GENETIC ENGINEERING TECHNIQUES

RECENT DEVELOPMENTS

Edited by
P. C. HUANG,
T. T. Kuo,
and Ray Wu

GENETIC ENGINEERING TECHNIQUES: RECENT DEVELOPMENTS

EDITED BY

P. C. HUANG
DEPARTMENT OF BIOCHEMISTRY
THE JOHNS HOPKINS UNIVERSITY
SCHOOL OF HYGIENE AND PUBLIC HEALTH
BALTIMORE, MARYLAND

T. T. KUO
ACADEMIA SINICA
NANKANG, TAIPEI
TAIWAN, REPUBLIC OF CHINA

RAY WU
CORNELL UNIVERSITY
SECTION OF BIOCHEMISTRY
ITHACA, NEW YORK

1982

ACADEMIC PRESS
A Subsidiary of Harcourt Brace Jovanovich, Publishers

New York London
Paris San Diego San Francisco São Paulo Sydney Tokyo Toronto

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

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

Library of Congress Cataloging in Publication Data
Main entry under title:

Genetic engineering.

Proceedings of a symposium held Jan. 11-12, 1982, in Taiwan.
Includes index.
1. Genetic engineering--Congresses. 2. Genetic engineering--Technique--Congresses. I. Huang, Pien-Chien, Date. II. Kuo, T. T., Date.
III. Wu, Ray.
QH442.G457 1982 574.87'328 82-20687
ISBN 0-12-358250-4

PRINTED IN THE UNITED STATES OF AMERICA

82 83 84 85 9 8 7 6 5 4 3 2 1

CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Thomas R. Broker (57, 325), *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724*

Jean Burckhardt (23), *Cancer Biology Program, NCI-Frederick Cancer Research Facility, Frederick, Maryland 21701*

Nancy T. Chang (299), *Centocor, Incorporated, 244 Great Valley Parkway, Malvern, Pennsylvania 19355*

Sheng-Yung Chang (243), *Cetus Corporation, 600 Bancroft Way, Berkeley, California 94710*

Shing Chang (243, 333), *Cetus Corporation, 600 Bancroft Way, Berkeley, California 94710*

Tse-Wen Chang (263, 299), *Centocor, Incorporated, 244 Great Valley Parkway, Malvern, Pennsylvania 19355*

Louise T. Chow (57, 325), *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724*

Craig Crowley (251), *Department of Molecular Biology, Genentech, Inc., South San Francisco, California 94080*

Jeffrey A. Engler (57), *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724*

Oanh Gray (243), *Cetus Corporation, 600 Bancroft Way, Berkeley, California 94710*

Richard A. Guilfoyle (57), *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724*

Li-He Guo (3), *Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853*

Daniel L. Hamilton (23), *Cancer Biology Program, NCI-Frederick Cancer Research Facility, Frederick, Maryland 21701*

Diana Ho (243), *Cetus Corporation, 600 Bancroft Way, Berkeley, California 94710*

P. C. Huang (285), *Department of Biochemistry, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland 21205*

Ru-chih C. Huang (93), *Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218*

David R. Hyde (203), *Department of Biochemistry, Microbiology, Molecular and Cell Biology, Paul M. Althouse Laboratory, The Pennsylvania State University, University Park, Pennsylvania 16802*

James Kroyer (243), *Cetus Corporation, 600 Bancroft Way, Berkeley, California 94710*

Maria-Regina Kula (159), *Gesellschaft fur Biotechnologische Forschung, 3300 Braunschweig, Stockheim, West Germany*

Ching-Juh Lai (189), *Molecular Viral Biology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205*

Robert A. Lamb (189), *Rockefeller University, 1230 York Avenue, New York, New York*

Arthur D. Levinson (251), *Department of Molecular Biology, Genentech, Inc., South San Francisco, California 94080*

Bor-Chian Lin (189), *Molecular Viral Biology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Disases, National Institutes of Health, Bethesda, Maryland 20205*

Yuan Lin (111, 337), *Biological Carcinogenesis Program, NCI-Frederick Cancer Research Facility, Frederick, Maryland 21701*

Werner Lindenmaier (159), *Gesellschaft fur Biotechnologische Forschung, 3300 Braunschweig, Stockhein , West Germany*

Chung-Cheng Liu (251), *Department of Molecular Biology, Genentech, Inc., South San Francisco, California 94080*

Mark M. Manak (225), *Biotech Research Laboratories, Inc., Rockville, Maryland 20850*

