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Linear-after-the-exponential (LATE)-PCR: Improved asymmetric PCR for quantitative DNA-analysis

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ABSTRACT

Linear-after-the-exponential (LATE)-PCR describes a novel approach to asymmetric PCR which uses adjusted melting temperatures of the limited primer to increase PCR efficiency. In this proof-of-principle study we show that linear amplification is possible over a wide range of amplification cycles. The curve characteristics of the real time PCR show that the plateau effect of conventional PCR can be avoided by this innovative technique.

1. Introduction

Linear-after-the-exponential (LATE)-PCR describes a novel approach to asymmetric PCR [1]. Conventional PCR is known to plateau stochastically due to competition of binding between primers and PCR products: The more PCR products bind to the target, the less efficient the reaction becomes, because no *de novo* synthesis takes place. Consequently, an increase in the number of PCR cycles does not necessarily lead to an increase in the total amount of PCR product, once the plateau phase has been reached. Furthermore, there is no correlation between the total amount PCR product and the amount of starting target.

To avoid the plateau, asymmetric PCR was described in which massively different primer concentrations are used to transform the exponential enrichment of PCR products into a linear, single stranded synthesis. Previous attempts to asymmetric PCR showed limited PCR efficiency due to differences in the optimal annealing temperature between the limited and the excess primer. To overcome this limitation, primers were designed with adjusted melting temperatures, namely by an increased length of the limited primer. This leads to increased amplification efficiencies as described in [2].

Here we present a proof-of-principle study for this innovative technique using the STR system humTH01. To test the exact curve characteristic of LATE-PCR, all reactions were performed as real time PCR. Due to the fact that linear amplification leads to the formation of a single stranded PCR product, the real time PCR signal detection was performed using a molecular beacon dye.

Material and methods

Primer design

Primers and molecular beacon probes were designed using primer3 (http://primer3.sourceforge.netsoftware), BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and AutoDimer [3]. Primer and probe sequences are given in Table 1.

DNA analysis

Real-time-PCR was performed in an ABI PRISM® 7500 Real-time PCR System (Life Technologies). PCR-Mastermix contains 1,85 µl HPLC, 1,75 µl $MgCl_2$ (25 mM), 0,5 µl BSA (20 mg/ml), 1,25 µl buffer (10×), 2 µl dNTP (10 mM), 0,15 µl AmpliTaq Gold (5 U/µl).

Results and discussion

The optimal temperature for hybridization of the molecular beacon has been determined by melt curve analysis and was found

Table 1
Primer and probe sequences for TH01.

Sequence (5′-3′)	T_m [°C]	
ATT CAA AGG GTA TCT GGG CTC TGG GGT GA	62.5	Limited primer
GTG GGC TGA AAA GCT CCC GAT TAT	58	Excess primer
CGCGA-CCA TGG AGT CTG TGT TCC CTG TGA CCT -TCGCG	72	Molecular beacon

