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Expression and Analysis of Recombinant Ion Channels; Contents; Preface; List of Contributors; Color Plates; 1 Expression of Ion Channels in *Xenopus* Oocytes; 1.1 Introduction; 1.2 Advantages and Disadvantages of *Xenopus* Oocytes; 1.3 Procedures for Using Oocytes; 1.4 Types of Analyses; 1.4.1 Electrophysiological Analysis; 1.4.1.1 Two-electrode Whole Cell Voltage-clamp; 1.4.1.2 Cut-open Oocyte Voltage-clamp; 1.4.1.3 Macropatch Clamp; 1.4.1.4 Single Channel Analysis; 1.4.2 Biochemical Analysis; 1.4.3 Compound Screening; 1.4.3.1 Serial Recording Using the Roboocyte; 1.4.3.2 Parallel Recording Using the OpusXpress; 1.5 Examples of Use; 1.5.1 Characterization of cDNA Clones for a Channel; 1.5.2 Structure-Function Correlations; 1.5.3 Studies of Human Disease Mutations; 1.6 Conclusions; Acknowledgments; References; 2 Molecular Biology Techniques for Structure - Function Studies of Ion Channels; 2.1 Introduction; 2.2 Methods for cDNA Subcloning; 2.2.1 Conventional Sub-cloning Using Restriction Enzymes and DNA Ligase; 2.2.2 PCR-based cDNA Sub-cloning; 2.2.3 Sub-cloning cDNA through Site-specific Recombination; 2.3 Generation of Chimeric Channel cDNAs; 2.3.1 Use of Restriction Enzymes to Generate Chimeric Channel cDNAs; 2.3.2 PCR-mediated Overlap Extension for Chimera Generation; 2.3.3 PCR-mediated Integration or Replacement of cDNA Fragments; 2.4 Site-directed Mutagenesis; 2.4.1 Examples of the Use of Site-directed Mutagenesis; 2.4.2 Modification of the QuikChange Method for the Replacement of cDNA Fragments; 2.5 Epitope-tagged Channels and Fusion Partners; 2.6 Channel Subunit Concatamers; 2.7 Concluding Remarks; References; 3 Unnatural Amino Acids as Probes of Ion Channel Structure - Function and Pharmacology; 3.1 Introduction; 3.2 Unnatural Amino Acid Mutagenesis Methodology; 3.3 Unnatural Amino Acid Mutagenesis for Ion Channel Studies; 3.4 Structure-Function Example Studies; 3.4.1 Nicotinic Acetylcholine Receptor; 3.4.2 Drug Interactions with the hERG Voltage-gated Potassium Ion Channel; 3.5 Other Uses of Unnatural Amino Acids as Probes of Protein Structure and Function; 3.6 Conclusions; Acknowledgements; References; 4 Functional Expression of Ion Channels in Mammalian Systems; 4.1 Introduction; 4.2 cDNA Cloning and Manipulation; 4.3 Choice of Host Cell Background; 4.4 Post-translational Processing of Heterologous Expressed Ion Channels; 4.5 Cytotoxicity; 4.6 Transient Expression Systems; 4.6.1 "Standard" Transient Expression; 4.6.2 Viral Expression Systems; 4.7 Stable Expression of Ion Channels; 4.7.1 Bicistronic Expression Systems; 4.7.2 Stable Expression of Multiple Subunits; 4.7.3 Inducible Expression; 4.8 Summary; Acknowledgements; References; 5 Analysis of Electrophysiological Data; 5.1 Overview; 5.2 Introduction; 5.3 Expression Systems and Related Recording Techniques; 5.3.1 Expression in *Xenopus* Oocytes; 5.3.2 Expression in Mammalian Cells; 5.3.3 Leak and Capacitance Subtraction

Sommario/riassunto

Filling the gap created over the past five years, during which many new techniques have entered the market, this book is directed at both the new and the experienced ion channel researcher wishing to learn more about the considerations and methods for studying recombinant ion channels. These latest developments are covered here for the first time, contributed by editors and authors working for major pharmaceutical companies and who routinely apply these techniques in their daily work. The first three chapters cover the use of the *Xenopus* oocyte expression system for structure-function stud

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