

Homogeneous Fluorescent Chemistries for Real-Time PCR

M. A. Lee, D. J. Squirrell, D. L. Leslie,
and T. Brown

Abstract

The development of fluorescent methods for a closed tube polymerase chain reaction has greatly simplified the process of quantification. Current approaches use fluorescent probes that interact with the amplification products during the PCR to allow kinetic measurements of product accumulation. These probe methods include generic approaches to DNA quantification such as fluorescent DNA binding dyes. There are also a number of strand-specific probes that use the phenomenon of Fluorescent Resonance Energy Transfer (FRET). In this chapter we describe these methods in detail, outline the principles of each process, and describe published examples. This text has been written to provide an impartial overview of the utility of different assays and to show how they may be used on various commercially available thermal cyclers.

Introduction

A fluorescent real-time polymerase chain reaction (PCR) (Saiki *et al.*, 1985; Mullis *et al.*, 1987) can provide both qualitative and quantitative analysis for various applications. Real-time PCR differs from earlier methods of analysis in that additional components are required to carry out the process. These components include an optical system integrated into the thermal cycler, and a probe that reports amplification during the course of the PCR process. The real-time PCR thermal cycler is discussed in chapter 2 in detail. In this chapter we discuss the probe technologies or “reporting chemistries.”

In order to give the reader a complete understanding of current technology, the principles of real-time analysis will initially be presented outside the context of any specific commercial platform. The number of these is increasing and it is certain that their capabilities on offer will undergo continued improvement and allow new fluorescent approaches to be realised. At this point it is important to highlight to new users the relationship between the choice of probe system and the instrument. These are inextricably linked: the optical specification and the analysis tools on any given platform greatly influence the applicability and utility of different probe systems. Whilst the main factors for the choice of instrument are often driven by throughput requirements and the initial purchase cost, careful consideration of the reporting chemistry for the required application should be made before purchase since the operation of one chemistry or another may be greatly compromised on some platforms. Equally important is the technical support provided from suppliers. This may be limited for non-supported chemistries and so called “open platforms” from manufacturers that do not support any one chemistry. These suppliers may not be able to provide technical advice for the chemistry of choice for the required application. In this chapter we break down probe technology into a number of components to enable the reader to understand how the different assay systems may be used on current and future fluorimetric thermal cyclers. For new users this will facilitate the implementation of various assays on commercial platforms.

REAL-TIME PCR

Current Technology and Applications

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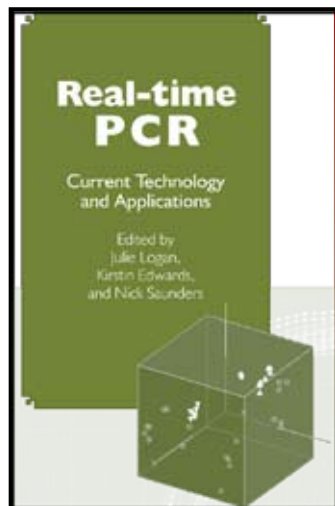
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Real-time PCR (RT-PCR) technology is highly flexible and many alternative instruments and fluorescent probe systems have been developed recently. The decreased hands-on time, increased reliability and improved quantitative accuracy of RT PCR methods 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.



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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*

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