Absolute Quantification Of Gene Expression Using Sybr Green

The books Molecular Diagnostics Part 1 and 2 provide a comprehensive and practical overview of the state-of-the-art molecular biological diagnostic strategies that are being used in a wide variety of disciplines. The editors and experts in their respective fields have combined their knowledge to write these two books. Many years of experience in the development, application and quality control of molecular diagnostic methods is reflected herewith. Molecular Diagnostics Part 1 is dedicated to the theoretical backgrounds of the technologies often applied in molecular diagnostics, in which nucleic acid amplification methods (such as real-time PCR), sequencing and bioinformatics are the basic tools. The assay design and -development, combined with items of trouble-shooting are described in detail. As a foundation of reliable molecular diagnostic assays, the quality control required for validation, implementation and performance of molecular diagnostic assays is thoroughly discussed. This book also provides extensive information for those working with molecular techniques in a wide variety of research applications using conventional and real-time PCR technology, Sanger and high throughput sequencing techniques, and bioinformatics. Molecular Diagnostics Part 2 highlights the applications of the molecular diagnostic methods in the various diagnostic laboratories, comprising: - Clinical microbiology - Clinical chemistry - Clinical genetics - Clinical pathology - Molecular hematopathology - Veterinary health - Plant health - Food safety Both full-colour and well-illustrated books are particularly valuable for students, clinicians, scientists and other professionals who are interested in (designing) molecular diagnostic methods and for those who wish to broaden their knowledge on the current molecular biological revolution. The information in the books highlights the trend of the integration of multiple (clinical) disciplines into one universal molecular laboratory.

Aquatic Ecotoxicology: Advancing Tools for Dealing with Emerging Risks presents a thorough look at recent advances in aquatic ecotoxicology and their application in assessing the risk of well-known and emerging environmental contaminants. This essential reference, brought together by leading experts in the field, guides users through existing and novel approaches to environmental risk assessment, then presenting recent advances in the field of ecotoxicology, including omics-based technologies, biomarkers, and reference species. The book then demonstrates how these advances can be used to design and perform assays to discover the toxicological endpoints of emerging risks within the aquatic environment, such as nanomaterials, personal care products, PFOS and chemical mixtures. The text is an invaluable reference for any scientist who studies the effects of contaminants on organisms that live within aquatic environments. Provides the latest perspectives on emerging toxic risks to aquatic environments, such as nanomaterials, pharmaceuticals, chemical mixtures, and perfluorooctane sulfonate (PFOS) Offers practical guidance on recent advances to help in choosing the most appropriate toxicological assay Presents case studies and information on a variety of reference species to help put the ecotoxicological theory into practical risk assess

With a variety of detection chemistries, an increasing number of platforms, multiple choices for analytical methods and the jargon emerging along with these developments, real-time PCR is facing the risk of becoming an intimidating method, especially for beginners. Real-time PCR provides the basics, explains how they are exploited to run a real-time PCR assay, how the assays are run and where these assays are informative in real life. It addresses the most practical aspects of the techniques with the emphasis on 'how to do it in the laboratory'. Keeping with the spirit of the Advanced Methods Series, most chapters provide an experimental protocol as an example of a specific assay.

In joint replacement surgery with suboptimal bone, allograft materials are often used to achieve biological fixation of the metallic implant to the host bone and reducing the implant fixation time. The most commonly used techniques are cemented and hydroxyapatite (HA)-coated metallic implants. Typically, HA coatings are suggested for patients with better bone stock, whereas recommended implant fixation process for most other osteoporotic patients is bone cements. In general, there is a long-standing need to improve the performance of hip and other devices for longer in vivo implant lifetime that can help in reducing the number of revision surgeries, as well as minimizing physical and mental trauma to the patient. To achieve these goals, it is important to understand the mechanical and biological properties of coatings that can influence not only its short- and long-term bioactivity but also life span in vivo. Over the years, it has been recognized that the stability of a coated implant is governed by its physical and mechanical properties. A coating that separates from the implant provides no advantage over an uncoated implant and undesirable due to problems with debris materials, which can lead to osteolysis. Therefore, it is important to properly characterize the coated implants in terms of its physical and mechanical properties. In this chapter, specific details on coating characterization techniques including sample dimensions, sample preparation, experimental procedure and data interpretation are discussed. In particular, the standards and requirements of regulatory organizations are presented elucidating the significance and use of each characterization. It is important to appreciate that mechanical properties of coatings can only be determined with certain coating specification such as coating thickness. This chapter is designed even for non-experts to follow mechanical property characterizations of coatings on medical implants.

