Lonza

White Paper:

StellARray™ Gene Expression System

Revealing Profiles with Unbiased Significance

Application Examples

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Abstract: In this paper, we present three application examples demonstrating the utility of the StellARray™ Gene Expression System to reveal gene expression level changes in diverse biological contexts such as toxicology, cancer, and stem cell differentiation. By combining Clonetics® and Poietics® Primary Human Cells with the StellARray™ Gene Expression System, all from Lonza, the researcher is provided with a synergistic system to reveal gross and subtle changes in gene expression when analyzing *in vitro* models of human tissues. This is accomplished easily in 96- and 384-well formatted StellARray™ qPCR Arrays using a standard qPCR instrument and a generic SYBR® Green-based Reagent Master Mix. The Global Pattern Recognition™ (GPR) Data Analysis Tool is optimally suited to generate a ranked list of significantly changed genes within a qPCR dataset. GPR overcomes the inconsistencies associated with conventional single gene normalization procedures by eliminating *a priori* normalizer selection. Overall, the results show how the StellARray™ Gene Expression System eliminates false positives and provides TRUE results that are backed by a rigorous statistical analysis.

Introduction

Molecular profiling is widely regarded to be integral for advancing life science research and clinical diagnosis in the 21st century. It is increasingly recognized that subsets of genes (hundreds rather than thousands) provide the most reliable gene profile definitions of any specific biological process.

We have developed a novel solution – The StellARray™ Gene Expression System – to meet the need of biomedical scientists for reliable profiling of biologically focused gene sets. It is based on quantitative polymerase chain reaction

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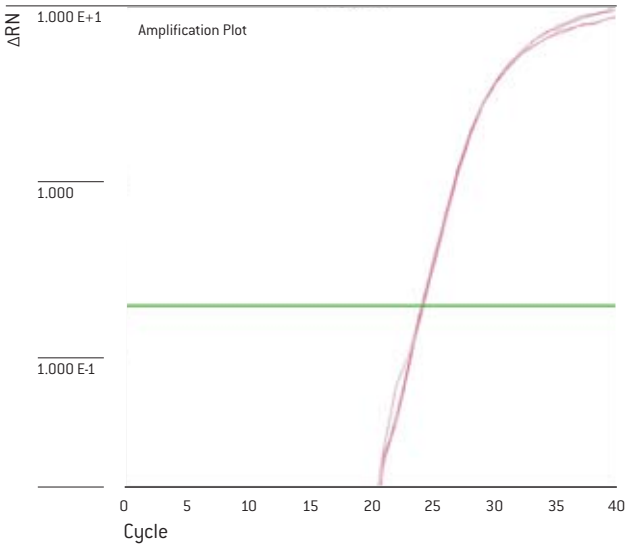

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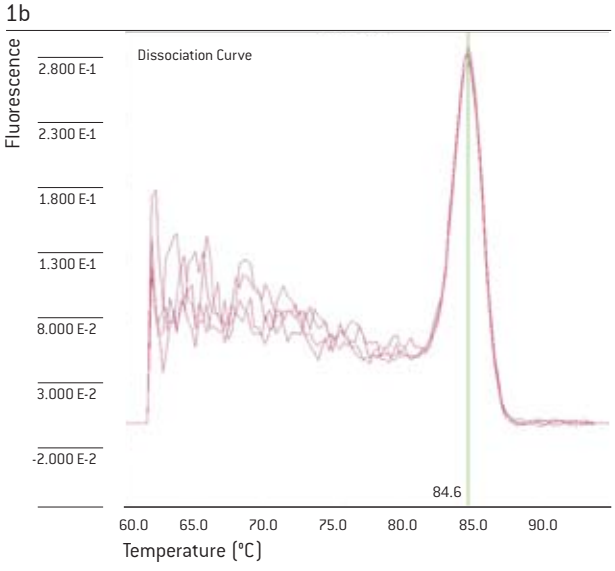

Figures 1a & b. Real-time PCR data showing amplification curves of quadruplicate wells from a StellARray™ qPCR Array under standardized reaction conditions. **1a**. Example of highly reproducible amplification curve data. **1b**. A single peak indicating a single PCR product.

(qPCR), the gold standard for accurate measurements of gene expression, having an inherently wider dynamic range when compared to microarrays.

The StellARray™ Gene Expression System

The StellARray™ Gene Expression System is comprised of three parts:

1) StellARray™ qPCR Array
2) Global Pattern Recognition™ (GPR) Data Analysis Tool
3) GeneSieve™ Query

Each StellARray™ qPCR Array is compiled to provide the highest information content with a scientifically relevant context. Each qPCR array is either a 96- or 384-well formatted, 'wet lab' validated group of gene primer sets, focused on specific biological processes. A single gene quality control example illustrating the accuracy and reproducibility of amplification using the StellARray™ qPCR Array is shown in (Figures 1a & b). StellARray™ qPCR Arrays are used with standard qPCR instruments and SYBR® Green-based Reagents. They provide a streamlined pathway to successful molecular profiling experiments.

