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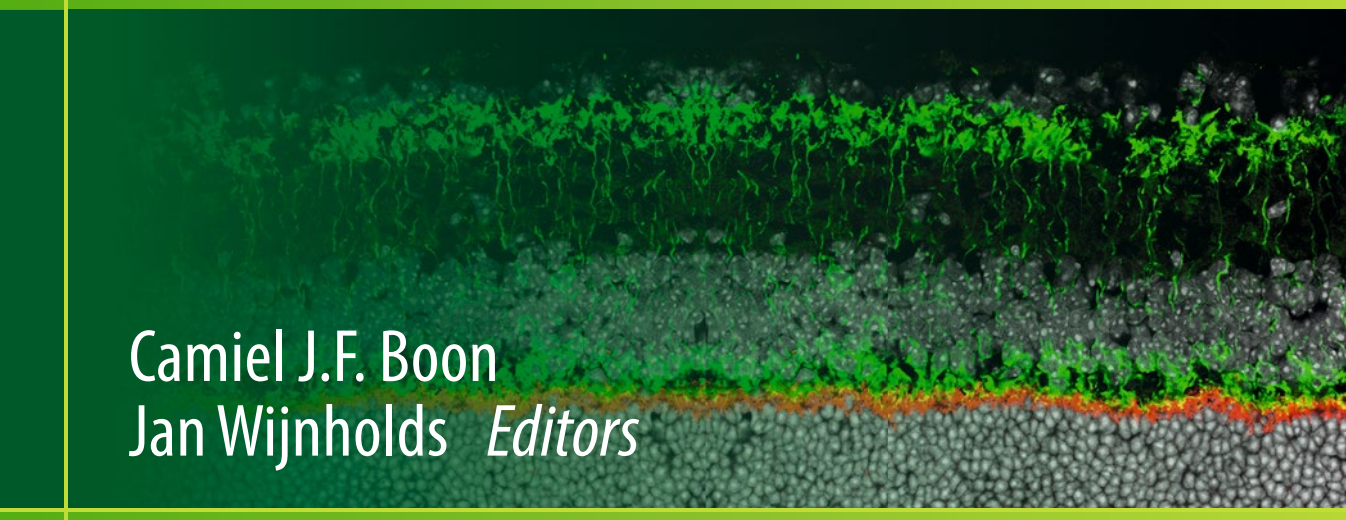

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Retinal Gene Therapy

Methods and Protocols

Humana Press

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Retinal Gene Therapy

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Preface

The eye is in the frontline of gene therapy development. As a relatively closed compartment, it is on the one hand relatively isolated and immune-privileged from the rest of the body, yet at the same time well-accessible to surgical intervention and a broad range of clinical examinations of its structure and function. The eye is therefore an attractive target organ for the application of gene therapy. With more than 2000 clinical trials to date for a wide range of genetic diseases, ocular gene therapy offers a promising perspective for the more than currently known 250 retinal disease genes. A preferred delivery system are the adeno-associated viral (AAV) gene therapy vectors for gene augmentation, gene editing, and miRNA delivery, next to the antisense oligonucleotide delivery system. As in preclinical studies, ocular clinical gene therapy studies know their successes and disappointments. The first successful ocular clinical studies for the orphan drug AAV-*RPE65* for Leber congenital amaurosis and retinitis pigmentosa were reported in the literature in 2008. More than a decade later, AAV-*RPE65* retinal gene therapy approaches are evaluated in phase 3 clinical studies and reached market approval. Clinical development of retinal gene therapy is yet a time-consuming process for technological as well financial reasons, but many more successes are expected in the coming decade for ocular gene augmentation, gene editing, miRNA delivery, as well as the antisense oligonucleotide delivery system.

This volume of *Methods in Molecular Biology* describes a spectrum of methods and protocols that can be used for the bench-to-bedside development and evaluation of retinal gene therapy. Methods for the successful delivery of these gene therapy vector systems to the retina are reviewed, as well as assays to test the efficacy in vitro in cell cultures; in vivo on rodents, pigs, and monkey retinas; and on human retinal explants as well as in human clinical studies. Chapters in this book are organized into three major parts: Part I elaborates on the production of retinal gene therapy vectors and testing these in biological assays in vitro. Part II describes assays for gene augmentation and gene editing in vivo on rodent, pig, and macaque retina. Part III highlights clinical protocols and retinal gene therapy vector testing on human retina. Written in the highly successful *Methods in Molecular Biology* series format, chapters include introductions to their respective topics, with step-by-step lists of the necessary materials and reagents, readily reproducible laboratory protocols, and tips on troubleshooting and how to avoid known pitfalls.

