

# An Overview of Real-Time PCR Platforms

J. M. J. Logan and K. J. Edwards

## **Abstract**

Real-time PCR continues to have a major impact across many disciplines of the biological sciences and this has been a driver to develop and improve existing instruments. From the first two commercial platforms introduced in the mid 1990s, there is now a choice in excess of a dozen instruments, which continues to increase. Advances include faster thermocycling times, higher throughput, flexibility, expanded optical systems, increased multiplexing and more user-friendly software. In this chapter the main features of each instrument are compared and factors important to weigh up when deciding on a platform are highlighted.

## **History of Real-time PCR**

Today it is clear that few techniques have had such a powerful impact on biology than the development of the polymerase chain reaction (PCR). More recently the PCR has become even more sophisticated with the introduction of real-time PCR. Initial work by Higuchi and

colleagues (Higuchi *et al.*, 1992) first demonstrated the simultaneous amplification and detection of specific DNA sequences in real-time by simply adding ethidium bromide (EtBr) to the PCR reaction so that the accumulation of PCR product could be visualised at each cycle. When EtBr is bound to double-stranded DNA and excited by UV light it fluoresces, therefore an increase in fluorescence in such a PCR indicates positive amplification. Soon afterwards they introduced the idea of real-time PCR product quantitation or ‘kinetic PCR’, by continuously measuring the increase in EtBr intensity during amplification with a charge-coupled device camera (Higuchi *et al.*, 1993). By creating amplification plots of fluorescence increase versus the cycle number, they demonstrated that the kinetics of EtBr fluorescence accumulation during thermocycling was directly related to the starting number of DNA copies. Fewer cycles are needed to produce a detectable signal, when a greater number of target molecules are present. Kinetic monitoring also provided a means whereby the efficiency of amplification under different conditions could be determined, providing for the first time insight into the fundamental PCR processes. Therefore, the principle underlying real-time PCR can simply be defined as the monitoring of fluorescent signal from one or more PCRs cycle-by-cycle to completion, where the amount of product produced during the exponential amplification phase can be used to determine the amount of starting material.

The approach described above was not ideal since EtBr binds non-specifically to DNA duplexes and non-specific amplification products, such as primer–dimers, can contribute to the fluorescent signal and result in quantification inaccuracies. Subsequent refinements, the most significant of which was the introduction of fluorogenic probes to monitor product accumulation, added a greater element of specificity to real-time PCR and provided greater quantitative precision and dynamic range than previous methods.

These significant advances to the basic PCR technique not surprisingly led to the development of a new generation of PCR platforms and reagents, which allowed simultaneous amplification and quantification of specific nucleic acid sequences cycle-by-cycle. Indeed a few years after Higuchi coined the term ‘kinetic’ or ‘real-time PCR’ the first

# REAL-TIME PCR

## Current Technology and Applications

Edited by: **Julie Logan, Kirstin Edwards and Nick Saunders**

c. 262 pp., January 2009

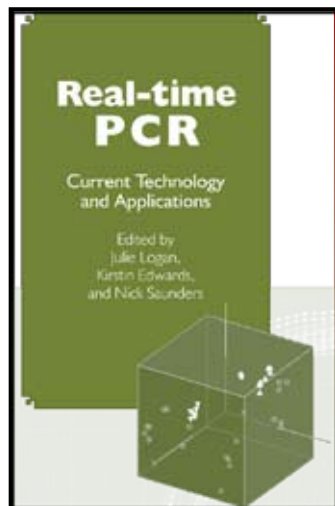
ISBN 978-1-904455-39-4 \$310/£150

Published by: **Caister Academic Press** [www.caister.com](http://www.caister.com)

Real-time PCR (RT-PCR) technology is highly flexible and many alternative instruments and fluorescent probe systems have been developed recently. The decreased hands-on time, increased reliability and improved quantitative accuracy of RT PCR methods have contributed to the adoption of RT PCR for a wide range of new applications.

This essential manual presents 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. Renowned experts in the field describe and discuss the latest PCR platforms, fluorescent chemistries, validation software, data analysis, and internal and external controls. This timely and authoritative volume also discusses a wide range of RT-PCR applications including: clinical diagnostics, biodefense, RNA expression studies, validation of array data, mutation detection, food authenticity and legislation, NASBA, molecular halotyping, and much more.

An essential book for all laboratories using PCR.



[www.caister.com](http://www.caister.com)

### Table of Contents

- An Introduction to Real-Time PCR *N. A. Saunders*
- An Overview of PCR Platforms *J. M. J. Logan and K. J. Edwards*
- Homogeneous Fluorescent Chemistries for Real-Time PCR *M. A. Lee, D. J. Squirrell, D. L. Leslie and T. Brown*
- Reference Gene Validation Software for Improved Normalization *J. Vandesompele, M. Kubista and M. W. Pfaffl*
- Data Analysis Software *M. W. Pfaffl, J. Vandesompele, M. Kubista*
- Performing Real-time PCR *K.J. Edwards and J.M.J. Logan*
- Internal and External Controls for Reagent Validation *M. A. Lee, D. L. Leslie and D. J. Squirrell*
- Introduction to the Applications of Real-Time PCR *N.A. Saunders*
- Analysis of mRNA Expression by Real-Time PCR *Stephen A. Bustin and Tania Nolan*
- Validation of Array Data *Elisa Wurmbach*
- Mutation Detection by Real-Time PCR *Elaine Lyon, Rong Mao and Jeffrey Swensen*
- Real-Time NASBA *Julie D. Fox, Catherine Moore and Diana Westmoreland*
- Applications in Clinical Microbiology *Andrew David Sails*
- Diagnosis of Invasive Fungal Infections *D.S. Perlin*
- Biodefense *Christina Egan, Nick M. Cirino and Kimberlee A. Musser*
- Real-Time PCR: Application to Food Authenticity and Legislation *Gordon Wiseman*
- Molecular Haplotyping by Real-time PCR *Genevieve Pont-Kingdon, Alison Millson and Elaine Lyon*

#### Order from:

- ISBS, Inc., 920 NE 58th Avenue, Suite 300, Portland, OR 97213-3786, **USA** Tel: 503 287-3093; Fax: 503 280-8832 <http://usa.caister.com>
- Book Systems Plus, BSP Hse, Station Road, Linton, Cambs, CB1 6NW, **UK** Tel: 01223 894870; Fax: 01223 894871 <http://uk.caister.com>

Quantity	Title	ISBN	Cost
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Name \_\_\_\_\_

Address \_\_\_\_\_

E-mail \_\_\_\_\_

Tel. \_\_\_\_\_ Fax. \_\_\_\_\_

UK £5; Europe £8; USA \$5.50; Rest of World please call \_\_\_\_\_

Visa  Mastercard  Bill me

Exp. date [ ][ ]/[ ][ ] Security number [ ][ ][ ][ ]

Cardholder \_\_\_\_\_

Signature \_\_\_\_\_ Date \_\_\_\_\_