Supplementary Data 2

Real-time PCR Methods

The ABI 7700 ® real-time PCR system (Applied Biosystems, USA) was used for analysis. Multiple gene markers distributed around the genome and three housekeeping genes were used for real-time PCR analysis using the SYBR® GreenER™ qPCR SuperMix for ABI PRISM® (Invitrogen, CA). The sequence information of all the primers is listed in Supplementary Data **Table 2**. 5 ng total cDNA isolated from each stable cell line was added to a 20 µl reaction containing SYBR® GreenER™ qPCR SuperMix for ABI PRISM®, and 200 nM of each primer. Triplicate reactions were performed for each marker in a 384-well plate using a two-step amplification program of initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 20 s and 60°C for 30 s. A melting curve analysis step was carried out at the end of the amplification, consisting of denaturation at 95°C for 1 min and re-annealing at 55°C for 1 min. Standard curves were generated from each experimental plate using serial 5-fold dilutions of untreated cDNA. The geometric mean of Ct-value for each reaction was calculated. Amplification efficiencies were calculated according to the equation $E = 10^{(-1/\text{slope})}$ (Heid *et al.*, 1996) and ranged from 90–104% for all gene markers; no unspecific amplification or primer dimmer was observed in any of the reactions as confirmed by the melt curve analysis. To compensate for potential differences in between markers, the relative expressions was computed, based on the efficiency (*E*) , normalized by a panel of housekeeping genes, *β-actin*, *HPRT*, and *GAPDH* and the Ct difference (Δ) of sample versus control ($\Delta Ct_{\text{sample-control}}$).

$$\textbf{Relative expression} = 2^{-(Ct,\text{Sample} - Ct,\text{HKG}) - (Ct,\text{Control} - Ct,\text{HKG})}$$

$$\textbf{Relative expression} = 2^{-ddCt}$$