Tom Maniatis (129), *Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02137*

Lewis J. Markoff (189), *Molecular Viral Biology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205*

Jane McLaughlin (243), *Cetus Corporation, 600 Bancroft Way, Berkeley, California 94710*

Mara Rossini (57), *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724*

Richard C. Scarpulla (3), *Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853*

Che-Kun James Shen (129, 329), *Department of Genetics, University of California, Davis, California 95616*

Thomas Y. Shih (175), *Laboratory of Tumor Virus Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205*

Michael M. Sveda (189), *Molecular Viral Biology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205*

Kurt P. Timmerman (203), *Department of Biochemistry, Microbiology, Molecular and Cell Biology, Paul M. Althouse Laboratory, The Pennsylvania State University, University Park, Pennsylvania 16802*

Robert C. Ting (225), *Biotech Research Laboratories, Inc., Rockville, Maryland 20850*

Hsin Tsai (159), *Gesellschaft fur Biotechnologische Forschung, 3300 Braunschweig, Stockheim, West Germany*

Chen-Pei D. Tu (203), *Department of Biochemistry, Microbiology, Molecular and Cell Biology, Paul M. Althouse Laboratory, The Pennsylvania State University, University Park, Pennsylvania 16802*

Mark P. van Bree (57), *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724*

James C. Wang (41), *Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138*

Ray Wu (3), *Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853*

Robert Yuan (23, 337), *Cancer Biology Program, NCI-Frederick Cancer Research Facility, P. O. Box B, Frederick, Maryland 21701*

PREFACE

A plethora of symposia have been held during the last few years on the subject of genetic engineering for various purposes. The one which took place in Taipei during the week of January 11, 1982, was truly unique and we felt the content should be recorded, hence this volume.

The event is noteworthy in at least three aspects. (1) It helped to inoculate the seed of genetic engineering research and development in Taiwan whose agriculture and industry would be benefited by this new technology. (2) It experimented with a format of meeting in which formal presentations were followed by laboratory workshops. Such an arrangement received a most encouraging response by an audience attempting to master a field which is yet novel. (3) It was able to gather overseas compatriots to provide a truly international feedback and collaboration. The speakers' admirable attempts to supplement English, the common scientific language, with Chinese, a native tongue begging for appropriate scientific counterparts, helped to make the presentation widely appreciated. Thus the extended form of the symposium is not an ordinary one. It not only reported and reviewed advances in this field but might have also set an example for others who wish to organize such a program.

The meeting was held in four sessions, basic tools, cloning, expression, and potentials. It lasted for two days and was attended by over 800 participants. A total of seventeen speakers delivered these papers that are presented in full in this volume.

The laboratory sessions were organized for five groups, twelve participants and an equal number of observers in each. One session was on the hybridoma techniques for the elicitation and isolation of anti-mouse IgG monoclonal antibody. Another was on the use of electron microscopy as a mapping technique. RNA:DNA heteroduplexes were examined. Two cloning workshops took place concurrently; one focused on cDNA and the other on the construction of a genomic library. Globin gene was used as an example. It was possible for the session on restriction enzyme purification and DNA sequencing to start with the preparation of P-32 labeled nucleoside triphosphates and to end with a comparison of the conventional chemical versus the new enzymatic exonuclease-polymerase method of sequencing. Since the laboratory sessions were held at separate sites, a joint meeting was called at the conclusion for report and discussion (followed by a banquet, of course). In general, the participants agreed that practical exercises are very helpful and stimulating when coupled with symposium talks. While space does not allow us to present each laboratory exercise, portions of the workshop protocols are reproduced in this volume as they were proven practical.

The uniqueness of combining formal talks and laboratory exercises required detailed coordination involving particularly the logistics of allocating supplies of enzymes, chemicals, cells and animals, and even micropipettes. In this case, such a collaboration was enjoyed. We have included a list of items for each workshop so that local availability could facilitate planning and ordering by the instructors. This is particularly important if relatively little prior work on the subject has been done at the locality.

It has been a pleasure to be able to record this event and with it our appreciation is due to the chairpersons of the sessions: Professors C. C. Yang (Chin Hwa University), H. P. Wu (Academia Sinica), T. C. Lin (Preventive Medicine Institute), and J. Y. Lin, Y. C. Su, and C. S. Yang (all of Taiwan University), for their assurance as former mentors and colleagues; to members of the local arrangement committee Drs. K. C. Hsieh, C. L. Tien, and S. S. Pei, for their hospitality and dedication; to the organizers of the meeting, Professor Ru-chih C. Huang and Dr. T. T. Kuo for their good judgment.