Refinement in sequencing technologies and potential of genomic research resulted in meteoric growth of biological information such as sequences of DNA, RNA and protein requiring databases for efficient storage, management and retrieval of the biological information. Also, computational algorithms for analysis of these colossal data became a vital aspect of biological sciences. The work aims to show the process of turning bioscience innovation into companies and products, covering the basic science, the translation of science into technology. Due to rapid developments, there seems to be no basic difference between the pharmaceutical industry and the biotechnological industry. However, approved products in the pipeline and renewed public confidence make it one of the most promising areas of economic growth in the near future. India offers a huge market for the products as well as cheap manufacturing base for export. The book is a sincere work of compilation of new and recent advances in the topic of concern through various innovative researches and scientific opinion therefrom. The book is dedicated to the readers who will definitely find it interesting and knowledgeable in carrying out their respective researches in different aspects of applied microbiology and biotechnology.

The next generation sequencing technology, RNA-sequencing (RNA-seq), has an increasing popularity over traditional microarrays in transcriptome analyses. Statistical methods used for gene expression analyses with these two technologies are di erent because the array-based technology measures intensities using continuous distributions, whereas RNA-seq provides absolute quantification of gene expression using counts of reads. There is a need for reliable statistical methods to exploit the information from the rapidly evolving sequencing technologies and limited work has been done on expression analysis of time-course RNA-seq data. Functional clustering is an important method for examining gene expression patterns and thus discovering co-expressed genes to better understand the biological systems. Clusteringbased approaches to analyze repeated digital gene expression measures are in demand. In this dissertation, we propose a model-based clustering method for identifying gene expression patterns in time-course RNA-seq data. Our approach employs a longitudinal negative binomial mixture model to postulate the over-dispersed time-course gene count data. The e ectiveness of the proposed clustering method is assessed using simulated data and is illustrated by real data from time-course genomic experiments. Due to the complexity and size of genomic data, the choice of good starting values is an important issue to the proposed clustering algorithm. There is a need for a reliable initialization strategy for cluster-wise regression specifically for time-course discrete count data. We modify existing common initialization procedures to suit our model-based clustering algorithm and the procedures are evaluated through a simulation study on artificial datasets and are applied to real genomic examples to identify the optimal initialization method. Another common issue in gene expression analysis is the presence of missing values in the datasets. Various treatments to missing values in genomic datasets have

been developed but limited work has been done on RNA-seq data. In the current work, we examine the performance of various imputation methods and their impact on the clustering of time-course RNA-seq data. We develop a cluster-based imputation method which is specifically suitable for dealing with missing values in RNA-seq datasets. Simulation studies are provided to assess the performance of the proposed imputation approach.

Understanding PCR: A Practical Bench-Top Guide gives you all of the information you need to plan your first PCR, from reagents to conditions to analysis and beyond. It is a user friendly book that has step-by-step basic protocols, which can be adapted to your needs. Includes helpful information such as where to order your reagents and basic troubleshooting hints and tips. Includes resources for reagents Explains basic laboratory preparation Provides straightforward experimental protocols Incorporates fundamental analytical techniques Contains a troubleshooting guide

Biocompatibility is an essential criteria for a dental material as to ensure safety for the patients before it is placed into the oral cavity. Glass Ionomer Cement (GIC) is one of the most biocompatible dental restorative material and it has been used widely in clinical application. Nevertheless, conventional GIC has some drawbacks such as poor physical and mechanical properties hence lead to the development of nano-hydroxyapatite-silica (nano-HA-silica) fillers. These filler is added to conventional GIC to increase the material's strength and it has undergone some evaluation in terms of its properties. We introduce this special book to give information regarding this novel material and discuss about its cytotoxicity, cell attachment and dentinogenic differention properties in response to Dental Pulp Stem Cells and selected odontogenic gene markers. It is hope that this book will provide new insight about this novel material which has the potential to be an alternative material for use in restorative dentistry.

"Recent studies have discovered new known and characterized cytokines, allowing for advancement in miniaturization of micro-analytical methods as well as the extensive development of bio-informatics and nanotechnology. These advancements have allowed researchers to reduces sample sizes making for more accurate determinations then previously possible. In Cytokine Protocols: Second Edition, expert researchers in the field detail many of the methods which are now commonly used to study cytokines. These methods and techniques for studying cytokines include historical importance and the importance of researchers using bioassay, quantification, and characterization of cytokine related RNAs, posttranscriptional modifications of RNA, either naturally or artificially, and observations at the protein level. Written in the highly successful Methods in Molecular BiologyTM series format, the chapters include the kind of detailed description and implementation advice that is crucial for getting optimal results in the laboratory. Authoritative and practical, Cytokine Protocols: Second Edition seeks to aid scientists in furthering the crucially important advancement of cytokine research."

