

# METHODS IN MOLECULAR BIOLOGY

*Series Editor*

**John M. Walker**

**School of Life and Medical Sciences**

**University of Hertfordshire**

**Hatfield, Hertfordshire, AL10 9AB, UK**

For further volumes:

<http://www.springer.com/series/7651>

# Mammalian Synthetic Promoters

Edited by

**David Gould**

*Department of Biochemical Pharmacology, William Harvey Research Institute,  
Queen Mary University of London, London, UK*

 Humana Press

*Editor*

David Gould  
Department of Biochemical Pharmacology  
William Harvey Research Institute  
Queen Mary University of London  
London, UK

ISSN 1064-3745                      ISSN 1940-6029 (electronic)  
Methods in Molecular Biology  
ISBN 978-1-4939-7221-0              ISBN 978-1-4939-7223-4 (eBook)  
DOI 10.1007/978-1-4939-7223-4

Library of Congress Control Number: 2017946166

© Springer Science+Business Media LLC 2017

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Printed on acid-free paper

This Humana Press imprint is published by Springer Nature  
The registered company is Springer Science+Business Media LLC  
The registered company address is: 233 Spring Street, New York, NY 10013, U.S.A.

---

## **Preface**

It is nearly 40 years since the TATA box was first identified. Since this time there has been a revolution in genetics research and an increase in the ways that promoters are utilized. Current applications of promoters include their use in biotechnology for expression of recombinant proteins; as switches or logic gates in synthetic biology; for spatiotemporal expression in gene targeting; disease, tissue-specific, and constitutive expression in gene therapy; and monitoring transcription factor activity and numerous applications for expression of genes of interest in basic biological research. In this volume a number of methods are outlined that will aid in the selection of promoter sequences and vector components, methods for the assembly and testing of synthetic promoters, and examples of their application. Depending upon the application there can be different requirements from the strength and promoter length to specificity and regulation. My own interest in the use of promoters stems from the potential to achieve long-term pharmacologically regulated expression of therapeutic molecules in gene therapy approaches. Indeed, this interest has led to some bias of the volume toward regulated promoters but most of the information should be pertinent to all areas of synthetic promoter research. Hopefully this volume will aid researchers that are new to the field of synthetic promoters and inspire new developments.

*London, UK*

*David Gould*

---

# Contents

<i>Preface</i> .....	<i>v</i>
<i>Contributors</i> .....	<i>ix</i>
1 Initial Considerations Before Designing a Promoter Construct .....	1
<i>David Gould</i>	
PART I MONITORING PROMOTER INTERACTIONS AND ACTIVITY	
2 Demonstrating Interactions of Transcription Factors with DNA by Electrophoretic Mobility Shift Assay .....	11
<i>Nasim Yousaf and David Gould</i>	
3 Chromatin Immunoprecipitation and Quantitative Real-Time PCR to Assess Binding of a Protein of Interest to Identified Predicted Binding Sites Within a Promoter .....	23
<i>Jordan E. Read</i>	
4 Secreted Reporters for Monitoring Multiple Promoter Function .....	33
<i>Ghazal Lashgari, Rami S. Kantar, and Bakhos A. Tannous</i>	
5 Bioluminescence Monitoring of Promoter Activity In Vitro and In Vivo .....	49
<i>Juliette M.K.M. Delhove, Rajvinder Karda, Kate E. Hawkins, Lorna M. FitzPatrick, Simon N. Waddington, and Tristan R. McKay</i>	
6 Monitoring Promoter Activity by Flow Cytometry .....	65
<i>Taber E.I. Taber</i>	
PART II PROMOTER DESIGN AND APPLICATIONS	
7 Functional Screening of Core Promoter Activity .....	77
<i>Dan Y. Even, Adi Kedmi, Diana Ideses, and Tamar Juven-Gershon</i>	
8 Bioinformatically Informed Design of Synthetic Mammalian Promoters .....	93
<i>Michael L. Roberts, Polyxeni Katsoupi, Vivian Tseveleki, and Era Taoufik</i>	
9 Synthetic Tumor-Specific Promoters for Transcriptional Regulation of Viral Replication .....	113
<i>Maria Veronica Lopez, Eduardo G. Cafferata, Diego L. Viale, and Osvaldo L. Podhajcer</i>	
10 Constructing Strong Cell Type-Specific Promoters Through Informed Design .....	131
<i>Adam J. Brown and David C. James</i>	
11 PCR Assembly of Synthetic Promoters .....	147
<i>Hodan Mohamed and David Gould</i>	

