Springer Science+Business Media, B.V.

Dear Reader,

We would very much appreciate receiving your suggestions and criticisms for the *Plant Molecular Biology Manual*, 2nd Edition. They will prove to be most helpful during our preparations for future supplements.

Would you please answer the questions listed below, and send your comments with any further suggestions you may have, to *Drs. Gilles Jonker* at the abovementioned address.

Thank for your assistance!

Drs. Gilles Jonker
Publisher

PLANT MOLECULAR BIOLOGY MANUAL

1. What errors have you found? (list page numbers and describe mistakes)
2. What protocols do you find to be confusing or lacking in detail? (list chapter numbers and page numbers and describe problems)
3. What protocols do you feel should be replaced in future supplements with newer (better) methods?
4. What new topics or other material would you like to see included in future supplements?

Please print or type your answers in the space below, and continue overleaf.

Name:
Address:

Date:

PLANT MOLECULAR BIOLOGY MANUAL

PLANT MOLECULAR BIOLOGY MANUAL

Second edition

Edited by

STANTON B. GELVIN

*Purdue University, West Lafayette
Indiana, USA*

ROBBERT A. SCHILPEROORT

*Leiden State University, Leiden
The Netherlands*

Springer Science+Business Media, B.V.

A C.I.P. Catalogue record for this book is available from the Library of Congress.

ISBN 978-94-011-7654-5 ISBN 978-94-011-0511-8 (eBook)
DOI 10.1007/978-94-011-0511-8

Neither Kluwer Academic Publishers nor any person acting on its behalf is responsible for the use which might be made of the information contained herein.

Printed on acid-free paper

All Rights Reserved

© 1994 Springer Science+Business Media Dordrecht

Originally published by Kluwer Academic Publishers in 1994

No part of the material protected by this copyright notice may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying, recording, or by any information storage and retrieval system, without written permission from the copyright owners.

Contents

SECTION A: In vitro methods of gene transfer to plant cells

1. PEG-mediated direct gene transfer and electroporation
Roland Bilang, Andreas Klöti, Martin Schrott & Ingo Potrykus
2. Gene transfer to plants via particle bombardment
Paul Christou

SECTION B: Agrobacterium-mediated gene transfer to plant cells

1. *Agrobacterium*-mediated gene transfer to plant cells: cointegrate and binary vector systems
Cindy R. Walkerpeach & J. Velten
2. Specialized vectors for gene tagging and expression studies
Csaba Koncz, Norbert Martini, László Szabados, Milan Hroudá, Andreas Bachmair & Jeff Schell
3. *Agrobacterium* molecular genetics
Paul J.J. Hooykaas & Teresa Mozo
4. Genetic manipulation of *Agrobacterium tumefaciens* strains to improve transformation of recalcitrant plant species
Stanton B. Gelvin & Chang-Nong Liu
5. Transient expression assays using GUS constructs and fluorometric detection for analysis of T-DNA transfer
Linda A. Castle & Roy O. Morris
6. *Agrobacterium* inoculation techniques for plant tissues
Nancy L. Mathis & Maud A.W. Hinchey

SECTION C: Selectable and screenable markers for plant transformation

1. Antibiotic-resistance markers for plant transformation
Geert Angenon, Willy Dillen & Marc van Montagu
2. Reporter genes for plants
Luis Herrera-Estrella, Patricia León, Olof Olsson & Teemu H. Teeri

3. Opines as screenable markers for plant transformation
Yves Dessaux & Annik Petit

SECTION D: Nucleic acid extraction from plant tissue

1. Extraction of total cellular DNA from plants, algae and fungi
Scott O. Rogers & Arnold J. Bendich
2. Isolation and characterization of nuclear scaffolds
Gerald E. Hall, Jr. & Steven Spiker
3. Isolation of plant mitochondria and mitochondrial nucleic acids
Sally A. Mackenzie
4. Isolation of chloroplasts and chloroplast DNA
C.A. Price, Nouredine Hadjeb, Lee Newman & Ellen M. Reardon
5. Isolation of total, poly (A) and polysomal RNA from plant tissues
Katharina Pawlowski, Reinhard Kunze, Sacco de Vries & Ton Bisseling

SECTION E: Transcription and translation systems

1. Assay for gene expression using run-on transcription in isolated nuclei
Imre E. Somssich
2. Preparation of an *in vitro* transcription system of plant origin, with methods and templates for assessing its fidelity
Yuhki Yamaguchi, Fujio Mukumoto, Hidemasa Imaseki & Ken-Ichi Yamazaki