The Global Pattern Recognition™ (GPR) Analysis Tool has solved one of the most fundamental problems facing experimentation using qPCR.

"How do I analyze the data and determine REAL changes in gene expression?"

The answer to this question is found in the GPR algorithm, which makes gene expression analysis simple, fast and reliable.

The StellARray™ Gene Expression System also includes the GeneSieve™ Query, bringing a new level of search capability to researchers by merging databases containing gene-centric biological information in a new and innovative way. GeneSieve™ Query can filter or "sieve" thousands of genes for a custom population of real-time PCR arrays that have a unifying biological theme, such as a particular disease or pathway of interest.

Why is there a need for the Global Pattern Recognition™ (GPR) Algorithm?

The traditional approach to measure gene expression changes from qPCR data has been to normalize the results of a gene of interest with respect to a housekeeping gene (a.k.a.

a reference or normalizer gene). The general assumption is that the level of expression of the normalizer gene does not change in the context of the experiment and can be used to normalize the variability in RNA quantity between individual samples. By normalizing to a housekeeping gene, in theory, a magnitude of change can be calculated between groups of samples for a gene of interest. However, this mode of analysis is greatly complicated by the fact that housekeeping genes commonly used as normalizers (e.g., GAPDH, ß-actin, and HPRT) can themselves change in apparent expression when comparing tissues or cells in different states (Bustin 2000; Schmittgen et al. 2000; Goidin et al. 2001; Hamalainen et al. 2001).

18S rRNA is another normalizer that intuitively and experimentally seems more stable, but even 18S can vary in comparison to other genes when analyzed by sensitive qPCR techniques (Bustin 2000, Akilesh et al., 2003). Additionally, the use of 18S rRNA preempts the use of oligo-dT as a first-strand cDNA synthesis primer as 18S rRNA is not poly-adenylated and will yield varying amounts when using purified mRNA via oligo-dT based mRNA purification systems. Any small variation in the normalizer amplification would therefore compromise the analysis of the complete qPCR data set.

(Figure 2) illustrates the vagaries of reliance on single gene normalization. In this example, the p-value distribution for 384 genes is compared by normalizing to either 18S or GAPDH. It can be clearly seen that there is statistical discordance with respect to many of the genes determined to be statistically significant changes by either method alone. This illustrates the disparity that can result from single gene normalization.

Global Pattern Recognition™ (GPR) Algorithm, modified for the StellARray™ Gene Expression System

To combat the above mentioned problems, access to a modified, proprietary GPR algorithm is provided as part of the StellARray™ Gene Expression System, which is optimally suited to generate a ranked list of significantly changed genes within a qPCR dataset. This unique algorithm and accompanying software overcomes the problem of identifying invariant normalizers by globally positioning changes in gene expression with respect to all genes within an experiment.

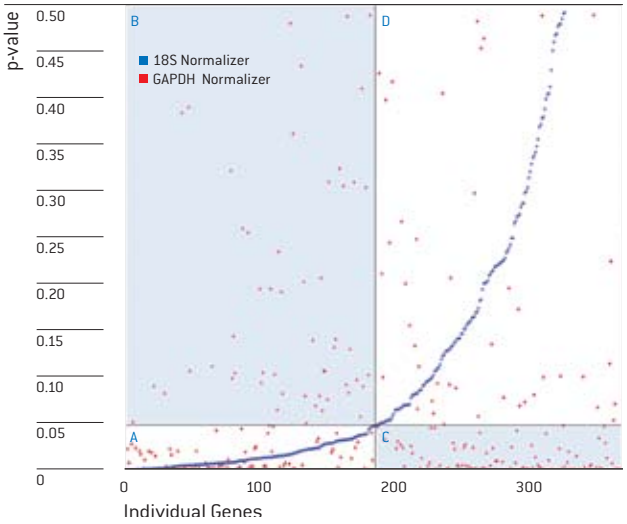

Figure 2. Comparison of the p-value distributions of genes normalized to either 18S or GAPDH illustrates the disparity of results produced by single gene normalization. This example shows values from the Human Lymphoma and Leukemia 384 StellARray™ qPCR Array (Cat. No. 00188333) for cDNA derived from Human Lymphoma compared to Normal Adjacent Tissue RNA (Applied Biosystems, Inc., Part No. AM7268). Genes on the X-axis are sorted according to p-values in ascending order (left to right) relative to the 18S normalization. Data points for each unique gene, normalized to either 18S or GAPDH, are located in the same vertical plane. Overall, quadrants containing data points for both 18S and GAPDH (blue and red) represent genes in statistical agreement between both normalization routines. Specifically, quadrant A (lower left) shows those genes that are significantly different between the two groups when normalized relative to either 18S or GAPDH. Quadrant B (upper left) shows those genes determined to be significantly different when normalized to 18S, but not by GAPDH. Quadrant C (lower right) shows those genes determined to be significantly different when normalized to GAPDH, but not by 18S. Quadrant D (upper right) shows those genes that are not significantly different when normalized to either GAPDH or 18S.