Rapid Cycle Real-Time PCR is a powerful technique for nucleic acid quantification and analysis that takes less than 30 minutes to complete. Fluorescence is automatically monitored each cycle and the amount of template quantified by advanced analytical methods, such as the second derivative maximum method. Immediately following rapid cycle PCR, melting curve analysis is performed to verify product purity with SYBR Green I and/or genotype with fluorescently-labeled hybridization probes(HybProbes or SimpleProbes). Rapid cycle real-time PCR is often cited as the most versatile, efficient method for nucleic acid quantification in research and climical studies. Molecular analysis has never been easier!

Methylmalonyl-CoA mutase (MCM, E.C. 5.4.99.2), a coenzyme B12-dependent enzyme, catalyses the. inter conversion of succinyl-CoA and methylmalonylCoA. The gene (sbm) encoding this enzyme is found in Escherichia coli (E. coll) at 62.3min on the E. coli chromosome. However, the metabolic role· of this enzyme in the organism is not kno~n. This project involves an investigation into this metabolic obscurity. The sbln gene is part of a four gene operon which also includes argK (or ygfD) that codes for a protein kinase catalysing the phosphorylation of two periplasmic binding proteins involved in cationic amino acid transport, ygfG that codes for methylmalonyl-CoA decarboxylase and ygfH that codes for propionyl-CoA: succinyl-CoA transferase. From existing literature we suspect that this operon, including the sbln gene, could be involved in the utilisation of unusual carbon sources such as succinate and propionate. An insertion mutant of the sbln gene created by transposon mediated mutagenesis was used for investigating the role of this gene. The wild type E. coli Kl2 strain, E. coli TR6524 and the mutant E. coli Kl2 (sbln::MudJ) were used in this study. Growth of the two strains (E. coli TR6524 and FAIPI) in minimal media with three different concentrations (0.05, 0.5, 5.0/lglmL) of vitamin B12 and in the presence succinate, propionate or glucose as the sole source of carbon, was studied. Growth was typical in media with glucose with no major differences in the growth pattern of the wild type and mutant strain. However, the two strains exhibited a differential growth pattern in media containing succinate, with the wild type growing faster than the mutant, indicating the role of the sbm gene in the utilisation of this carbon source. Growth in media containing propionate as the sole carbon source indicated only marginal differences in t1}.e growth pattern of the wild type and mutapt strain. This result possibly suggests that the other pathways for propionate utilisation in E. coli compensate for the lack of a functional Sbm protein in the mutant strain. Promoter analysis indicated the presence of a promoter induced by as, a transcription factor involved in the expression of proteins under stress or stationary phase growth conditions. Reverse transcription polymerase chain reaction (RT-PCR) studies of the genes of the sblll operon (sbm-argK-ygfGygfH) under the same growth conditions were carried out. Densitometric analysis of the PCR products suggested that the transcription level of sblll was higher in E. coli grown in succinate as compared to when grown in glucose and not as much when grown in propionate indicating a transcriptional level control of the sblll gene expression during the utilisation of succmate. RT-PCR studies also indicated a higher level of transcription of the gene in the stationary phase of the culture during the utilisation of succinate. Real time reverse transcription PCR (QPCR) analysis was used for the absolute quantification of the transcription of the genes of the sblll operon. An increase in the mRNA levels corresponding to the sbm, argK and ygfG genes was observed as E. coli TR6524 growth reached stationary phase, in the presence of succinate or propionate as the sole source of carbon as compared to glucose, In contrast, the highest mRNA levels corresponding to the ygfH gene were observed in the early log-phase of growth. This indicated a differential transcriptional level control of the genes within the operon. This study further established the possible role of this operon in the utilisation of succinate and propionate. The MCM enzyme activity measurement in the whole cell extracts of the wild type E. coli K12, grown under the above mentioned conditions, led to the fIrst ever measurement of MCM activity in wild type E. coli. These measurements also revealed a four fold increase of the MCM specific activity in the case of growth in succinate (4.76xlO-3U/mg) and a two fold increase for growth in propionate (2.79xlO-3U/mg) compared to that observed with growth in glucose (1.37xlO-3U/mg), indicating a significant level of involvement of the enzyme in succinate utilisation, and to a lesser extent in propionate utilisation. The proteomic analysis to understand the gene expression pattern of E. coli TR6524 was carried out using cells harvested at the stationary phase. The results showed that growth conditions induced the expression of transport related (HisJ, DppA) and energy generating proteins (PckA, AceF) required by E. coli to cope with the stressful growth conditions. However, Sbm was not identified among the limited protein spots that were analysed. Finally, E. coli K12 shm gene was successfully cloned into B. cereus Spy leading to the development of a metabolically engineered polyhydroxyalkanoate producing strain of B. cereus. The intention was to provide the bacteria with a natural intracellular source of propionyl-CoA, leading to the production of the P(3HB-co-3HV) copolymer from structurally non related carbon sources like glucose. Hence, this work has initiated investigation into the metabolic role of the shm gene product in E. coli. In addition, it has also led to the use of this gene product in metabolic engineering applications.