SECTION F: Blotting and gene detection systems

1. Southern, Northern and Western blot analysis
Johan Memelink, Kathleen M.M. Swords, L. Andrew Staehelin & J. Harry C. Hoge
2. Screening of cDNA expression libraries with synthetic oligonucleotides for DNA-binding proteins
Wolfgang Werr, Bärbel Überlackner & Bettina Klinge
3. Non-radioactive nucleic acid detection systems
Susan J. Karcher

SECTION G: In situ hybridization and immunodetection

1. RNA *in situ* hybridization in plants
Nicholas B. Duck
2. *In situ* hybridization to plant metaphase chromosomes using digoxigenin labeled nucleic acid sequences
S. Hinnisdaels, I. Farbos, J. Del-Favero, J. Veuskens, M. Jacobs & A. Mouras

SECTION H: Cloning and detection of DNA sequences from large DNA molecules

1. Methods for generating plant genomic libraries
Marjory A. Snead, Patricia L. Kretz & Jay M. Short
2. Construction of plant yeast artificial chromosome libraries
Gregory B. Martin
3. Preparation of high molecular weight plant DNA and analysis by pulsed field gel electrophoresis
Raymond A.J.J. van Daelen & Pim Zabel
4. Random amplified polymorphic DNA (RAPD) markers
Antoni Rafalski, Scott Tingey & John G.K. Williams

SECTION I: Protein-nucleic acid interaction analyses

1. Gel mobility shift assay
Koji Mikami, Hisabumi Takase & Masaki Iwabuchi
2. Optimization of DNase I footprinting experiments
Susan J. Martino-Catt & Steve A. Kay
3. Analyses of plant chromatin and *in vivo* protein-DNA interactions
Anna-Lisa Paul & Robert J. Ferl
4. Expression and characterization of recombinant plant trans-acting factors
Lee Meisel & Eric Lam

SECTION J: Subcellular targeting of proteins

1. *In vitro* import of proteins into chloroplasts
Barry D. Bruce, Sharyn Perry, John Froehlich & Kenneth Keegstra
2. *In vitro* targeting of proteins to mitochondria
Marc A. Boutry, Didier Thomas & François Chaumont
3. Targeting of proteins to the vacuole
James E. Dombrowski, Luis Gomez, Maarten J. Chrispeels & Natasha V. Raikhel
4. Visualizing protein import into the plant cell nucleus
Vitaly Citovsky

SECTION K: Gene tagging using transposons

1. Gene tagging by endogenous transposons
Wolf-Ekkehard Lönnig & Peter Huijser
2. Heterologous transposon tagging as a tool for the isolation of plant genes
Erik A. van der Biezen, Mark J.J. van Haaren, Bert Overduin, H. John J. Nijkamp & Jacques Hille

List of Contributors

<i>Author</i>	<i>Chapter</i>	<i>Address</i>
Angenon, G.	C1	Laboratorium voor Genetica, Universiteit Gent, Ledeganckstraat 35, B-9000 Gent, Belgium
Bachmair, A.	B2	Max-Planck Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, D-50829 Köln 30, Germany
Bendich, A.J.	D1	Botany Dept. KB-15, University of Washington, Seattle WA 98195, USA
Bilang, R.	A1	Institute of Plant Sciences, Swiss Federal Institute of Technology, ETH-Zentrum, CH-8092 Zürich, Switzerland
Bisseling, T.	D5	Dept. of Molecular Biology, Transitarium, Agricultural University Wageningen, Dreyenlaan 3, 6703 HA Wageningen, The Netherlands
Boutry, M.A.	J2	Unité de Biochemie Physiologique, University of Louvain, Place Croix du Sud 2-20, 1348 Louvain-la-Neuve, Belgium
Bruce, B.D.	J1	Department of Botany, University of Wisconsin, Madison, WI 53706, USA
Castle, L.A.	B5	Dept. of Plant Biology, University of California, Berkeley, CA 94720, USA
Chaumont, F.	J2	Unité de Biochimie Physiologique, University of Louvain, Place Croix du Sud, 2-20, B-1348 Louvain-la-Neuve, Belgium
Chrispeels, J.	J3	Department of Biology, University of California – San Diego, La Jolla, CA 92093–1116, USA
Christou, P.	A2	Agracetus Inc., Research & Development, 8520 University Green, Middleton WI 53562, USA
Citovsky, V.	J4	Dept. of Biochemistry and Cell Biology, State University of New York, Stony Brook, NY 11794, USA
Del-Favero, J.	G2	Free University of Brussels, Institute for Molecular Biology, Paardenstraat 65, B-1640 St.-Genesius-Rode, Belgium
Dessaux, Y.	C3	Institut des Sciences Végétales, Bâtiment 23 C.N.R.S., Avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France
De Vries, S.	D5	Department of Molecular Biology, Agricul-

