

Monoclonals and DNA Probes In Diagnostic and Preventive Medicine

Editors

Robert C. Gallo

Giuseppe Della Porta

Alberto Albertini



1990年7月一日

6/M751

Monoclonals and DNA Probes In Diagnostic and Preventive Medicine

Editors

Robert C. Gallo, M.D.

*Chief Laboratory of Tumor Cell Biology
Department of Health and Human Services
National Cancer Institute
National Institutes of Health
Bethesda, Maryland*

Giuseppe Della Porta, M.D.

*Istituto Nazionale per lo
Studio e la Cura dei Tumori
Milan, Italy*

Alberto Albertini, M.D.

*Chair of Chemistry
Faculty of Medicine
University of Brescia
Brescia, Italy*



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

© 1987 by Raven Press Books, Ltd. All rights reserved. This book is protected by copyright. No part of it may be reproduced, stored in a retrieval system, or transmitted, in any form or recording, or otherwise, without the prior written permission of the publisher.

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.

I. 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

The material contained in this volume was submitted as previously unpublished material, except in the instances in which credit has been given to the source from which some of the illustrative material was derived.

Great care has been taken to maintain the accuracy of the information contained in the volume. However, Raven Press cannot be held responsible for errors or for any consequences arising from the use of the information contained herein.

Materials appearing in this book prepared by individuals as part of their official duties as U.S. Government employees are not covered by the above-mentioned copyright.

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.

Robert C. Gallo
G. Della Porta
A. Albertini

Contributors

C. M. Alberini

*Istituto di Chimica e Dipartimento
Materno-Infantile
Università degli Studi di Brescia
25100 Brescia, Italy*

A. Albertini

*Istituto di Chimica
Università degli Studi di Brescia
25100 Brescia, Italy*

B. Arveiler

*Laboratoire de Génétique Moléculaire
des Eucaryotes du CNRS
Unité 184 de l'INSERM
Faculté de Médecine
67085 Strasbourg Cedex, France*

C. Aubert

*Department de Virologie
Institut Pasteur
75724 Paris Cedex 15, France*

E. Bakker

*Department of Human Genetics
State University Leiden
2333 AL Leiden, The Netherlands*

M. Baldi

*Division of Gastroenterology
San Giovanni Battista Hospital
Corso Bramante 88
10126 Torino, Italy*

G. Barbanti-Brodano

*Institute of Microbiology
University of Ferrara
I-44100 Ferrara, Italy*

A. A. B. Bergen

*Department of Human Genetics
State University Leiden
2333 AL Leiden, The Netherlands*

T. L. J. Boehm

*Zentrum der Biologischen Chemie
Zentrum der Kinderheilkunde
Klinikum der J. W. Goethe-Universität
Frankfurt am Main
Theodor Stern-Kai 7
6000 Frankfurt a.M. 70, F.R.G.*

F. Bonino

*Division of Gastroenterology
San Giovanni Battista Hospital
Corso Bramante 88
10126 Torino, Italy*

E. Bonten

*Department of Human Genetics
State University Leiden
2333 AL Leiden, The Netherlands*

Jean Claude Boulain

*Centre d'Etude Nucléaire de Saclay
91191 Gif Sur Yvette, France*

M. Brahic

*Department de Virologie
Institut Pasteur
75724 Paris Cedex 15, France*

E. Brocchi

*Istituto Zooprofilattico Sperimentale
25100 Brescia, Italy*

C. Bruck

*Department of Molecular Biology
University of Brussels
1640 Rhode-St. Genèse, Belgium*

M. R. Brunetto

*Division of Gastroenterology
San Giovanni Battista Hospital
Corso Bramante 88
10126 Torino, Italy*

R. Buffa

*Department of Human Pathology
Ospedale Multizonale
21100 Varese, Italy*

M. Burmeister

*EMBL
D-6009 Heidelberg, FRG*

A. Burny

*Department of Molecular Biology
University of Brussels
1640 Rhode-St. Genèse, Belgium*

G. Camerino

*Dipartimento di Genetica e
Microbiologia
Università di Pavia
I-27100 Pavia, Italy*

S. Canevari

*Istituto Nazionale per lo Studio e la
Cura dei Tumori
20133 Milano, Italy*

A. Cao

*Istituto di Clinica e Biologia dell'Eta'
Evolutiva
Università degli di Studi
09100 Cagliari, Italy*

C. Capella

*Istituto di Anatomia Patologica
Università di Pavia
27100 Pavia, Italy*

Michael C. Carroll

*Division of Immunology
Children's Hospital
Department of Pediatrics
Harvard Medical School
Boston, MA 02115*

