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Quantification of Primer Efficiency of the Studies on Severe combined immunodeficiency (SCID) Genotypes Profiles Using real-time PCR Method Based on SYBR Green

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Abstract

The study of the molecular etiologies of severe combined immunodeficiency (SCID) has yielded valuable insights into immune cell development and regulation with the use of gene expression analysis tools that unravel how specific genes respond thus allow us to infer the consequences on the organism physiology, development and survival. Real-time quantitative PCR (qPCR) is an efficient and frequently used tool to quantify gene expression that enables for the detection of the PCR product directly during the exponential phase of the reaction in a single step. Two most common detection method used in quantitative gene expression analysis are by using SYBR Green and TaqMan probe. SYBR Green is a non-specific dsDNA-binding dyes technically based on binding the fluorescent dye to double-stranded deoxyribonucleic acid (dsDNA) and comparatively less expensive while TaqMan probe is highly specific but more expensive. When using SYBR Green, the most important factor to consider is specificity. In this study, we aimed at quantifying primer efficiency of SCID genotypes by using qPCR to determine genetic expression of SCID genotypes profiles with great sensitivity and specificity using SYBR green as a binding dyes for detection. The results using this technique provides a specific and sensitive method to quantify gene expression for a future sequencing-based strategy for the diagnosis of patients with SCID. By using this technique, the use of high performance primer and proper protocols will aid precise detection of gene and the usage of expensive specific probe can be avoided.

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