

SHORT REPORT

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Hot start reverse transcriptase: an approach for improved real-time RT-PCR performance



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Abstract

Background: Reverse transcriptase is an indispensable enzyme for real-time reverse transcriptase (RT)-PCR, a standard method in molecular diagnostics for detection and quantification of defined RNA molecules. The prevention of non-specific products due to elongation of misprimed oligonucleotides by the enzyme at temperatures beneath the specific annealing temperature is one of the biggest challenges in real-time RT-PCR.

In the present study, an aptamer directed against the reverse transcriptase was analyzed for its potential to attain a temperature-dependent reverse transcriptase ("hot start" RT).

Findings: The hot start effect was investigated in a one-step real-time RT-PCR assay for the detection of Middle East respiratory syndrome coronavirus (MERS-CoV). Results with aptamer revealed a reduced RT activity at low temperatures while achieving full activity at the specific annealing temperature of 55 °C. Sensitivity (limit of detection (LoD) 95 %) of the MERS-CoV assay was increased by about two times in the presence of aptamer.

Conclusions: The study demonstrates the potential of aptamer-dependent hot start RT for the improvement of diagnostic real-time RT-PCR assays.

Keywords: Real-time RT-PCR; Reverse transcriptase; Hot start; MERS-CoV

Findings

Introduction

Real-time RT-PCR is the method of choice in molecular diagnostics for detection and quantification of defined RNA molecules (Mackay 2004). This technique utilizes reverse transcriptase (RT) to convert RNA into complementary DNA (cDNA), a thermostable DNA-dependent DNA polymerase for the amplification of specific target sequences and target specific probes (oligonucleotides) labelled with fluorophores for the detection of amplified DNA (Gibson et al. 1996).

Real-time RT-PCR is regarded as a method with high sensitivity and specificity (Martel et al. 2002). However, this is challenged by non-specific products generated by elongation of misprimed primer that competes with the synthesis of specific amplification products in each cycle (Chou et al. 1992; Li et al. 1990). The probability of non-specific product formation increases with the complexity

of the real-time RT-PCR system and the background nucleic acid in the specimen (Browne et al. 1997; Handschlar et al. 2009). Ultimately, non-specific products can severely decrease sensitivity as well as specificity of real-time RT-PCR assays (Sharkey et al. 1994; Birch et al. 1996; Jayasena 1999).

Assays for the detection of RNA viruses are often highly complex (high quantity of different oligonucleotides) due to low sequence conservation of the RNA genome (Gardner et al. 2004; Browne et al. 1997). In general, mispriming occurs at temperatures below the specific annealing temperature of the oligonucleotides (Jayasena 1999). Thus, the formation of non-specific products can be reduced by using hot start variants of the enzymes, which are inactive at low temperatures and activated at higher temperatures, appropriate for specific primer annealing to the target nucleic acid (Birch et al. 1996).

Several biological or chemical hot start concepts exist for *Taq* polymerase, a thermostable DNA-dependent DNA polymerase. The *Taq* polymerase can be inactivated by binding of specific antibodies or aptamers, by incubation with chemicals or by altered molecular kinetics

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Cumulated Index Medicus, 2000

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PCR Methods and Applications ,1995 *Investigative Ophthalmology & Visual Science* ,2002 *Products for Life Science Research* Sigma-Aldrich Corporation,2008 PCR Primer Carl W. Dieffenbach,Gabriela S. Dveksler,2003 The Polymerase Chain Reaction PCR technique was invented nearly 20 years ago Its subsequent variations and applications were many and varied and today molecular biology clinical and forensic laboratories make almost daily use of PCR This second edition of the much praised PCR Primer A Laboratory Manual updates the tried and true methods and presents the advances made in the 10 years since the first edition After introducing the basics for PCR and methods of sample preparation PCR Primer provides laboratory tested protocols for RT PCR methods detection of PCR products analysis of differential expression cloning and mutagenesis These step by step methods include extensive background information as well as valuable troubleshooting information provided by the leading experts in this technology This manual is a comprehensive and reliable source of the full range of PCR methods for novices and experienced investigators alike The Scientist ,1999 Textbook of Pulmonary Diseases Gerald L. Baum,1998 New edition of a comprehensive overview of pulmonary diseases that supplies enough detail to appeal to the specialist and enough basic medical science and background discussion to keep a good beginning medical student from feeling over his her head Emphasizing accurate diagnosis this edition reflects the current focus on a genetic basis for many pulmonary abnormalities and the relevance of public health concerns when diagnosing and treating pulmonary infections The contributors approach to infectious diseases extrapolates from established standards of clinical bacteriology to diagnose and treat viral rickettsial and fungal diseases Such discussions also integrate recent progress in chemotherapy and rehabilitation techniques To further aid the clinician s skills many chapters end with diagnostic tables that summarize that chapter s findings Annotation copyrighted by Book News Inc Portland OR

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summarizes the information and knowledge of environmental microbiology in a single source. It details the natural fate of microorganisms in the environment as well as the intentional attempts to eliminate from the environment microorganisms that are pathogenic to humans or to plants and animals. The basic principles of environmental microbiology and general analytical methodologies common across the range of the environments covered are presented first. The core sections are structured with regard to the type of environmental medium being discussed. This landmark effort defines the study of environmental microbiology as we know it today and serves as an essential contribution to the literature. **Proceedings of the Indian National Science Academy**, Indian National Science Academy, 1970. *Bacteria*, Paul Singleton, 1995-08-08. It continues with applied aspects: recombinant DNA technology, medicine, food, ecology, waste water treatment, etc. and also covers classification, identification, disinfection, sterilization, culture, microscopy, and other areas of practical bacteriology. The Appendix contains mini-descriptions of 70 genera of bacteria useful for rapid orientation and is helpful to those new to bacterial names. *The Journal of Immunology*, 1995. **Cancer Research**, 2008-04. *Proceedings*, Indian National Science Academy, 2004.

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