Methods in Molecular Biology™

Series Editor
John M. Walker
School of Life Sciences
University of Hertfordshire
Hatfield, Hertfordshire, AL10 9AB, UK

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Preface

The huge potential for gene therapy to cure a wide range of diseases has led to high expectations and a great increase in research efforts in this area. The first human gene therapy protocol was conducted in 1990 by W. French Anderson and showed promising results. Over the following years, more than 1,500 gene therapy protocols were approved for clinical trials, illustrating the rapid growth of this field. Furthermore, with the sequencing of the human genome and the development of advanced technologies for the identification of genes and their function, the number of candidate diseases for gene therapy has continued to increase. However, the efficient transfer of a therapeutic gene into human cells depends upon the technology used for gene therapy. A number of delivery systems are in use, which either involve physical delivery of naked DNA or the use of viral vectors. The protocols of the latter system are the subject of this book.

There is a large and rapidly growing body of literature on methods for gene delivery involving the use of viral vectors. This is because genes are delivered more efficiently by viral vectors, compared to DNA transfection. Vectors derived from retroviruses and adenoviruses are used in the majority of gene therapy clinical trials to date. However, vectors derived from adeno-associated viruses, poxviruses, herpes simplex viruses, and baculoviruses are receiving increasingly more attention in the field of gene therapy. The properties of each of these vectors are described in Chapter 1, while Chapter 2 gives answers based on examples of clinical trials to the question of why gene therapy has not yet become an effective treatment for genetic disease.

*Methods in Molecular Biology: Viral Vectors for Gene Therapy* brings together the knowledge and experience of those who are employing methodology of virus production, transferring protocols, and evaluating the efficacy of gene product. This is a comprehensive methods book that provides basic principles for the development of gene therapy viral products that are safe and effective. Chapters presenting protocols in readily reproducible, step-by-step fashion, opening with an introductory overview, a list of the materials and reagents needed to complete the experiment, and followed by a detailed procedure that is supported with a helpful notes section offering tips and tricks of the trade as well as troubleshooting advice. There are chapters on production, purification, and characterization of the most popular viral vector systems of adenovirus, retrovirus, and adeno-associated virus (Chapters 5–11). The methodologies are in most cases simple, tested, and robust processes. The protocols for the less common viral vector systems of baculovirus, herpes virus, and measles virus are presented in Chapters 12–14. The growing interest in these vectors has created a strong demand for large-scale manufacturing and purification procedures.

In view of the interest of many laboratories and practitioners in the preclinical and clinical application of gene therapy vectors, it seems appropriate to include chapters to describe protocols on the in vivo gene delivery into CD34 and mesenchymal cells as non-exhaustive examples for in vivo gene transfer (Chapters 15 and 16). In this context, we have also included Chapter 11 on characterization and quality control testing of in vivo gene delivery of AAV viral vectors for the treatment of muscular and eye diseases to present an example on a subject which is still today very much en vogue for most scientists.
Chapter 3 presents basic considerations concerning the characterization of cell banks for the production of viral vectors. It describes the advantages and disadvantages of the most widely used cell lines, HEK293. The importance of viral purification in manufacturing is now widely recognized, and information is presented here (Chapter 4) on the most commonly used purification methods and chromatographic options available for large-scale processes.

Gene therapy raises many unique ethical concerns. Although germ line gene therapy is controversial, somatic gene therapy is morally acceptable for treating diseases since all effects of therapy end with the life of the patient, at the very latest. Chapter 17 explores some of the ethical issues surrounding human gene therapy. The final chapter (Chapter 18) presents examples of clinical trials and examines the processes of good clinical practice, good manufacturing practice, and regulations for conducting gene therapy trials.

Protocols in gene therapy are not well understood by many scientists who will find this book to be of interest. The material is addressed primarily to those interested in viral gene therapy, but topics will also be of interest to scientists in virology, biomedicine, molecular biology, cell culture, preclinical and clinical trials, cell banking, manufacturing, quality control as well as medical practitioners. It will provide an invaluable resource for students and researchers involved in the development of expression systems, gene delivery systems, and therapeutic products. The editors come from industrial gene therapy (O.-W. Merten) and academic bioprocessing (M. Al-Rubeai) backgrounds and are therefore well placed to ensure that the contents are addressed to and understandable by a wide range of readers. We are enthusiastic for the cause of gene therapy – we hope that our readers find inspiration to explore further its potential themselves and that this work helps their rapid progress.

Finally, we thank all the contributors, the series editor John Walker, and Humana Press for their efforts which made this volume possible.