Perfectly timed, this handbook covers many important aspects of the topic that have only recently been understood -- making this a truly comprehensive work. With its extensive use of color, it surveys the most important proteins involved in photosynthesis, discussing the structural information we have at our disposal. Most chapters are dedicated to one protein, while a few also summarize general associated concepts. The book also has an accompanying website that contains data files and animations to allow readers to visualize many of the complicated proteins presented. A must for anyone studying

photosynthesis and structural biology, as well as those working in the plant and crop biotechnology industry.
Do you want to know the details that should be taken into consideration in order to have accurate conventional and real-time PCR results? If so, this book is for you. Polymerase Chain Reaction for Biomedical Applications is a collection of chapters for both novice and experienced scientists and technologists aiming to address obtaining an optimized real-time PCR result, simultaneous processing of a large number of samples and assays, performing PCR and RT-PCR on cell lysate without extraction of DNA or RNA, detecting false-positive PCR results, detecting organisms in viral and microbial diseases and hospital environment, following safety assessments of food products, and using PCR for introduction of mutations. This is a must-have book for any PCR laboratory.
Next generation sequencing (NGS) has surpassed the traditional Sanger sequencing method to become the main choice for large-scale, genome-wide sequencing studies with ultra-high-throughput production and a huge reduction in costs. The NGS technologies have had enormous impact on the studies of structural and functional genomics in all the life sciences. In this book, Next Generation Sequencing Advances, Applications and Challenges, the sixteen chapters written by experts cover various aspects of NGS including genomics, transcriptomics and methylomics, the sequencing platforms, and the bioinformatics challenges in processing and analysing huge amounts of sequencing data. Following an overview of the evolution of NGS in the brave new world of omics, the book examines the advances and challenges of NGS applications in basic and applied research on microorganisms, agricultural plants and humans. This book is of value to all who are interested in DNA sequencing and bioinformatics across all fields of the life sciences.
A cutting-edge collection of updated and core techniques for the neurological study of drugs of abuse. These readily reproducible protocols cover a wide variety of coherent methods for gathering information on quantitative changes in protein and mRNA at both tissue and cellular levels. There are various methods for detecting single and multiple alterations in single and multiple gene expression, for analyzing the functional roles of genes and proteins, for studying the release kinetics of striatal dopamine, and for the quantitative measurement of such neurotransmitters as acetylcholine.
This essential manual presents a comprehensive guide to the most appropriate and up-to-date technologies and applications as well as providing an overview of the theory of this important technique. Written by recognized experts in the field this timely and authoritative volume is an essential requirement for all laboratories using PCR. Topics covered include: Real-time PCR instruments and probe chemistries, set-up, controls and validation, quantitative real-time PCR, analysis of mRNA expression, mutation detection, NASBA, application in clinical microbiology and diagnosis of infection.
Essential manual providing a comprehensive guide to the most up-to-date technologies and applications as well as providing an overview of the theory of this increasingly important technique.
Calculations for Molecular Biology and Biotechnology: A Guide to Mathematics in the Laboratory, Second Edition, provides an introduction to the myriad of laboratory calculations used in molecular biology and biotechnology. The book begins by discussing the use of scientific notation and metric prefixes, which require the use of exponents and an understanding of significant digits. It explains the mathematics involved in making solutions; the characteristics of cell growth; the multiplicity of infection; and the quantification of nucleic acids. It includes chapters that deal with the mathematics involved in the use of radioisotopes in nucleic acid research; the synthesis of oligonucleotides; the polymerase chain reaction (PCR) method; and the development of recombinant DNA technology. Protein quantification and the assessment of protein activity are also discussed, along with the centrifugation method and applications of PCR in forensics and paternity testing. Topics range from basic scientific notations to complex subjects like nucleic acid chemistry and recombinant DNA technology Each chapter includes a brief explanation of the concept and covers necessary definitions, theory and rationale for each type of calculation Recent applications of the procedures and computations in clinical, academic, industrial and basic research laboratories are cited throughout the text New to this Edition: Updated and increased coverage of real time PCR and the mathematics used to measure gene expression More sample problems in every chapter for readers to practice concepts
Cardiac Gene Expression: Methods and Protocols presents both cutting-edge and established methods for studying cardiac gene expression. The protocols provide a template for solid research, and cover the process through screening, analysis, characterization, and functional confirmation of novel genes or known genes with a new function.
This volume provides experimental and bioinformatics approaches related to different aspects of gene expression analysis. Divided in three sections chapters detail wet-lab protocols, bioinformatics approaches, single-cell gene expression, highly multiplexed amplicon sequencing, multi-omics techniques, and targeted sequencing. Written in the highly successful Methods in Molecular Biology series format, chapters include introductions to their respective topics, lists of the necessary materials and reagents, step-by-step, readily reproducible laboratory protocols, and tips on troubleshooting and avoiding known pitfalls. Authoritative and cutting-edge, Gene Expression Analysis: Methods and Protocols aims provide useful information to researchers worldwide.
This book is a comprehensive manual to allow both the novice researcher and the expert to set up and carry out quantitative PCR assays from scratch. However, this book also sets out to explain as many features of qPCR as possible, provide alternative viewpoints, methods, and aims to simulate the researchers into generating, interpreting, and publishing data that are reproducible, reliable, and biologically meaningful
Gene QuantificationSpringer Science & Business Media
A comprehensive account of recent research in translational control and the molecular mechanisms involved, focusing on the numerous control mechanisms observed in eukaryotes. Subjects include basic mechanisms; the role of phosphorylation; regulation by trans-acting proteins; effects of viral infection; and mRNA stability. Other topics include translational control mediated by upstream AUG codons; a comparative view of initiation site selection mechanisms; and genetics of mitochondrial translation. For researchers with interests in gene expression, RNA biology, and protein synthesis. Annotation copyright by Book News, Inc., Portland, OR
Epigenetics and Systems Biology highlights the need for collaboration between experiments and theoretical modeling that is required for successful application of systems biology in epigenetics studies. This book breaks down the obstacles which exist between systems biology and epigenetics researchers due to information barriers and segmented research, giving real-life examples of successful combinations of systems biology and epigenetics experiments. Each section covers one type of modeling and one set of epigenetic questions on which said models have been successfully applied. In addition, the book highlights how modeling and systems biology relate to studies of RNA, DNA, and genome instability, mechanisms of DNA damage signaling and repair, and the effect of the environment on genome stability. Presents original research in a wider perspective to reveal potential for synergies between the two fields of study Provides the latest experiments in primary literature for the modeling audience Includes chapters written by experts in systems biology and epigenetics who have vast experience studying clinical applications
The large potential of RNA sequencing and other "omics" techniques has contributed to the production of a huge amount of data pursuing to answer many different questions that surround the science's great unknowns. This book presents an overview about powerful and cost-efficient methods for a comprehensive analysis of RNA-Seq data, introducing and revising advanced concepts in data analysis using the most current algorithms. A holistic view about the entire context where transcriptome is inserted is also discussed here encompassing biological areas with remarkable technological advances in the study of systems biology, from microorganisms to precision medicine.

