Establishment of a Quantitative PCR System for Discriminating Chitinase-Like Proteins

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Outline

Recently we established quantitative real-time PCR (qPCR) using a single standard DNA to quantify the expression levels of chitinases and reference genes. In this study, we applied our methodology to the chitinase-like proteins' (CLPs') levels analysis (Figure 1). Ym2 primers could be cross-reactions between Ym1/AMCase and Ym2. We prepared a linear standard DNA containing the pGEM-T Easy sequence. We overcame the problems, and through the validation of the mouse Refs/CLPs standard DNA with pGEM-T Easy, we could individually quantify Ym1 and Ym2 using the standard DNA. Exponential amplification was maintained over a wide range of cycles, yielding a dynamic range of seven orders of magnitude (Figure 2).

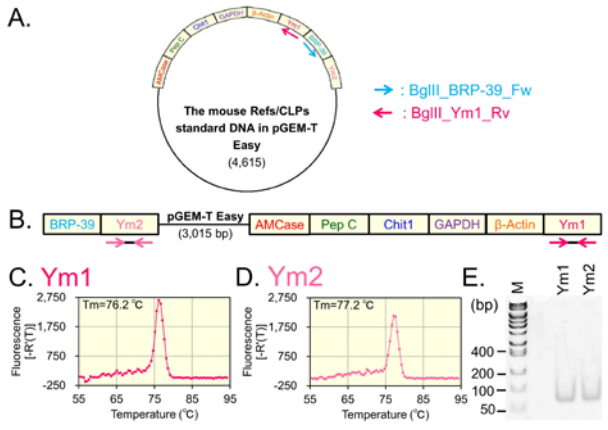

Figure 1. Construction of mouse Refs/CLPs standard DNA with pGEM-T Easy

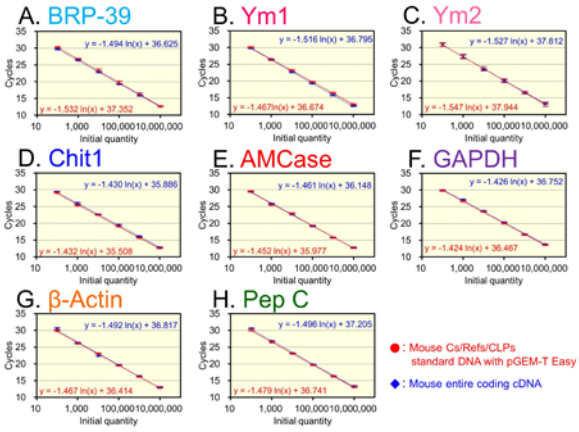

Figure 2. Standard curves of standard DNA

Novelty

To evaluate the expression levels of CLPs in mouse tissues, we developed a quantification system using a mouse Refs/CLPs standard DNA with pGEM-T Easy. We could individually quantify Ym1, Ym2 and BRP-39.

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., Kida, Y., Sakaguchi, M., Sugahara, Y. and Oyama. F. (2012) Establishment of a quantitative PCR system for discriminating chitinase-like proteins: catalytically inactive breast regression protein-39 and Ym1 are constitutive genes in mouse lung. ***BMC Molecular Biology*** **15:** 23.

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