Otto-Wilhelm Merten
Mohamed Al-Rubeai
Contents

Preface ................................................................. v
Contributors ......................................................... ix

1 Introduction to Viral Vectors ..................................... 1
   James N. Warnock, Claire Daigre, and Mohamed Al-Rubeai

2 Introduction to Gene Therapy: A Clinical Aftermath .......... 27
   Patrice P. Denèfle

3 Host Cells and Cell Banking ....................................... 45
   Glyn N. Stacey and Otto-Wilhelm Merten

4 Overview of Current Scalable Methods for Purification of Viral Vectors 89
   Maria Mercedes Segura, Amine A. Kamen, and Alain Garnier

5 Methods to Construct Recombinant Adenovirus Vectors ......... 117
   Miguel Chillon and Ramon Alemany

6 Manufacturing of Adenovirus Vectors: Production and Purification
   of Helper Dependent Adenovirus ................................ 139
   Edwige Dormond and Amine A. Kamen

7 Manufacturing of Retroviruses .................................... 157
   Pedro E. Cruz, Teresa Rodrigues, Marlene Carmo, Dagmar Wirth,
   Ana I. Amaral, Paula M. Alves, and Ana S. Coroadinha

8 Lentiviral Vectors ................................................. 183
   Marc Giry-Laterrière, Els Verhoeyen, and Patrick Salmon

9 Adeno-Associated Viruses ........................................ 211
   Mauro Mezzina and Otto-Wilhelm Merten

10 Manufacturing of Adeno-Associated Viruses, for Example: AAV2 .... 235
    Haifeng Chen

11 Vector Characterization Methods for Quality Control Testing of
   Recombinant Adeno-Associated Viruses ......................... 247
   J. Fraser Wright and Olga Zelenaia

12 Baculoviruses Mediate Efficient Gene Expression in a Wide
   Range of Vertebrate Cells ........................................ 279
   Kari J. Airenne, Kaisa-Emilia Makkonen, Anssi J. Mäbönen,
   and Seppo Ylä-Herttuala

13 Herpes Simplex Virus Type 1-Derived Recombinant and Amplicon Vectors 303
    Cornel Fraefel, Peggy Marconi, and Alberto L. Epstein

14 Manufacture of Measles Viruses ................................ 345
    Kirsten K. Langfield, Henry J. Walker, Linda C. Gregory,
    and Mark J. Federspiel

15 In Vivo Gene Delivery into hCD34+ Cells in a Humanized Mouse Model .... 367
    Cecilia Frecha, Floriane Fusil, François-Loïc Cosset, and Els Verboeyen
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>In Vivo Evaluation of Gene Transfer into Mesenchymal Cells (In View of Cartilage Repair)</td>
<td>391</td>
</tr>
<tr>
<td></td>
<td><strong>Kolja Gelse and Holm Schneider</strong></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Ethical Consideration</td>
<td>407</td>
</tr>
<tr>
<td></td>
<td><strong>Michael Fuchs</strong></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Clinical Trials of GMP Products in the Gene Therapy Field</td>
<td>425</td>
</tr>
<tr>
<td></td>
<td><strong>Kathleen B. Bamford</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Index*                                                                 | 443   |
Contributors

KARI J. AIRENNE • Department of Molecular Medicine, A.I. Virtanen Institute, University of Eastern Finland, Kuopio, Finland
RAMON ALEMANY • Laboratori de Recerca Trasacional, Institut Català d’Oncologia – IDIBELL, L’Hospitalet de Llobregat, Barcelona, Spain
MOHAMED AL-RUBEAI • School of Chemical & Bioprocess Engineering and Conway Institute for Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland
PAULA M. ALVES • IBET/ITQB-UNL, Oeiras, Portugal
ANA I. AMARAL • IBET/ITQB-UNL, Oeiras, Portugal
KATHLEEN B. BAMFORD • Department of Microbiology, Imperial College Healthcare NHS Trust, London, UK; Department of Infectious Diseases and Immunity, Imperial College London, London, UK
MARLENE CARMO • ICH, UCL, London, UK
HAIFENG CHEN • Virovek, Inc., San Francisco, CA, USA
MIGUEL CHILLON • Biochemistry and Molecular Biology Department, Laboratory of Gene Therapy for Autoimmune Diseases, CBATEG, Universitat Autònoma Barcelona, Barcelona, Spain
ANA S. COROADINHA • Animal Cell Technology Laboratory, IBET/ITQB-UNL, Oeiras, Portugal
FRANÇOIS-LOÏC COSSET • Human Virology Department, INSERM U758, Ecole Normale Supérieure de Lyon, and Université de Lyon 1, Lyon, France
PEDRO E. CRUZ • Animal Cell Technology Laboratory, IBET/ITQB-UNL, and ECBIO, Oeiras, Portugal
CLAIRE DAIGRE • Department of Agricultural and Biological Engineering, Mississippi State University, Starkville, MS, USA
PATRICE P. DENEFLE • Translational Sciences, IPSEN, and Biotherapies, ParisTech Institute, Paris-Descartes University, Paris, France
EDWIGE DORMOND • Baxter Bioscience Manufacturing SARL, Neuchâtel, Switzerland
ALBERTO L. EPSTEIN • Université Lyon 1, Lyon, France, and Centre de Génétique et Physiologie Moléculaire et Cellulaire, CNRS, UMR5534, Villeurbanne, France
MARK J. FEDERSPIEL • Department of Molecular Medicine, Gene and Virus Therapy Shared Resource, Viral Vector Production Laboratory, Mayo Clinic Comprehensive Cancer Center, Mayo Clinic, Rochester, MN, USA
CORNEL FRAEFEL • University of Zurich, Institute of Virology, Zurich, Switzerland
CECILIA FRECHA • Human Virology Department, INSERM U758, Ecole Normale Supérieure de Lyon, and Université de Lyon 1, Lyon, France
MICHAEL FUCHS • Institut für Wissenschaft und Ethik, University Bonn, Bonn, Germany