Biomaterials for Oral and Dental Tissue Engineering examines the combined impact of materials, advanced techniques and applications of engineered oral tissues. With a strong focus on hard and soft intraoral tissues, the book looks at how biomaterials can be manipulated and engineered to create functional oral tissue for use in restorative dentistry, periodontics, endodontics and prosthodontics. Covering the current knowledge of material production, evaluation, challenges, applications and future trends, this book is a valuable resource for materials scientists and researchers in academia and industry. The first set of chapters reviews a wide range of biomaterial classes for oral tissue engineering. Further topics include material characterization, modification, biocompatibility and biotoxicity. Part Two reviews strategies for biomaterial scaffold design, while chapters in parts three and four review soft and hard tissues. Connects materials science with restorative dentistry Focuses on the unique field of intraoral tissues Highlights long-term biocompatibility and toxicity of biomaterials for engineered oral tissues

Focusing on Saccharomyces cerevisiae, the second edition of Yeast Gene Analysis represents a major reworking of the original edition, with many completely new chapters and major revisions to all previous chapters. Originally published shortly after completion of the yeast genome sequence, the new edition covers many of the major genome-wide strategies that have been developed since then such as microarray analysis of transcription, synthetic gene array studies, protein microarrays and chemical genetic approaches. It represents a valuable resource for any research laboratory using budding yeast as their experimental system in which to identify new yeast gene functions. The chapters are written in a readable style with useful background information, technical tips and specific experimental protocols included as appropriate, enabling both the novice and the experienced yeast researcher to adopt new procedures with confidence. New chapters on: Strain construction; genome-wide two-hybrid approaches; use of microarrays for transcript analysis; real-time analysis of chromosome behaviour and FRET; synthetic gene array technology and protein arrays; chemical genomics and yeast prions; RNA gene analysis and mitochondrial gene function analysis; phylogenetic footprinting; discovering human gene function and predicting yeast gene function

Expert overviews of Bayesian methodology, tools and software for multi-platform high-throughput experimentation.

