

Mutation Detection by Real-Time PCR

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

Abstract

Real-time PCR is ideally suited for analysis of single nucleotide polymorphisms (SNPs) and has been increasingly used for this purpose since the advent of real-time PCR and as whole genome sequences have become available. It requires methods that are rapid, sensitive, specific and inexpensive, and several real-time methods have evolved which fulfil these requirements. Additionally real-time PCR is a technique that is readily amenable to automation and no post-PCR handling is required. Different formats have been applied including hybridisation probes with melting curve analysis, hydrolysis probes, molecular beacons and scorpion primers. SNP detection by real-time PCR has found applications in diagnosis of human disease, pharmacogenetics, clinical microbiology and drug development, and has replaced techniques such as sequencing, single strand conformation polymorphism and restriction enzyme digestion.

Principles of Mutation Detection

The detection of mutations is a fast growing field of increasing importance for many areas of science including diagnosis of human disease, pharmacogenetics, drug development and microbiology. Mutations are classed as one or more changes in the DNA bases and can include large re-arrangements such as translocations, inversions and gene insertions/deletions or small alterations such as point mutations and base insertions/deletions (SNPs). SNPs are the commonest type of DNA sequence variations and can occur once every 100-300 bases. As the requirement for rapid, reliable, sensitive and inexpensive methods for SNP detection grow the number of techniques available also increases, each with inherent strengths and weaknesses. Most of these techniques can be divided into hybridisation-based or enzyme-based methods and have been extensively reviewed (Syvanen, 2001; Kirk *et al.*, 2002; Kwok, 2002).

Real-time PCR, a hybridisation-based method, has become widely used for mutation detection. The systems are flexible with a number of different probe systems that can be used and there is additional flexibility in the design of the probes. Several formats have evolved including, hybridisation probes, hydrolysis probes, molecular beacons and scorpion primers. These methods are sensitive and specific, inexpensive, rapid (some assays can be performed in as little as 30 minutes) and they are both easy to perform and interpret the results. All of the available platforms are semi-automated and none require additional post-PCR handling for example, agarose gel analysis. Depending on the platform used real-time PCR is suitable for low to medium sample throughput. The greatest advantage of these systems is the quality of the data that is generated, known mutations are easily detected and there are possibilities with some of the systems to detect new mutations. Due to increased demand there is now a number of commercially available tests developed, especially for the Applied Biosystems Sequence Detection Systems (ABI 7700 and 7000) and the LightCycler.

REAL-TIME PCR

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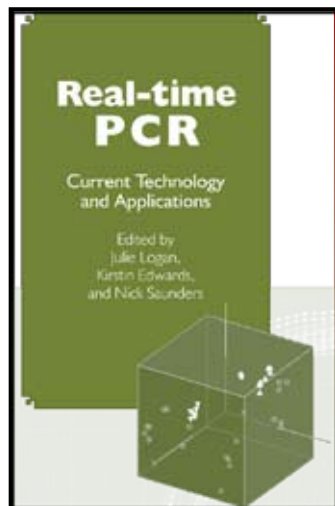
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Real-time PCR (RT-PCR) technology is highly flexible and many alternative instruments and fluorescent probe systems have been developed recently. The decreased hands-on time, increased reliability and improved quantitative accuracy of RT PCR methods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