

### Reverse Transcription

Taqman RT-PCR reagents (Applied Biosystems) were used for the generation of cDNA for all samples.

A master mix consisting of 1x Taqman RT buffer, 5.5 mM MgCl<sub>2</sub>, 500  $\mu$ M of each dNTP, 2.5  $\mu$ M random hexamer, 0.4U/  $\mu$ l of RNase inhibitor, and 1.25U/  $\mu$ l of Reverse Transcriptase was made that was sufficient for all tumor RNA preparations. In a 96-well plate, 90  $\mu$ l of the master mix was added to 2  $\mu$ g of total RNA from each tumor (suspended in 10  $\mu$ l of sterile nuclease free water) and run under the following thermal cycler parameters: 25°C for 10 min, 48°C for 30 min, and 95°C for 5 min. Each sample was then collected, mixed thoroughly, aliquoted, and frozen at -80°C. The identical procedure was utilized to generate cDNA from total mouse liver RNA that was subsequently utilized to clone each amplicon.

### Primer Design and Preparation of Templates

All primers were designed and blasted using IDT Primerquest software ([www.idtdna.com/primerquest](http://www.idtdna.com/primerquest)) from imported sequences obtained from NCBI. The sequences of the primers, respective accession numbers, and product sizes are listed in supplementary data. In order to test the primers and generate templates for the real time PCR quantitation, 4  $\mu$ l of mouse liver cDNA was amplified using 12.5  $\mu$ l 2X SYBR Green master mix reagent (Applied Biosystems), 0.4  $\mu$ M of each primer, and water to a total reaction volume of 25  $\mu$ l. The conditions for the amplification were as follows: 10 min at 95 °C followed by 35 cycles of 15 s at 95°C, 30 s at 60°C, and 30s at 72°C and a final extension of 1 cycle at 72°C for 7 min. The products were run on a gel and melting curve analysis was performed. An aliquot of each reaction was cloned into pCR2.1-Topo (Invitrogen) using standard manufacturers protocol, transformed into Top10® cells, and plated on LB-agar containing ampicillin (100  $\mu$ g/ml) and X-gal (40  $\mu$ g/ml). White colonies were screened by PCR with the appropriate primer set, analyzed on a 2% agarose gel, and each positive clone was sequenced using the universal M13 forward primer. A single colony from each clone, containing the appropriate amplicon, was grown up in 5 ml of LB broth overnight. The plasmids were then isolated using the Qiagen spin-miniprep kit and ultimately eluted with 50  $\mu$ l of 10mM Tris buffer. The concentrations were determined by spectrophotometric analysis at 260nm and 1  $\mu$ l of each plasmid was further analyzed by gel electrophoresis on a 1% agarose gel. Dilutions of the plasmids were created based on the combined molecular weight of pCR 2.1 and the respective cloned amplicon to calculate copy number. Each plasmid set ( $1 \times 10^6$  copies) was initially amplified as described above in order to evaluate the minor differences in

concentration. On average these differences resulted in a  $C_T$  value that varied by less than 0.5 cycles, which likely represents the variation from spectrophotometric reading or dilutions.

#### Real Time Quantitative PCR

All real time runs were performed under identical conditions using the Opticon 2 Monitor (MJ Research) with 2X SYBR Green master mix reagent (Applied Biosystems) from the same lot number. Real-time PCR runs were performed in 96-well optical plates in triplicate, each containing 1X SYBR Green master mix (Applied Biosystems), 0.4 pmol/ $\mu$ l of appropriate forward and reverse primer and 1  $\mu$ l template DNA (either cDNA or appropriate plasmid DNA at ten-fold dilutions ranging from  $10^7$  to  $10^2$  copies/ $\mu$ l in a final volume of 25  $\mu$ l). The conditions for the amplification were as follows: 1 cycle of 2 min at 50°C and 1 cycle of 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, 30 s at 60°C, and 30s at 72°C. Data was acquired at the end of each 72°C cycle. Following the final cycle, the samples were subjected to melting curve analysis over a temperature range of 65°C to 95°C in 0.2°C increments with data acquisition at each increment for 0.1 s. At the completion of each run the baseline was subtracted over the appropriate cycle range and the  $C_T$  adjusted accordingly in order to generate the highest efficiency with ideal linearity for each standard curve. Quantitative values (represented as number of copies) were determined for each well using the equation of the standard curve in each run. Triplicates were averaged and normalized to the  $\beta$ -actin expression by taking the ratio of gene expression to  $\beta$ -actin expression for each appropriate sample. The actin ratios were then averaged for each sample in each tumor group and graphed (error bars represent SEM where n=3-5).