The advent of technologies specifically designed to capture glimpses of gene expression on a systems-wide scale has led to a revolution in our understanding of cellular dynamics, identifying the contributions and interactions of families of genes involved in cell development, dysfunction, and death. Broadly classified into count-based "digital" or signal-based "analogue" approaches, these technologies have permitted "portraits" of the transcriptome to be generated through comparative measurements of gene expression, enabling, for example, the generation of qualitative models of disease. However, truly predictive models of cellular function that can enhance our ability to discover new pharmaceuticals, detect and monitor disease, evaluate treatments, and ultimately, predict and prevent illness, require platforms that can provide detailed and accurate measurements of transcript abundances on an absolute scale. Unfortunately, inherent limitations preclude these technologies from providing this level of quantitative information. This thesis examines design issues associated with a popular digital approach to transcriptomics, serial analysis of gene expression (SAGE), that diminish us utility as a tool for absolute transcriptomics. Careful analysis of the processing steps involved in converting the starting mRNA population into short sequence tags (SST5) and subsequently into a format amenable to interrogation via sequencing technology reveals the introduction of strong biases and artifacts that limit reproducible abundance measurements in SAGE to transcripts present within the highest 2 orders of magnitude in the original sample. As a large number of steps are involved in formatting SSTs for analysis via sequencing, an alternative strategy is presented that utilizes a microarray-based analogue approach for the interrogation of SSTs. Termed the Universal Sequence Tag Array (U-STAR) platform, this platform is able to provide accurate quantitative measurements over a 3-deca.

This monograph equips clinicians with the knowledge required to detect oral cancer at the earliest possible stage while simultaneously inspiring researchers to work on novel methods of detection. All the methods employed in the oral cancer context are considered, from simple ones like oral screening to more complex emerging optical methods and biomarker identification strategies. Individual chapters focus on conventional oral screening and application of vital stains, optical methods like white light based fluorescence-reflectance imaging, narrow band imaging, direct-oral-microscopy, and more advanced methods like optical coherence tomography, an in-vivo optical biopsy technique, and photo-acoustic imaging that allows visualization of deeper tissue changes. Novel electrical methods like bio-impedance assessment, occult biophysical methods like crystallization test, and the most promising salivary biomarkers and point-of-care opportunities are covered. Helpful information is also provided on essential topics including, oral potentially malignant disorders, biological aspects and molecular mechanisms underlying oral cancer progression, global epidemiology, concept of diagnostic delays, traditional imaging, and classic histopathology and microscopic features. The newer techniques are currently of active research interest, and can soon become powerful chair-side tools with potential to reduce diagnostic delays and improve survival.

Quantitative Real-Time PCR: Methods and Protocols focuses on different applications of qPCR ranging from microbiological detections (both viral and bacterial) to pathological applications. Several chapters deal with quality issues which regard the quality of starting material, the knowledge of the minimal information required to both perform an assay and to set the experimental plan, while the others focus on translational medicine applications that are ordered following an approximate logical order of their medical application. The last part of the book gives you an idea of an emerging digital PCR technique that is a unique qPCR approach for measuring nucleic acid, particularly suited for low level detection and to develop non-invasive diagnosis. Written for the Methods in Molecular Biology series, most chapters include introductions to their respective topics, lists of the necessary materials and reagents, step-by-step, laboratory protocols and tips on troubleshooting and avoiding known pitfalls. Practical and authoritative, Quantitative Real-Time PCR: Methods and Protocols aims to aid researchers seeking to devise new qPCR-based approaches related to his or her area of investigation.

Rapid-Cycle Real-Time PCR is a powerful technique for nucleic acid amplification and analysis that often requires less than half an hour to perform. Samples are amplified by rapid-cycle PCR followed by immediate melting curve analysis in the same instrument. Melting curve analysis of PCR products with SYBR Green I often allows product identification without gel electrophoresis. Furthermore, in the presence of fluorescent hybridization probes, melting curves provide "dynamic dot blots" for fine sequence analysis, including single nucleotide polymorphisms (SNPs). The method is often cited as the most versatile, efficient method for nucleic acid analysis in research and diagnostics in the fields of genetics and oncology. Molecular diagnostics has never been easier!