The symposium and workshop were sponsored jointly by the National Science Council and Academia Sinica at Taipei.

P. C. Huang
T. T. Kuo
Ray Wu
July 1982

CONTENTS

II. CLONING

III. EXPRESSION

IV. POTENTIALS

V. SUMMARY

VI. WORKSHOP

SECTION I THE BASIC TOOLS

GENETIC ENGINEERING TECHNOLOGY - AN OVERVIEW AND SOME RECENT ADVANCES

Ray Wu[a]
Li-He Guo
Richard C. Scarpulla

Department of Biochemistry, Molecular and Cell Biology
Cornell University
Ithaca, New York

I. INTRODUCTION

As is often the case with scientific discoveries, technological advances preceded the acquisition of new knowledge. The remarkable advances in our understanding of gene structure and function have been based on the discovery of DNA ligase and restriction endonucleases, their application in recombining genes, the availability of plasmids to serve as cloning vehicles, and the development of rapid methods of sequencing DNA. It is the ability of the scientists to couple recombinant DNA research with DNA sequencing techniques that has proven to be the powerful cutting edge in modern molecular biology. Together with bioengineers, biochemists have laid the foundation for genetic engineering technology.

The purpose of this article is first to summarize briefly the major strategies underlying genetic engineering technology. Other authors in this volume will provide more detailed information on specific areas later. More information can also be found in several books (1,2) and review articles (3,4). In the second part of this paper, we will present a new rapid method for sequencing DNA. Finally, the rat cytochrome c gene will be used as an example of how some of these techniques can be applied to cloning and structural analysis.

---

[a]Supported by NIH grants GM27365 and GM29179, and the Damon Runyon-Walter Winchell Cancer Fund.

ISBN 0-12-358250-4

II. BASIC STEPS IN GENETIC ENGINEERING TECHNOLOGY

Step 1. Isolation and Specific Cleavage of DNA

DNA molecules from various cells or viruses are isolated and purified by standard procedures (5,6). The DNA molecule is then cleaved with a specific restriction enzyme to give fragments varying in size between a few hundred to over ten thousand base pairs. When a restriction enzyme (7,8) that recognizes a specific hexanucleotide sequence is used to cleave DNA from a virus, bacterium or animal cell, the number of fragments ranges from 10 to 1,000,000. Only 4 such fragments (X_1, X_2, X_3 and X_4) are depicted in Figure 1 for simplicity. A number of restriction enzymes produce DNA fragments with cohesive ends, which can be joined to other DNA fragments with identical cohesive ends. Other restriction enzymes produce even ends, and they can be joined by T_4 DNA ligase (9). Alternatively, chemically synthesized oligonucleotide adapters can be ligated to the blunt-ended DNA to create cohesive ends (10,11). It is also possible to add homopolymeric tails to the blunt-ended DNA to be cloned by using terminal transferase (12,13) in the presence of a deoxynucleoside triphosphate; complementary tails are added to the cloning vehicle. The cloning vehicle, such as a circular plasmid DNA, is cleaved with the same restriction enzyme to produce a linear molecule with cohesive ends or blunt ends. This cloning vehicle has the following important characteristics. (a) It is a circular, self-replicating DNA molecule that can maintain itself within a cell. (b) It is cut in only a single place by a given restriction enzyme such as BamHI yielding a linear molecule (step 1 righthand side). (c) It confers upon a host bacterium resistance to antibiotics, which in this example are tetracycline (tet) and ampicillin (amp). Note, however, that cleavage of the plasmid with BamHI destroys the tetracycline resistance gene.

Derivatives of bacteriophage λ have been especially suitable for making gene libraries of DNA from eukaryotic sources where about a million clones are needed for a complete library (14). The filamentous bacteriophage M13 has been used for cloning short segments of DNA (15) that can be sequenced directly by the dideoxynucleotide chain termination method (16).