<i>Author</i>	<i>Chapter</i>	<i>Address</i>
		tural University Wageningen, NL-6703 HA Wageningen, The Netherlands
Dillen, W.	C1	Laboratorium voor Genetica, Universiteit Gent, Ledeganckstraat 35, B-9000 Gent, Belgium
Dombrowski, E.	J3	MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312, USA
Duck, N.	G1	Monsanto Company, 700 Chesterfield Village Parkway, St. Louis MO 63198, USA
Farbos, I.	G2	Université de Bordeaux II, Laboratoire de Biologie Cellulaire, Av. des Facultés, F-33405 Talence-Cedex, France
Ferl, R.J.	I3	Dept. of Horticultural Sciences, University of Florida, Gainesville FL 32611, USA
Froehlich, J.	J1	DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA
Gelvin, S.B.	B4	Dept. of Biological Sciences, Purdue University, Lilly Hall of Life Sciences, West Lafayette IN 47907, USA
Gomez, L.	J3	Department of Biology, University of California – San Diego, La Jolla, CA 92093-1116, USA
Hadjeb, N.	D4	Waksman Institute, Rutgers University, Piscataway, NJ 08855-0759, USA
Hall, G.E., Jr.	D2	Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614, USA
Herrera-Estrella, L.	C2	Department of Plant Genetic Engineering, CINVESTAV del I.P.N., Unidad Irapuato, Apartado Postal 629, 36500 Irapuato, Gto, México
Hille, J.	K2	Dept. of Genetics, Institute for Molecular Biological Sciences, BioCentrum Amsterdam, Free University, De Boelelaan 1007, 1001 HV Amsterdam, The Netherlands
Hinchee, M.	B6	Crop Transformation, Monsanto, Plant Protection Improvement, 700 Chesterfield VP, St. Louis MO 63198, USA
Hinnisdaels, S.	G2	Free University of Brussels, Institute for Molecular Biology, Paardenstraat 65, B-1640 St.-Genesius-Rode, Belgium
Hoge, J.H.C.	F1	Institute of Molecular Plant Sciences, Leiden

<i>Author</i>	<i>Chapter</i>	<i>Address</i>
Hooykaas, P.J.J.	B3	University, Clusius Laboratory, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands Institute of Molecular Plant Sciences, Clusius Laboratory, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands
Hrouda, M.	B2	Research Institute for Crop Production, Drnovska 507, Prague 6, Ruzyne, 161 06 Czech Republic
Huijser, P.	K1	Max-Planck Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, 5000 Köln 30, Germany
Imaseki, H.	E2	Research Institute for Biochemical Regulation, School of Agricultural Science, Nagoya University, Chikusa, Nagoya 464-01, Japan
Iwabuchi, M.	I1	Kyoto University, Faculty of Science, Dept. of Botany, Ktrashirikawa, Kyoto 606-01, Japan
Jacobs, M.	G2	Institute for Molecular Biology, Free University of Brussels, Paardenstraat 65, St. Genesius Rode, 1640 Belgium
Karcher, S.J.	F3	Dept. of Biological Sciences, Purdue University, B-315 Lilly Hall, West Lafayette, IN 47907-13902, USA
Kay, S.A.	I2	NSF Center for Biological Timing, Dept. of Biology, Gilmer Hall, University of Virginia, Charlottesville VA 22903, USA
Keegstra, K.	J1	University of Wisconsin, Dept. of Botany, 430 Lincoln Drive, Madison WI 53706, USA
Klinge, B.	F2	Institut für Genetik, Universität zu Köln, Weyertal 121, 50931 Köln, Germany
Klöti, A.	A1	Institute of Plant Sciences, Swiss Federal Institute of Technology, ETH-Zentrum, CH-8092 Zürich, Switzerland
Koncz, C.	B2	Max-Planck Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, D-50829 Köln 30, Germany
Kretz, P.L.	H1	Stratagene Cloning Systems, La Jolla, CA 92037, USA
Kunze, R.	D5	Institute of Genetics, Universität zu Köln, D-50931 Köln, Germany
Lam, E.	I4	Rutgers University, AgroBiotech Center and