The preparation of AAVs is a relatively simple task for the ones skilled in molecular biology, but robust protocols are needed to obtain high-quality high-titer stocks of AAV. In Part I, we have included three of such chapters, Chapters 1–3, describing detailed various techniques to produce microscale and small-scale batches of AAVs with useful notes on the various steps in the production. AAVs have a limited packaging capacity compared to lentiviral vectors, but vectors with multiple gene cassettes enabling cell-specific co-expression of microRNA (miRNA) and protein factors are of high interest. In Chapter 4, we have included such an example that may contribute to the development of combination therapies for various ocular diseases. Other promising retinal gene therapy methods such as the use of antisense oligonucleotides (AONs) to correct pre-mRNA splicing defects involved in inherited retinal dystrophies are described in Chapter 5. Bioavailability and bioactivity of retinal gene delivery system products can be tested in three-dimensional co-culture assays, and these methods and protocols are described in Chapter 6. To test new gene therapy AAV gene

augmentation vectors, there is a need for reliable and sensitive in vitro assays to determine the expression of delivered proteins, which is covered in Chapter 7. In Part II, we highlight assays for gene augmentation and editing in vivo on rodent, pig, and macaque retina. Chapter 8 describes techniques to study the expression of gene therapy vectors upon in vivo electroporation of the developing mouse retina. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated protein or CRISPR/Cas gene editing of the adult retina is a powerful technique for correcting inherited ocular disease, and Chapters 9 and 13 describe methods to apply these techniques in preclinical models. Chapters 10 and 11 present protocols to detect the expression of therapeutic protein by fluorescence immunohistochemistry, histological studies using ultrathin sections, and immuno-electron microscopy and confocal laser scanning microscopy. Application techniques to deliver AAV vectors either subretinally or intravitreally into the murine retinas are described in Chapters 9–13 and 15. Very large genes cannot be expressed in a single AAV vector, but some can be expressed using dual-vector technology approaches as described in Chapter 11. Chapter 12 highlights methods for optogenetic retinal gene therapy to express light-sensitive G protein-coupled receptors (GPCRs) in retinal neurons, recording light responses in retinal explants in vitro by multi-electrode array (MEA), recording cortical light responses in vivo by visually evoked response (VEP), and testing visually guided behavior in open field test and water maze task in treated mice. Noninvasive diagnostic methods to assess retinal function and morphology in vivo in rodents by electroretinography (ERG) and optical coherence tomography (OCT) are discussed in Chapters 9, 13, and 14. Neutralizing antibodies (NABs) to AAVs may limit the infection capacity when administered intravitreal to the retina, and therefore, Chapters 16 and 17 are included to describe methods to screen rodent and nonhuman primate (NHP) serum for pre-existing NABs, providing useful tips and tricks. Chapters 11 and 18 focus on techniques for subretinal and intravitreal retinal injections in large animal models such as pigs and monkeys. Part III deals with clinical protocols and retinal gene therapy vector testing on human retinal explants and in vivo evaluation of the human retina in the context of retinal gene therapy. Natural as well as recombinant AAVs need to be tested on human tissue for their infection and expression efficacy. Chapter 19 describes iPS-derived human retina production protocols as well as methods to infect retinal cell types with AAVs. Chapters 20 and 21 highlight ex vivo validation on cultured retinal explants obtained from donor retina or from retinal surgery, respectively. Chapters 22–26 describe clinical protocols that can be used in retinal gene therapy studies such as visual acuity testing, electroretinography, visual field testing by Goldmann perimetry, central visual field sensitivity testing by fundus-driven perimetry also known as microperimetry, and spectral domain optical coherence tomography (SD-OCT) and OCT angiography. Methods to test vector shedding have become increasingly important with the number of AAV gene therapy trials that have started, and Chapter 27 describes standard operating protocols for these.

Retinal gene therapy is a broad field of research using various methods and protocols. This book therefore provides a wide range of readers from students to research experts with useful information on ocular gene therapy vector technology, in vitro and in vivo biological assays, and clinical protocols. We hope that the book finds its way in the scientific community to promote further studies for the benefit of children and adults with inherited retinal disease.

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Leiden, The Netherlands

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