

transcription and six PCR reactions, the relative ratio obtained through the LightCycler Relative Quantification Software was compared to one whereby copy number determinations from standard curves (Table 1) were used. In support of this selection, the coefficient of variations for a calibrated CK20 relative ratio were 17% and 22%, respectively, with the larger error of the latter likely to be introduced by the variations in standard curve construction between PCR runs. Without factoring PBGD quantities and a calibrator ratio, the error from a method determining absolute CK20 copy numbers per nanogram RNA via a standard curve was 40%.

Summary

This study describes the new LightCycler-CK20 Quantification Kit that may prove a valuable tool for

Product	Pack Size	Cat. No.
LightCycler-CK20 Quantification Kit	1 kit (96 reactions)	3 118 835
LightCycler Relative Quantification Software	1 software package	3 158 527



micrometastases detection in research applications. The assay is quantitative, rapid, convenient-to-use, flexible, specific, sensitive and amenable to large-scale routine analysis. A PCR quantification model is used that maximises reproducibility and provides a platform for standardisation across laboratories. This assay provides a more informative analysis and may help to unravel reasons behind discrepancies between studies on micrometastases detection. Such a standardised, quantitative assay amenable to large scale analysis potentially opens the possibility to determine thresholds for background expression and investigate the potential significance of measuring tumor load. Further intensive studies are required to clarify this potential.

References

1. Van Houten V. M. et al. (2000), Clin. Cancer Research 6: 3803-3816.
2. Moll R. et al. (1993), Differentiation 53: 75-93.
3. Soeth E. et al. (1997), Cancer Research 57: 3106-3110.
4. Funaki N. O. et al. (1998), Brit. J. Cancer 77: 1327-32.
5. Weitz J. et al. (1998), Clin. Cancer Research 4: 343-348.
6. Wyld D. K. et al. (1998), Int. J. Cancer 79: 288-293.
7. Champelovier P. et al. (1999), Anticancer Res. 19: 2073-2078.
8. Wittwer C. T. et al. (1997), Biotechniques 22: 130-138.
9. Kellogg D. E. et al. (1994), Biotechniques 16: 1134-1137.



Efficient Amplification of a Low-abundance Template with FastStart Taq DNA Polymerase

Li Xu and Shi-Yuan Cheng*; University of Pittsburgh Cancer Institute, Pittsburgh/USA
*corresponding author: chengs@msx.upmc.edu

Introduction

An important step in a biological study is the examination of the expression pattern of a gene of interest. Due to the low abundance of some transcripts, researchers usually use RT-PCR to analyze their expression. Therefore, it is critical to optimize the PCR reaction to allow efficient amplification of weak signals. The DNA polymerase used in PCR is the most important factor in determining PCR efficiency. One of the research projects of the group of Dr. Shi-Yuan Cheng at the University of Pittsburgh Cancer Institute is to determine expression levels of endothelial growth factors and their receptors in cells and tissues. While performing RT-PCR to investigate the expression levels of one endothelial receptor, the vascular endothelial growth factor (VEGF) receptor, it was found that

FastStart Taq DNA Polymerase is an excellent enzyme for detecting expression of a gene that has low copy numbers. In comparison with Taq Polymerase from other commercial sources, FastStart Taq DNA Polymerase increased PCR reaction sensitivity dramatically without losing the specificity of gene amplification.

Materials and Methods

Amplification enzymes

FastStart Taq DNA Polymerase was purchased from Roche Molecular Biochemicals and used according to the manufacturer's instruction, using 0.4 µl (2 units) per 50 µl reaction. Supplier A's modified hot start polymerase was used in their buffer II under the amplification conditions specified by its manufacturer.

Thermal cycler

A PCR express thermal cycler purchased from Hybaid Corporation was used for all amplifications.

PCR amplification conditions

PCR reactions were performed on a PCR express thermal cycler in 0.2 ml thin-walled tubes. A 298-bp (wild type) or a 527-bp mutant fragment of the VEGF receptor gene was amplified using the following conditions: The FastStart Taq DNA Polymerase 50 µl reactions contained 5 µl of 10x FastStart Taq PCR buffer containing 2 mM MgCl₂ (final concentration), 200 µM of each dNTP, 2 units of FastStart Taq DNA Polymerase, 20 pmol of forward primer and reverse primer. The other reactions contained the same components, except another supplier's modified hot start DNA polymerase and its supplied buffer II were used instead of FastStart Taq DNA Polymerase and its buffer. As template cDNA from total RNA of an immortalized cell line was used. Cycling conditions began with a 4-minutes activation of FastStart Taq DNA Polymerase at 95 °C based on manufacturer's instructions, then 30 seconds at 95 °C, 30 seconds at 52 °C (G:2)* and 1 minute at 72 °C for 40 cycles, with a final extension at 72 °C for 7 minutes. The supplier A's modified Taq Polymerase was initially activated for 4 minutes.

