

The Quantitative Amplification Refractory Mutation System

P. Punia and N. Saunders

Abstract

The amplification refractory mutation system (ARMS), which has also been described as allele-specific PCR (ASP) and PCR amplification of specific alleles (PASA), is a PCR-based method of detecting single base mutations (Newton *et al.*, 1989). ARMS has been applied successfully to the analysis of a wide range of polymorphisms, germline mutations and somatic mutations. The technique has the ability to discriminate low-levels of the mutant sequence in a high background of wild-type DNA (Billadeau *et al.*, 1991). In an ARMS PCR the terminal 3' nucleotide of one of the PCR primers coincides with the target mutation. Most applications of the method rely on 'end-point' analysis, utilising the classic gel-electrophoresis method. However, end-point analysis can only assess the presence or absence of mutant or wild-type sequences and does not give an indication of the ratio of mutant to wild-type in a mixed population of DNA. Here we describe a real-time PCR adaptation of ARMS, quantitative ARMS, that allows measurement of the size of the population of each variant

in a mixture. A method for the detection of human hepatitis B virus mutations that confer resistance to the antiviral lamivudine is described as an example.

Introduction

PCR can be adapted in many ways to facilitate the analysis of DNA. Several different strategies are used for the detection of single point mutations. Current methods rely on target amplification, usually by PCR, followed by the identification of DNA variants using probes, restriction endonucleases, ligases or polymerases.

In ARMS, the primer pair is designed so that one of the 3' ends coincides with a variant nucleotide in the target sequence. When the primer mismatches the template the frequency of extension is very low and consequently the effective number of sequence copies available for amplification is greatly reduced. ARMS PCR exploits a thermostable polymerase that lacks 3' exonuclease activity (usually *Taq* polymerase). Such enzymes extend primers bound to their target sequences very inefficiently when the 3' base is mismatched. Because the 3' exonuclease activity required for mismatch repair is not present, the extension of such primers in PCR is a rare event and amplification is retarded (Figure 1). However, once mismatch extension has occurred amplification proceeds normally from the newly synthesised target strand. Thus an important principle of ARMS is that non-matching (*i.e.* non-matching at only the 3' end of one primer) template may be amplified. The factors that contribute to this apparent 'failure' of ARMS are that when very high numbers of template molecules are added to the reaction, amplification is likely to proceed because the small proportion of mismatched primer extensions reach the sensitivity threshold of the reaction. Second, certain mismatches are extended more efficiently than others so that proportion extended in each PCR cycle varies.

Detection of a single nucleotide mutation at a predetermined point in a target sequence can be achieved by running the ARMS PCR with a single primer pair and then scoring the production of amplicon as

REAL-TIME PCR

Current Technology and Applications

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

c. 262 pp., January 2009

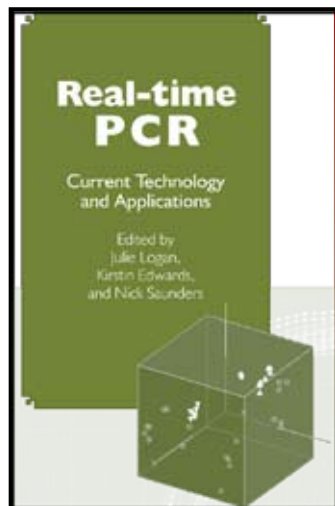
ISBN 978-1-904455-39-4 \$310/£150

Published by: **Caister Academic Press** www.caister.com

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

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 _____