Contents

Foreword ............................................................................................................... v
Preface ............................................................................................................... vii
Contributors .................................................................................................... xiii

PART I. PROTEIN-BASED IDENTIFICATION OF ANTIGENS
1 Proteomic Approaches to Antigen Discovery
Karen M. Dobos, John S. Spencer, Ian M. Orme,
and John T. Belisle .................................................................................... 3
2 Immunoproteomics
Alexander Krah and Peter R. Jungblut .................................................... 19
3 Immunoprecipitation and Blotting: The Visualization
of Small Amounts of Antigens Using Antibodies and Lectins
Stephen Thompson .................................................................................... 33

PART II. DNA-BASED IDENTIFICATION OF ANTIGENS
4 Representational Difference Analysis of cDNA
Lucas D. Bowler ....................................................................................... 49
5 Microarray Data Analysis and Mining
Silvia Saviozzi, Giovanni Iazzetti, Enrico Caserta,
Alessandro Guffanti, and Raffaele A. Calogero ................................. 67
6 Expression Cloning
Michael J. Lodes, Davin C. Dillon, Raymond L. Houghton,
and Yasir A. W. Skeiky ........................................................................ 91

PART III. IDENTIFICATION OF EPITOPES
AND IMMUNOMODULATORY COMPONENTS
7 Determination of Epitopes by Mass Spectrometry
Christine Hager-Braun and Kenneth B. Tomer ..................................... 109
8 Identification of T Cell Epitopes Using ELISpot
and Peptide Pool Arrays
Timothy W. Tobery and Michael J. Caulfield ................................. 121
9 Virus-like Particles: A Novel Tool for the Induction
and Monitoring of Both T-Helper and Cytotoxic
T-Lymphocyte Activity
Ludwig Deml, Jens Wild, and Ralf Wagner ....................................... 133
10 Application of Single-Cell Cultures of Mouse Splenocytes as an Assay System to Analyze the Immunomodulatory Properties of Bacterial Components
   Ludwig Deml, Michael Aigner, Alexander Eckhardt, Jochen Decker, Norbert Lehn, and Wulf Schneider-Brachert ..... 159

PART IV. EXPRESSION OF RECOMBINANT PROTEINS
11 High-Throughput Expression and Purification of 6xHis-Tagged Proteins in a 96-Well Format
   Jutta Drees, Jason Smith, Frank Schäfer, and Kerstin Steinert ........ 179
12 Production of Antigens in Chlamydomonas reinhardtii: Green Microalgae as a Novel Source of Recombinant Proteins
   Markus Fuhrmann ................................................................. 191
13 Codon-Optimized Genes that Enable Increased Heterologous Expression in Mammalian Cells and Elicit Efficient Immune Responses in Mice after Vaccination of Naked DNA
   Marcus Graf, Ludwig Deml, and Ralf Wagner ......................... 197

PART V. PURIFICATION, MODIFICATION, AND RENATURATION OF RECOMBINANT PROTEINS
14 Purification and Immunological Characterization of Recombinant Antigens Expressed in the Form of Insoluble Aggregates (Inclusion Bodies)
   Udo Reischl ................................................................. 213
15 Purification of Recombinant Proteins with High Isoelectric Points
   Raffaele A. Calogero and Anna Aulicino .................................. 225
16 Refolding of Inclusion Body Proteins
   Marcus Mayer and Johannes Buchner ...................................... 239
17 Small-Molecule–Protein Conjugation Procedures
   Stephen Thompson .............................................................. 255

PART VI. CHARACTERIZATION OF RECOMBINANT PROTEINS
18 Structural Characterization of Proteins and Peptides
   Rainer Deutzmann .............................................................. 269
19 Determination of Kinetic Data Using Surface Plasmon Resonance Biosensors
   Claudia Hahnefeld, Stephan Drewianka, and Friedrich W. Herberg ................................................. 299
PART VII. EVALUATION OF RECOMBINANT PROTEINS IN IMMUNOLOGICAL TEST SYSTEMS

21 Solid Supports in Enzyme-Linked Immunosorbent Assay and Other Solid-Phase Immunoassays
   John E. Butler .......................................................... 333

22 Design and Preparation of Recombinant Antigens as Diagnostic Reagents in Solid-Phase Immunosorbent Assays
   Alan Warnes, Anthony R. Fooks, and John R. Stephenson .......... 373

23 Basic Problems of Serological Laboratory Diagnosis
   Walter Fierz ............................................................. 393

24 Molecular Diagnostics Resources on the Internet
   Larry Winger .......................................................... 429

PART VIII. RECOMBINANT RECEPTOR MOLECULES

25 Cloning Single-Chain Antibody Fragments (scFv) from Hybridoma Cells
   Lars Toleikis, Olaf Broders, and Stefan Dübel ....................... 447

Index ................................................................. 459