# Preface

With the completion of sequencing projects and the advancement of analytical tools for protein identification, proteomics—the study of the expressed part of the genome—has become a major region of the burgeoning field of functional genomics. High-resolution 2-D gels can reveal virtually all proteins present in a cell or tissue at any given time, including posttranslationally modified proteins. Changes in the expression and structure of most cellular proteins caused by differentiation or external stimuli can be displayed and eventually identified using 2-D protein gels.

*2-D Proteome Analysis Protocols* covers all aspects of the use of 2-D protein electrophoresis for the analysis of biological problems. The contributors include many of the leaders in the fields of biochemistry and analytical chemistry who were instrumental in the development of high-resolution 2-D gels, immobilized pH gradients, computer analysis, and mass spectrometry-based protein identification methodologies.

This book is intended as a benchtop manual and guide both for novices to 2-D gels and for those aficionados who wish to try the newer techniques. Any group using protein biochemistry—especially in the fields of molecular biology, biochemistry, microbiology, and cell biology—should find this book eminently useful.

*2-D Proteome Analysis Protocols* takes the researcher through the complete process of working with 2-D protein gels from making the protein extract to finally identifying the proteins of interest. It includes protocols for generating 2-D protein extracts from most of the standard model organisms, including bacteria, yeast, nematode, *Drosophila*, plants, mouse, and human. This book covers the traditional methods of using carrier ampholytes in the first dimension and the growing movement in the field toward immobilized pH gradients. A brief description of the advantages and disadvantages of each method is given. Analytical and preparative 2-D gels, including the lastest protocols for casting IPG gradients and in-gel rehydration of IPG strips, are covered. For the second dimension, methods for running flatbed or vertical gels, including homogeneous and gradient gels, are given. After running the 2-D gel, there are protocols for protein detection that include autoradiogra-

phy, and Coomassie, silver, and reversible metal chelate stains. *2-D Proteome Analysis Protocols* covers image acquisition of the 2-D gel and has a detailed protocol for computer analysis of the 2-D gel image. With the growing importance of the Internet, this book also includes protocols that enable readers to compare their results with other 2-D databases over the Internet or to construct their own 2-D resolved proteins, focusing on the latest mass spectrometry methods including MALDI-TOF-based peptide mapping, automated tandem mass spectrometry, and nanospray electrospray ionization technology.

Each chapter opens with a description of the protocol and the basic theory behind the method. The Materials section lists all the equipment and reagents required for carrying out the protocol. In the Methods section, each step of the protocol is listed in sufficient detail to successfully execute the experiment. Finally, the Notes section provides invaluable hints and alternatives for dealing with any problems or difficulties that may occur with the protocol.

Many people have contributed time and energy to make *2-D Proteome Analysis Protocols* possible. A special thanks goes to all the contributors, each of whom made many important suggestions and improvements. I wish especially to thank Jenny Fichmann, Reudi Aebersold, Thierry Rabilloud, Mary Lopez, Wayne Patton, Lou Ramagli, Jean-Charles Sanchez, Marc Wilkins, Ron Appel, Angelike Görg, Vitaliano Pallini, and Denis Hochstrasser for invaluable advice and help in preparing this book.

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