

# Relative, Label-free Protein Quantitation: Spectral Counting Error Statistics from Nine Replicate MudPIT Samples

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Nine replicate samples of peptides from soybean leaves, each spiked with a different concentration of bovine apotransferrin peptides, were analyzed on a mass spectrometer using multidimensional protein identification technology (MudPIT). Proteins were detected from the peptide tandem mass spectra, and the numbers of spectra were statistically evaluated for variation between samples. The results corroborate prior knowledge that combining spectra from replicate samples increases the number of identifiable proteins and that a summed spectral count for a protein increases linearly with increasing molar amounts of protein. Furthermore, statistical analysis of spectral counts for proteins in two- and three-way comparisons between replicates and combined replicates revealed little significant variation arising from run-to-run differences or data-dependent instrument ion sampling that might falsely suggest differential protein accumulation. In these experiments, spectral counting was enabled by PANORAMICS, probability-based software that predicts proteins detected by sets of observed peptides. Three alternative approaches to counting spectra were also evaluated by comparison. As the counting thresholds were changed from weaker to more stringent, the accuracy of ratio determination also changed. These results suggest that thresholds for counting can be empirically set to improve relative quantitation. All together, the data confirm the accuracy and reliability of label-free spectral counting in the relative, quantitative analysis of proteins between samples. (*J Am Soc Mass Spectrom* 2010, 21, 1534–1546) © 2010 American Society for Mass Spectrometry

**M**ultidimensional protein identification technology (MudPIT), a qualitative mass spectrometry (MS) method for the detection of many proteins in a sample, is becoming ever more quantitative [1, 2]. Instrumentation improvements for mass precision, sensitivity, selectivity, and speed, along with advances in software and computational approaches, are changing the ways that amounts of proteins are measured relative to one another in a single sample or between samples [1]. These quantitative applications are enabling systems biology studies and have led to broader understandings of protein dynamics in cells [3–6].

Relative protein quantitation in MudPIT takes two forms: labeled and label-free. Labels include isotopes or isobaric tags that shift the mass of a labeled protein or peptide. If two differentially labeled samples are analyzed concurrently, an expected shift in the mass spectrum can be observed for a parent or daughter ion from one of the samples and the relative amounts of proteins or peptides determined from the differential ion peak

heights (ion signal strength), ion peak areas, or numbers of tandem mass spectra produced [3, 7–13]. In the label-free approaches, parent ion peaks, parent ion mass counts, or numbers of tandem mass spectra can also be used to determine amounts of protein or peptide in a sample [4, 14–20]. However, label-free samples cannot be analyzed concurrently in a mass spectrometer because there is no predictable mass shift to enable a direct comparison. Therefore, while the label-free methods may be more convenient in that they save financial or time costs associated with labeling, they are thought to be less accurate than labeling methods [1]. In reality, label and label-free methods are independently supported by empirical data that confirm their quantitative accuracy within a relative context.

In this light, we have independently pursued a label-free method of counting tandem mass spectra for deducing protein accumulation changes between samples. Our decision to pursue a label-free method over labeling was based on the difficulty of incorporating isotopic labels into the metabolic cycles of live agricultural pathogens and plants that we study and our lack of resources for developing and using labeling chemistries. Instead, we invented a software platform, PANORAMICS, that allows us to analyze large amounts

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# Relative Label Protein Quantitation Spectral

**JE Gale**



## **Relative Label Protein Quantitation Spectral:**

**Precision Molecular Pathology of Prostate Cancer** Brian D. Robinson, Juan Miguel Mosquera, Jae Y. Ro, Mukul Divatia, 2018-02-13 This volume focuses on our current understanding of the molecular underpinnings of prostate cancer and their potential application for precision medicine approaches The emergence and applications of new technologies has allowed for a rapid expansion of our understanding of the molecular basis of prostate cancer and has revealed a remarkable genetic heterogeneity that may underlie the clinically variable behavior of the disease The book consists of five sections which provide insight about the following 1 General principles 2 Molecular signatures of primary prostate cancer 3 Molecular signatures of advanced prostate cancer 4 Key molecular pathways in prostate cancer development and progression 5 and Precision medicine approach Diagnosis treatment prognosis Precision Molecular Pathology of Prostate Cancer is an important resource for the practicing oncologist urologist and pathologist and will also be useful for researchers in the prostate cancer community

