

XenoQ™ Assays



XenoQ™ Assays have been designed to detect human tumor xenografts in a mouse background and to identify gene expression changes that are crucial to malignant transformation, invasion and metastasis. These assays utilize the sensitivity of quantitative PCR and SYBR® Green I to detect gene expression in a minimal amount of human tumor cells that are circulating in the mouse bloodstream without interference from mouse cell gene expression. Previous studies involving the use of mouse tumor models have often raised the question of whether human tumor data obtained from a xenograft model is impacted by gene expression changes within the mouse. Those studies have also required that the mice be sacrificed in order to remove and analyze the response of the implanted human tumor to therapeutic interventions.

Benefits:

- Low copy number detection
- Minimize host vehicle costs
- Higher clinical transition success rates

XenoQ™ Assays offer several advantages over previous xenograft experimental methodologies. Because XenoQ™ Assays are effective at detecting minimal numbers of human cells found in the bloodstream of the mouse, a researcher has the option of taking serial blood draws as opposed to sacrificing the mouse in order to study tumor responses to therapy over time. This minimizes cost and variability by eliminating the need to sacrifice multiple mice. In addition, preclinical xenograft studies are all too often followed by failure of a particular drug/therapy in clinical trials as a result of analyzing and detecting both human and mouse genetic changes. XenoQ™ Assays will minimize these failures by allowing researchers to specifically focus on how the drugs/therapies are impacting the human tumors without cross-detection of genetic changes in the mouse models. Measuring the response of a human tumor to drugs/therapies will be done faster and more accurately using XenoQ™ Assays while significantly reducing the experimental costs of the preclinical trials.

Technical Advantages:

- Gene Expression and Copy Number Detection
- No cross-reactivity guaranteed
- Full customization of genes and format

XenoQ™ Assays have dual functionality; they can be used to quantitate gene copy number variation as well as gene expression. XenoQ™ Assays have been designed and tested using strict protocols. A method for primer design has been developed that results in the amplification of human targets and these primer sets have no specificity for mouse targets. Each primer set has been rigorously tested against both genomic DNA (gDNA) and complementary DNA (cDNA). When tested in a 384-well format, each primer set can successfully detect gene expression in as little as ≥ 60 ng of human

gDNA in a background of up to $\leq 1\mu\text{g}$ of mouse gDNA; equivalent to $\geq 0.14\text{ng}$ Hs gDNA in a background of $\leq 2.33\text{ng}$ Mm gDNA per $10\mu\text{L}$ reaction. Ct values for the gDNA-validated human primers fall in the range of 23-35. When tested against cDNA in a 384-well format, each primer set can detect human cDNA derived from an equivalent of $\geq 125\text{ ng}$ of RNA with a background of mouse cDNA derived from an equivalent of $\leq 2\mu\text{g}$ of RNA; equivalent to $\geq 0.3\text{ng}$ Hs cDNA with a background of $\leq 4.65\text{ng}$ Mm cDNA per $10\mu\text{L}$ reaction. Ct values for the cDNA-validated primers fall in the range of 22 - 36.9. cDNA was derived from Stratagene QPCR Human Reference Total RNA (CAT. #750500) and Stratagene QPCR Mouse Reference Total RNA (CAT. #750600).

XenoQ™ Assays are available as fully customizable assays and can be arranged in 48, 96 and 384-well formats. Validated Primer sets have been designed with a special focus in cancer, toxicology and immunology. We will continue to expand our database and will design primer sets that researchers would like to add to their XenoQ™ Assays.

Analysis:

- Global Pattern Recognition™ included for FREE

Global Pattern Recognition™ Software is extremely simple to use and reliably tabulates statistical significance (p-value) of gene expression changes on the fly allowing you to immediately focus on the real biology. Simply log into GPR™, select the XenoQ™ Assay that you ran on your real-time PCR instrument, upload your data and submit for analysis. An HTML or Excel® formatted file will be generated that gives a ranked list of genes by p-value, fold change value, and links to MGI and NCBI gene pages. Analysis by Global Pattern Recognition™ (GPR™) is included at no cost.

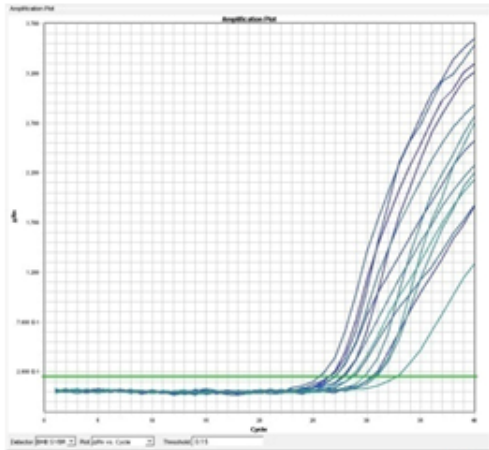
- Tabulates statistical significance (p-value) of gene expression changes

GPR™ analyzes the data generated by the XenoQ™ Assays and determines what the real changes in gene expression are. GPR™ Software provides a true statistical analysis of results based on consistency in the data and accordingly it is optimally suited to detect small, but reproducible changes. Only after the genes are statistically ranked is the magnitude of the change calculated. A typical XenoQ™ Assay experiment would utilize 'biological replicates' (Bio-Reps). Bio-Reps are defined as samples collected from separate and closely matched biological samples. They are processed individually under closely matched conditions. GPR™ Software processes the data derived from groups and reveals the 'constellation' of changing genes. Each constellation can be evaluated for the most likely biological context providing the researcher with a better understanding of the overall results. Each constellation may also provide the researcher with a step-wise treatment plan that can revert the tumor phenotype back to a more normal state.

