Comparison of Real-time PCR fluorescent and non-fluorescent quenchers in standard amplification plots of delta-6 desaturase gene in PANC-1 cell line culture

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Abstract

Background: Employing non-fluorescent quenchers in Real-time PCR is appropriate for gene expression examination.

Objective: The objective of this study was to compare Real-time PCR fluorescent and non-fluorescent quenchers in standard amplification plots of delta-6 desaturase gene in PANC-1 cell line culture.

Methods: This analytical study was conducted in the Reference Laboratory affiliated to Qazvin University of Medical Sciences in 2012. Human pancreatic cancer cells (PANC-1) were cultured in 75 cm² flasks, 3x10⁶ cells were seeded in 6-well plates and were treated with specific intracellular signaling drugs. Changes in expression of delta-6 desaturase gene were examined with fluorescent and non-fluorescent quenchers using Real-time PCR in equal conditions, separately. Data were analyzed using student T-test and gene expression results were analyzed using ΔΔCT method with the assumption of 100% efficiency.

Findings: Employed quenchers showed different absorption of the fluorescence emitted by the reporter and caused different results in Real-time PCR. Using non-fluorescent quencher, the amplification plot was more precise and its baseline was lower. Therefore the signal to noise ratio (S/N) was decreased. Also, the Threshold cycle (Ct) value was lower because of increased Tm (melting temperature) and decreased non-specific bindings.

Conclusion: With regards to the results, non-fluorescent quencher is more appropriate compared to fluorescent quencher and can be a better alternative for current quenchers especially in allelic discrimination and SNP (single nucleotide polymorphism) studies.

Keywords: Real-Time PCR, Delta-6 Desaturase, Cell line

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Received: 24 Apr 2013
Accepted: 11 Sep 2013