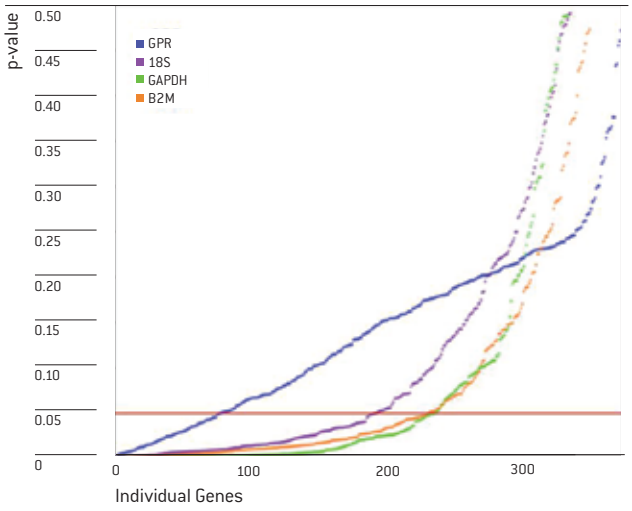

Figure 3. P-value distribution of single gene normalized values for three commonly used 'Normalizers' compared to GPR. Each data point represents a single p-value with respect to a single gene. Genes for each method have been independently sorted by p-value. The data was derived from the Human Lymphoma and Leukemia 384 StellARray™ qPCR Array (Cat. No. 00188333) by determining the expression level changes in cDNA derived from Human Lymphoma compared to Normal Adjacent Tissue RNA (Applied Biosystems, Inc., Part No. AM7268). For illustrative purposes, data above a p-value of 0.5 has been excluded from the graph. The GPR global normalization process produces a linear p-value distribution with a lower false positive rate resulting in more accurate data and solid gene leads.

Rank	Gene Name	p-value	Fold Change
1	Gene A	0.0001	29.4376
2	Gene B	0.0003	19.8958
3	Gene C	0.0007	6.2012

Figure 4. An example of GPR data formatting for data analysis purposes. The magnitude of fold change is calculated after statistical ranking by p-value has been performed.

GPR provides a true statistical analysis of results based on internal consistency of the data, which makes GPR optimally suited to detect small, but reproducible, changes in gene expression. GPR also eliminates data skewing that results from the variability associated with single gene normalizers. Compared to conventional analysis methods, the data skew correction provided by the GPR global normalization process produces a linear p-value distribution with a lower false positive rate resulting in more accurate data (Figure 3).

Only after the genes are statistically evaluated for replicate consistency is the magnitude of the change calculated. An example of the GPR output is illustrated in (Figure 4).

Application of the StellARray™ Gene Expression System

Materials and Methods

Since true biological replicates are not necessarily available, it is acceptable to generate samples from split cultures of cells that have been treated as individual samples. This includes RNA purifications and cDNA syntheses and are referred to as biotechnical replicates ('biotech reps', for short). In this example, the toxicology and stem cell applications, we used quadruplicate biotech replicates per group. In some cases, for example, it is acceptable to generate data sets derived from technical replicates. In this context, a technical replicate refers to data sets that are derived from single or pooled cDNA's that are then split into triplicates (at least) and analyzed as individual replicates.

For the cancer application the data sets were derived from technical triplicates.

Sample Preparation

RNA purification

All RNA purifications were carried out using the RNeasy Mini Kit (RNeasy Mini Kit, Cat. No. 74104, Qiagen, Inc.) and following the manufacturer's recommendations for cultured cells. One microliter (1µl) of SUPERase-In™ ([20 U/µl] Cat. No AM2694, Applied Biosystems, Inc.) was added to the bottom of the RNA elution receiver tube (just prior to elution) to improve RNA stability. The RNA quantity was determined using the RiboGreen® Assay Kit (Quant-iT™ RiboGreen® RNA Assay Kit, Cat. No. R11490, Invitrogen, Inc.). The RNA quality was determined using the BioAnalyzer 2100 microfluidics-based

platform (2100 Bioanalyzer System, Cat. No. G2940CA, Agilent, Inc.).

cDNA Synthesis

Approximately 200ng total RNA was used as template per cDNA reaction using the qScript cDNA Synthesis Kit (qScript cDNA Synthesis Kit, Cat. No. 95047, Quanta Biosciences, Inc.) following the manufacturer's recommendations.