**Protein and Peptide Mass Spectrometry in Drug Discovery** Michael L. Gross, Guodong Chen, Birendra Pramanik, 2011-09-26 The book that highlights mass spectrometry and its application in characterizing proteins and peptides in drug discovery An instrumental analytical method for quantifying the mass and characterization of various samples from small molecules to large proteins mass spectrometry MS has become one of the most widely used techniques for studying proteins and peptides over the last decade Bringing together the work of experts in academia and industry Protein and Peptide Mass Spectrometry in Drug Discovery highlights current analytical approaches industry practices and modern strategies for the characterization of both peptides and proteins in drug discovery Illustrating the critical role MS technology plays in characterizing target proteins and protein products the methods used ion mobility and the use of microwave radiation to speed proteolysis the book also covers important emerging applications for neuroproteomics and antigenic peptides Placing an emphasis on the pharmaceutical industry the book stresses practice and applications presenting real world examples covering the most recent advances in mass spectrometry and providing an invaluable resource for pharmaceutical scientists in industry and academia analytical and bioanalytical chemists and researchers in protein science and proteomics

**Agricultural Proteomics Volume 1** Ghasem Hosseini Salekdeh, 2016-08-20 This book will cover several topics to elaborate how proteomics may enhance agricultural productivity These include crop and food proteomics farm animal proteomics aquaculture microorganisms and insect proteomics It will also cover several technical advances which may address the current need for comprehensive proteome analysis An emerging field of the proteomics aim is to integrate knowledge from basic sciences and to translate it into agricultural applications to solve issues related to economic values of farm animals crops food security health and energy sustainability Given the wealth of information generated and to some extent applied in agriculture there is the need for more efficient and broader channels to freely disseminate the information to the scientific community

**Mass Spectrometry in Chemical**

**Biology** Norberto Peoporine Lopes, Ricardo Roberto da Silva, 2017-11-16 Mass spectrometry is one of the most widespread technologies in chemistry and has been increasingly used in biology with the rise of omics sciences This book summarizes some important methodological approaches in mass spectrometry and applications in the field of chemical biology The core chapters build on basic concepts introduced in the opening chapter and explore established fields such as high throughput screening proteomics and metabolomics Emerging applications of mass spectrometry in elucidating biosynthetic pathways enzyme mechanisms and protein protein interactions are then presented Connections between these diverse research fields are highlighted throughout The book concludes with a discussion of databases and future perspectives This book will be a useful tool to early chemical biology researchers wishing to incorporate mass spectrometry as a tool in their research

**Translational Biomedical Informatics** Bairong Shen, Haixu Tang, Xiaoqian Jiang, 2016-10-31 This book introduces readers to essential methods and applications in translational biomedical informatics which include biomedical big data cloud computing and algorithms for understanding omics data imaging data electronic health records and public health data The storage retrieval mining and knowledge discovery of biomedical big data will be among the key challenges for future translational research The paradigm for precision medicine and healthcare needs to integratively analyze not only the data at the same level e g different omics data at the molecular level but also data from different levels the molecular cellular tissue clinical and public health level This book discusses the following major aspects the structure of cross level data clinical patient information and its shareability and standardization and privacy It offers a valuable guide for all biologists biomedical informaticians and clinicians with an interest in Precision Medicine Informatics

*Reviews in Pharmaceutical and Biomedical Analysis* Paraskevas D. Tzanavaras, Constantinos K. Zacharis, 2010 *Reviews in Pharmaceutical and Biomedical Analysis* contains coverage and review of new trends and applications in all areas of pharmaceutical biomedical and analytical chemistry Authors have contributed review articles according to their expertise on various topics

