# Contents

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

## PART I. METHODS FOR STUDYING POTASSIUM CHANNEL TRANSCRIPTION AND mRNA SPlicing  

1. Identifying Transcriptional Regulatory Regions Using Reporter Genes and DNA–Protein Interactions by Chromatin Immunoprecipitation ................................. 3  
   Lexanne Ooi and Ian C. Wood  
2. Quantitative RT-PCR Methods for Investigation of Low Copy Potassium Channel Gene Expression in Native Murine Arteries .................................................. 19  
   Alex Cheong, Samuel J. Fountain, and David J. Beech  
3. Cloning of Potassium Channel Splice Variants from Tissues and Cells .................. 35  
   Lie Chen and Michael J. Shipston  

## PART II. METHODS FOR STUDYING THE CELL BIOLOGY OF POTASSIUM CHANNEL PROTEINS  

4. Chemiluminescence Assays to Investigate Membrane Expression and Clathrin-Mediated Endocytosis of K<sub>ATP</sub> Channels ............................................ 63  
   Andrew J. Smith and Asipu Sivaprasadarao  
5. Investigation of K<sub>ATP</sub> Channel Endocytosis by Immunofluorescence ............. 69  
   Andrew J. Smith and Asipu Sivaprasadarao  
6. Investigation of K<sub>ATP</sub> Channel Endocytosis and Cell Surface Density by Biotinylation and Western Blotting ..................................................... 79  
   Andrew J. Smith and Asipu Sivaprasadarao  
7. Lipid Microdomains and K<sup>+</sup> Channel Compartmentation: Detergent and Non-Detergent-Based Methods for the Isolation and Characterisation of Cholesterol-Enriched Lipid Rafts ........................................... 91  
   Laura J. Sampson and Caroline Dart  
8. Determination of Phosphoinositide Binding to K<sup>+</sup> Channel Subunits Using a Protein–Lipid Overlay Assay ..................................................... 103  
   Alison M. Thomas and Andrew Tinker  
9. Protein Complex Analysis of Native Brain Potassium Channels by Proteomics .......... 113  
   Guillaume Sandoe and Florian Lesage  

## PART III. ELECTROPHYSIOLOGICAL TECHNIQUES FOR THE STUDY OF POTASSIUM CHANNEL FUNCTION  

10. *Xenopus* Oocytes as a Heterologous Expression System for Studying Ion Channels with the Patch-Clamp Technique .................................................. 127  
    Paolo Tammaro, Kenju Shimomura, and Peter Proks
11. Whole-Cell Recording Using the Perforated Patch Clamp Technique ................. 141
   Jonathan D. Lippiat
12. Recording the Activity of ATP-Sensitive K⁺ Channels in Open-Cell
Cell-Attached Configuration .............................................................. 151
   Andrei I. Tarasov
13. Planar Patch Clamp: Advances in Electrophysiology ................................. 165
   Andrea Brüggemann, Cecilia Farre, Claudia Haarmann, Ali Haythornthwaite,
   Mohamed Kreir, Sonja Stoelzel, Michael George, and Niels Fertig
14. Analysing Steroid Modulation of BKCa Channels Reconstituted
into Planar Lipid Bilayers ................................................................. 177
   Heidi de Wet, Jonathan D. Lippiat, and Marcus Allen

PART IV. OPTICAL TECHNIQUES FOR THE STUDY OF POTASSIUM
CHANNEL FUNCTION AND INTERACTIONS
15. Using Bioluminescence Resonance Energy Transfer to Measure
Ion Channel Assembly ................................................................. 189
   Gina M. Whitaker and Eric A. Accili
16. The Use of FRET Microscopy to Elucidate Steady State Channel
Conformational Rearrangements and G Protein Interaction
with the GIRK Channels ................................................................. 199
   Adi Raveh, Inbal Riven, and Eitan Reuveny
17. The Voltage-Clamp Fluorometry Technique ......................................... 213
   Chris S. Gandhi and Riccardo Olcese

PART V. METHODS FOR STUDYING POTASSIUM CHANNELOPATHIES
AND PHARMACOLOGICAL MODULATORS
18. Identification of Mutations in the Kir6.2 Subunit of the KATP Channel .......... 235
   Sarah E. Flanagan and Sian Ellard
19. Modulation of Potassium Ion Channel Proteins Utilising Antibodies .......... 247
   Mark L. Dallas, Susan A. Deuchars, and Jim Deuchars
20. Fluorescence-Based Tl⁺-Influx Assays as a Novel Approach
for Characterization of Small-Conductance Ca²⁺-Activated
K⁺ Channel Modulators ................................................................. 257
   Susanne Jögensen, Tina H. Johansen, and Tino Dyhring
21. Rubidium Efflux as a Tool for the Pharmacological Characterisation
of Compounds with BK Channel Opening Properties .............................. 267
   Neil G. McKay, Robert W. Kirby, and Kim Lawson
22. Recording hERG Potassium Currents and Assessing the Effects
of Compounds Using the Whole-Cell Patch-Clamp Technique .................. 279
   Ray M. Helliwell

Index ................................................................. 285
Potassium Channels
Methods and Protocols
Lippiat, J.D. (Ed.)
2009, X, 302 p. 59 illus., Hardcover
A product of Humana Press