E. Cash

*Department de Virologie
Institut Pasteur
75724 Paris Cedex 15, France*

M. Chamorro

*Department de Virologie
Institut Pasteur
75724 Paris Cedex 15, France*

Alain Charbit

*Unité de Programmation Moléculaire
et Toxicologie Génétique
(CNRS UA271, INSERMU163)
Institut Pasteur
75015 Paris, France*

E. Chiaberge

*Division of Gastroenterology
San Giovanni Battista Hospital
Corso Bramante 88
10126 Torino, Italy*

M. Cianfriglia

*Laboratory of Cellular Biology
Istituto Superiore Sanità
00100 Rome, Italy*

D. Cirillo

*Department of Biomedical Sciences
& Oncology
University of Torino Medical School
10126 Torino, Italy*

M. I. Colnaghi

*Istituto Nazionale per lo Studio e la
Cura dei Tumori
20133 Milano, Italy*

P. M. Comoglio

*Department of Biomedical Sciences
& Oncology
University of Torino Medical School
10126 Torino, Italy*

F. Conde

*Centro Ramon Y. Cajal
28000 Madrid, Spain*

A. Corallini

*Institute of Microbiology
University of Ferrara
I-44100 Ferrara, Italy*

M. Cornaggia

*Department of Human Pathology
Ospedale Multizonale
21100 Varese, Italy*

J. R. Crowther

*Animal Virus Research Institute
Pirbright, Woking
Surrey GU24 0NF, England*

F. de Simone

*Istituto Zooprofilattico Sperimentale
25100 Brescia, Italy*

M. F. Di Renzo

*Department of Biomedical Sciences
& Oncology
University of Torino Medical School
10126 Torino, Italy*

D. Drahovsky

*Zentrum der Biologischen Chemie
Klinikum der J. W. Goethe-Universität
Frankfurt am Main
Theodor Stern-Kai 7
6000 Frankfurt a.M. 70, F.R.G.*

R. Ferracini

*Department of Biomedical Sciences
& Oncology
University of Torino Medical School
10126 Torino, Italy*

S. Ferrone

*Department of Microbiology
& Immunology
New York Medical College
Valhalla, New York 10595*

G. Filippi

*Cattedra di Genetica Medica
Università di Trieste
34100 Trieste, Italy*

R. Fontanelli

*Istituto Nazionale per lo Studio e la
Cura dei Tumori
20133 Milano, Italy*

L. Frati

*Institute of General Pathology
University of Rome
00100 Rome, Italy*

D. Gamba

*Istituto di Chimica
Università degli Studi di Brescia
25100 Brescia, Italy*

G. Gaudino

*Department of Biomedical Sciences
& Oncology
University of Torino Medical School
10126 Torino, Italy*

Glen N. Gaulton

*Department of Pathology
Division of Immunobiology
University of Pennsylvania
School of Medicine
Philadelphia, Pennsylvania 19104*

G. Gerna

*Institute of Infectious Diseases
University of Pavia
I-27100 Pavia, Italy*

S. Ghielmi

*Istituto di Chimica
Università degli Studi di Brescia
25100 Brescia, Italy*

S. Giordano

*Department of Biomedical Sciences
& Oncology
University of Torino Medical School
10126 Torino, Italy*

Mark I. Greene

*Department of Pathology
Division of Immunobiology
University of Pennsylvania
School of Medicine
Philadelphia, Pennsylvania 19104*

D. Grégoire

*Department of Molecular Biology
University of Brussels
1640 Rhode-St. Genèse, Belgium*

R. Heilig

*Laboratoire de Génétique Moléculaire
des Eucaryotes du CNRS
Unité 184 de l'INSERM
Faculté de Médecine
67085 Strasbourg Cedex, France*

M. H. Hofker

*Department of Human Genetics
State University Leiden
2333 AL Leiden, The Netherlands*

Maurice Hofnung

*Unité de Programmation Moléculaire
et Toxicologie Génétique
(CNRS UA271, INSERMU163)
Institut Pasteur
75015 Paris, France*

R. Kettmann

*Department of Molecular Biology
University of Brussels
1640 Rhode-St. Genèse, Belgium*

M. Kusama

*Department of Microbiology
& Immunology
New York Medical College
Valhalla, New York 10595*

Antonio Lanzavecchia

*Basel Institute for Immunology
Grenzacherstrasse 487
Postfach
CH-4005 Basel, Switzerland*

