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Quantitative Real-Time PCR Real-time PCR Gene Quantification Rapid Cycle Real-Time PCR — Methods and Applications Quantitative Real-time RT-PCR Based Transcriptomics Quantitative Real-time PCR in Applied Microbiology Real-time PCR Quantitative Real-time Pcr Quantitative Real-time RT-PCR Based Transcriptomics Rapid Cycle Real-Time PCR Polymerase Chain Reaction for Biomedical Applications PCR Technology RT-PCR Protocols Standardization and Quality Assurance in Fluorescence Measurements II Quantitative Real-time Polymerase Chain Reaction (QPCR) Assay as a Molecular Tool to Assess Rickettsial Replications in Tick Hosts Single Cell Methods Accurate Normalization of Real-time Quantitative Rt-pcr Data by Geometric Averaging of Multiple Internal Control Genes Quantitative Real-Time

Polymerase Chain Reaction (qPCR) of Filamentous Fungi in Carpet A Rapid Quantitative Real-time PCR (qPCR)-based DNA Quantification Assay Coupled with Species-determination Capabilities for Two Hybridizing Macaca Species The Reliability and Validity of Real-time PCR Data in Biomedical Sciences Early, rapid and sensitive veterinary molecular diagnostics - real time PCR applications Development and Use of a Real-time Quantitative Reverse Transcriptase PCR System for Analyzing Gene Expression in Listeria Monocytogenes Quantitative Real-time PCR Analysis of Oxidative Stress Related Genes in Enteric Pathogens Development of Multiplex Quantitative Real-Time PCR for Detection of Common Viral Infections of Central Nervous System Development of a Quantitative Real-Time Polymerase Chain Reaction Assay Specific for Orientia Tsutsugamushi Real Time Quantitative RT-PCR Quantification of Cryptosporidium Parvum and Enteroviruses by Quantitative Real-time PCR (qPCR) in Environmental Samples RT-PCR Protocols Quantitative Analysis and Modeling of Earth and Environmental Data A-Z of Quantitative PCR Laboratory Methods in Enzymology: DNA A Quantitative Real Time PCR Assay for Detecting BK Virus in Serum, Plasma and Urine The PCR Revolution Detection and Pathogenesis Study of CSFV Using a Quantitative Real Time RT-PCR Assay Quantitative Real Time PCR Assay for Detecting EBV Virus in Multiple Sample

Types Forecasting: principles and practice Comparison of Quantitative Real-time PCR (qPCR) and Droplet Digital PCR (ddPCR) for Quantification of Lactobacillus Reuteri in Human Feces Verification of Next Generation Sequencing Gene Expression Data by Quantitative Real-time Polymerase Chain Reaction in Vertebral Bone Marrow Stromal Cells from Modic Changes Quantitating Retroviral Titer with Quantitative Real-time Polymerase Chain Reaction Specific PCR and Quantitative Real Time PCR in Ocular Samples from Acute and Delayed-onset Post Operative Endophtalmitis

The human gut microbiota, a complicated microbial community consisting of trillions of microorganisms, has been recognized as of great importance for maintaining human health. To establish direct linkages between a certain group of gut microorganisms and the physiological status of the host, it is critical to quantify their abundance with high sensitivity, accuracy, and reproducibility. Quantitative real-time PCR (qPCR) has been widely used in the absolute quantification of microorganisms by comparing PCR cycle numbers with those of a standard curve. Recently, the development of droplet digital PCR (ddPCR) has demonstrated the potential to handle samples with a complex background without standard curves. The goal of this work was to compare absolute quantification of a specific bacterial strain (Lactobacillus reuteri DSM 17938) in

