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Homologous Recombination

Methods and Protocols

Edited by

Andrés Aguilera

Centro Andaluz de Biología Molecular y Medicina Regenerativa, CABIMER, Universidad de Sevilla, Sevilla, Spain

Aura Carreira

Institut Curie, PSL Research University, CNRS, UMR3348, F-91405, Orsay, France; University Paris Sud, Paris-Saclay University, CNRS, UMR3348, F-91405, Orsay, France

Humana Press

Editors
Andrés Aguilera
Centro Andaluz de Biología Molecular
y Medicina Regenerativa, CABIMER
Universidad de Sevilla
Sevilla, Spain

Aura Carreira
Institut Curie, PSL Research University, CNRS
UMR3348, F-91405
Orsay, France

University Paris Sud, Paris-Saclay University, CNRS
UMR3348, F-91405
Orsay, France

ISSN 1064-3745 ISSN 1940-6029 (electronic)
Methods in Molecular Biology
ISBN 978-1-0716-0643-8 ISBN 978-1-0716-0644-5 (eBook)
https://doi.org/10.1007/978-1-0716-0644-5

Cover Caption: This image was prepared by Aura Carreira and Teresa Moreno Rodriguez.

This Humana imprint is published by the registered company Springer Science+Business Media, LLC, part of Springer Nature.
The registered company address is: 1 New York Plaza, New York, NY 10004, U.S.A.

Preface

DNA double-strand breaks (DSBs) are the most harmful lesions to DNA in the cell. To cope with these insults, all organisms have devised two main types of evolutionary conserved mechanisms for their repair, homologous recombination (HR), and non-homologous end joining (NHEJ). The first one operates predominantly during the S/G2 phase of the cell cycle, when the sister chromatid is available for repair. Because HR requires homology for repair, this pathway is considered essentially error-free. NHEJ is the pathway of choice in the other phases of the cell cycle, including G1. This pathway is generally faithful but can be prone to errors.

Most spontaneous DNA breaks arising in somatic cells occur randomly as a consequence of DNA replication failure caused by either DNA lesions or generated by obstacles that impede the progression of the replication fork (e.g., protein-bound to DNA, DNA secondary structures, replication–transcription conflicts, etc.). For this reason, HR is a major DNA repair pathway during S/G2 phases of the cell cycle. Thus, HR is intimately ligated to the prevention of genome instability in replicating somatic cells. In meiotic cells however, DSBs are developmentally controlled by the action of specific endonucleases where HR is essential; gametogenesis is not possible in the absence of HR.

Genome instability and in particular defective HR is a common feature of a number of genetic diseases including cancer. Defects in HR in meiotic cells can lead to birth defects such as Down syndrome. Considering the relevance of HR as one of the major DSB repair pathways in mitotically cycling cells, as well as its essential role in meiosis, understanding the molecular mechanisms and factors that participate in HR is of key importance in Molecular Biology and Biomedicine. In this book, we compile a series of laboratory protocols covering the analysis of different steps of the homologous recombination process from the genetic, molecular biology, and cell biology perspectives. As these steps are very well conserved through evolution, taking advantage of different model organisms have led to accelerated discoveries in this field. Thus, when appropriate, some of the protocols we present here are explained in the context of more than one model system. We hope this book will facilitate the use of both classical and more recent approaches to answer specific questions on HR mechanisms as well as to decipher the function of novel factors involved in HR. We expect that this compilation of protocols elaborated by leading experts in the field will be useful not only to the scientific community working in genome integrity but also to scientists working in other areas such as cancer biology or cell cycle with renovated interests in HR and DSB repair.