Results and Applications

The expression pattern of the VEGF receptor was first examined with another supplier's modified Taq Polymerase for hot start PCR. No desired amplification products were detected, and sometimes non-specific amplification occurred. The expected target, even the positive control, seemed to be consistently elusive. After reconfirmation of RNA integrity, RT reaction efficiency, and PCR primers, a trial was performed with the FastStart Taq DNA Polymerase advertised on Roche's website.

Figure 1 shows the comparison of PCR amplification efficiency between FastStart Taq Polymerase from Roche Molecular Biochemicals and Supplier A's modified Taq preparation designed for hot start PCR. A gradient of annealing temperatures was used to optimize PCR amplification conditions. Lane 1 contains a positive control for the wild-type fragment (298 bp), and lanes 2 to 9 contain PCR products from the same tumor cell RT reaction in which the inserted fragment (527 bp) was found. The amplification conditions for lanes 7 to 9, which contained Supplier A's modified hot start polymerase, were the same as that for lane 6, which was the one optimized for

*(G:2) is PCR gradient nomenclature: to optimize the PCR reaction conditions, a basal temperature (e.g., 52 °C) is set and is increased 2 ° along each row on the cycler's rack.

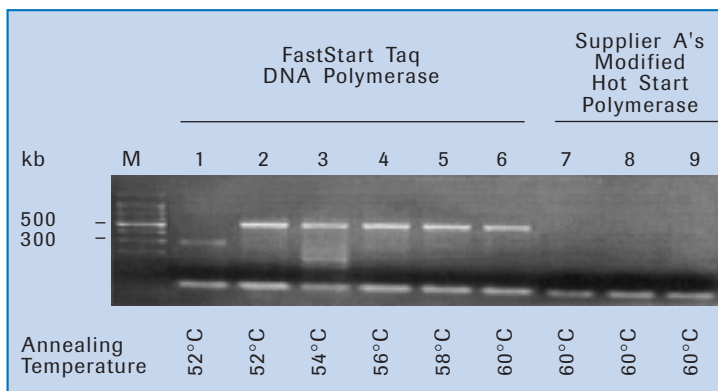


Figure 1: PCR examination of VEGF receptor expression with different DNA polymerases. Lane 1: positive control (298 bp). Lanes 2 to 9: amplification results from the same tumor cell RT reaction. Gradient annealing temperatures were used to optimize conditions. FastStart Taq DNA polymerase was used in lanes 1 to 6; Supplier A's modified hot start polymerase was used in lanes 7 to 9. A 527-bp fragment was detected (lanes 2 to 6) with FastStart Taq DNA polymerase, but Supplier A's modified hot start polymerase failed to amplify the signal (lanes 7 to 9)

this PCR. Our data clearly demonstrated that FastStart Taq DNA Polymerase has a higher efficiency and excellent specificity. FastStart Taq DNA Polymerase also successfully detected the expression of VEGF receptor in many cancer cell lines, cancer tissues, as well as normal tissues.

Summary

Efficient Taq DNA Polymerase is crucial for amplifying targets from templates. In our case, we used FastStart Taq DNA Polymerase to detect weak signals of a low-abundance receptor. The efficiency and sensitivity is obvious. The FastStart Taq DNA Polymerase is only activated after heating, providing a natural hot start and offering the convenience of being able to organize reaction components at room temperature without worrying about introducing non-specific amplification. In contrast, to avoid non-specific amplification with unmodified Taq Polymerase, working on ice is necessary (data not shown). The high efficiency of FastStart Taq DNA Polymerase is due to its high specificity, which allows the efficient generation of a product without producing interfering non-specific amplification.

<http://biochem.roche.com/pcr>



Product	Pack Size	Cat. No.
FastStart Taq DNA Polymerase	50 units	2 158 264
	100 units	2 032 902
	500 units	2 032 929
	1000 units	2 032 937
	2500 units	2 032 945
	5000 units	2 032 953