human stool between qPCR and ddPCR with three commonly used DNA extraction methods (QIAamp Fast Stool DNA Kit [QK], phenol chloroform [PC], protocol Q [PQ]). DNA extracted using QK and PQ had acceptable quantity and high quality while PC produced DNA with highest concentration but lower purity. Compared to the other two methods, PQ recovered the most substantial proportion of L. reuteri cells from feces. Generally, reproducibility was better in ddPCR than qPCR with methods QK and PC, but comparable with PQ. Within the detectable range, both qPCR and ddPCR presented better linearity with DNA extracted using methods QK and PQ than PC. For qPCR, the limit of detection (LOD) was 3.95 Log10, 4.86 Log10, 4.11 Log10 CFU/g feces with QK, PC and PQ. ddPCR exhibited a lower limit of quantification (LOQ) when compared to qPCR. The LOQ was 4.30 Log10 CFU/g feces when QK and ddPCR were combined and was slightly higher in use of PQ and qPCR (4.50 Log10 CFU/g feces). However, the cost of ddPCR per unit was 3 times higher than qPCR and is more time-consuming (6.5 h vs. 2.5 h). Therefore, the combination of PQ and qPCR is suggested as the best to detect L. reuteri in fecal samples. The second goal of my project was to use the information above and design strain/lineage-specific qPCR systems for the absolute quantification of L. reuteri PB-W1 and DSM 20016T in fecal samples. To achieve this, strain/lineage-specific primers were designed using a

database approach. The LOD for PB-W1 measurement was 3.05 Log10 CFU/g feces, which was the lowest level reached in the field, and for DSM 20016T, it was 2.96 Log10 CFU/g feces, which was equally decent. Overall, this work successfully established approaches for a quantitative, selective, accurate, and sensitive quantification of bacterial strains in human fecal samples based on qPCR and ddPCR and provided information to select the appropriate methods for measurement and DNA extraction. Examines the latest innovations and the overall impact of PCR on areas of molecular research. PCR's simplicity as a molecular technique is, in some ways, responsible for the huge amount of innovation that surrounds it, as researchers continually think of new ways to tweak, adapt, and re-formulate concepts and applications. PCR Technology: Current Innovations, Third Edition is a collection of novel methods, insights, and points of view that provides a critical and timely reference point for anyone wishing to use this technology. Topics in this forward-thinking volume include: The purification and handling of PCR templates The effect of the manufacture and purification of the oligonucleotide on PCR behavior Optimum buffer composition Probe options The design and optimization of qPCR assays Issues surrounding the development and refinement of instrumentation Effective controls to protect against uncertainties due to reaction variability Covering all aspects of PCR and

real-time PCR, the book contains detailed protocols that make it suitable as both a reference and an instruction manual. Each chapter presents detailed guidelines as well as helpful hints and tips supplied by authors who are recognized experts in their fields. In addition to descriptions of current technology and best practices, the book also provides information about new developments in the PCR arena. The first comprehensive treatise on Rapid Cycle Real-Time PCR. With amplification times of 15-30 minutes of on-line detection and analysis, nucleic acid quantification of mutation analysis finally becomes a routine, powerful and rapid method. Focusing primarily on the LightCycler, an instrument that combines Rapid Cycle PCR with fluorescent monitoring, this technology provides convenient analysis by melting temperatures. PCR products can be identified by product Tm, and single base mismatches can easily be genotyped by probe Tm. Methods chapters detail the theory behind quantification of mutation analysis; the design of synthesis of fluorescent hybridization probes of the preparation of template DNA. Application chapters apply nucleid acid quantification to infectious organisms of intracellular messengers and mutation detection to somatic of acquired mutations. 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. Seminar paper from the year 2014 in the subject Statistics, grade: B, National University of Modern Languages, Islamabad (NUML), course: Statistics, language: English, abstract: Despite a fairly broad implementation and application of real-time PCR, there still exists a vacuum in determining the correct procedures for the examination of quantitative real-time PCR; more explicitly, there is a need to determine appropriate procedures to attain the right kind of statistical treatment. In today’s various methods of data analysis, the key statistical inferences are not as exclusive as required like confidence interval. This paper presents and tends to relate four statistical models and approaches on the basis of standard curve method and methods for data analysis. The first approach developed a multiple regression analysis model for the determination of ??Ct directly from the approximation of interface of gene and treatment paraphernalia. The second approach