PART I Molecular Biology 1. Molecular Biology and Genetic Engineering Definition, History and Scope 2. Chemistry of the Cell: 1. Micromolecules (Sugars, Fatty Acids, Amino Acids, Nucleotides and Lipids) Sugars (Carbohydrates) 3. Chemistry of the Cell . 2. Macromolecules (Nucleic Acids; Proteins and Polysaccharides) Covalent and Weak Non-covalent Bonds 4. Chemistry of the Gene: Synthesis, Modification and Repair of DNA DNA Replication: General Features 5. Organisation of Genetic Material 1. Packaging of DNA as Nucleosomes in Eukaryotes Techniques Leading to Nucleosome Discovery 6. Organization of Genetic Material 2. Repetitive and Unique DNA Sequences 7. Organization of Genetic Material: 3. Split Genes, Overlapping Genes, Pseudogenes and Cryptic Genes Split Genes or .Interrupted Genes 8. Multigene Families in Eukaryotes 9. Organization of Mitochondrial and Chloroplast Genomes 10. The Genetic Code 11. Protein Synthesis Apparatus Ribosome, Transfer RNA and Aminoacyl-tRNA Synthetases Ribosome 12. Expression of Gene . Protein Synthesis 1. Transcription in Prokaryotes and Eukaryotes 13. Expression of Gene: Protein Synthesis: 2. RNA Processing (RNA Splicing, RNA Editing and Ribozymes) Polyadenylation of mRNA in Prokaryotes Addition of Cap (m7G) and Tail (Poly A) for mRNA in Eukaryotes 14. Expression of Gene: Protein Synthesis: 3. Synthesis and Transport of Proteins (Prokaryotes and Eukaryotes) Formation of Aminoacyl tRNA 15. Regulation of Gene Expression: 1. Operon Circuits in Bacteria and Other Prokaryotes 16. Regulation of Gene

Expression . 2. Circuits for Lytic Cycle and Lysogeny in Bacteriophages 17. Regulation of Gene Expression 3. A Variety of Mechanisms in Eukaryotes (Including Cell Receptors and Cell Signalling) PART II Genetic Engineering 18. Recombinant DNA and Gene Cloning 1. Cloning and Expression Vectors 19. Recombinant DNA and Gene Cloning 2. Chimeric DNA, Molecular Probes and Gene Libraries 20. Polymerase Chain Reaction (PCR) and Gene Amplification 21. Isolation, Sequencing and Synthesis of Genes 22. Proteins: Separation, Purification and Identification 23. Immunotechnology 1. B-Cells, Antibodies, Interferons and Vaccines 24. Immunotechnology 2. T-Cell Receptors and MHC Restriction 25. Immunotechnology 3. Hybridoma and Monoclonal Antibodies (mAbs) Hybridoma Technology and the Production of Monoclonal Antibodies 26. Transfection Methods and Transgenic Animals 27. Animal and Human Genomics: Molecular Maps and Genome Sequences Molecular Markers 28. Biotechnology in Medicine: l.Vaccines, Diagnostics and Forensics Animal and Human Health Care 29. Biotechnology in Medicine 2. Gene Therapy Human Diseases Targeted for Gene Therapy Vectors and Other Delivery Systems for Gene Therapy 30. Biotechnology in Medicine: 3. Pharmacogenetics / Pharmacogenomics and Personalized Medicine Phannacogenetics and Personalized 31. Plant Cell and Tissue Culture' Production and Uses of Haploids 32. Gene Transfer Methods in Plants 33. Transgenic Plants . Genetically Modified (GM) Crops and Floricultural Plants 34. Plant Genomics: 35. Genetically Engineered Microbes (GEMs) and Microbial Genomics References

A Top 25 CHOICE 2016 Title, and recipient of the CHOICE Outstanding Academic Title (OAT) Award. How much energy is released in ATP hydrolysis? How many mRNAs are in a cell? How genetically similar are two random people? What is faster, transcription or translation?Cell Biology by the Numbers explores these questions and dozens of others provid

Microbial Endophytes: Functional Biology and Applications focuses on endophytic bacteria and fungi, including information on foundational endophytes and the latest advances in relevant genomics, proteomics and nanotechnological aspects. The book provides insights into the molecular aspects of plant endophytes and their interactions and applications, also exploring the potential commercialization of endophytic microorganisms and their use as bio fertilizers, in biocontrol, and as bioactive compounds for other sustainable applications. Coverage of important and emerging legal considerations relevant to those working to implement these important bacteria in production processes is also included. Presents discussion on entry, colonization and the distribution of endophytic microorganisms Explores the phyto immunological functions of endophytic microorganisms Provides genomic insights on plant endophyte interaction Identifies bio-commercial aspects of microbial endophytes for sustainable agriculture, including potential legal issues and IPR in microbial research

This book is intended to present current concepts in molecular biology with the emphasis on the application to animal, plant and human pathology, in various aspects such as etiology, diagnosis, prognosis, treatment and prevention of diseases as well as the use of these methodologies in understanding the pathophysiology of various diseases that affect living beings.