C. Lavarini

*Division of Gastroenterology
San Giovanni Battista Hospital
Corso Bramante 88
10126 Torino, Italy*

F. Leoni

*Istituto Nazionale per lo Studio
e la Cura dei Tumori
20133 Milano, Italy*

J. Lindmark

*The National Veterinary Institute
Department of Virology
Biomedical Center
S-751 23 Uppsala, Sweden*

K. Lövgren

*The National Veterinary Institute
Department of Virology
Biomedical Center
S-751 23 Uppsala, Sweden*

D. Majoor-Krakauer

*Erasmus University
3000 DR Rotterdam, The Netherlands*

J. L. Mandel

*Laboratoire de Génétique Moléculaire
des Eucaryotes du CNRS
Unité 184 de l'INSERM
Faculté de Médecine
67085 Strasbourg Cedex, France*

G. Mantero

*Istituto di Chimica
Università degli Studi di Brescia
25100 Brescia, Italy*

E. Maran

*Division of Gastroenterology
San Giovanni Battista Hospital
Corso Bramante 88
10126 Torino, Italy*

K. C. McCullough

*Ciba-Geigy AG, Biovet Unit
Centre de Recherches Agricoles
CH-1566 Saint Aubin FR, Switzerland*

S. Ménard

*Istituto Nazionale per lo Studio
e la Cura dei Tumori
20133 Milano, Italy*

G. Milanesi

*Institute of Biochemical and
Evolutionary Genetics
National Research Council
University of Pavia
I-27100 Pavia, Italy*

J. P. Moisan

*Laboratoire de Génétique Moléculaire
des Eucaryotes du CNRS
Unité 184 de l'INSERM
Faculté de Médecine
67085 Strasbourg Cedex, France*

B. Morein

*The National Veterinary Institute
Department of Virology
Biomedical Center
S-751 23 Uppsala, Sweden*

M. Mottes

*Institute of Biochemical and
Evolutionary Genetics
National Research Council
University of Pavia
I-27100 Pavia, Italy*

L. Naldini

*Department of Biomedical Sciences
& Oncology
University of Torino Medical School
10126 Torino, Italy*

P. G. Natali

*Laboratory of Immunology
Regina Elena Tumor Institute
00100 Rome, Italy*

F. Negro

*Division of Gastroenterology
San Giovanni Battista Hospital
Corso Bramante 88
10126 Torino, Italy*

L. D. Notarangelo

*Istituto di Chimica e
Dipartimento Materno-Infantile
Università degli Studi di Brescia
25100 Brescia, Italy*

M. Nuti

*Institute of General Pathology
University of Rome
00100 Rome, Italy*

I. Oberlé

*Laboratoire de Génétique Moléculaire
des Eucaryotes du CNRS
Unité 184 de l'INSERM
Faculté de Médecine
67085 Strasbourg Cedex, France*

T. Obi

*Animal Virus Research Institute
Pirbright, Woking
Surrey GU24 0NF, England*

R. Orlandi

*Istituto Nazionale per lo Studio
e la Cura dei Tumori
20133 Milano, Italy*

M. Pagnani

*Institute of Microbiology
University of Ferrara
I-44100 Ferrara, Italy*

P. Panina

*Istituto di Chimica e Dipartimento
Materno-Infantile
Università degli Studi di Brescia
25100 Brescia, Italy*

P. L. Pearson

*Department of Human Genetics
State University Leiden
2333 AL Leiden, The Netherlands*

S. Pecorelli

*Istituto di Chimica e Dipartimento
Materno-Infantile
Università degli Studi di Brescia
25100 Brescia, Italy*

F. Perosa

*Department of Microbiology
& Immunology
New York Medical College
Valhalla, New York 10595*

M. Pirastu

*Istituto di Ricerche sulle
Talassemie ed Anemie
Mediterranee-CNR
Università degli Studi
09100 Cagliari, Italy*

P. Pizzetti

*Istituto Nazionale per lo Studio
e la Cura dei Tumori
20133 Milano, Italy*

G. Porro

*Istituto Nazionale per lo Studio
e la Cura dei Tumori
20133 Milano, Italy*

L. Primi

*Sclavo S.p.A.
20100 Milano, Italy*

M. Purrello

*Memorial Sloan Kettering
Cancer Center
New York, NY 10021*

Francesco Ramirez

*Department of Microbiology
and Immunology
Morse Institute of Molecular Genetics
SUNY Health Center at Brooklyn
Brooklyn, New York 11203*