used the analysis of covariance i.e. ANCOVA model where the derivation of ??Ct could be made through the sequential evaluation and analysis of effects of concurrent variables. The remainder of the models chiefly involves the calculation of ?Ct subsequently connected through the non-parametric comparable Wilcoxon test and a two group T-test. Moreover, a data quality control model was established, which was then applied through the SAS programs determined for all of the aforementioned approaches; analyzed data output was also presented for a sample set. The SAS programs were used to put forward practical statistical solutions for real-time PCR data while the programs were also utilized to analyze a sample dataset. After a comprehensive analysis conducted through the approaches and models mentioned above, similar results were obtained. Forecasting is required in many situations. Stocking an inventory may require forecasts of demand months in advance. Telecommunication routing requires traffic forecasts a few minutes ahead. Whatever the circumstances or time horizons involved, forecasting is an important aid in effective and efficient planning. This textbook provides a comprehensive introduction to forecasting methods and presents enough information about each method for readers to use them sensibly. Methods in Enzymology volumes provide an indispensable tool for the researcher. Each volume is carefully written and edited by experts to contain state-

of-the-art reviews and step-by-step protocols. In this volume, we have brought together a number of core protocols concentrating on DNA, complementing the traditional content that is found in past, present and future Methods in Enzymology volumes. Indispensable tool for the researcher Carefully written and edited by experts to contain step-by-step protocols In this volume we have brought together a number of core protocols concentrating on DNA 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 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! 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. This dissertation, "Development of Multiplex Quantitative Real-time PCR for Detection of Common Viral Infections of Central Nervous System" by Ho-yan, Mandy, Chan, ???, was obtained from The University of Hong Kong (Pokfulam, Hong Kong) and is being sold pursuant to Creative Commons: Attribution 3.0 Hong Kong License. The content of this dissertation has not been altered in any way. We have altered the formatting in order to facilitate the ease of printing and reading of the dissertation. All rights not granted by the above license are retained by the author. DOI: 10.5353/th_b4663185 Subjects: Central nervous system - Infections - Diagnosis Gene-expression analysis is increasingly important in biological research, with real-time reverse transcription PCR

(RT-PCR) becoming the method of choice for high-throughput and accurate expression profiling of selected genes. Given the increased sensitivity, reproducibility and large dynamic range of this methodology, the requirements for a proper internal control gene for normalization have become increasingly stringent. Although housekeeping gene expression has been reported to vary considerably, no systematic survey has properly determined the errors related to the common practice of using only one control gene, nor presented an adequate way of working around this problem. We outline a robust and innovative strategy to identify the most stably expressed control genes in a given set of tissues, and to determine the minimum number of genes required to calculate a reliable normalization factor. We have evaluated ten housekeeping genes from different abundance and functional classes in various human tissues, and demonstrated that the conventional use of a single gene for normalization leads to relatively large errors in a significant proportion of samples tested. The geometric mean of multiple carefully selected housekeeping genes was validated as an accurate normalization factor by analyzing publicly available microarray data. The normalization strategy presented here is a prerequisite for accurate RT-PCR expression profiling, which, among other things, opens up the possibility of studying the biological relevance of small expression differences. Proceeds from the sale of this book go to the support of an elderly disabled

person. This book is written for both the beginner and the expert in real–time PCR. It starts with a comprehensive background explaining real–time PCR technology, as well as reverse transcription and immuno–QPCR. The book focuses on the quantitative aspects of real–time PCR, including experimental design, and data pre–processing and analysis. It covers the areas of absolute quantification, relative quantification, and expression profiling. Descriptive statistics, univariate statistics, and basic multivariate statistics are also discussed. Several chapters have expert co–authors to ensure the book is a comprehensive, authoritative whole. Quantitative real-time polymerase chain reaction (qRT-PCR) is a new method for reliable quantification of low-abundance mRNA in biological samples. Since the strength of the fluorescence signal emitted by the report dye is proportional to the produced DNA amount, the fluorescence monitoring enables visualisation of the full reaction trajectory. The reaction trajectory can be then extrapolated back to an input concentration. RNA extraction can introduce unwanted contaminants into the sample, inhibiting the reverse transcription (RT) as well as the PCR reaction. These inhibitions cause then the reaction to precede sample-specific. In addition, the amplification efficiency varies not only between samples, but also along the recorded amplification trajectory of a single sample. Consequently, a correct determination of each probe’s PCR efficiency as well as a good standardization

