Quantification of mRNA Levels by PCR Using the Single Standard DNA

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Outline

Recently, real-time RT-PCR has been used to quantify mRNA levels in many gene expression studies. Real-time PCR commonly involves the normalization of the expression levels of the gene of interest with those of the housekeeping genes that are thought to be consistently expressed in all of the samples. However, this quantification method fails to compare the levels of the different gene transcripts on the same scale.

In the present investigation, we developed a quantitative real-time PCR system that uses standard DNA produced by ligating the cDNA fragments of the target genes [Chit1, AMCase, housekeeping gene (GAPDH and β-actin) and reference gene (pepsinogen C)] (Figure 1). Exponential amplification was maintained over a wide range of cycles, yielding a dynamic range of seven orders of magnitude (Figure 2 and Figure 3).

This system enabled us to quantify and compare the expression levels of the chitinases and the reference genes on the same scale.

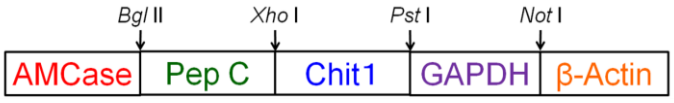

Figure 1. Construction of mouse standard DNA

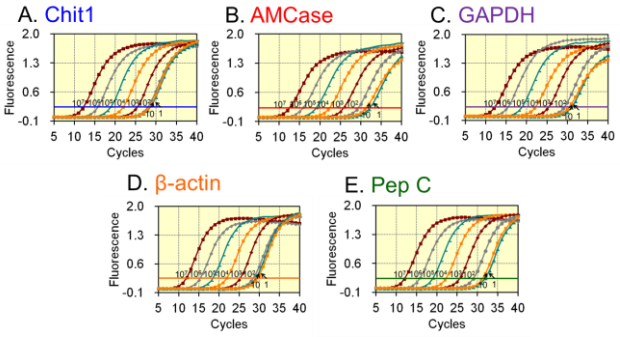

Figure 2. Amplification curve of standard DNA

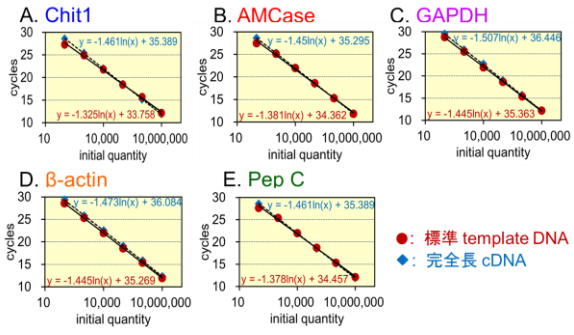

Figure 3. Standard curves of standard DNA

Novelty

We established a quantitative real-time PCR system to evaluate the expression levels of multiple genes using a laboratory-constructed standard DNA. Although our method requires multiple steps associated with the construction of the standard DNA and with the validation processes (Figure 2 and Figure 3), this method can provide gene expression data that are directly comparable between genes.

Application

This technique is very well suited to the quantification and comparison of mRNA levels across multiple genes using the same scale. Therefore our method is applicable to biomedical engineering as well as to clinical and practical uses.

Related information

●Original paper　Ohno, M., Tsuda, K., Sakaguchi, M., Sugahara, Y. and Oyama. F. (2012) Chitinase mRNA levels by quantitative PCR using the single standard DNA: acidic mammalian chitinase is a major transcript in the mouse stomach. ***PLoS ONE*** **7**: e50381.

●URL　http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0050381

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