METHODS IN MOLECULAR BIOLOGY

Series Editor
John M. Walker
School of Life and Medical Sciences
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Plant Cytogenetics

Methods and Protocols

Edited by

Shahryar F. Kianian

University of Minnesota, St. Paul, MN, USA

Penny M.A. Kianian

University of Minnesota, St. Paul, MN, USA

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Editors
Shahryar F. Kianian
University of Minnesota
St. Paul, MN, USA

Penny M.A. Kianian
University of Minnesota
St. Paul, MN, USA

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Foreword: The Modern Cytogenetics Tool Box—A Picture Is Still Worth a Thousand Words

Cytogenetics is a stepping stone toward a full understanding of genetics. The report of in situ hybridization by Pardue and Gall in 1969 introduced a new era combining cytogenetics and molecular biology. An important feature was that allelic variation was not required for placing important genes to chromosome—and their place could be directly visualized with the light microscope. The narrative of this book takes the reader from the neoclassical to modern technologies for visualizing chromosomes, chromosome segments, and DNA strands. Many of the techniques require modest equipment and other resources. The book addresses complex situations including polyploidy in species such as oat and wheat. The chapters in this book are clearly written and provide useful protocols and the appropriate references. The description of C banding shows how such a slight modification of standard cytogenetic techniques can provide previously inaccessible information relative to deletions, translocations, and other chromosome structural changes and can be used in place of more extensive—and expensive—molecular technologies. It is an easy jump from C banding to the use of genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH). Certainly, these techniques show that "a picture is worth a thousand words". From distinguishing ancestral genomes to placing single-copy sequences relative to each other has yielded important insights in many cases, such as detecting gaps between BACs. Fiber FISH further advanced resolution by identifying each individual chromosome and localizing sequences on individual chromatin fibers. This technique is now being enhanced through tyramide signal amplification (TSA-FISH) of low copy sequences which increases the sensitivity of FISH perhaps by 1000-fold.

Recombination is the basis of genetic compositions selected by the breeder. Further understanding of the frequency and distribution of recombination points could become the basis of major advances in breeding. Genes exist that control homologous and homoeologous recombination. The use of such genes affecting recombination can lead to unique combinations of chromosome segments: ideas for achieving such goals are described in this book.

Analysis of genomes is sometimes made simpler by using radiation to cause chromosome breakages. The creation of Radiation Hybrids has provided not only information on physical linkages via high-resolution physical maps but also many new cytogenetic stocks for use in genetic experimentation. Further resolution can be achieved through cutting chromosome fibers by restriction enzymes and the placement of sequences by optical mapping (also described in this book).

The flow sorting of chromosomes simplifies genomic analysis by separating out specific chromosomes or chromosome segments. This approach was important for the sequencing of the wheat genome in that BAC libraries were made from individual flow-sorted chromosomes or segments almost free of organellar DNA. A complete set of chromosome arm-specific BAC libraries were constructed for wheat. Chromosome microdissection is another method described in this book for reducing the complexity of subsequent genome analysis. Isolated interphase nuclei also avoid some of the complications of tissue isolation that may provide an unwanted mixture of cell types. This approach can be followed by subsequent

immunolocalization of proteins to show spatial and temporal relationships, such as the described RAD51 involved in the repair of double-stranded breaks. Epigenomic modifications of the genome are now well-established events and also can be increasingly understood by chromosome visualization techniques involving chromatin immunoprecipitation techniques.

The realm of cytogenetics—as described in this book—continues to expand and provides clear insights for genomic analyses.

St. Paul, MN, USA ***Ronald L. Phillips***

Preface

Cytogenetic studies have contributed greatly to our understanding of genetics, biology, reproduction, and evolution. From early studies in basic chromosome behavior, the field has expanded enabling whole genome analysis to the manipulation of chromosomes and their organization. This book covers a range of methods used in cytogenetics, beginning with basic analysis of chromosomes and visualizing gene locations (Chapters 1–6), to manipulating and dissecting chromosomes (Chapters 7–12), and then focusing on less-understood features of chromosomes such as recombination initiation sites and epigenomic marks (Chapters 13–15). The methods described are detailed and built on each other, assisting those new to the field a comprehensive platform to support their research endeavor, while introducing advanced techniques to experienced researchers. We hope this book starts you on an adventure into the field of cytogenetics, while you discover the wonderment of the complexity of nature and beauty of the biologically important chromosomes through your microscope eyepiece.