PART III REGULATED PROMOTER APPROACHES

12 The Tetracycline Responsive System..... 159  
*Nasim Yousaf and David Gould*

13 Light-Responsive Promoters ..... 173  
*Maximilian Hörner, Konrad Müller, and Wilfried Weber*

14 A Simple Method for Constructing Artificial Promoters  
Activated in Response to Ultrasound Stimulation ..... 187  
*Ryohei Ogawa, Go Kagiya, Akihiko Watanabe, Akihiro Morii,  
Zheng-Guo Cui, and Takashi Kondo*

15 Promoter Activation with Electromagnetism ..... 205  
*Abraham O. Rodríguez-De la Fuente, J. Antonio Heredia-Rojas,  
Juan M. Alcocer-González, Laura E. Rodríguez-Flores,  
Cristina Rodríguez-Padilla, and Reyes S. Taméz-Guerra*

16 Application of Synthetic Tumor-Specific Promoters Responsive  
to the Tumor Microenvironment ..... 213  
*Eduardo G. Cafferata, Maria Veronica Lopez, Felipe J. Nuñez,  
Maria A.R. Maenza, and Osvaldo L. Podhajcer*

PART IV COMBINING PROMOTERS IN SYNTHETIC CIRCUITS

17 A Modular Approach to Building Complex Synthetic Circuits..... 231  
*Yingqing Li and Ron Weiss*

18 Computational Sequence Design with R2oDNA Designer ..... 249  
*James T. MacDonald and Velia Siciliano*

19 Design of Synthetic Promoters for Gene Circuits in Mammalian Cells..... 263  
*Pratik Saxena, Daniel Bojar, and Martin Fussenegger*

*Index* ..... 275

---

## Contributors

- JUAN M. ALCOCER-GONZÁLEZ • *Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, UANL, Av. Universidad S/N Ciudad Universitaria, San Nicolás de los Garza, Nuevo León CP, México*
- DANIEL BOJAR • *Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland*
- ADAM J. BROWN • *Department of Chemical and Biological Engineering, University of Sheffield, Sheffield, UK*
- EDUARDO G. CAFFERATA • *Laboratory of Molecular and Cellular Therapy, Leloir Institute—Conicet, Buenos Aires, Argentina*
- ZHENG-GUO CUI • *Public Health, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan*
- JULIETTE M.K.M. DELHOVE • *Cardiovascular and Cell Sciences Research Institute, St. George's University of London, London, UK; Wits/SAMRC Antiviral Gene Therapy Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa*
- DAN Y. EVEN • *The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel*
- LORNA M. FITZPATRICK • *Cardiovascular and Cell Sciences Research Institute, St. George's University of London, London, UK; School of Healthcare Science, Manchester Metropolitan University, Manchester, UK*
- MARTIN FUSSENEGGER • *Faculty of Science, University of Basel, Basel, Switzerland; Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland*
- DAVID GOULD • *Department of Biochemical Pharmacology, William Harvey Research Institute, Queen Mary University of London, London, UK*
- KATE E. HAWKINS • *Cardiovascular and Cell Sciences Research Institute, St. George's University of London, London, UK*
- J. ANTONIO HEREDIA-ROJAS • *Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, UANL, Av. Universidad S/N Ciudad Universitaria, San Nicolás de los Garza, Nuevo León CP, México*
- MAXIMILIAN HÖRNER • *Faculty of Biology, University of Freiburg, Freiburg, Germany; Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, Freiburg, Germany; Centre for Biological Signalling Studies (BIOSS), University of Freiburg, Freiburg, Germany*
- DIANA IDESES • *The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel*
- DAVID C. JAMES • *Department of Chemical and Biological Engineering, University of Sheffield, Sheffield, UK*
- TAMAR JUVEN-GERSHON • *The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel*
- GO KAGIYA • *School of Allied Health Sciences, Kitasato University, Sagamihara, Japan*
- RAMI S. KANTAR • *Experimental Therapeutics and Molecular Imaging Laboratory, Department of Neurology, Neuroscience Center, Massachusetts General Hospital, Boston, MA, USA; Program in Neuroscience, Harvard Medical School, Boston, MA, USA*