Sevilla, Spain — *Andrés Aguilera*
Orsay, France — *Aura Carreira*

Contents

Preface . . . . . *v*
Contributors . . . . . *xi*

1 Detection of DNA Double-Strand Breaks by γ-H2AX Immunodetection 1
Sonia I. Barroso and Andrés Aguilera

2 END-seq: An Unbiased, High-Resolution, and Genome-Wide Approach to Map DNA Double-Strand Breaks and Resection in Human Cells 9
Nancy Wong, Sam John, André Nussenzweig, and Andres Canela

3 Resection of a DNA Double-Strand Break by Alkaline Gel Electrophoresis and Southern Blotting 33
Erika Casari, Elisa Gobbini, Michela Clerici, and Maria Pia Longhese

4 Analysis of DNA Double-Strand Break End Resection and Single-Strand Annealing in *S. pombe* 47
Zhenxin Yan, Sandeep Kumar, and Grzegorz Ira

5 Quantifying DNA End Resection in Human Cells 59
Yi Zhou and Tanya T. Paull

6 Genetic and Molecular Approaches to Study Chromosomal Breakage at Secondary Structure–Forming Repeats 71
Anissia Ait Saada, Alex B. Costa, and Kirill S. Lobachev

7 Biochemical Analysis of D-Loop Extension and DNA Strand Displacement Synthesis 87
Youngho Kwon and Patrick Sung

8 DNA Strand Exchange to Monitor Human RAD51-Mediated Strand Invasion and Pairing 101
Sudipta Lahiri and Ryan B. Jensen

9 Monitoring Homologous Recombination Activity in Human Cells 115
Domagoj Vugic, Åsa Ehlén, and Aura Carreira

10 Interhomolog Homologous Recombination in Mouse Embryonic Stem Cells 127
Fabio Vanoli, Rohit Prakash, Travis White, and Maria Jasin

11 Branch Migration Activity of Rad54 Protein 145
Olga M. Mazina and Alexander V. Mazin

12 Holliday Junction Resolution 169
Raquel Carreira, F. Javier Aguado, Tomas Lama-Diaz, and Miguel G. Blanco

13 Identification and Analysis of Different Types of UFBs 187
Simon Gemble and Mounira Amor-Guéret

14 Intrachromosomal Recombination in Yeast 193
Anastasiya Epshtein, Lorraine S. Symington, and Hannah L. Klein

15 Genome-Wide Analysis of Mitotic Recombination in Budding Yeast 201
Lydia R. Heasley, Nadia M. V. Sampaio, and Juan Lucas Argueso

16 Monitoring Gene Conversion in Budding Yeast by Southern Blot Analysis 221
Miyuki Yamaguchi and James E. Haber

17 DNA Double-Strand Break-Induced Gene Amplification in Yeast 239
Tomas Strucko, Michael Lisby, and Uffe Hasbro Mortensen

18 Measuring Chromosome Pairing During Homologous Recombination in Yeast 253
Fraulin Joseph, So Jung Lee, Eric Edward Bryant, and Rodney Rothstein

19 Cytological Monitoring of Meiotic Crossovers in Spermatocytes and Oocytes 267
Yan Yun, Masaru Ito, Sumit Sandhu, and Neil Hunter

20 Detection of DSBs in *C. elegans* Meiosis 287
Tatiana García-Muse

21 Methods to Map Meiotic Recombination Proteins in *Saccharomyces cerevisiae* 295
Aurore Sanchez and Valérie Borde

22 Investigation of Break-Induced Replication in Yeast 307
Beth Osia, Rajula Elango, Juraj Kramara, Steven A. Roberts, and Anna Malkova

23 Measurement of Homologous Recombination at Stalled Mammalian Replication Forks 329
Nicholas A. Willis and Ralph Scully

24 Super-Resolution Imaging of Homologous Recombination Repair at Collapsed Replication Forks 355
Donna R. Whelan and Eli Rothenberg

25 The Analysis of Recombination-Dependent Processing of Blocked Replication Forks by Bidimensional Gel Electrophoresis 365
Karol Kramarz, Anissia Ait Saada, and Sarah A. E. Lambert