of the raw expression estimators is of great importance for a correct interpretation of results. To find a solution to above problems a series of biological experiments with RNA extracted from various ovine and bovine tissues and from cultured leukocytes was carried out. Constant amount of RNA was then reverse–transcribed to cDNA. All PCR runs were performed on a LightCycler instrument and Fluorescence data was saved in the LightCycler software. Based on this data, mathematical models together with statistical procedures were developed and validated. These can investigate the optimal quantification range and exactly determine its real-time PCR efficiency. Additionally, methods were developed to disclose heterogeneity between probes. All these procedures contribute to better quality of results obtained. Resulting from these standardisations, a decision algorithm for a proper analysis of the qRT-PCR data was designed. Objective: To evaluate the contribution of specific PCR targeting S. aureus and S. pneumonia for the identification of these species and the quantitative PCR for endophthalmitis due to S. epidermidis in ocular samples from patients with acute or delayed-onset post-operative endophthalmitis. Methods: 153 consecutive patients presenting with acute or delayed-onset postoperative endophthalmitis were included in three university hospitals between 2008 and 2016. A total of 284 ocular samples were obtained from aqueous humor (AH) or vitreous fluids (VF): 151 samples at admission

and 133 after one intravitreal injection of antibiotics. Microbiological techniques include bacterial culture after inoculation into pediatric blood culture bottle, 16S PCR, real-time PCR assays for the detection of specific sequence regions of S. aureus femA gene and S. pneumoniae lytA gene. Quantitative real-time PCR assay targeting the tuf gene of Staphylococcus spp was used for 25 patients infected with S. epidermidis. Results: At the time of admission; the detection rate was not significantly different between PCR and cultures (38% versus 30% in AH samples; 66% versus 63% in vitreous fluid, p=0.6). ln contrast, after one intravitreous injection of antibiotics, the identification rate was higher using PCR (62%) in VF than using culture (48%, p=0.05). Bacteria was identified in 70% with a predominance of Gram-positive bacteria (93%). Specific PCR targeting S. aureus and S. pneumonia were notable to make additional diagnosis. Quantitative PCR performed before and after treatment found no significant changes in bacterial load after one intravitreal injection (51803 ±47161 DNA copies/ml at admission versus 95737 ±147431 after antibiotic treatment, p=0.6). Conclusion: Identification rate was better in vitreous fluid than in aqueous humor. 16s PCR is more effective than cultures in detecting bacteria in vitreous samples after intravitreal administration of antibiotics. PCR targeting S. aureus and S. pneumonia were specific but, with the low frequency of endophthalmitis due to these virulent

bacteria, sensitivity was not determined. Real-time quantitative PCR targeting the tuf gene of Staphylococcus spp showed high level of bacterial load and no change of DNA copies after one intravitreal injection of antibiotics. Once a tedious, highly skilled operation, reverse-transcription polymerase chain reaction (RT-PCR) has become a routine and invaluable technique used in most laboratories. In RT-PCR Protocols, Second Edition, expert researchers fully update the technologies presented in the popular previous edition, such as competitive RT-PCR, nested RT-PCR, RT-PCR from single cells, and RT-PCR for cloning. In addition, newer technologies are also explored, including multiplex RT-PCR, RT-LATE-PCR, and the greatly advanced field of real-time quantitative RT-PCR, while recent advances in creating the optimum RT-PCR reaction, e.g. RNA extraction, primer design, and reverse transcription, end the book with their indispensable input. Written in the highly successful Methods in Molecular BiologyTM series format, chapters include brief introductions to their respective topics, lists of the necessary materials and reagents, step-by-step, readily reproducible protocols, and notes sections, highlighting tips on troubleshooting and avoiding known pitfalls. User friendly and up-to-date, RT-PCR Protocols, Second Edition acts as a handy companion to scientists from numerous diverse backgrounds who wish to explore further the marvels of gene expression. 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.

Rhesus and long-tailed macaques are the most frequently used non-human primate models for grant-funded and private industry biopharmaceutical research. Studies of regional populations of rhesus and long-tailed macaques indicate a fundamental difference in mitochondrial DNA (mtDNA), short tandem repeat (STR) and single nucleotide polymorphism (SNP) variation among insular (e.g., Indonesia, Sarawak, the Philippines) and mainland (e.g., Indochina, Thailand, Burma and Peninsular Malaysia population. Therefore, the suitability of a given population may vary according to the particular type of research. Some of these studies have revealed genetic admixture between these species due to natural hybridization as well as human-assisted intercrosses. For these reasons, a quantitative real-time PCR (qPCR) assay was developed to efficiently determine the species of origin of a macaque biological sample, as well as to quantify the species-specific template DNA prior to downstream research. Prior knowledge of species identity and DNA concentrations are both very important for maintaining cost-effective methods and accurate DNA analysis. One hundred and sixteen rhesus (from Burma, China and India) and long-tailed macaque (Indonesia, Mauritius, the Philippines and Vietnam) DNA samples were amplified using qPCR to determine the species and quantity of DNA. Unlabelled oligonucleotide primers and species-specific TaqMan[superscript TM] probes were designed and each