<i>Author</i>	<i>Chapter</i>	<i>Address</i>
León, P.	C2	Graduate Program in Microbiology, Waksman Institute, Piscataway NJ 08854, USA
Liu, C.-N.	B4	Instituto de Biotecnología UNAM, Apartado Postal 510-3, Cuernavaca, Morelos, México
Lönnig, W.E.	K1	Dept. of Biological Sciences, Lilly Hall of Life Sciences, Purdue University, West Lafayette, IN 47907 USA
MacKenzie, S.	D3	Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, 50829 Köln, Germany
Martin, G.B.	H2	Dept. of Agronomy, Lilly Hall of Life Sciences, Purdue University, West Lafayette, IN 47907, USA
Martini, N.	B2	Purdue University, Dept. of Agronomy, 1150 Lilly Hall, West Lafayette IN 47907-1150, USA
Martino-Catt, S.J.	I2	Max-Planck Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, D-50829 Köln 30, Germany
Mathis, N.L.	B6	NSF Center for Biological Timing, Department of Biology, University of Virginia, Charlottesville, VA 22903, USA
Meisel, L.	I4	Crop Transformation, Monsanto Co., Plant Protection Improvement, 700 Chesterfield VP, St. Louis, MO 63198, USA
Memelink, J.	F1	AgBiotech Center and Graduate Program in Microbiology, Rutgers University, Waksman Institute, P.O. Box 759, Piscataway, NJ 08854, USA
Mikami, K.	I1	Clusius Laboratory, Molecular Plant Sciences Institute, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands
Morris, R.O.	B5	Division of Developmental Biology, National Institute for Basic Biology, Okazaki 444, Japan
Mouras, A.	F2	Dept. of Biochemistry, University of Missouri-Columbia, Columbia, MO 65211, USA
Mozo, T.	B3	Université de Bordeaux II, Laboratoire de Biologie Cellulaire, Av. des Facultés, F-33405 Talence-Cedex, France
		Institute of Molecular Plant Sciences, Clusius

<i>Author</i>	<i>Chapter</i>	<i>Address</i>
Mukumoto, F.	E2	Laboratory, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands Research Institute for Biochemical Regulation, School of Agricultural Science, Nagoya University, Chikusa, Nagoya 464-01, Japan
Newman, L.	D4	Waksman Institute, Rutgers University, Piscataway, NJ 08855-0759, USA
Nijkamp, H.J.J.	K2	Department of Genetics, Institute for Molecular Biological Sciences, BioCentrum Amsterdam, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands
Olsson, O.	C2	Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-90187 Umeå, Sweden
Overduin, B.	K2	Department of Genetics, Institute for Molecular Biological Sciences, BioCentrum Amsterdam, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands
Paul, A.-L.	I3	Department of Horticultural Sciences, University of Florida, Gainesville, FL 32611, USA
Pawlowski, K.	D5	Department of Molecular Biology, Agricultural University Wageningen, NL-6703 HA Wageningen, The Netherlands
Perry, S.	J1	Department of Botany, University of Wisconsin, Madison, WI 53706, USA
Petit, A.	C3	Institut des Sciences Végétales, Bâtiment 23, CNRS, Avenue de la Terrasse, F-91198 Gif-sur-Yvette, France
Potrykus, I.	A1	Institute of Plant Sciences, Swiss Federal Institute of Technology, ETH Zentrum LfV-E20, 8092 Zürich, Switzerland
Price, C.A.	D4	Waksman Institute, Rutgers State University, Piscataway NJ 08855-0759, USA
Rafalski, A.	H4	DuPont Co. Agricultural Products & Biotechnology, P.O. Box 80402, Wilmington DE 19880-0402, USA
Raikhel, N.	J3	Michigan State University, MSU-DOE Plant Research Laboratory, East Lansing MI 48824-1321, USA
Reardon, E.M.	D4	Waksman Institute, Rutgers University, Piscataway, NJ 08855-0759, USA