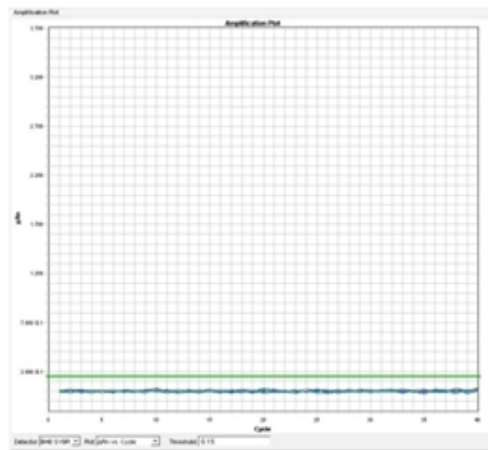
- GPR™ identifies normalizers within the experiment

GPR™ Software globally positions the expression level of each gene with respect to all genes within an experiment. This can be done without prior assumption that a gene (normalizer) has an invariant expression level. GPR™ Software is unbiased in that it enables the experimental data to define the invariant normalizer genes, not the experimenter. The use of any gene as a potential normalizer also maximizes the use of the limited real-estate on XenoQ™ Assays by eliminating the loss of wells used to contain potentially erroneously predefined normalizers.

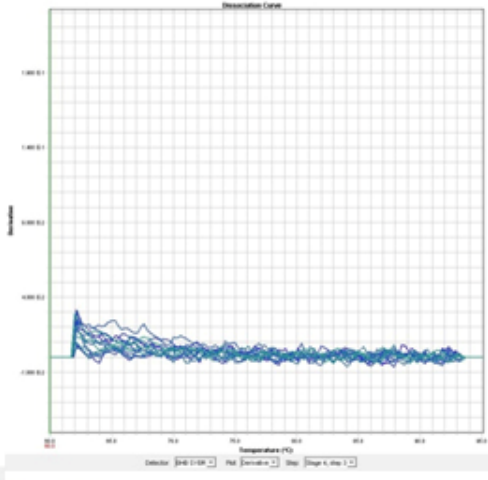
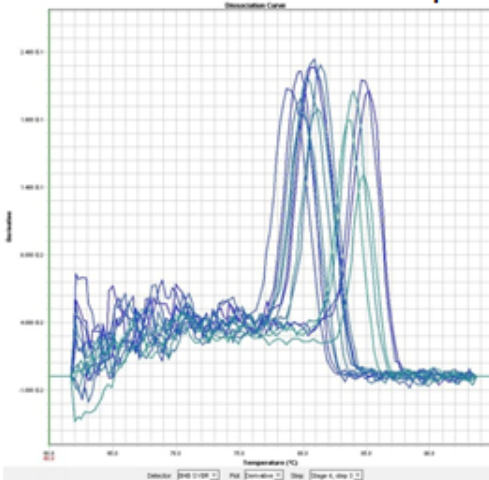
Human cDNA



Mouse cDNA



Amplification Plots



Dissociation Curves

XenoQ™ Assay primers were QA/QC tested using both genomic and complementary DNA. The above figures depict amplification plots and dissociation curves derived from XenoQ™ Assays performed using human or mouse cDNA. XenoQ™ Assays guarantee no mouse cDNA cross-reactivity.

XenoQ™ Assays: Technical Notes

XenoQ™ Assays were validated using the following procedures.

Human gDNA template was run against primer sets alone or with a background of mouse gDNA in quadruplicate reactions using a hot-start based SYBR® Green I detection system. The reactions are run using the following Applied Biosystems 7900HT Real-Time PCR System conditions: one (1) cycle of 50°C for 2 minutes, one (1) cycle of the recommended Master Mix Specific Hot Start Temperature and Time, forty-five (45) cycles of: 95°C for 10 seconds, 67°C for 20 seconds and 72°C for 40 seconds (data acquired at 72°C). The run is complete after the acquisition of a standard dissociation curve.

The first round of evaluation begins with validation of primer pairs in gDNA. Primer pairs that generate a multi-peak dissociation curve (DC test) when run against human gDNA are eliminated. Once primer pairs pass the DC test, they are then evaluated using the Ct test. If the mean Ct of the quadruplicates is ≥ 37 cycles, they are eliminated. In addition, primer pairs run against mouse gDNA whose mean Ct values are ≤ 37 cycles are also eliminated. Finally, human gDNA and mouse gDNA were added simultaneously over a range of concentrations in order to determine threshold requirements for human gDNA input and threshold allowances for background mouse gDNA. It is recommended to use ≥ 60 ng human gDNA with a background of ≤ 1 μ g mouse gDNA.

The second round of evaluation involves testing the primer pairs in cDNA. Human and mouse RNA are DNase-treated followed by the validation of its integrity using an Agilent BioAnalyzer 2100. Samples with (a) genomic contamination or (b) RNA degradation are not used. It is critical that the RNA that is used for XenoQ™ Assays is of the highest quality. Following Agilent analysis of RNA, RNA is transcribed into cDNA using the following kit: MessageSensor RT Kit (Cat#1745, AB/Ambion). Primer pairs are subjected to the same Applied Biosystems 7900HT Real-Time PCR System conditions as above. Primer pairs are eliminated as a result of two occurrences: First, any primer pair with a Ct value of > 37 cycles when run against human cDNA is eliminated. Second, any primer pair with a Ct value of ≤ 37 cycles when run against mouse cDNA is eliminated. It is recommended to use human cDNA derived from an equivalent of ≥ 125 ng of RNA and mouse cDNA derived from an equivalent of ≤ 2 μ g of RNA.

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