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

Syarifah Masyitah Habib Dzulkarnain[1,2], Ilie Fadzilah Hashim[1], Zarina Thasneem Zainudeen[1], Intan Juliana Abd Hamid[*1]
Universiti Sains Malaysia[1], Universiti Teknologi MARA[2], Penang, Malaysia

Online 7th ASID Congress October 1st-2nd 2021

Background

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 is the reliable detection and measurement of products generated during each cycle of the polymerase chain reaction (PCR) process which are directly proportionate to the amount of template prior to the start of the PCR process. The two most common detection method used in quantitative gene expression analysis are by using SYBR Green and TaqMan probe.

Purpose:

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. 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.

Methods:

A qPCR assay with SYBR Green was developed for detecting primer efficiency for Interleukin 7 receptor (IL7R), one of the SCID genotypes for determining the defect in signalling pathway. We performed one dilution series consisting of 10-fold dilution of 3 concentrations, each was analysed using 3 qPCR replicates (3 × 3) on Applied Biosystem StepOnePlus™ Real-Time PCR System with following parameters: initial denaturation of 95^{0}C for 3min, followed by 38 cycles of denaturation at 95^{0}C and annealing at 60^{0}c for 10s and extension at 72^{0}C for 5s.

Results:

A standard plot was made from the dilution series and efficiency was calculated from the slope. The efficiency of 91.79% was achieved with $R^2$of 0.9911 showing that 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.

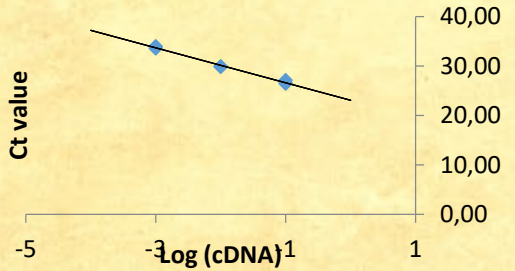

Figure 1: The standard curve plotted for the specific primer efficiency of IL7R.

Results	
Slope	-3.536666667
R Squared	0.9911
Amplification factor (E)	1.92
Efficiency (%)	91.76

Table 1: The efficiency of 91.79% was achieved with R^2 of 0.9911

Conclusion:

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. The assessment of QPCR efficiency by means of a standard curve is a quality benchmark for the assay, reliable and is recommended in the MIQE guidelines.

Acknowledgement:

The authors wish to acknowledge USM for the Short Term Research Grant (USM: 304.CIPPT.6315309) and Ministry of Higher Education Malaysia for sponsoring the student.

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