<i>Author</i>	<i>Chapter</i>	<i>Address</i>
Rogers, S.O.	D1	Environmental and Forest Biology, State University of New York, College of Environmental Science and Forestry, Syracuse, NY 13210, USA
Schell, J.	B2	Max-Planck Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, D-50829 Köln 30, Germany
Schrott, M.	A1	Institute of Plant Sciences, Swiss Federal Institute of Technology, ETH-Zentrum, CH-8092 Zürich, Switzerland
Short, J.M.	H1	Stratagene Cloning Systems, La Jolla, CA 92037, USA
Snead, M.	H1	Stratagene Cloning Systems, La Jolla CA 92037, USA
Sommich, I.E.	E1	Max-Planck Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, 5000 Köln 30, Germany
Spiker, S.	D2	Dept. of Genetics, 3530 Gardner Hall, North Carolina State University, Raleigh NC 27695-7614, USA
Stachelin, L.A.	F1	Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309-0347, USA
Swords, K.M.M.	F1	Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309-0347, USA
Szabados, L.	B2	Institute of Plant Physiology, Biological Research Center of Hungarian Academy of Sciences, Temesvári krt 62, P.O. Box 521, H-6701 Szeged, Hungary
Takase, H.	I1	Division of Developmental Biology, National Institute for Basic Biology, Okazaki 444, Japan
Teeri, T.	C2	Dept. of Genetics, Institute of Biotechnology, University of Helsinki, Arkadiankatu 7, 00100 Helsinki, Finland
Thomas, D.	J2	Unité de Biochimie Physiologique, University of Louvain, Place Croix du Sud, 2-20, B-1348 Louvain-la-Neuve, Belgium
Tingey, S.	H4	DuPont Co. Agricultural Products, Biotechnology, P.O. Box 80402 Wilmington, DE 19880-0402, USA

<i>Author</i>	<i>Chapter</i>	<i>Address</i>
Überlackner, B.	F2	Institut für Genetik, Universität zu Köln, Weyertal 121, 50931 Köln, Germany
Van Daelen, R.A.J.J.	H3	Wageningen Agricultural University, Department for Molecular Biology, Dreyenlaan 3, 6703 HA Wageningen, The Netherlands
Van der Biezen, E.A.	K2	Department of Genetics, Institute for Molecular Biological Sciences, BioCentrum Amsterdam, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands
Van Haaren, M.J.J.	K2	Department of Genetics, Institute for Molecular Biological Sciences, BioCentrum Amsterdam, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands
Van Montagu, M.	C1	Laboratorium voor Genetica, Universiteit Gent, Ledeganckstraat 35, 9000 Gent, België
Velten, J.	B1	USDA-ARS, New Mexico State University, Box 3GL, Las Cruces NM 88003, USA
Veuskens, J.	G2	Free University of Brussels, Institute for Molecular Biology, Paardenstraat 65, B-1640 St.-Genesius-Rode, Belgium
Walkerpeach, C.R.	B1	Plant Sciences, Monsanto Co., 700 Chesterfield Village Parkway, St. Louis, MO 63198, USA
Werr, W.	F2	Institut für Genetik, Universität zu Köln, Weyertal 121, 50931, Köln, Germany
Williams, J.G.K.	H4	DuPont Co. Agricultural Products, Biotechnology, P.O. Box 80402 Wilmington, DE 19880-0402, USA
Yamaguchi, Y.	E2	Research Institute for Biochemical Regulation, School of Agricultural Science, Nagoya University, Chikusa, Nagoya 464-01, Japan
Yamazaki, K.	E2	Research Institute for Biochemical Regulation, School of Agricultural Sciences, Nagoya University, Chikusa, Nagoya, 46401, Japan
Zabel, P.	H3	Dept. of Molecular Biology, Agricultural University Wageningen, Dreyenlaan 3, 6703 HA Wageningen, The Netherlands

Preface

Five years ago, the first edition of the Plant Molecular Biology Manual appeared. At that time, the editors felt that the field of plant molecular biology had matured to a point that the publication of a series of protocols in plant molecular biology was warranted. During the past five years, the field of plant molecular biology has expanded rapidly. This expansion is, among other things, reflected by the presence of several journals in the plant sciences, as well as by the increasing amount of plant sciences articles that are published in the more general journals. In 1991 approximately 3000 people attended the Third International Congress of Plant Molecular Biology in Tucson, Arizona, where more than 2000 posters were presented. It is also remarkable to see that nowadays botanical and physiological meetings pay a considerable amount of attention to plant molecular biology.

Since the first edition of this manual appeared, we have published, yearly, a series of supplements to the original volume. These supplements covered new subjects and described new methods that had been developed. With time, however, the editors realized that the original manual plus supplements had become cumbersome to use, and we decided to publish a reorganized version of the manual. This newly organized edition eliminates much of the duplication of procedures found previously, and incorporates new techniques (such as specialized transformation vectors, particle bombardment, nuclear scaffolds, *in vitro* transcription systems, non-radioactive detection systems, *in situ* hybridization, YAC library construction, protein-nucleic interaction assays, and transposon tagging). We again plan to publish supplements to this second edition, to keep the work up-to-date. We hope that this manual will continue to help researchers and students in the field of plant molecular biology by clearly describing up-to-date techniques. We welcome suggestions for supplementary chapters.

As before, the editors thank the authors for the speed with which they contributed their chapters. Special thanks go to Ms. Janet Hollister (Purdue University) for her secretarial assistance.