*Integrative Omics* Manish Kumar Gupta, Pramod Katara, Sukanta Mondal, Ram Lakhan Singh, 2024-05-03 *Integrative Omics Concepts Methodology and Applications* provides a holistic and integrated view of defining and applying network approaches integrative tools and methods to solve problems for the rationalization of genotype to phenotype relationships The reference includes a range of chapters in a systemic step by step manner which begins with the basic concepts from Omic to Multi Integrative Omics approaches followed by their full range of approaches applications emerging trends and future trends All key areas of Omics are covered including biological databases sequence alignment pharmacogenomics nutrigenomics and microbial omics integrated omics for Food Science and Identification of genes associated with disease clinical data integration and data warehousing translational omics as well as omics technology policy and society research *Integrative Omics Concepts Methodology and Applications* highlights the recent concepts methodologies advancements in technologies and is also well suited for researchers from both academic and industry background undergraduate and graduate students

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### **Current Developments in**

**Biotechnology and Bioengineering** Swati Joshi,Ashok Pandey,Ranjna Sirohi,Sung Hoon Park,2022-04-29 Designer Microbial Cell Factories Metabolic Engineering and Applications the latest release in the Current Developments in Biotechnology and Bioengineering series provides a detailed overview of the biotechnological approaches and strategies used to generate engineered microbes and to facilitate the acceleration modulation and diversion of metabolic pathways to get desired output such as production of value added compound or biodegradation of xenobiotic pollutant The book also highlights applied aspects of designer microbes in fields as diverse as agriculture pharmaceuticals and bioremediation Designer microbes generated through reprogramming the microbial cell factories MCFs provide an edge over their natural counterparts in terms of increased molecular diversity and selective chemistry These bugs are becoming instrumental in several areas including agriculture environment and human health Engineering microbes through directed evolution not only gives freedom from evolutionary constrains but also allow introduction of regulated and foreseeable functions into MCFs Dedicated to the designing of microbes covering state of the art technological advancements in the field Includes applications of metabolic engineering in the field of agriculture bioremediation value added products therapeutics and more Contains chapters dedicated to innovative approaches surrounding engineered microbial consortia Provides comprehensive details and helps users understand concepts

[Basic Science of Oncology, Fifth Edition](#) Ian F. Tannock,Richard P. Hill,Robert G. Bristow,Lea Harrington,2013-09-22 NOW IN FULL COLOR Discover the science of cancer with this newly revised essential introduction to cancer biology and genetics Here in one well organized reader friendly volume you ll find everything you must know about the biology underlying cancer and its treatment supported by the latest peer reviewed research Written by preeminent oncology researchers and clinicians the book highlights the full range of important oncology topics and takes you through the biological basis of current and future biological therapy as well as more traditional approaches to cancer treatment Presented in full color the Fifth Edition of The Basic Science of Oncology is thoroughly updated and refreshed to reflect the latest critical thinking in oncology For graduate students oncologists residents and fellows there can be no more useful guide to the bedrock science and practice of oncology than this all in one reference FEATURES The most current

