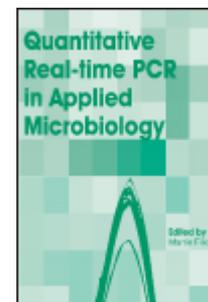


# Quantitative Real-time PCR in Applied Microbiology



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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*

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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.

A real-time polymerase chain reaction (real-time PCR) is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR). It monitors the amplification of a targeted DNA molecule during the PCR (i.e., in real time), not at its end, as in conventional PCR. Real-time PCR can be used quantitatively (quantitative real-time PCR) and semi-quantitatively (i.e., above/below a certain amount of DNA molecules) (semi-quantitative real-time PCR). More particularly, real-time quantitative PCR (qPCR) is considered as a method of choice for the detection and quantification of microorganisms. One of its major advantages is to be faster than conventional culture-based methods. It is also highly sensitive, specific and enables simultaneous detection of different microorganisms. Application of reverse-transcription-qPCR (RT-qPCR) to study population dynamics and activities through quantification of gene expression in food, by contrast with the use of qPCR, is just beginning. Provided that appropriate controls are included in the analyses, qPCR Quantitative real-time polymerase chain reaction (PCR) assays (CFX96 Real-Time system; Bio-rad, USA) were performed with the SYBR Green Supermix (QPK-201, Toyobo Co., LTD., Tokyo, Japan), following Denman and McSweeney [14] and Denman et al [11]. Abundance of these microbes was determined by the following equation. In particular, real-time polymerase chain reaction indicated that the methanogenic archaea and *Fibrobacter succinogenes* populations were significantly reduced, while the *Ruminococcus flavefaciens* populations were significantly increased at 24 h, when supplemented with *G. amansii* extracts as compared with controls.