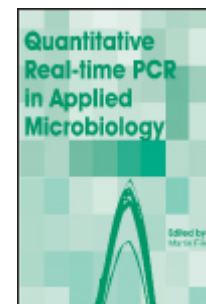


Quantitative Real-time PCR in Applied Microbiology



Edited by: **Martin Filion**

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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 affordable than ever before.

Written by experts in the field and aimed specifically at microbiologists, this volume describes and explains the most important aspects of current qPCR strategies, instrumentation and software. Renowned authors cover the application of qPCR technology in various areas of applied microbiology and comment on future trends. Topics covered include instrumentation, fluorescent chemistries, quantification strategies, data analysis software, environmental microbiology, water microbiology, food microbiology, gene expression studies, validation of microbial microarray data and future trends in qPCR technology.

The editor and authors have produced an outstanding book that will be invaluable for all microbiologists. A recommended book for all microbiology laboratories.

Chapter 1. An Introduction to the Real-time Polymerase Chain Reaction (qPCR). *Stephen A Bustin, Sara Zaccara and Tania Nolan*

Chapter 2. Instrumentation and Fluorescent Chemistries Used in qPCR. *Mathilde H. Josefsen, Charlotta Löfström, Trine Hansen, Eyjólfur Reynisson and Jeffrey Hoorfar*

Chapter 3. Quantification Strategies in Real-time RT-PCR (RT-qPCR). *Michael W. Pfaffl*

Chapter 4. Genex: Data Analysis Software. *Mikael Kubista, Vendula Rusnakova, David Svec, Björn Sjögreen and Ales Tichopad*

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Chapter 6. Quantification of Microorganisms Using a Functional Gene Approach. *Lia C.R.S. Teixeira and Etienne Yergeau*

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Chapter 10. RT-qPCR for Validating Microbial Microarray Data. *Dan Tulpan, Michelle Davey and Mark Laflamme*

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In real-time quantitative PCR (qPCR), PCR product is measured at each cycle. By monitoring reactions during the exponential-amplification phase of the reaction, users can determine the initial quantity of the target with great precision. PCR theoretically amplifies DNA exponentially, doubling the number of target molecules with each amplification cycle. Real-time PCR is a variation of the standard PCR technique that is commonly used to quantify DNA or RNA in a sample. Using sequence-specific primers, the number of copies of a particular DNA or RNA sequence can be determined. By measuring the amount of amplified product at each stage during the PCR cycle, quantification is possible. A quantitative Real-Time PCR assay was applied using specific primers to estimate the numbers and population changes of representative rumen bacteria (Table 3). Reactions were performed in the LightCycler 480II Real-Time PCR system (Roche) using SYBRGreen I master mix (Roche, 04707516001) with slight modifications of a previously described method [46]. The effects of plant metabolites on rumen metabolism vary greatly depending on their antimicrobial spectrum and applied doses. In this study, the minimum inhibitory concentrations (MICs) of commercial aldehydes, trans-2-hexenal (T2H), cis-3-hexenal (C3H), trans-2-nonenal (T2N), and trans-2-decenal (T2D) from green leaf volatiles, were tested on rumen bacteria. Real-time PCR also called quantitative PCR (qPCR) is a variant of standard polymerase chain reaction in which amplification and simultaneous quantitation of a target DNA is done in the same PCR machine, using commercially available fluorescence-detecting thermocyclers. Fluorescent dyes specifically label DNA of interest and the amount of fluorescence generated is proportional to the quantity of DNA present. Applications. Real-time PCR enables calculation of the starting template concentration and is, therefore, a frequently used analytical tool in evaluating DNA copy number, viral load, SNP detection, and allelic discrimination. Professor and Microbiologist at Department of Microbiology and Immunology, Patan Academy of Health Sciences, Nepal. Real time PCR (quantitative PCR, qPCR) is now a well-established method for the detection, quantification, and typing of different microbial agents in the areas of clinical and veterinary diagnostics and food safety. Although the concept of PCR is relatively simple, there are specific issues in qPCR that developers and users of this technology must bear in mind. These include the use of correct terminology and definitions, understanding of the principle of PCR, difficulties with interpretation and presentation of data, the limitations of qPCR in different areas of microbial diagnostics and par Quantitative real-time PCR (RTqPCR) has become a technique of choice for studying such bio-processes, due to its unique ability to both detect and quantify a target transcript in real-time. Challenges in extracting inhibitor-free, structurally intact RNA, amenable for a sensitive technique like RT-qPCR, has however proved to be a major impediment in our ability to rigorously implement this highly versatile technology. Chapter 11: Future Trends in RT-qPCR Technology and Their Implication in Applied Microbiology -Vijay J. Gadkar and Martin Fillion Real time-quantitative PCR (RT-qPCR) technology has revolutionized the detection landscape in every area of molecular biology.

