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Quantification of infectious recombinant murine MSCV-based retroviruses using PCR approach.

ABSTRACT

The current available molecular method to detect the retroviral-mediated gene expression is reverse transcriptase-polymerase chain reaction (RT-PCR). However, the conventional PCR is time consuming, prone to error and not sensitive. In this paper, we performed a real-time PCR assay to quantify retroviral mediated gene expression in the supernatant of infectious recombinant virus. We documented an optimized methodology for accurate and reproducible quantification of retrovirus-based gene expression which enables rapid and quantitative determination of sample gene expression. We have also compared the performance of the assay with our routine RT-PCR assay. RNA was extracted from infectious recombinant retrovirus at 48 h post-infection and assayed for VP3 gene. The standard curve from linear DNA standards showed high sensitivity and good linearity (R2 = 0.9902 for VP3 standards graphs), ranged from 102 to 108 copies of comparable accuracy to current quantification real-time PCR methods. The present study presumes a copy number of transgene expression to equal a molecule of infectious recombinant virus particle. Another advantage, this concept could determine vector stability by comparison of infectious particles, total particles and particles with RNA. In comparison to gel-based RTPCR, we conclude that real-time PCR as a better approach in gene expression quantification.

Keyword: Infectious; Retrovirus; Real-time PCR.