26 The Sister-Chromatid Exchange Assay in Human Cells 383
Emanuela Tumini and Andrés Aguilera

27 Analysis of Recombination at Yeast Telomeres 395
Marie-Noelle Simon, Dmitri Churikov, and Vincent Géli

28 Gel Electrophoresis Analysis of rDNA Instability in *Saccharomyces cerevisiae* 403
Mariko Sasaki and Takehiko Kobayashi

29 Analyzing Homologous Recombination at a Genome-Wide Level 427
Coline Arnould, Vincent Rocher, and Gaëlle Legube

30 CRISPR/Cas9-Induced Breaks in Heterochromatin, Visualized by Immunofluorescence 439
Ioanna Mitrentsi and Evi Soutoglou

31 In Vivo Binding of Recombination Proteins to Non-DSB DNA Lesions and to Replication Forks 447
Román González-Prieto, María J. Cabello-Lobato, and Félix Prado

32 Live Cell Imaging of Nuclear Actin Filaments and Heterochromatic Repair foci in *Drosophila* and Mouse Cells 459
Colby See, Deepak Arya, Emily Lin, and Irene Chiolo

33 In Vitro Characterization of Sumoylation of HR Proteins 483
Veronika Altmannova and Lumir Krejci

34 High-Throughput Analysis of Heteroduplex DNA in Mitotic Recombination Products 503
Dionna Gamble, Yee Fang Hum, and Sue Jinks-Robertson

35 Fluorescence Microscopy for Analysis of Relocalization of Structure-Specific Endonucleases 521
Carl P. Lehmann, Irene Saugar, and José Antonio Tercero

36 Physical and Genetic Assays for the Study of DNA Joint Molecules Metabolism and Multi-invasion-Induced Rearrangements in *S. cerevisiae* 535
Aurèle Piazza, Pallavi Rajput, and Wolf-Dietrich Heyer

Index 555

Contributors

F. Javier Aguado • *Departamento de Bioquímica e Bioloxía Molecular, CIMUS, Universidade de Santiago de Compostela-Instituto de Investigación Sanitaria (IDIS), Santiago de Compostela, Spain*

Andrés Aguilera • *Centro Andaluz de Biología Molecular y Medicina Regenerativa, CABIMER, University of Seville-CSIC-UPO, Seville, Spain*

Anissia Ait Saada • *School of Biological Sciences, Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, USA*

Veronika Altmannova • *Department of Biology, Masaryk University, Brno, Czech Republic; International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic*

Mounira Amor-Guéret • *Institut Curie, PSL Research University, CNRS UMR 3348, Orsay, France; CNRS UMR 3348, Paris Saclay University, Institut Curie, Research Center, Orsay, France*

Juan Lucas Argueso • *Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO, USA; Cell and Molecular Biology Graduate Program, Colorado State University, Fort Collins, CO, USA*

Coline Arnould • *LBCMCP, Centre de Biologie Integrative (CBI), CNRS, Université de Toulouse, UT3, Toulouse, France*

Deepak Arya • *Molecular and Computational Biology Department, University of Southern California, Los Angeles, CA, USA*

Sonia I. Barroso • *Centro Andaluz de Biología Molecular y Medicina Regenerativa, CABIMER, University of Seville-CSIC-UPO, Seville, Spain*

Miguel G. Blanco • *Departamento de Bioquímica e Bioloxía Molecular, CIMUS, Universidade de Santiago de Compostela-Instituto de Investigación Sanitaria (IDIS), Santiago de Compostela, Spain*

Valérie Borde • *Institut Curie—Research Center, UMR3244 CNRS, Pavillon Trouillet Rossignol, PSL Research University, Paris Cedex 05, France; Paris Sorbonne Université, Paris, France*

Eric Edward Bryant • *Department of Biological Sciences, Columbia University, New York, NY, USA*