sample was examined using qPCR. Of the 19 Vietnamese long-tailed macaques, three samples were discovered to be hybrids. A standard dilution curve was assembled from two high concentration reference samples. By using this assay, primatologists and biomedical researchers will have the ability to rapidly quantify and speciate rhesus and long-tailed macaque DNA samples obtained from biomaterial discovered in the wild as well those derived from primate products, including live animals imported into the US for biomedical research. Until the mid 1980s, the detection and quantification of a specific mRNA was a difficult task, usually only undertaken by a skilled molecular biologist. With the advent of PCR, it became possible to amplify specific mRNA, after first converting the mRNA to cDNA via reverse transcriptase. The arrival of this technique—termed reverse transcription-PCR (RT-PCR)—meant that mRNA suddenly became amenable to rapid and sensitive analysis, without the need for advanced training in molecular biology. This new accessibility of mRNA, which has been facilitated by the rapid accumulation of sequence data for human mRNAs, means that every biomedical researcher can now include measurement of specific mRNA expression as a routine component of his/her research plans. In view of the ubiquity of the use of standard RT-PCR, the main objective of RT-PCR Protocols is essentially to provide novel, useful applications of RT-PCR. These include some useful adaptations

and applications that could be relevant to the wider research community who are already familiar with the basic RT-PCR protocol. For example, a variety of different adaptations are described that have been employed to obtain quantitative data from RT-PCR. Quantitative RT-PCR provides the ability to accurately measure changes/imb-ances in specific mRNA expression between normal and diseased tissues. Two specific and sensitive polymerase chain reaction (PCR) assays were developed to detect and quantitate Orientia tsutsugamushi, the agent of scrub typhus, using a portion of the 47-kD outer membrane protein antigen/ high temperature requirement A gene as the target. A selected 47-kD protein gene primer pair amplified a 118-basepair fragment from all 26 strains of O. tsutsugamushi evaluated, but it did not produce amplicons when 17 Rickettsia and 18 less-related bacterial nucleic acid extracts were tested. Similar agent specificity for the real-time PCR assay, which used the same primers and a 31-basepair fluorescent probe, was demonstrated. This sensitive and quantitative assay determination of the content of O. tsutsugamushi nucleic acid used a plasmid containing the entire 47-kD gene from the Kato strain as a standard. Enumeration of the copies of O. tsutsugamushi DNA extracted from infected tissues from mice and monkeys following experimental infection with Orientia showed 27 5,552 copies/ L of mouse blood, 14,448 86,012 copies/ L of mouse liver/spleen homogenate, and 3 21

copies/ L of monkey blood. In this study a real time RT-PCR diagnostic assay for Classical Swine Fever (CSFV) was developed. The developed Taqman assay together with the MagNaPure LC could be a valuable tool in monitoring the CSFV free status of Australia and assist in diagnosis of a possible outbreak. This book gives a comprehensive account of the practical aspects of Real time PCR and its application to veterinary diagnostic laboratories. The optimisation of assays to help diagnose livestock diseases is stressed and exemplified through assembling standard operating procedures from many laboratory sources. Theoretical aspects of PCR are dealt with as well as quality control features necessary to maintain an assured testing system. The book will be helpful to all scientists involved in diagnostic applications of molecular techniques, but is designed primarily to offer developing country scientists a collection of working methods in a single source. The book is an adjunct to the Molecular Diagnostic PCR Handbook published in 2005. 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. 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. 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. Analytical chemists and materials scientists will find this a useful addition to their armory. The contributors have sought to highlight the present state of affairs in the validation and quality assurance of fluorescence measurements, as well as the need for future standards. Methods included range from steady-state fluorometry and microfluorometry, microscopy, and micro-array technology, to time-resolved fluorescence and fluorescence depolarization