- RAJVINDER KARDA • *Gene Transfer Technology Group, Institute for Women's Health, University College London, London, UK*
- POLYXENI KATSOUPH • *Synpromics Ltd, Edinburgh, UK*
- ADI KEDMI • *The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel*
- TAKASHI KONDO • *Department of Radiological Sciences, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan*
- GHAZAL LASHGARI • *Experimental Therapeutics and Molecular Imaging Laboratory, Department of Neurology, Neuroscience Center, Massachusetts General Hospital, Boston, MA, USA; Program in Neuroscience, Harvard Medical School, Boston, MA, USA*
- YINQING LI • *Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, MA, USA*
- MARIA VERONICA LOPEZ • *Laboratory of Molecular and Cellular Therapy, Leloir Institute—Conicet, Buenos Aires, Argentina*
- JAMES T. MACDONALD • *Centre for Synthetic Biology and Innovation, Imperial College, London, UK; Department of Medicine, Imperial College, London, UK*
- MARIA A.R. MAENZA • *Laboratory of Molecular and Cellular Therapy, Leloir Institute - Conicet, Buenos Aires, Argentina*
- TRISTAN R. MCKAY • *Cardiovascular and Cell Sciences Research Institute, St. George's University of London, London, UK; School of Healthcare Science, Manchester Metropolitan University, Manchester, UK*
- HODAN MOHAMED • *Public Health England, London, UK*
- AKIHIRO MORII • *Department of Urology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan; Urology Department, Kurobe Municipal Hospital, Kurobe, Japan*
- KONRAD MÜLLER • *Faculty of Biology, University of Freiburg, Freiburg, Germany; Centre for Biological Signalling Studies (BIOSS), University of Freiburg, Freiburg, Germany; Novartis Pharma AG, Biologics Technical Development and Manufacturing, Basel, Switzerland*
- FELIPE J. NÚÑEZ • *Laboratory of Molecular and Cellular Therapy, Leloir Institute—Conicet, Buenos Aires, Argentina*
- RYOHEI OGAWA • *Department of Radiological Sciences, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan*
- OSVALDO L. PODHAJECER • *Laboratory of Molecular and Cellular Therapy, Leloir Institute—Conicet, Buenos Aires, Argentina*
- JORDAN E. READ • *Department of Biochemical Pharmacology, William Harvey Research Institute, Queen Mary University of London, London, UK*
- MICHAEL L. ROBERTS • *Synpromics Ltd, Edinburgh, UK*
- ABRAHAM O. RODRÍGUEZ-DE LA FUENTE • *Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, UANL, Av. Universidad S/N Ciudad Universitaria, San Nicolás de los Garza, Nuevo León CP, México*
- LAURA E. RODRÍGUEZ-FLORES • *Facultad de Medicina, Universidad Autónoma de Nuevo León, UANL, Av. Francisco I. Madero pte. S/N, Col. Mitras Centro, Monterrey, Nuevo León CP, México*
- CRISTINA RODRÍGUEZ-PADILLA • *Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, UANL, Av. Universidad S/N Ciudad Universitaria, San Nicolás de los Garza, Nuevo León CP, México*



- PRATIK SAXENA • *Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland*
- VELIA SICILIANO • *Centre for Synthetic Biology and Innovation, Imperial College, London, UK; Department of Medicine, Imperial College, London, UK*
- TAHER E.I. TAHER • *Experimental Medicine and Rheumatology, William Harvey Research Institute, Charterhouse Square, London, UK*
- REYES S. TAMÉZ-GUERRA • *Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, UANL, Av. Universidad S/N Ciudad Universitaria, San Nicolás de los Garza, Nuevo León CP, México*
- BAKHOS A. TANNOUS • *Experimental Therapeutics and Molecular Imaging Laboratory, Department of Neurology, Neuroscience Center, Massachusetts General Hospital, Boston, MA, USA; Program in Neuroscience, Harvard Medical School, Boston, MA, USA; Neuroscience Center, Massachusetts General Hospital, Charlestown, MA, USA*
- ERA TAOUFIK • *Hellenic Pasteur Institute, Athens, Greece*
- VIVIAN TSEVELEKI • *Hellenic Pasteur Institute, Athens, Greece*
- DIEGO L. VIALE • *Laboratory of Molecular and Cellular Therapy, Leloir Institute—Conicet, Buenos Aires, Argentina*
- SIMON N. WADDINGTON • *Wits/SAMRC Antiviral Gene Therapy Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; Gene Transfer Technology Group, Institute for Women's Health, University College London, London, UK*
- AKIHIKO WATANABE • *Department of Urology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan*
- WILFRIED WEBER • *Faculty of Biology, University of Freiburg, Freiburg, Germany; Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, Freiburg, Germany; Centre for Biological Signalling Studies (BIOS), University of Freiburg, Freiburg, Germany*
- RON WEISS • *Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, MA, USA; Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA*
- NASIM YOUSAF • *Bone and Joint Research Unit, William Harvey Research Institute, Queen Mary University of London, London, UK*