St. Paul, MN, USA

Shahryar F. Kianian
Penny M.A. Kianian

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Contributors

Jan Bartoš • *Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Olomouc, Czech Republic*

Xiwen Cai • *Department of Plant Sciences, North Dakota State University, Fargo, ND, USA*

Petr Cápal • *Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Olomouc, Czech Republic*

Changbin Chen • *Department of Horticultural Science, University of Minnesota, St. Paul, MN, USA*

Jarmila Číhalíková • *Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Olomouc, Czech Republic*

Karin R. Deal • *Department of Plant Sciences, University of California, Davis, CA, USA*

Daryna Dechyeva • *Institute of Botany, Technische Universität Dresden, Dresden, Germany*

Chuan-Liang Deng • *Henan Normal University, Xinxiang, China*

Jaroslav Doležel • *Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Olomouc, Czech Republic*

Stefanie Dukowic-Schulze • *Department of Horticultural Science, University of Minnesota, St. Paul, MN, USA*

Esther Ferrer • *Department of Biomedicine and Biotechnology, University of Alcalá, Alcalá de Henares, Madrid, Spain*

Araceli Fominaya • *Department of Biomedicine and Biotechnology, University of Alcalá, Alcalá de Henares, Madrid, Spain*

Juan M. González • *Department of Biomedicine and Biotechnology, University of Alcalá, Alcalá de Henares, Madrid, Spain*

Anthony Harris • *Department of Horticultural Science, University of Minnesota, St. Paul, MN, USA*

Alex R. Hastie • *BioNano Genomics, San Diego, CA, USA*

Yan He • *National Maize Improvement Center of China, Beijing Key Laboratory of Crop Genetic Improvement, China Agricultural University, Beijing, China*

Justin B. Hegstad • *Department of Plant Sciences, North Dakota State University, Fargo, ND, USA*

Zan-Min Hu • *Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China*

Chao-Chien Jan • *USDA-ARS, Sunflower and Plant Biology Research Unit, Northern Crop Science Laboratory, Fargo, ND, USA*

Eric N. Jellen • *Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT, USA*

Miroslava Karafiátová • *Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Olomouc, Czech Republic*

Penny M.A. Kianian • *Department of Horticultural Science, University of Minnesota, St. Paul, MN, USA*

Shahryar F. Kianian • *USDA-ARS, Cereal Disease Laboratory, Department of Plant Pathology, University of Minnesota, St. Paul, MN, USA*

Marie Kubaláková • *Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Olomouc, Czech Republic*
Katie L. Liberatore • *USDA-ARS, Cereal Disease Laboratory, Department of Plant Pathology, University of Minnesota, St. Paul, MN, USA*
Zhao Liu • *Department of Plant Sciences, North Dakota State University, Fargo, ND, USA*
Yolanda Loarce • *Department of Biomedicine and Biotechnology, University of Alcalá, Alcalá de Henares, Madrid, Spain*
Adam J. Lukaszewski • *Department of Botany and Plant Sciences, University of California, Riverside, CA, USA*
Ming-Cheng Luo • *Department of Plant Sciences, University of California, Davis, CA, USA*
Marisa E. Miller • *Department of Horticultural Science, University of Minnesota, St. Paul, MN, USA*
Armond Murray • *Department of Plant Sciences, University of California, Davis, CA, USA*
Zhixia Niu • *USDA-ARS, Cereal Crops Research Unit, Northern Crop Science Laboratory, Fargo, ND, USA*
Wojciech P. Pawlowski • *Department of Plant Breeding and Genetics, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA*
Ali Pendle • *Department of Cell and Developmental Biology, John Innes Centre, Norwich, UK*
Gernot Presting • *Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, HI, USA*
Henry Sadowski • *BioNano Genomics, San Diego, CA, USA*
Jan Šafář • *Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Olomouc, Czech Republic*
Michael Saghbini • *BioNano Genomics, San Diego, CA, USA*
Thomas Schmidt • *Institute of Botany, Technische Universität Dresden, Dresden, Germany*
Peter Shaw • *Department of Cell and Developmental Biology, John Innes Centre, Norwich, UK*
Hana Šimková • *Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Olomouc, Czech Republic*
Will Stedman • *BioNano Genomics, San Diego, CA, USA*
Qi Sun • *Institute of Biotechnology, Biotechnology Resource Center, Cornell University, Ithaca, NY, USA*
Jan Vrána • *Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Olomouc, Czech Republic*
Minghui Wang • *Institute of Biotechnology, Biotechnology Resource Center and Section of Plant Biology in School of Integrative Plant Science, Cornell University, Ithaca, NY, USA*
Zidian Xie • *Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, HI, USA*
Steven S. Xu • *USDA-ARS, Cereal Crops Research Unit, Northern Crop Science Laboratory, Fargo, ND, USA*
Ying-Xin Zhang • *Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China; Graduate University of Chinese Academy of Sciences, Beijing, P. R. China*
Qijun Zhang • *Department of Plant Sciences, North Dakota State University, Fargo, ND, USA*
Tingting Zhu • *Department of Plant Sciences, University of California, Davis, CA, USA*