imaging techniques. Abstract: Listeria monocytogenes is one of the most dangerous foodborne pathogens, capable of causing severe illness and death in susceptible individuals. The bacterium uses several virulence factors to invade host cells and cause disease; one of the key players is internalin. L. monocytogenes uses internalins to adhere to and invade human intestinal epithelial cells, making the expression of these virulence genes a critical early step in its pathogenecity .However, proper quantitative and rapid evaluation of gene expression is still a major challenge in L. monocytogenes studies. Due to the lack of standard quantitative analysis platforms, revealing the contribution of food ingredients and processing conditions on L. monocytogenes virulence is extremely difficult. The primary aim of this study was to develop a quantitative real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR) platform to study the impact of environmental factors on L. monocytogenes virulence. A rapid semi-quantitative gene expression platform was established using the L. monocytogenes ribosomal protein L4 encoding gene as an internal standard. Using this platform, the effect of environmental factors on L. monocytogenes virulence was assessed by monitoring the expression of the inlA gene, which encodes for the L. monocytogenes virulence factor internalin A protein. The impact of conditions found in both the host and the food processing and storage environment, including lower

temperatures and higher acidity, was evaluated. Results of this study show that inlA expression is significantly upregulated at pH 5.0 in the presence of HCl at 370C (110% increase) and 250C (42% increase), while lactic and acetic acids have less effect (81% increase at 370C and no statistically significant difference at 250C). None of the acids caused a significant increase in expression at 40C. These fmdings show that HCl, found in the host stomach and small intestine, may play an important role triggering L. monocytogenes virulence; acids commonly found in foods also significantly enhanced the expression of inlA. The results suggest that certain food processing or storage conditions might have contributed to the increased L. monocytogenes virulence. The rapid quantitative gene expression analysis method developed in the study enables future studies to effectively screen for food ingredients or storage conditions that might inhibit L. monocytogenes virulence, which is important for food safety and public health. This volume provides a comprehensive overview for investigating biology at the level of individual cells. Chapters are organized into eight parts detailing a single-cell lab, single cell DNA-seq, RNA-seq, single cell proteomic and epigenetic, single cell multi-omics, single cell screening, and single cell live imaging. 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, Single Cell Methods: Sequencing and Proteomics aims to make each experiment easily reproducible in every lab. Quantitative Analysis and Modeling of Earth and Environmental Data: Space-Time and Spacetime Data Considerations introduces the notion of chronotopologic data analysis that offers a systematic, quantitative analysis of multi-sourced data and provides information about the spatial distribution and temporal dynamics of natural attributes (physical, biological, health, social). It includes models and techniques for handling data that may vary by space and/or time, and aims to improve understanding of the physical laws of change underlying the available numerical datasets, while taking into consideration the in-situ uncertainties and relevant measurement errors (conceptual, technical, computational). It considers the synthesis of scientific theory-based methods (stochastic modeling, modern geostatistics) and data-driven techniques (machine learning, artificial neural networks) so that their individual strengths are combined by acting symbiotically and complementing each other. The notions and methods presented in Quantitative Analysis and Modeling of Earth and Environmental Data: Space-Time and Spacetime Data Considerations cover a wide range of data in various forms and sources, including hard measurements, soft observations, secondary

information and auxiliary variables (ground-level measurements, satellite observations, scientific instruments and records, protocols and surveys, empirical models and charts). Including real-world practical applications as well as practice exercises, this book is a comprehensive step-by-step tutorial of theory-based and data-driven techniques that will help students and researchers master data analysis and modeling in earth and environmental sciences (including environmental health and human exposure applications). Explores the analysis and processing of chronotopologic (i.e., space-time and spacetime) data that varies spatially and/or temporally, which is the case with the majority of data in scientific and engineering disciplines Studies the synthesis of scientific theory and empirical evidence (in its various forms) that offers a mathematically rigorous and physically meaningful assessment of real-world phenomena Covers a wide range of data describing a variety of attributes characterizing physical phenomena and systems including earth, ocean and atmospheric variables, environmental and ecological parameters, population health states, disease indicators, and social and economic characteristics Includes case studies and practice exercises at the end of each chapter for both real-world applications and deeper understanding of the concepts presented

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