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1875-1768/

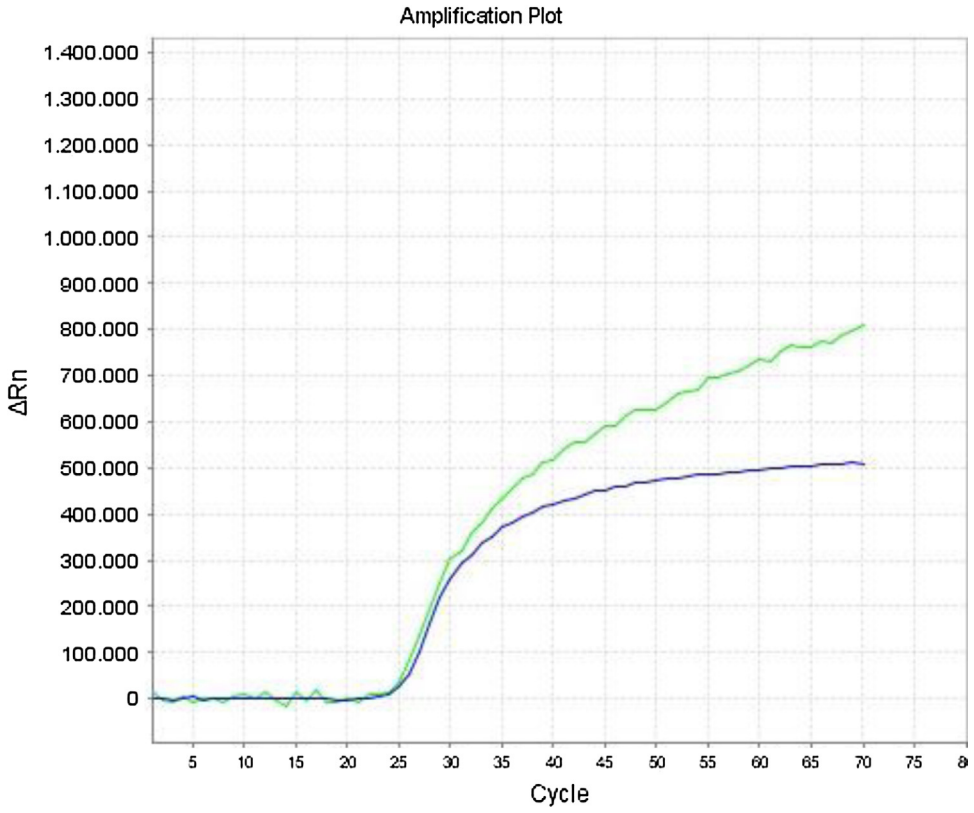

Fig. 1. Comparison of conventional PCR (blue) and LATE-PCR of TH01 (green). The plateau phase is clearly visible for conventional PCR while the LATE-PCR shows constant increase of the PCR product amount up to 70 cycles.

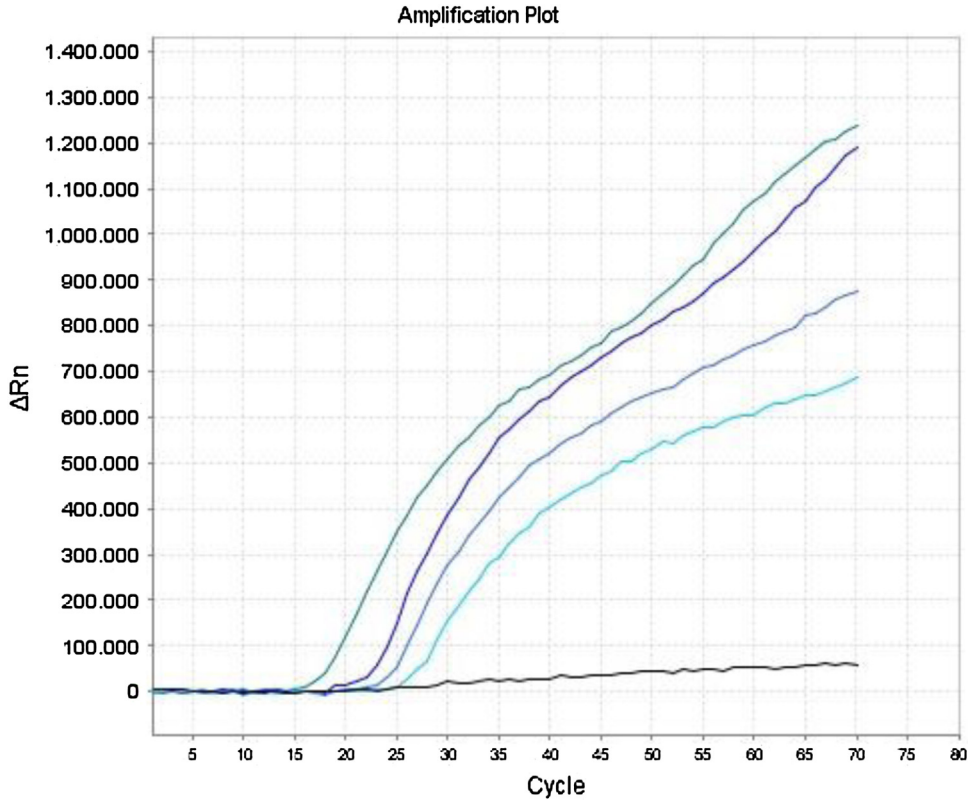

Fig. 2. Comparison of different DNA-concentrations in LATE-PCR of TH01. A clear relationship between end point quantities and target quantities seem to exist; however, further optimization is needed to improve the correlation coefficient.

to be 70 °C. Consequently, for all subsequent reactions this temperature was used for signal detection.

LATE-PCR does not reach the characteristic plateau like conventional PCR, but ends in a nearly linear phase (Fig. 1). This is an indication that there is no more product annealing, because after the first cycles the limited primer is depleted. Thus, more PCR cycles are possible without the creation of additional artefacts. Consequently, the amplification of samples with low DNA content is more successful with the regard to the final concentration of the amplicon.

Furthermore, the fluorescence signal at the end point of the amplification seems to correspond with the target amount (Fig. 2). However, further optimization is needed to obtain full correlation between end point fluorescence and DNA concentration.

The main benefit of LATE-PCR is that for samples comprising low quantity and/or quality of DNA, additional cycles can be used for amplification without the problem of plateauing. Furthermore, the amplification of typical STR artefacts, such as the incorporation of a non-matching base, is avoided because in LATE-PCR, linear amplification is more likely to use the original DNA strand as template compared to conventional PCR.

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