Methods in Molecular Biology

Volume 4

New Nucleic Acid Techniques

Edited by

John M. Walker

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Biological Methods

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edited by ***John M. Walker***

Volume I: **Proteins,** *1984*
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Liquid Chromatography in Clinical Analysis
edited by ***Pokar M. Kabra*** and ***Laurence J. Marton,*** *1981*

Metal Carcinogenesis Testing
Principles and In Vitro Methods
by ***Max Costa,*** *1980*

Preface

In recent years there has been a tremendous increase in our understanding of the functioning of the cell at the molecular level. This has been achieved in the main by the invention and development of new methodology, particularly in that area generally referred to as "genetic engineering."

Although this revolution has been taking place in the field of nucleic acids research, the protein chemist has at the same time developed fresh methodology to keep pace with the requirements of present day molecular biology. Today's molecular biologists can no longer be content with being experts in one particular area alone. They need to be equally competent in the laboratory at handling DNA, RNA, and proteins, moving from one area to another as required by the problem that is being solved. Although many of the new techniques in molecular biology are relatively easy to master, it is often difficult for a researcher to obtain all the relevant information necessary for setting up and successfully applying a new technique. Information is of course available in the research literature, but this often lacks the depth of description that the new user requires. This requirement for in-depth practical details has become apparent by the considerable demand for places on our Molecular Biology Workshops held at Hatfield each summer.

Volume 2 of this series described practical procedures for a range of nucleic acid techniques frequently used by research workers in the field of molecular biology. Because of the limitations on length necessarily inherent in producing any

book, one obviously had to be selective in the choice of titles for Volume 2. The production of Volume 4 therefore allows the development of the theme initiated in Volume 2. This volume contains a further selection of detailed protocols for a range of analytical and preparative nucleic acid techniques and should be seen as a continuation of Volume 2. In particular we have introduced protocols for the rapidly developing area of plant molecular biology. Companion Volumes 1 and 3 provide protocols for protein methodology.

Each method is described by an author who has regularly used the technique in his or her own laboratory. Not all the techniques described necessarily represent the state of the art. They are, however, dependable methods that achieve the desired result.

Each chapter starts with a description of the basic theory behind the method being described. The main aim of this book, however, is to describe the practical steps necessary for carrying out the method successfully. The Methods section, therefore, contains a detailed step-by-step description of a protocol that will result in the successful execution of the method. The Notes section complements the Methods section by indicating any major problems or faults that can occur with the technique, and any possible modifications or alterations.

This book should be particularly useful to those with no previous experience of a technique, and, as such, should appeal to undergraduates (especially project students), postgraduates, and research workers who wish to try a technique for the first time.

John M. Walker

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Contents

Chapter 1

Electrophoresis of RNA Denatured with Glyoxal or Formaldehyde

Christopher F. Thurston, Caroline R. Perry, and Jeffrey W. Pollard

1. Introduction

The first successful method for electrophoretic analysis of the full size range of cellular RNA molecules was described by Loening (*1*), and its introduction allowed for major advances, most particularly in the molecular biology of eukaryotic organisms. The method had, nevertheless, two significant disadvantages in that the gels (composed of acrylamide at very low concentrations) were mechanically fragile, and the migration of RNA molecules did not necessarily reflect their size because RNA secondary structure was not disrupted.

More modern methods have consequently sought to incorporate conditions under which RNA is fully denatured and that avoid the use of very fragile gels. Fragility of the gel was overcome by use of agarose either in combination with, or in place of, acrylamide. The problem of RNA denaturation is, however, more complex. It is necessary both for analysis of the