METHODS IN VIROLOGY

EDITED BY

KARL MARAMOROSCH

AND

HILARY KOPROWSKI

Volume VIII

METHODS IN VIROLOGY

EDITED BY

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WAKSMAN INSTITUTE OF MICROBIOLOGY
RUTGERS UNIVERSITY
NEW BRUNSWICK, NEW JERSEY

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THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA, PENNSYLVANIA

Volume VIII

1984

ACADEMIC PRESS, INC.
(Harcourt Brace Jovanovich, Publishers)

Orlando San Diego New York London
Toronto Montreal Sydney Tokyo

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 66-30091

ISBN 0-12-470208-2

PRINTED IN THE UNITED STATES OF AMERICA

84 85 86 87 9 8 7 6 5 4 3 2 1

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Preface

In a letter to Dr. John L. Dorsey dated May 23, 1804, Benjamin Rush said that "A wide field opens for medical investigations in the United States. The walls of the Old School are daily falling about the ears of its masters and scholars. Come, and assist your Uncle and his friends in erecting a new fabric upon its ruins." In the seventeen years since the publication of the first volume of *Methods in Virology,* many "walls of the Old School" have fallen down to be replaced by "new fabric." The editors, being cognizant of these events, have tried in these two volumes to present the reader with up-to-date, modern revisions of techniques applied to animal, plant, and insect virology.

The early volumes in this series were published at the dawn of the era of molecular virology. Today, techniques applied to the study of molecular virology are becoming standard household techniques, and the contents of Volumes VII and VIII reflect the existence of the "new world" of virology. A series of books in existence for seventeen years may be regarded as a "venerable" one. We feel, however, that none of the preceding volumes can be classified as obsolete. New techniques and methods must be considered as improvements of previously described techniques, but not necessarily as their replacements. Moreover, one never knows what surprises the future holds for students of virology.

Volumes VII and VIII will be of considerable usefulness to all who are engaged in virus research, including graduate students interested in becoming familiar with modern techniques. Infectious disease specialists, bacteriologists, immunologists, vertebrate, invertebrate, and plant pathologists, parasitologists, biochemists, veterinarians, geneticists, and biotechnicians will find these volumes of interest. These two books are an important addition not only to the series but to the rapidly growing list of works dealing with viruses as well.

We express our thanks and appreciation to those who have contributed chapters to Volumes VII and VIII. The authors were chosen on the basis of their outstanding knowledge of given methods, as recognized authorities in

their specialized fields, or as creators of new techniques. We also wish to express our appreciation to the staff of Academic Press for continuous encouragement and advice throughout the planning and completion of these volumes.

KARL MARAMOROSCH
HILARY KOPROWSKI

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1 *Hybridization of Viral Nucleic Acids: Newer Methods on Solid Media and in Solution*

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I. Introduction

The technology of nucleic acid hybridization was the forerunner of recombinant DNA biotechnology, which in turn has spread the use of hy-

ISBN 0-12-470208-2

bridization techniques into many areas of molecular biology. Now developments in recombinant DNA research have had an impact on hybridization technology, primarily by providing pure, well-defined probes of reduced complexity. Although the basic techniques of molecular hybridization have changed little since the previous review of the methodology in Chap. 13, Vol. VI of this series (Huang and Pagano, 1977), the emphasis on different techniques has changed. Also, the application of the techniques to pathobiology has widened with the increasing appreciation of the contributions that molecular biological technology can make toward understanding mechanisms of disease on the cellular, molecular, and epidemiological level.

The concept of nucleic acid hybridization is based on the homology between the two hydrogen-bonded strands of nucleic acid either in double-stranded DNA or in hybrid molecules that consist of strands of RNA and DNA. In all cases a single-stranded nucleic acid probe that has been labeled in some fashion is allowed to form a hybrid with homologous sequences. The double-stranded labeled material is then either quantitated, visualized, or further analyzed. The principal uses of nucleic acid hybridization techniques are for detection of specific genomes or portions of them, determination of homology between genomes, characterization of DNA structure, and detection and analysis of RNA transcripts.

The purposes of this chapter are to provide in one place a compendium of the most useful techniques of molecular hybridization in a practical rather than a theoretical form. We present here a brief recapitulation of some of the basic techniques that were presented in detail in Vol. VI of this series with some examples of areas in which they have continued utility, and then we expand with greater detail on methods largely based on restriction enzyme technology. As most scientific questions can be approached in several ways, the choice of a particular method is largely governed by the availability of material in a particular system. However, the emergence of recombinant DNA and restriction enzyme technology dictates the choice of methods that rely on abundant DNA and provide an analysis of DNA fine structure in accurate detail.

In this chapter, we use Epstein–Barr virus (EBV) systems to provide examples and illustrations. EBV is a herpesvirus containing double-stranded DNA with a molecular weight of approximately 115×10^6, or 170,000 base pairs. The genome of the virus has a complex structure with several regions of repetitive DNA interspersed with unique DNA. Much of the current knowledge of the biochemistry of EBV and its identification as a pathologic agent in several malignant and benign disease states have been dependent on hybridization technology. However, the same techniques as applied to

detection and characterization of EBV genomes and transcripts can be utilized in most other viral and nonviral systems.

II. Purification of Nucleic Acid

A. PURIFICATION OF VIRAL DNA

In those types of hybridization analyses in which a test DNA is analyzed for the presence of viral sequences, purity of the nucleic acid used as a radiolabeled probe is essential for specific hybridization. However, the availability of specific viral sequences in recombinant DNA, which is propagated as a plasmid in bacteria, has largely circumvented purification difficulties. When nucleic acid purified from virus must be used for radiolabeling, a rigorous purification scheme is called for. After purification of virions, the nucleic acid extracted from them can be separated from contaminating DNA on the basis of physical properties of the viral DNA such as sedimentation coefficient, buoyant density, or physical configuration.

For example, in the EBV system virus is harvested from the extracellular fluid from producer lymphocyte lines. The supernatant fluids are clarified by centrifugation at 4000 *g* for 15 min. The virus can then be pelleted from the clarified fluids by centrifugation at 15,000 *g* for 90 min followed by banding in a 10–30% dextran sulfate gradient. The light-scattering viral band is aspirated from the gradient with a syringe fitted with an 18-gauge needle, and this virus is centrifuged through 0.5 m*M* sodium phosphate, pH 6.5. The pellet is treated with 2% sodium dodecyl sulfate (SDS) for 5 min at 60°C followed by phenol/chloroform extraction. The nucleic acid is then purified to equilibrium through a cesium chloride gradient, and the fractions corresponding to the buoyant density of EBV, 1.718 g/cm^3, are pooled, dialyzed, and concentrated (Dolyniuk *et al.*, 1976).

An alternative method, essentially that of Adams and Lindahl (1975), is to pellet the virus from the clarified supernatant fluid by treatment with polyethylene glycol (PEG). To each liter of supernatant fluid 20 g of sodium chloride is added and stirred until dissolved, followed by 200 ml of PEG solution [polyethylene glycol 6000, 50% (w/v) in 0.5 *M* NaCl]. This suspension is placed at 4°C overnight. The PEG complexes are centrifuged at 8000 *g* for 15 min and then suspended in 2% RPMI. The virus is pelleted at 25,000 rpm for 90 min in an SW27 rotor. The virus pellet is suspended in phosphate-buffered saline solution (PBS) with 5 m*M* $MgCl_2$ and incubated with pancreatic DNase, 50 μg/ml, for 2 hr at room temperature. The