María J. Cabello-Lobato • *Division of Cancer Sciences, Manchester Cancer Research Center, University of Manchester, Manchester, UK*

Andres Canela • *Laboratory of Genome Integrity, National Cancer Institute, NIH, Bethesda, MD, USA; The Hakubi Center for Advanced Research and Radiation Biology Center, Graduate School of Biostudies, Kyoto University, Kyoto, Japan*

Aura Carreira • *Institut Curie, PSL Research University, CNRS, UMR3348, F-91405, Orsay, France; University Paris Sud, Paris-Saclay University, CNRS, UMR3348, F-91405, Orsay, France*

Raquel Carreira • *Departamento de Bioquímica e Bioloxía Molecular, CIMUS, Universidade de Santiago de Compostela-Instituto de Investigación Sanitaria (IDIS), Santiago de Compostela, Spain*

Erika Casari • *Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano-Bicocca, Milano, Italia*

Irene Chiolo • *Molecular and Computational Biology Department, University of Southern California, Los Angeles, CA, USA*
Dmitri Churikov • *Marseille Cancer Research Center (CRCM), U1068 Inserm, UMR7258 CNRS, Institut Paoli-Calmettes, Aix Marseille University, Marseille, France*
Michela Clerici • *Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano-Bicocca, Milano, Italia*
Alex B. Costa • *School of Biological Sciences, Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, USA*
Åsa Ehlén • *Institut Curie, PSL Research University, CNRS, UMR3348, F-91405, Orsay, France; University Paris Sud, Paris-Saclay University, CNRS, UMR3348, F-91405, Orsay, France*
Rajula Elango • *Department of Medicine, Division of Hematology-Oncology, Cancer Research Institute, Harvard Medical School, Boston, MA, USA; Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA*
Anastasiya Epshtein • *Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, NY, USA*
Dionna Gamble • *Department of Molecular Genetics and Microbiology, Duke University, Durham, NC, USA; University Program in Genetics and Genomics, Duke University, Durham, NC, USA*
Tatiana García-Muse • *Centro Andaluz de Biología Molecular y Medicina Regenerativa-CABIMER, Universidad de Sevilla-CSIC-UPO, Seville, Spain*
Vincent Géli • *Marseille Cancer Research Center (CRCM), U1068 Inserm, UMR7258 CNRS, Institut Paoli-Calmettes, Aix Marseille University, Marseille, France*
Simon Gemble • *Institut Curie, PSL Research University, CNRS UMR144, Paris, France*
Elisa Gobbini • *Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano-Bicocca, Milano, Italia*
Román González-Prieto • *Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, The Netherlands*
James E. Haber • *Department of Biology, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA, USA*
Lydia R. Heasley • *Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO, USA*
Wolf-Dietrich Heyer • *Department of Microbiology and Molecular Genetics, University of California, Davis, CA, USA; Department of Molecular and Cellular Biology, University of California, Davis, CA, USA*
Yee Fang Hum • *Department of Cancer Biology, Abramson Family Cancer Research Institute Basser Center for BRCA, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Neil Hunter • *Howard Hughes Medical Institute, Department of Microbiology and Molecular Genetics, Davis, University of California, Davis, CA, USA*
Grzegorz Ira • *Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA*
Masaru Ito • *Howard Hughes Medical Institute, Department of Microbiology and Molecular Genetics, Davis, University of California, Davis, CA, USA*
Maria Jasin • *Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Ryan B. Jensen • *Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT, USA*

