RNA	230
<b>PART THREE</b>	
<b>PROTEINS</b>	<b>233</b>
Section 1. Protein Electrophoresis	235
1.1 Polyacrylamide Gel Electrophoresis	235
1.2 Denaturing Gel Electrophoresis (SDS-PAGE)	236
1.3 Nondenaturing Gel Electrophoresis (Native PAGE)	240
1.4 Immunoelectrophoresis in Agarose Gel	244
1.5 Rocket Immunoelectrophoresis	245
1.6 Two Dimensional Polyacrylamide Gel Electrophoresis	246
1.7 Staining of Protein Gels	251
1.7.1 Coomassie Blue Staining	251
1.7.2 Silver Staining	252
1.7.3 Staining of Proteins on Membranes	253
1.8 Gel Drying	254

Section 2. Proteins Isolation . . . . .	256
Introduction . . . . .	256
2.1 Preparation of Conditioned Medium . . . . .	256
2.2 Membrane Protein Extraction . . . . .	257
2.3 Preparing Cell Lysate for Protein Detection . . . . .	258
Section 3. Proteins Detection and manipulation . . . . .	260
3.1 Enzyme-Linked Immunosorbent Assays (ELISA) . . . . .	260
3.2 Immunoprecipitation . . . . .	262
3.3 Western Blotting . . . . .	264
3.4 Enhanced Chemiluminescence (ECL) Western Immunoblotting . . . . .	265
3.5 Western Ligand Blotting . . . . .	267
3.6 Affinity Cross-Linking of Ligands and Binding Proteins . . . . .	268
3.7 Immunomobility-Shift Assay for Protein and Ligand Binding . . . . .	270
3.8 Electrophoretic Detection of Protease Activity with <sup>125</sup> I Labeled Substrates . . . . .	272
3.9 Zymography Assay for Detection of Proteases . . . . .	273
3.10 Immunostaining of Cells for Flow Cytometry Analysis . . . . .	275
I. Indirect Labeling of Cells for Single Color Analysis . . . . .	275
II. Direct Labeling of Cells for Single or Double Color Analysis . . . . .	276
3.11 Labeling of Cellular Proteins . . . . .	278
3.12 Automated Protein Microsequencing . . . . .	280
<b>PART FOUR</b>	
<b>COMPUTER AIDED TECHNIQUES . . . . .</b>	<b>283</b>
Section 1. Computer Programs for Molecular Biology Applications . . . . .	285
1.1 Genetics Computer Group (GCG) Package . . . . .	285
1.2 MacVector Computer Sequence Analysis Software . . . . .	286
1.3 DNASTar-Lasergene . . . . .	287
1.4 Primer Designer . . . . .	287
1.5 Entrez Sequences and References . . . . .	288
Section 2. Computer Network in Molecular Biology . . . . .	290
2.1 GenBank Retrieval and Sequence Similarity Searching . . . . .	290
2.2 Other Network Data Bases or Servers for Molecular Biology . . . . .	292
Section 3. Bio-Information in Computer Network . . . . .	293
Section 4. Design PCR Primers with GCG Program . . . . .	296
Appendix . . . . .	302
Suppliers . . . . .	307
Index . . . . .	310

## **Part One**

# **DEOXYRIBONUCLEIC ACID (DNA)**





## Section 1. Electrophoresis

### 1.1 Agarose Gel Electrophoresis

**Contributors:** *Yi-Yang Li and Bin Yang*

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  $\mu\text{g/ml}$ ) 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<sub>2</sub>O, stir vigorously, bring to 1000 ml with dH<sub>2</sub>O].

Ethidium bromide stock (10 mg/ml): add 1 g of ethidium bromide to 100 ml of H<sub>2</sub>O, 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: