METHODS IN MOLECULAR BIOLOGY™

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Difference Gel Electrophoresis (DIGE)

Methods and Protocols

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Preface

Protein analysis is increasingly becoming a cornerstone in deciphering the molecular mechanisms of life. Proteomics, the large-scale and high-sensitivity analysis of proteins, is already pivotal to the new life sciences such as Systems Biology and Systems Medicine. Proteomics, however, relies heavily on the past and future advances of protein purification and analysis methods. In-depth protein and protein interaction analysis would be impossible without these advances. It is this progress being made available through techniques such as DIGE that is enormously important for pushing the boundaries in the analysis of the cellular machinery and larger biological systems even further.

Apart from mere "stamp collections", most analytical measurements have to be somewhat quantitative. This is particularly true for the analysis of proteins and protein contents in order to find out at what level these small molecular engines are involved in the fundamental processes of life. Thus, the proteomic research community has become increasingly aware of the fact that most proteomic analyses have to fulfill strict requirements with regard to their quantitative aspects. Protein or peptide identification and characterization without quantification might just be sufficient in areas such as Proteogenomics where this information can be used to curate genomic data or delineate function. However, once a protein is characterized and known to be present, it is essential to obtain quantitative information. Only this quantitative data obtained from different time points and conditions will enable a comprehensive understanding of the dynamics of protein contents in biological systems and, thus, the elucidation of how these systems function and react.

Consequently, many quantitative proteomic analysis methods were devised shortly after Proteomics was born. Most of these are based on quantification using the mass spectral readout from methods of metabolic isotope labeling (e.g., SILAC), chemical isotope labeling (e.g., iTRAQ or TMT), and increasingly from simple non-labeling methods such as MS ion signal comparison in so-called label-free approaches. However, virtually all MS-based quantitative proteomic analyses favor the analysis at the peptide level which inherently excludes quantification at the protein level, i.e., of protein isoforms. Thus, one often-cited strength of proteomics, the analysis of posttranscriptional and -translational changes, is fundamentally hampered through the inadequacy of these methods in protein isoform quantification. DIGE, being able to quantify proteins in their intact form, is one of a few methods that can facilitate this type of analysis and still provides the protein isoforms in an MS-compatible state for further identification and characterization with high analytical sensitivity.

This volume introduces the concept of DIGE and its advantages in quantitative measurements with the specific focus on proteomic analyses. It provides detailed protocols and important notes on the practical aspects of DIGE with both generic and specific applications in the various areas of quantitative protein analysis. As such this volume can be used by novices with some background in biochemistry or molecular biology, who want to widen their portfolio of quantitative techniques in protein analysis, as well as by experts in proteomics, who would like to deepen their understanding of DIGE and its employment in many hyphenations and application areas. With its many protocols, applications, and methodological variants,

it is also a unique reference for all who seek fundamental details on the working principle of DIGE and ideas for a possible future use of DIGE in novel analytical approaches.

The chapters in this volume have been divided into four categories. Starting with the basics of DIGE the reader acquires a sound background in the technique and its practical details with a focus on the planning of a DIGE experiment and its data analysis. The next chapters introduce various DIGE methods. While most of these have been employed by scientists world-wide, some are more novel and provide a glance at what is at the horizon in the DIGE world. The final sets of chapters provide a good overview of the wide range of DIGE applications from Clinical Proteomics to Animal, Plant and Microbial Proteomics applications.

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