Sue Jinks-Robertson • *Department of Cancer Biology, Abramson Family Cancer Research Institute Basser Center for BRCA, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Sam John • *Laboratory of Genome Integrity, National Cancer Institute, NIH, Bethesda, MD, USA*
Fraulin Joseph • *Department of Genetics and Development, Columbia University Irving Medical Center, New York, NY, USA*
Hannah L. Klein • *Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, NY, USA*
Takehiko Kobayashi • *Laboratory of Genome Regeneration, Institute for Quantitative Biosciences, University of Tokyo, Tokyo, Japan*
Juraj Kramara • *Department of Biology, University of Iowa, Iowa City, IA, USA*
Karol Kramarz • *Institut Curie, CNRS, UMR3348, PSL Research University, Orsay, France; Institut Curie, Paris-Saclay University, Unite Mixte de Recherche, Centre National de la Recherche Scientifique, Orsay, France; Genome Integrity, RNA and Cancer Unit (UMR3348), Equipe Labelisée Ligue, Institut Curie—Research Center, Orsay, France*
Lumir Krejci • *Department of Biology, Masaryk University, Brno, Czech Republic; International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic; National Center for Biomolecular Research, Masaryk University, Brno, Czech Republic*
Sandeep Kumar • *Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA*
Youngho Kwon • *Department of Biochemistry and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA*
Sudipta Lahiri • *Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT, USA*
Tomas Lama-Diaz • *Departamento de Bioquímica e Bioloxía Molecular, CIMUS, Universidade de Santiago de Compostela-Instituto de Investigación Sanitaria (IDIS), Santiago de Compostela, Spain*
Sarah A. E. Lambert • *Institut Curie, CNRS, UMR3348, PSL Research University, Orsay, France; Institut Curie, Paris-Saclay University, Unite Mixte de Recherche, Centre National de la Recherche Scientifique, Orsay, France; Genome Integrity, RNA and Cancer Unit (UMR3348), Equipe Labelisée Ligue, Institut Curie—Research Center, Orsay, France*
So Jung Lee • *Department of Genetics and Development, Columbia University Irving Medical Center, New York, NY, USA*
Gaëlle Legube • *LBCMCP, Centre de Biologie Integrative (CBI), CNRS, Université de Toulouse, UT3, Toulouse, France*
Carl P. Lehmann • *Centro de Biología Molecular Severo Ochoa (CSIC/UAM), Madrid, Spain*
Emily Lin • *Molecular and Computational Biology Department, University of Southern California, Los Angeles, CA, USA*
Michael Lisby • *Department of Biology, University of Copenhagen, Copenhagen, Denmark*
Kirill S. Lobachev • *School of Biological Sciences, Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, USA*
Maria Pia Longhese • *Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano-Bicocca, Milano, Italia*

Anna Malkova • *Department of Biology, University of Iowa, Iowa City, IA, USA*
Alexander V. Mazin • *Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA, USA*
Olga M. Mazina • *Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA, USA*
Ioanna Mitrentsi • *Institut de Génétique et de Biologie Moléculaire et Celullaire, INSERM U964, CNRS, UMR7104, Illkirch, France; Université de Strasbourg, Strasbourg, France*
Uffe Hasbro Mortensen • *Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, Denmark*
André Nussenzweig • *Laboratory of Genome Integrity, National Cancer Institute, NIH, Bethesda, MD, USA*
Beth Osia • *Department of Biology, University of Iowa, Iowa City, IA, USA*
Tanya T. Paull • *The Department of Molecular Biosciences, The University of Texas at Austin, Austin, TX, USA*
Aurèle Piazza • *Spatial Regulation of Genomes, Institut Pasteur, UMR3525 CNRS, Paris, France; Department of Microbiology and Molecular Genetics, University of California, Davis, CA, USA; Univ Lyon, ENS, UCBL, CNRS, INSERM, Laboratory of Biology and Modelling of the Cell, UMR5239, Lyon, France*
Félix Prado • *Department of Genome Biology, Andalusian Molecular Biology and Regenerative Medicine Center (CABIMER), CSIC-University of Seville-UPO, Seville, Spain*
Rohit Prakash • *Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Pallavi Rajput • *Department of Microbiology and Molecular Genetics, University of California, Davis, CA, USA*
Steven A. Roberts • *School of Molecular Biosciences, Center for Reproductive Biology, Washington State University, Pullman, WA, USA*
Vincent Rocher • *LBCMCP, Centre de Biologie Integrative (CBI), CNRS, Université de Toulouse, UT3, Toulouse, France*
Eli Rothenberg • *Department of Biochemistry and Molecular Pharmacology, Perlmutter Cancer Center, New York University School of Medicine, New York, NY, USA*
Rodney Rothstein • *Department of Genetics and Development, Columbia University Irving Medical Center, New York, NY, USA*
Nadia M. V. Sampaio • *Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO, USA; Cell and Molecular Biology Graduate Program, Colorado State University, Fort Collins, CO, USA*
Aurore Sanchez • *Institut Curie—Research Center, UMR3244 CNRS, Pavillon Trouillet Rossignol, PSL Research University, Paris Cedex 05, France; Paris Sorbonne Université, Paris, France*
Sumit Sandhu • *Howard Hughes Medical Institute, Department of Microbiology and Molecular Genetics, Davis, University of California, Davis, CA, USA*
Mariko Sasaki • *Laboratory of Genome Regeneration, Institute for Quantitative Biosciences, University of Tokyo, Tokyo, Japan*
Irene Saugar • *Centro de Biología Molecular Severo Ochoa (CSIC/UAM), Madrid, Spain*
Ralph Scully • *Department of Medicine, Division of Hematology-Oncology, Cancer Research Institute, Harvard Medical School, Boston, MA, USA; Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA*