FLORIANE FUSIL • Human Virology Department, INSERM U758, Ecole Normale Supérieure de Lyon, and Université de Lyon 1, Lyon, France

ALAIN GARNIER • Department of Chemical Engineering, Centre de Recherche PROTEO, Université Laval, Laval, QC, Canada

KOLJA GELSE • Department of Pediatrics, Nikolaus Fiebiger Center of Molecular Medicine, University of Erlangen-Nürnberg, Erlangen, Germany

MARC GICY-LATERRIÈRE • Faculty of Medicine, Department of Neurosciences, CMU, Geneva, Switzerland

LINDA C. GREGORY • Department of Molecular Medicine, Gene and Virus Therapy Shared Resource, Viral Vector Production Laboratory, Mayo Clinic Comprehensive Cancer Center, Mayo Clinic, Rochester, MN, USA

AMINE A. KAMEN • Biotechnology Research Institute, NRC, Montreal, QC, Canada

KIRSTEN K. LANGFIELD • Department of Molecular Medicine, Gene and Virus Therapy Shared Resource, Viral Vector Production Laboratory, Mayo Clinic Comprehensive Cancer Center, Mayo Clinic, Rochester, MN, USA

ANSSI J. MÄHÖNEN • Department of Molecular Medicine, A.I. Virtanen Institute, University of Eastern Finland, and Ark Therapeutics Oy, Kuopio, Finland

KAISA-EMILIA MÄKKONEN • Department of Molecular Medicine, A.I. Virtanen Institute, University of Eastern Finland, Kuopio, Finland

PEGGY MARCONI • Department of Experimental and Diagnostic Medicine, Section of Microbiology, University of Ferrara, Ferrara, Italy

OTTO-WILHELM MERTEN • Génethon, Evry, France

MAURO MEZZINA • European Association for Scientific Career Orientation (CNRS/EASCO), Paris, France

TERESA RODRIGUES • Oxford Biomedica, Oxford, UK

PATRICK SALMON • Faculty of Medicine, Department of Neurosciences, CMU, Geneva, Switzerland

HOLM SCHNEIDER • Department of Pediatrics, Nikolaus Fiebiger Center of Molecular Medicine, University of Erlangen-Nürnberg, Erlangen, Germany

MĂRIA MERCEDES SEGURA • Department of Biochemistry and Molecular Biology, Center of Animal Biotechnology and Gene Therapy (CBATEG), Universitat Autònoma de Barcelona, Barcelona, Spain

GLYN N. STACEY • National Institute for Biological Standards and Control (An Operating Centre of the Health Protection Agency), South Mimms, UK

ELS VERHOEYEN • Human Virology Department, INSERM U758, Ecole Normale Supérieure de Lyon, and Université de Lyon 1, Lyon, France

HENRY J. WALKER • Department of Molecular Medicine, Gene and Virus Therapy Shared Resource, Viral Vector Production Laboratory, Mayo Clinic Comprehensive Cancer Center, Mayo Clinic, Rochester, MN, USA

JAMES N. WARNOCK • Department of Agricultural and Biological Engineering, Mississippi State University, Starkville, MS, USA

DAGMAR WIRTH • Helmholtz Centre for Infection Research, Braunschweig, Germany
J. Fraser Wright • Clinical Vector Core, Center for Cellular and Molecular Therapeutics, The Childrens Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Seppo Ylä-Herttuala • Department of Molecular Medicine and Department of Medicine and Gene Therapy Unit, A.I. Virtanen Institute, University of Eastern Finland, Kuopio, Finland

Olga Zelenia • Clinical Vector Core, Center for Cellular and Molecular Therapeutics, The Childrens Hospital of Philadelphia, Philadelphia, PA, USA