# Monoclonals and DNA Probes In Diagnostic and Preventive Medicine

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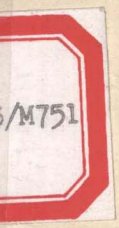

# Monoclonals and DNA Probes In Diagnostic and Preventive Medicine

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Raven Press New York

# Acknowledgments

We thank the contributors for their appreciable efforts in writing the papers for the BIOTECH RIA 86 Meeting, upon which the chapters in this volume are based. We are particularly indebted to the Giavanni Lorenzini Foundation for its help and generous support, which made the meeting possible.

**Raven Press, 1185 Avenue of the Americas, New York, New York 10036**



Made in the United States of America

**Library of Congress Cataloging-in-Publication Data**

Monoclonals and DNA probes in diagnostic and preventive medicine.

Based on the BIOTECH RIA 86 Meeting held in Florence, Italy on 8-10 April 1986.
Includes bibliographies and index.
1. Antibodies, Monoclonal—Diagnostic use—Congresses. 2. DNA probes—Diagnostic use—Congresses. I. Gallo, Robert C. II. Della Porta, Giuseppe. III. Albertini, Alberto. IV. BIOTECH RIA 86 Meeting (1986 : Florence, Italy) [DNLM: 1. Antibodies, Monoclonal—Diagnostic use—congresses. 2. DNA—immunology—congresses. 3. Immunoassay—congresses. QW 570 M7515 1986]
QR186.85.M664 1987 616.075 86-31635
ISBN 0-88167-286-6



9 8 7 6 5 4 3 2 1

# Preface

Recent advantages in biotechnology have effected a significant improvement in diagnostic and preventive medicine. While the application of recombinant DNA technology has presented the opportunity to detect inherited diseases (such as thalassemia, Duchenne muscular dystrophy, hemophilia) in the fetus, the use of monoclonal antibodies has enabled new possibilities in diagnostic medicine, as demonstrated by the investigation of solid tumors by means of immunoscintigraphy. Furthermore, both the use of DNA probes and the extensive application of monoclonal antibodies have contributed to the improvement of our knowledge of virus-mediated diseases.

Recently, progress has been made in the field of diagnostic and preventive medicine by means of biotechnology. This book provides the reader with up-to-date information on this continuously evolving field. This volume will be of interest to molecular biologists as well as physicians working in laboratories and in clinics.

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# Contents

*Monoclonals and DNA Probes in Diagnostic and Preventive Medicine*, edited by Robert C. Gallo, G. Della Porta, and A. Albertini: Raven Press, New York, © 1987.

# MOLECULAR APPROACH TO DIAGNOSTIC AND PREVENTIVE MEDICINE: THE STATUS OF THE ART

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## SUMMARY

The application of DNA recombinant technology and restriction enzyme analysis to the study of the fine molecular structure of eukaryotic genes has brought to the unexpected discovery of new and numerous genetic variants, which are inherited in a Mendelian fashion and are distributed in populations, as expected on the basis of the Hardy-Weinberg equilibrium law. From the first report in 1978, the list of such types of inherited polymorphisms has increased to a total of more than 300, 87 of which have been discovered with cloned genes and 245 with arbitrary DNA fragments. This harvest of genetic markers has brought within reach the goals of constructing a total genetic map of the human genome and of its application to diagnostic and preventive medicine. This lecture reviews the rationale and the latest methodological developments of this research endeavour and discusses the impact of this new type of genetic information on the understanding of basic biological phenomena, such as the recombination, differentiation and evolution of the human genome.

## INTRODUCTION

It is now well established that non-repeated fragments of human DNA (either in the form of a cloned gene, or a cDNA sequence or an arbitrary DNA genomic fragment) can be used as molecular probes to identify inheritable variation affecting the recognition sites of specific DNA restriction endonucleases. The individual variants (restriction fragment length variants or RFLV) are visualized as differences in the size of the restricted DNA fragment(s) containing the sequence of nucleotides homologous to the labeled molecular

probe used for their detection. The first human example of these types of genetic variants was found at a HpaI site flanking the 3' end of the beta globin gene and shown to be suitable for the indirect detection of the sickle cell mutation (16,17). Within months from this discovery it became clear that inheritable variation of this kind exists all over the genome within and between genes, including those DNA sequences that are not involved in the regular process of translation and/or transcription such as highly repetitive and spacer DNA, introns and pseudogenes. At the last workshop on human gene mapping the list of heritable polymorphisms at DNA restriction sites (restriction fragment length polymorphisms or RFLPs) has grown to the total of 332, among which 87 have been discovered with molecular probes that include cloned genes or gene fragments and 245 through cloned sequences of non-repeated DNA randomly derived from libraries of the total human genome or of individually isolated human chromosomes (6). Since the uniqueness of each individual genome - with the exception of the uniovular twins - is a fact of life, it is to be expected that this type of hidden DNA genetic variants will continue to grow in number for unlimited time. Thus far, however, the yield of human RFLPs seems to have been related to the combination of molecular probes and restriction endonucleases chosen for their detection. This is in part due to the well known circumstance that specific restriction sites may occur with a very different frequency in the human genome, as is the case with the common 4-base pair cleavage sequences TCGA & CCGG, respectively recognized by the restriction endonucleases TaqI and MspI, as opposed to the much less common 6-base cleavage sequences CAATTC and GTTAAC for the enzymes EcoRI and HpaI, the rare 8-base pair recognition sequence GCGGCCGC for NotI or the even rarer 13-base sequence GGCCNNNNNGGCC for SfiI.

The other main factor that influences the chance of RFLP finding is the primary structure of the chromosomal region which is scanned. Not surprisingly, the regions of the human genome that include obvious examples of gene duplication seem to be the ones yielding the largest number of RFLPs, when the restriction sites within or between the duplicated genes are screened with molecular probes that include the duplicated DNA sequence or a fraction of it. This has been found to be the case for the globin gene clusters on 11p and 16q, the MCH complex on 6p, the IgHC on 14q and in general all the chromosomal regions whose primary DNA structure can be best explained in terms of a deletion/insertion mechanism. The highly variable RFLPs identified with the use of random probes at the long arm subtelomeric region 14q32.1 (23,43), at the long arm subtelomeric region Xq28 (25) and at Yq11 (24) are striking examples of this type of genetic variation.

Soon after the report of the HpaI-RFLP flanking the beta globin gene (16) and of the common multiallelic EcoRI-RFLP detected by a random DNA unique sequence derived from the Maniatis library (43), Botstein et al. (2) estimated that a total of about 150 RFLPs of the same type at non-overlapping restriction sites scattered throughout the 24 human chromosomes (22 autosomes + the X & Y heterochromosomes) would probably be sufficient to construct a total