P. Reschiglian

*Institute of Microbiology
University of Ferrara
I-44100 Ferrara, Italy*

A. Rinaldi

*Istituto di Biologia Generale
Università di Cagliari
09100 Cagliari, Italy*

G. Rindi

*Department of Human Pathology
University of Pavia
I.R.C.C.S. Policlinico San Matteo
27100 Pavia, Italy*

M. Ripamonti

*Istituto Nazionale per lo Studio
e la Cura dei Tumori
20133 Milano, Italy*

C. Riva

*Department of Human Pathology
Ospedale Multizonale
21100 Varese, Italy*

Gillian Rumsby

*Department of Clinical Biochemistry
The Institute of Child Health
Hospitals for Sick Children
London, UK*

L. Sandkuyl

*Erasmus University
3000 DR Rotterdam, The Netherlands*

V. Sartorelli

*Istituto di Chimica e Dipartimento
Materno-Infantile
Università degli Studi di Brescia
25100 Brescia, Italy*

Doris Scheidegger

*Basel Institute for Immunology
Grenzacherstrasse 487
Postfach
CH-4005 Basel, Switzerland*

Peter M. Schneider

*Institut f. Rechtsmedizin
Universität Mainz
D-6500 Mainz, F.R.G.*

E. Silini

*Institute of Biochemical and
Evolutionary Genetics
National Research Council
University of Pavia
I-27100 Pavia, Italy*

M. P. J. M. Simons

*Department of Human Genetics
State University Leiden
2333 AL Leiden, The Netherlands*

M. Siniscalco

*Memorial Sloan Kettering
Cancer Center
New York, New York 10021*

M. I. Skraastad

*Department of Human Genetics
State University of Leiden
2333 AL Leiden, The Netherlands*

A. Smedile

*Division of Gastroenterology
San Giovanni Battista Hospital
Corso Bramante 88
10126 Torino, Italy*

M. Temponi

*Department of Microbiology
and Immunology
New York Medical College
Valhalla, New York 10595*

Petros Tsipouras

*Department of Pediatrics
UMDNJ-Rutgers Medical School
Piscataway, New Jersey 08854*

M. Tsujisaki

*Department of Microbiology
and Immunology
New York Medical College
Valhalla, New York 10595*

V. Turchi

*Institute of General Pathology
University of Rome
00100 Rome, Italy*

A. G. Ugazio

*Istituto di Chimica e Dipartimento
Materno-Infantile
Università degli Studi di Brescia
25100 Brescia, Italy*

A. Van den Broeke

*Department of Molecular Biology
University of Brussels
1640 Rhode-St. Genèse, Belgium*

Gert-Han B. Van Ommen

*Department of Human Genetics
State University of Leiden
2333 AL Leiden, The Netherlands*

H. Veenema

*Department of Human Genetics
State University of Leiden
2333 AL Leiden, The Netherlands*

J. M. H. Verkerk

*Department of Human Genetics
State University of Leiden
2333 AL Leiden, The Netherlands*

A. Werle

Zentrum der Biologischen Chemie
Klinikum der J. W. Goethe-Universität
Frankfurt am Main
Theodor Stern-Kai 7
6000 Frankfurt a.M. 70, F.R.G.

L. Willems

Department of Molecular Biology
University of Brussels
1640 Rhode-St. Genèse, Belgium

Arie Jeremy Zuckerman

Department of Microbiology
University of London
London School of Hygiene and
Tropical Medicine
London WC1E 7HT, Great Britain

Contents

- 1 Molecular Approach to Diagnostic and Preventive Medicine:
The Status of the Art
M. Siniscalco, G. Filippi, A. Rinaldi, and M. Purrello
- 23 Use of DNA Probes for Prenatal and Carrier Diagnosis of
Hemophilia and Fragile X Mental Retardation
*J.L. Mandel, B. Arveiler, G. Camerino, R. Heilig,
J.P. Moisan, and I. Oberlé*
- 33 The Application of DNA Probes to Diagnosis and Research of
Duchenne Muscular Dystrophy: Clinical Trial, New Probes
and Deletion Mapping
*Gert-Jan B. Van Ommen, E. Bakker, A.A.B. Bergen,
E. Bonten, M. Burmeister, M.H. Hofker,
D. Majoor-Krakauer, L. Sandkuyl, M.P.J.M. Simons,
M.I. Skraastad, H. Veenema, J.M.H. Verkerk, and
P.L. Pearson*
- 43 The Use of Synthetic Oligonucleotide Probes for
Antenatal Diagnosis of Beta-Thalassemia
M. Pirastu and A. Cao
- 55 Molecular Genetics of the Human Collagens
Francesco Ramirez and Petros Tsipouras
- 63 Molecular Genetics of Human Steroid 21-Hydroxylase
Genes
Michael C. Carroll, Peter M. Schneider, and Gillian Rumsby
- 73 The Enigma of Non-A, Non-B Hepatitis
Arie Jeremy Zuckerman
- 81 Detection of Hepatitis B Virus DNA and Hepatitis
Delta Virus RNA: Implications in Diagnosis and
Pathogenesis
*F. Bonino, A. Smedile, E. Chiaberge, M.R. Brunetto,
P. Negro, M. Baldi, C. Lavarini, and E. Maran*

