

Internal and External Controls for Reagent Validation

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

Abstract

PCR applications that require a high confidence in the result should be designed to control for the occurrence of false negatives. False negatives can occur from inhibition of one or more of the reaction components by a range of factors. While an external, or batch control is often used, the ideal control is one that is included in the reaction cocktail in a multiplex format. Early approaches used different sized amplicons combined with end-point analysis. Fluorescent homogenous real-time PCR methods have a number of advantages for implementing internal controls. Here we discuss the application and development of molecular mimics for use as controls in real-time PCR, and explain a number of concepts and experimental considerations that will aid in the optimisation of the controlled multiplexed assay.

Introduction

The confidence in assays based on the polymerase chain reaction (PCR) (Saiki *et al.*, 1985; Mullis *et al.*, 1987) may be compromised by the sporadic occurrence of either false positive or false negative results. False positives are a problem that is common to the general application of PCR. False positives occur mainly as a result of cross contamination from either positive samples or reaction products. A combination of preventative methods including good laboratory practice, delineated preparation/analysis areas, PCR cabinets with UV treatment, UV air scrubbers, closed tube assays, and uracil glycosylase carry-over prevention chemistry (Longo *et al.*, 1990), has effectively eliminated the occurrence of false positives for the majority of applications. However, there are a number of PCR applications where the avoidance of false negative results is of equal importance. False negatives occur through failure of one or more of the reagents, the presence of inhibitors, or the failure of the PCR thermal cycling process (Rossen *et al.*, 1992; Wilson *et al.*, 1997). The applications requiring high confidence in the PCR include pathogen detection in clinical diagnosis, food quality control and environmental analysis.

For most molecular tests the use of reference material in a batch test is the only control that can be implemented. However, PCR and other nucleic acid amplification techniques provide not only extremely sensitive detection, but also have the added advantage that they can be readily multiplexed to include an internal control (IC). An IC is a second target molecule that can be amplified with, but distinguished from, other products in the same tube. In an ideal assay this should be able to control for all of the reagents in a reaction cocktail, and for variations in machine operation parameters. This can be achieved by the use of a molecular mimic, a synthetic molecule that may be co-amplified using the same set of amplimers as the target species.

Early approaches for ICs used amplicons with different molecular masses and subsequent analysis using a separation method. For example, the molecular mimic could contain both priming sites, but an internal sequence changed by insertions or deletions (Ursi *et al.*, 1992). The products of amplification could be easily analysed by the

REAL-TIME PCR

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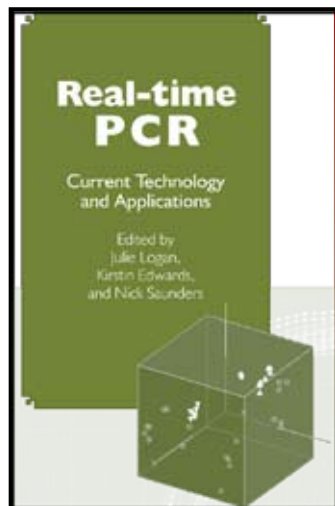
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Real-time PCR (RT-PCR) technology is highly flexible and many alternative instruments and fluorescent probe systems have been developed recently. The decreased hands-on time, increased reliability and improved quantitative accuracy of RT PCR methods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