

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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Molecular Diagnostics for Dermatology Gregory A. Hosler, Kathleen M. Murphy, 2014-04-30 Molecular diagnostics is an exploding field and recent advances in our understanding of the molecular basis of disease have provided a platform for the development of new diagnostic tests as well as tests to predict tumor behavior and potential response to targeted therapy This textbook provides a reference and practical guide to molecular diagnostics for dermatologists and dermatopathologists It outlines our current understanding of the molecular underpinnings of dermatologic disease describes the appropriate use of currently available molecular tests and explains the interpretation of these tests in the context of diagnosis and management Tests relating to various disorders are covered including but not confined to melanoma genodermatoses and infectious disease Pitfalls are highlighted and user friendly algorithmic approaches presented **Cumulated Index**

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Table of Contents Hot Start Reverse Transcriptase An Approach For Improved

1. Understanding the eBook Hot Start Reverse Transcriptase An Approach For Improved
 - The Rise of Digital Reading Hot Start Reverse Transcriptase An Approach For Improved
 - Advantages of eBooks Over Traditional Books
2. Identifying Hot Start Reverse Transcriptase An Approach For Improved
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Hot Start Reverse Transcriptase An Approach For Improved
 - User-Friendly Interface
4. Exploring eBook Recommendations from Hot Start Reverse Transcriptase An Approach For Improved

- Personalized Recommendations
 - Hot Start Reverse Transcriptase An Approach For Improved User Reviews and Ratings
 - Hot Start Reverse Transcriptase An Approach For Improved and Bestseller Lists
5. Accessing Hot Start Reverse Transcriptase An Approach For Improved Free and Paid eBooks
 - Hot Start Reverse Transcriptase An Approach For Improved Public Domain eBooks
 - Hot Start Reverse Transcriptase An Approach For Improved eBook Subscription Services
 - Hot Start Reverse Transcriptase An Approach For Improved Budget-Friendly Options
 6. Navigating Hot Start Reverse Transcriptase An Approach For Improved eBook Formats
 - ePub, PDF, MOBI, and More
 - Hot Start Reverse Transcriptase An Approach For Improved Compatibility with Devices
 - Hot Start Reverse Transcriptase An Approach For Improved Enhanced eBook Features
 7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Hot Start Reverse Transcriptase An Approach For Improved
 - Highlighting and Note-Taking Hot Start Reverse Transcriptase An Approach For Improved
 - Interactive Elements Hot Start Reverse Transcriptase An Approach For Improved
 8. Staying Engaged with Hot Start Reverse Transcriptase An Approach For Improved
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Hot Start Reverse Transcriptase An Approach For Improved
 9. Balancing eBooks and Physical Books Hot Start Reverse Transcriptase An Approach For Improved
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Hot Start Reverse Transcriptase An Approach For Improved
 10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
 11. Cultivating a Reading Routine Hot Start Reverse Transcriptase An Approach For Improved
 - Setting Reading Goals Hot Start Reverse Transcriptase An Approach For Improved
 - Carving Out Dedicated Reading Time
 12. Sourcing Reliable Information of Hot Start Reverse Transcriptase An Approach For Improved

- Fact-Checking eBook Content of Hot Start Reverse Transcriptase An Approach For Improved
 - Distinguishing Credible Sources
13. Promoting Lifelong Learning
- Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
14. Embracing eBook Trends
- Integration of Multimedia Elements
 - Interactive and Gamified eBooks

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