- 91 Reovirus Antiidiotypic Antibodies as Receptor Probes and Immunologic Mimics
Glen N. Gaulton and Mark I. Greene
- 101 Syngeneic Monoclonal Antiidiotypic Antibodies to Murine Anti Human High Molecular Weight-Melanoma Associated Antigen Monoclonal Antibodies
M. Kusama, M. Tsujisaki, M. Temponi, F. Perosa, and S. Ferrone
- 111 Evaluation of Discrepancies in Some Clinical Aspects Employing A Panel of hCG Monoclonal Antibodies in RIA and Immunohistochemistry
S. Ghielmi, G. Mantero, D. Gamba, C. Capella, L. Primi, and A. Albertini
- 119 Chromogranins and Neuropeptides as Histochemical Markets of Neuroendocrine Tumors
R. Buffa, C. Riva, M. Cornaggia, G. Rindi
- 129 Generation of a Monoclonal Antibody Recognizing a Novel Breast Tumor Associated Antigen
M. Nuti, V. Turchi, M. Cianfriglia, L. Frati, and P.G. Natali
- 137 Monoclonal Antibodies: Perspectives for Tumor Therapy
M.I. Colnaghi, S. Canevari, F. Conde, R. Fontanelli, F. Leoni, S. Ménard, R. Orlandi, P. Pizzetti, G. Porro, and M. Ripamonti
- 147 DNA Probes to Evaluate the Possible Association of Papovaviruses with Human Tumors
G. Barbanti-Brodano, E. Silini, M. Mottes, G. Milanesi, M. Pagnani, P. Reschiglian, G. Gerna, and A. Corallini
- 157 Phosphotyrosine Antibodies: A Probe for Class I Oncogene Products Expressed in Human Malignancies
P.M. Comoglio, D. Cirillo, M.F. DiRenzo, R. Ferracini, G. Gaudino, S. Giordana, and L. Naldini
- 173 Determination of Clonality and Lineage in Human Acute Leukaemias by Use of DNA Probes
T.L.J. Boehm, A. Werle, and D. Drahovsky

- 183 In Situ Hybridization: A Tool for the Study of Viral Pathogenesis
M. Brahic, E. Cash, C. Aubert, and M. Chamorro
- 189 Hybrid Monoclonal Antibodies as a Tool to Target Human Cytotoxic T Cells
Antonio Lanzavecchia and Doris Scheidegger
- 195 Evidence for Soluble Interleukin-2 Receptor (IL-2 R) as a New Immunosuppressive Factor in Pregnancy
L.D. Notarangelo, C.M. Alberini, A. Albertini, P. Panina, S. Pecorelli, V. Sartorelli, and A.G. Ugazio
- 201 Genetic Approaches to the Problem of Antigen Exposure by Vaccinal Strains
Alain Charbit, Jean Claude Boulain, and Maurice Hofnung
- 209 Application of Monoclonal Antibodies in Veterinary Medicine
K.C. McCullough, J.R. Crowther, E. Brocchi, F. de Simone, and T. Obi
- 219 Use of Bovine Leukemia Virus Proviral DNA Probes for the Analysis of BLV-Induced Leukemogenesis
R. Kettmann, L. Willems, C. Bruck, D. Grégoire, A. Van den Broeke, and A. Burny
- 227 Strategy of Antigenic Presentation of Small Molecules and Peptides
K. Lövgren, J. Lindmark, and B. Morein
- 233 Subject Index

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

M. Siniscalco¹, G. Filippi², A. Rinaldi³ and M. Purrello¹

1. Memorial Sloan Kettering Cancer Center, New York, N.Y., 10021, USA
2. Cattedra di Genetica Medica, Universita' di Trieste, Trieste, Italy
3. Istituto di Biologia Generale, Universita' di Cagliari, Cagliari, Italy

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