evidence based oncology primer one that encapsulates the science of cancer causation cancer biology and cancer therapy Key insights into molecular and genetic aspects of cancer familiarize you with cancer biology as applied to prognosis and personalized cancer medicine In depth focus on the discovery evaluation and biology of anti cancer drugs immunotherapy and molecularly targeted agents Up to date coverage of the basic science of radiation therapy Specific chapters sections on intratumor heterogeneity as it relates to potential new treatment strategies including tumor microenvironment and metabolism tumor stem cells and genomic proteomic heterogeneity NEW Chapter Essentials synthesize chapter content in a convenient list NEW Color diagrams and schematics summarize important data Novel Applications of Metabolic Labeling with Stable Isotopes for Proteomic Characterization of Multicellular Organisms Edward L. Huttlin,2008 *Biochemical and Physiological Network Responses Chlamydomonas Reinhardtii on Abiotic Stress* Do Yup Lee,2009 *Studia Universitatis Babeş-Bolyai* Universitatea "Babeş-Bolyai" .,2003 **Protein-lipid Interactions in Membranes** ,1982 **The Basic Science of Oncology, Sixth Edition** Lea Harrington,Robert E. Bristow,Ian F. Tannock,Richard Hill,2021-01-08 Complete coverage of the basis of cancer and molecular biology from globally recognized experts The Basic Science of Oncology is an accessible and thorough introduction to cancer causation cancer biology and the biology underlying cancer treatment You ll find everything you need to know about the latest critical thinking in oncology as well ready to apply information about state of the art science and therapeutic applications Written by leading oncology researchers and clinicians this is an essential resource for health professionals students advanced undergraduates and graduates in biological sciences and clinicians needing an understanding of cancer cells Presented in full color The Basic Science of Oncology reflects the latest research and developments in the field Features NEW chapters Epigenetics and Principles of Genome Regulation and Targeted Cancer Diagnosis and Treatment Thoroughly revised content with expanded coverage of key topics such as immune system and immunotherapy tumor growth and metabolism vaccine development methods of molecular analysis tumor environment and more The most current evidence based oncology primer one that encapsulates the science of cancer causation cancer biology and cancer therapy Key insights into molecular and genetic aspects of cancer familiarize you with cancer biology as applied to prognosis and personalized cancer medicine In depth focus on the discovery evaluation and biology of anti cancer drugs immunotherapy and molecularly targeted agents Up to date coverage of the basic science of radiation therapy

**Neuroproteomics** Andrew K. Ottens,Kevin K.W. Wang,2009-08-05 With the development of polypeptide ionization processes for mass spectrometry and the rapid processing of large scale datasets with bioinformatics researchers now have the tools necessary to propel neuroproteomics research to new levels In Neuroproteomics Methods and Protocols experts in the neurological and analytical sciences present experimental details for applying proteomics to the study of the central nervous system CNS and its dysfunction through trauma and disease Divided into four convenient sections this collection covers CNS animal models used for neuroproteomics research methods for separating and analyzing discrete subcomponents

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**Handbook of Drug Screening, Second Edition** Ramakrishna Seethala,Litao Zhang,2009-06-24 A presentation of screening techniques modern technologies and high capacity instrumentation for increased productivity in the development and discovery of new drugs chemical compounds and targeted delivery of pharmaceuticals It contains practical applications and examples of strategies in cell based and cell free screens as well as homogeneous fluorescence chemiluminescence and radioactive based technologies

**Thrombosis and Haemostasis** ,2007

**Protein Mass Spectrometry** Julian Whitelegge,2009 This book is designed to be a central text for young graduate students interested in mass spectrometry as it relates to the study of protein structure and function as well as proteomics It is a definite must have work for libraries at academic institutions with Master and Graduate programs in biochemistry molecular biology structural biology and proteomics individual laboratories with interests covering these areas libraries and individual laboratories in the pharmaceutical and biotechnology industries

*Trace Quantitative Analysis by Mass Spectrometry* Robert K. Boyd,Bob Boyd,Cecilia Basic,Robert A. Bethem,2008-05-27 This book provides a serious introduction to the subject of mass spectrometry providing the reader with the tools and information to be well prepared to perform such demanding work in a real life laboratory This essential tool bridges several subjects and many disciplines including pharmaceutical environmental and biomedical analysis that are utilizing mass spectrometry Covers all aspects of the use of mass spectrometry for quantitation purposes Written in textbook style to facilitate understanding of this topic Presents fundamentals and real world examples in a learning though doing style

**McGraw-Hill Yearbook of Science and Technology** McGraw-Hill,2005-12 From one of the most trusted sources for high quality information authoritative reviews of the most significant recent developments in science and technology Edited and illustrated for clarity and ease of understanding by non specialist and expert alike

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