

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 (Brownie 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 (Gardiner et al. 2004; Brownie 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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