Real time PCR (quantitative PCR, qPCR) is now a well-established method for the detection, quantification, and typing of different microbial agents in the areas of clinical and veterinary diagnostics and food safety. Although the concept of PCR is relatively simple, there are specific issues in qPCR that developers and users of this technology must bear in mind. These include the use of correct terminology and definitions, understanding of the principle of PCR, difficulties with interpretation and presentation of data, the limitations of qPCR in different areas of microbial diagnostics and par Real-time polymerase chain reaction allows researchers to estimate the quantity of starting material in a sample. It has a much wider dynamic range of analysis than conventional PCR. Quantitative real-time PCR can be readily applied to analysis of gDNA targets. Such studies may be genotyping/SNP determination, methylation analysis, screening transgenic sequences, or monitoring of insertions and deletions. Quantification and Analysis of mRNA Transcripts. A common application of qPCR is gene expression analysis, e.g., comparing the mRNA concentrations of a gene of interest between control and treated samples. The Polymerase Chain Reaction (PCR) is a commonly used method for amplifying DNA, which can then be used for gene cloning, sequencing, and for gene manipulation. The technique has also been applied to disease diagnosis. Credit: Vit Kovalcik/Shutterstock.com. It is important to study DNA to establish gene function and the effect of mutations on disease. DNA analysis also allows for the development of cures. In PCR, DNA is extracted from a cell and amplified, generating thousands of copies for analysis. Real-time Quantitative PCR is very sensitive to changes in DNA expression. This is advantageous compared to other current methods, such as microarrays, as the final result can be seen before the end point, giving more time for further analysis.

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 affordable than ever before. Renowned authors cover the application of qPCR technology in various areas of applied microbiology and comment on future trends. Real-time PCR assays, however, are limited by the quality of the primers which must be sensitive enough to target the organism of interest, yet specific enough to exclude all others. The primers for the gut bacterium *P. copri* currently used in the literature [12] work well on pure *P. copri* isolates. Their sensitivity and specificity is however lower in complex samples such as faeces, that contain DNA from e.g. plant, animal and microbial cells present in food, host cells and both living and degraded (micro) organisms, some of which - such as other *Prevotella* species- are closely related to the... Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clin Microbiol Rev. 2006;19:165-256
<https://doi.org/10.1128/CMR.19.1.165-256.2006>. The real-time PCR assay or the quantitative PCR is something different than the conventional PCR. We will cover all the information regarding the real-time PCR in the present article. The present article is huge, gigantic and with a lot of sub-topic on real-time PCR. So take a cup of coffee, notebook, pen and read the article till the end. The article is filled with graphics and easy to understand explanation on rtPCR. Quantitative PCR (Q-PCR or real-time PCR) approaches are now widely applied in microbial ecology to quantify the abundance and expression of taxonomic and functional gene markers within the environment. Q-PCR-based analyses combine 'traditional' end-point detection PCR with fluorescent detection technologies to record the accumulation of amplicons in 'real time' during each cycle of the PCR amplification. Secondly, we review the applications of (RT)-Q-PCR analyses in environmental microbiology and evaluate the contribution and advances gained from such approaches.