Colby See • *Molecular and Computational Biology Department, University of Southern California, Los Angeles, CA, USA*
Marie-Noelle Simon • *Marseille Cancer Research Center (CRCM), U1068 Inserm, UMR7258 CNRS, Institut Paoli-Calmettes, Aix Marseille University, Marseille, France*
Evi Soutoglou • *Institut de Génétique et de Biologie Moléculaire et Celullaire, INSERM U964, CNRS, UMR7104, Illkirch, France; Université de Strasbourg, Strasbourg, France; Genome Damage and Stability Center, School of Life Sciences, University of Sussex, Brighton, UK*
Tomas Strucko • *Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, Denmark*
Patrick Sung • *Department of Biochemistry and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA*
Lorraine S. Symington • *Department of Microbiology and Immunology, Columbia University Irving Medical Center, New York, NY, USA*
José Antonio Tercero • *Centro de Biología Molecular Severo Ochoa (CSIC/UAM), Madrid, Spain*
Emanuela Tumini • *Centro Andaluz de Biología Molecular y Medicina Regenerativa, CABIMER, Universidad de Sevilla-CSIC-UPO, Seville, Spain*
Fabio Vanoli • *Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Domagoj Vugic • *Institut Curie, PSL Research University, CNRS, UMR3348, F-91405, Orsay, France; University Paris Sud, Paris-Saclay University, CNRS, UMR3348, F-91405, Orsay, France*
Donna R. Whelan • *Department of Pharmacy and Biomedical Sciences, La Trobe Institute for Molecular Science, La Trobe University, Bundoora, VIC, Australia; Department of Biochemistry and Molecular Pharmacology, Perlmutter Cancer Center, New York University School of Medicine, New York, NY, USA*
Travis White • *Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Nicholas A. Willis • *Department of Medicine, Division of Hematology-Oncology, Cancer Research Institute, Harvard Medical School, Boston, MA, USA; Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA*
Nancy Wong • *Laboratory of Genome Integrity, National Cancer Institute, NIH, Bethesda, MD, USA*
Miyuki Yamaguchi • *Department of Biology, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA, USA*
Zhenxin Yan • *Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA*
Yan Yun • *Howard Hughes Medical Institute, Department of Microbiology and Molecular Genetics, Davis, University of California, Davis, CA, USA*
Yi Zhou • *The Department of Molecular Biosciences, The University of Texas at Austin, Austin, TX, USA*