Real time quantitative PCR (qPCR) technology has revolutionized almost all areas of microbiology, including clinical microbiology, food microbiology, industrial microbiology, environmental microbiology, and microbial biotechnology. Various modifications and improvements have enhanced the overall performance of this highly versatile technology and the qPCR instrumentation and strategies currently available are more sensitive, faster, and more affordable than ever before. Written by experts in the field and aimed specifically at microbiologists, this book describes and explains the most important aspects of current qPCR strategies, instrumentation, and software. Renowned scholars cover the application of qPCR technology in various areas of applied microbiology and comment on future trends. Topics include: instrumentation * fluorescent chemistries * quantification strategies * data analysis software * environmental microbiology * water microbiology * food microbiology * gene expression studies * validation of microbial microarray data * future trends in qPCR technology. This outstanding book will be invaluable for all microbiologists and is recommended for all microbiology laboratories.

Geneticists and molecular biologists have been interested in quantifying genes and their products for many years and for various reasons (Bishop, 1974). Early molecular methods were based on molecular hybridization, and were devised shortly after Marmur and Doty (1961) first showed that denaturation of the double helix could be reversed - that the process of molecular reassociation was exquisitely sequence dependent. Gillespie and Spiegelman (1965) developed a way of using the method to titrate the number of copies of a probe within a target sequence in which the target sequence was fixed to a membrane support prior to hybridization with the probe - typically a RNA. Thus, this was a precursor to many of the methods still in use, and indeed under development, today. Early examples of the application of these methods included the measurement of the copy numbers in gene families such as the ribosomal genes and the immunoglo bulin family. Amplification of genes in tumors and in response to drug treatment was discovered by this method. In the same period, methods were invented for estimating gene num bers based on the kinetics of the reassociation process - the so-called Cot analysis. This method, which exploits the dependence of the rate of reassociation on the concentration of the two strands, revealed the presence of repeated sequences in the DNA of higher eukaryotes (Britten and Kohne, 1968). An adaptation to RNA, Rot analysis (Melli and Bishop, 1969), was used to measure the abundance of RNAs in a mixed population.

Molecular Toxicology Protocols, Second Edition aims to bring together a series of articles describing validated methods to elucidate specific molecular aspects of toxicology, the emphasis being on the application of molecular methods to genetic toxicology. The volume is divided into ten parts, roughly corresponding to the spectrum of biomarkers intermediate between exposure and disease outcomes as proposed in molecular epidemiology models. Subjects of these new chapters range from preparation of fluid specimens for analysis of cellular inflammatory responses to genotoxic insults to sensitive methods for proteomic analysis and aberrant DNA methylation patterns. Written in the successful Methods in Molecular Biology series format, chapters include introductions to their respective topics, lists of the necessary materials and reagents, step-by-step, readily reproducible protocols, and notes on troubleshooting and avoiding known pitfalls. Authoritative and easily accessible, Molecular Toxicology Protocols, Second Edition addresses not only the needs of molecular biologists and toxicologists, but also those of individuals interested in applying molecular methods to clinical applications, such as geneticists, pathologists, biochemists, and epidemiologists.

Variations in gene expression are commonly considered the major determinants for dictating cell behavior. Accordingly, methods to measure gene expression, such as reverse-transcriptase (RT) PCR and DNA microarrays, have proven to be invaluable in regards to understanding cell regulatory processes and disease mechanisms. However, these methods generally provide only the relative change in gene expression for a population of cells with limited spatialtemporal information. We hypothesize that in order to acquire a more complete gene expression profile, a molecular imaging approach must be developed to allow for the absolute quantification of gene expression in single living cells. We have developed a novel molecular imaging probe, Quantitative Molecular Beacon (QMB), that allows for the absolute quantification of gene expression in single living cells with spatial and temporal resolution. Analogous to conventional MBs, QMBs consist of a hairpin-forming antisense oligonucleotide labeled with a reporter fluorophore and a quencher. Furthermore, QMBs are labeled with a second optically distinct "reference" dye/nanoparticle that remains unquenched regardless of the probe configuration. The reference signal allowed us to determine the intracellular distribution of QMBs, while the fluorescence ratio of the reporter dye to the reference dye (Freporter/Freference) provided us with a measure of the extent of probe hybridization. By comparing these QMB signals in single living cells with standardization curves constructed in vitro, we were able to obtain absolute measurements of RNA in single living cells. Additionally, we developed a method for the efficient cytosolic delivery of QMBs into living cells with low cytotoxicity. This allowed QMBs to be utilized for the high-throughput detection of gene expression via flow cytometry. With further refinement of the QMB design, it is envisioned that QMBs will become a valuable tool for diagnosing genetic abnormalities.