Step 2. Ligation of DNA Fragments to a Cloning Vehicle

The DNA fragments produced in step 1 are randomly associated by hydrogen bonding through their cohesive ends and joined covalently using DNA ligase (17) to a cloning

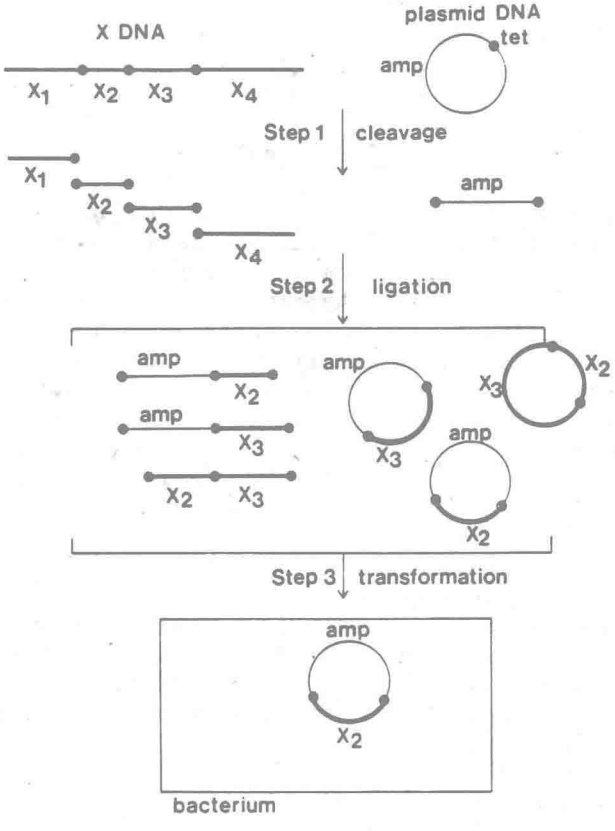

Figure 1: A scheme for cloning of DNA fragments. DNA to be cloned (heavy line) and plasmid DNA (thin line) are treated with a restriction enzyme. Sites at which the enzyme cleaves are indicated by filled circles. By treating the mixture of DNA fragments and the linearized plasmid DNA with DNA ligase (step 2), the fragments are joined with each other or with the plasmid DNA (only some hybrid molecular are shown). In step 3, the hybrid DNA molecules are introduced into bacterial cells by transformation. Bacteria which grow in the presence of ampicillin (amp) and tetracycline (tet) harbor unaltered plasmids, whereas those which grow in the presence of ampicillin but not tetracycline harbor plasmids into which a DNA fragment is inserted (hybrid DNA). Pure clones of the latter are obtained by single colony isolation (cloning). A cell from such a clone is shown.

vehicle, such as a plasmid molecule (step 2, Fig. 1). All possible combinations of fragments result (dimers, trimers, circular dimers and trimers, etc.), including circular molecules containing a plasmid (represented by a thin line) joined to a DNA fragment (represented by a thick line) to produce a hybrid DNA (e.g. plasmid - X_2).

Step 3. Transformation and Selection

The complex mixture of ligated DNA produced in step 2 is then presented to bacterial cells specially treated so that they take up DNA, a process called transformation (18) (step 3). Bacteria which contain hybrid DNA molecules are easily recognized as being resistant to ampicillin but sensitive to tetracycline. The cloned DNA fragment, X_2 in this example, can be isolated in quantity simply by growing large numbers of bacteria and isolating plasmid from them.

In cases where transformation-conferred antibiotic resistance cannot be used to select and identify the desired clone, several alternative methods are available: (a) by colony or plaque hybridization with radioactive labeled nucleic acid probes (19,20); (b) by size determination of the cloned DNA after cleavage with a restriction enzyme and fractionation of the DNA fragments by gel electrophoresis; (c) by genetic complementation of a mutation in the bacterial host with a gene carried on a cloning vehicle (21); (d) by immunological methods for the screening of the desired protein product synthesized in the bacteria (22-24); (e) by utilizing pools of the cloned DNA to purify a particular mRNA through DNA-RNA hybridization. The protein synthesized from the mRNA is then identified after *in vitro* translation (25) or after injecting oocytes; (f) by using pools of the cloned DNA to inhibit *in vitro* translation of a particular mRNA through formation of a DNA-RNA hybrid, hybrid-arrested translation (26).

In most cases, *E. coli* was the host organism for transformation. Recently, transformation in yeast (27) and *B. subtilis* (28,29) has been successful and there are certain advantages in using these alternative host cells.

Step 4. Confirming the Cloned Gene by Physical Mapping and DNA Sequencing

Once the desired clone was selected by one of the methods mentioned in step 3, the identity and the structure of the cloned gene must be confirmed by physical mapping (using several restriction enzymes) and by DNA sequence analysis. DNA sequencing may reveal the selection of a