RESEARCH NOTE

Asian Journal of Bio Science (April, 2010) Vol. 5 Issue 1 : 166-168

Real Time PCR – a robust molecular technique for ever

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(Accepted : March, 2010)

Key words : Real Time PCR, Molecular technique, Probe

INTRODUCTION

In recent years, real-time polymerase chain reaction (PCR) has emerged as a robust and widely used methodology for biological investigation because it can detect and quantify very small amounts of specific nucleic acid sequences. As a research tool, a major application of this technology is the rapid and accurate assessment of changes in gene expression as a result of physiology, clinical conditions, pathophysiology, or development. This method can be applied to model systems to measure responses to experimental stimuli and to gain insight into potential changes in protein level and function. Thus physiology can be correlated with molecular events to gain a better understanding of biological processes. Thus real-time PCR expands the influence of PCR-based innovations and presents intriguing directions for the future of biomedical sciences (especially molecular diagnostics and molecular physiology) and life science education (Walker, 2002). Present day real-time methods generally involve fluorogenic probes that "light up" to show the amount of DNA present at each cycle of PCR. "Quantitative PCR" refers to the ability to quantify the starting amount of a specific sequence of DNA. Consequently real-time PCR is quickly becoming the method of choice to quantify nucleic acids. Real-time PCR instrumentation was first made commercially available by company called Applied Biosystems in 1996, after which several other companies added new machines to the market. Presently, Applied Biosystems, BioGene, Bioneer, Bio-Rad, Cepheid, Corbett Research, Idaho Technology, MJ Research, Roche Applied Science, and Stratagene all offer instrumentation lines for real-time PCR.

The real-time PCR is to precisely distinguish and measure specific nucleic acid sequences in a sample even if there is only a very small quantity. Real-time PCR amplifies a specific target sequence in a sample then monitors the amplification progress using fluorescent technology. During amplification, how quickly the fluorescent signal reaches a threshold level correlates with the amount of original target sequence, thereby enabling quantification. In addition, the final product can be further characterized by subjecting it to increasing temperatures to determine when the double-stranded product "melts." This melting point is a unique property dependent on product length and nucleotide composition.

Fig.1 Real-time PCR technology (like normal PCR) uses DNA or cDNA as a template for amplification and is highly sensitive. Because the reaction is able to efficiently amplify DNA only up to a certain quantity before the plateau effect, there by measures product formation during "exponential phase" of PCR cycles (Fig.1). Real-time RT (Reverse Trancriptase)-PCR and has become the most popular method of quantitating steady-state mRNA levels (Bustin, 2000)). The precision and sensitivity of real-time RT-PCR, is such an extent that, even subtle changes in gene expression can be detected. Thus real-time PCR can be used to assess both DNA and RNA levels with great sensitivity and precision.

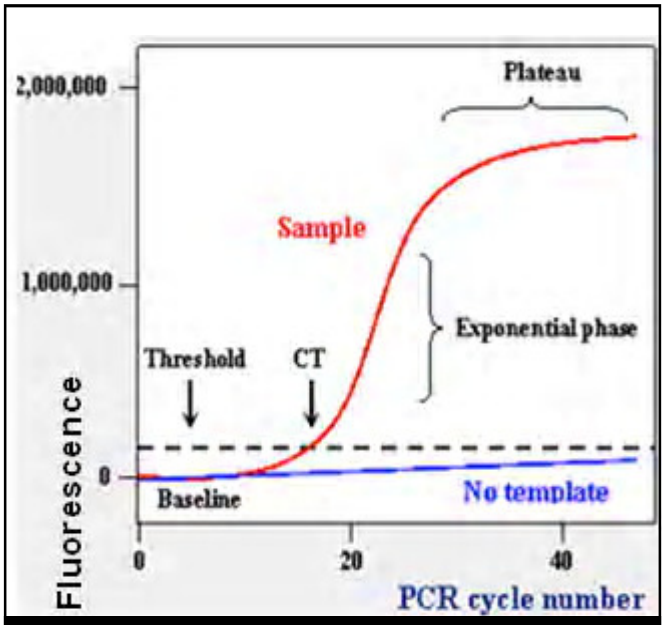

Fig. 1 : Real time PCR technology