Cell Culture

All cells were cultured using the specific growth and differentiation guidelines for each cell type.

Toxicology – Clonetics® Ready Heps™ Fresh Human Hepatocytes (Cat. No. CC-2703, Lonza) were cultured on collagen and allowed to recover from shipping for 24 hours in HCM™ Hepatocyte Culture Medium SingleQuots® Kit (Cat. No. CC-4182, Lonza) at 37°C with 5% (v/v) CO_2. A 1M stock solution (in ethanol) of Acetaminophen (Cat. No. A7085, Sigma-Aldrich) was used to produce medium containing a final concentration of 10mM Acetaminophen in fresh HCM™ Medium for the treatment plate. HCM™ Medium was removed from the cells and 2ml of medium, treated or untreated, was added to the cells. Cells were cultured at 37°C with 5% (v/v) CO_2 incubation for 4 hours. Cells were trypsinized, scraped and processed for RNA and cDNA as described above.

Cancer – FirstChoice® Human Tumor/ Normal Adjacent Tissue RNA (Cat. No. AM7268 Human Lymphoma/Normal Adjacent Tissue RNA, Applied Biosystems, Inc.) was used as the source for RNA and cDNA was synthesized as described above.

Stem Cells – hMSC Human Mesenchymal Stem Cells (Cat. No. PT-2501, Lonza) were cultured in MSC Growth Medium in T75 flasks for 1 week for expansion (MSCGM™ Mesenchymal Stem Cell Growth Medium BulletKit®, Cat. No. PT-3001, Lonza) at 37°C with 5% (v/v) CO_2 incubation. For stem cell osteogenic differentiation, hMSC's were plated in 24-well plates at a density of 3100 cells/cm^2 in Osteogenic Basal Medium using the hMSC Mesenchymal Stem Cell Osteogenic Differentiation BulletKit® (Cat. No, PT-3002, Lonza). Differentiation was initiated 2 days after initial plating using the supplied SingleQuots® Factors. Medium was changed every 2-3 days. Select wells were stained for positive mineralization with OsteoImage™ Mineralization Assay (Cat. No. PA-1503, Lonza) at day 21. Cells were harvested on day 22 of differentiation and processed for RNA and cDNA as described above.

Real-time PCR

Quantitative PCR data was collected using the three application-specific 384-well StellARray™ qPCR Arrays:
Toxicology Application - Human General Toxicology 384 StellARray™ qPCR Array (Lonza Cat. No. 00188331)
Cancer Application - Human Lymphoma & Leukemia 384 StellARray™ qPCR Array (Lonza Cat. No. 00188333)
Stem Cell Differentiation Application - Human Stem Cell 384 StellARray™ qPCR Array (Lonza Cat. No. 00188335)

Each StellARray™ qPCR Array well was loaded with 10 microliters of sample-specific, SYBR® Green Master Mix containing a chemically modified hot-start *Taq* Polymerase. The array was heat sealed, and run on a 7900HT Sequence Detection System (Applied Biosystems, Inc.) using default cycling parameters for 40 cycles (1 cycle of 50°C for 2 minutes, 1 cycle of 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute). Fluorescence data was acquired during the 60°C anneal/extension plateau. Post-run data collection involved the setting of a common threshold (Ct) across all arrays within an experiment, exportation and collation of the Ct values, and analysis via GPR.

GPR algorithm

Data input for GPR consists of a list of Ct values, derived directly from real-time PCR instruments, for each sample (control or experimental). After designating the control and experimental sets of Ct values, GPR filters expression data to separate genes into two groups – genes considered for analysis (G) and genes that can be used as potential normalizers (N). Genes that are not expressed in either sample are not considered further. After filtering the data into two sets, GPR performs a proprietary global normalization and statistical analysis by comparing each G to each N. The magnitude of change in expression ("fold change") for each gene is subsequently determined using the ten best N genes, as defined within each experiment.

Rank	Gene Name	p-value	Fold Change
1	MTTP	0.000003	25.6104
2	TNF	0.000005	22.2026
3	ABCG2	0.000035	6.9525
4	CCNA2	0.000093	3.8869
5	NDUFB7	0.000152	-3.6055
6	FOXM1	0.000386	2.7551
7	HBEGF	0.000410	3.4209
8	NF1	0.000451	3.3640
9	DAP	0.000866	-2.4263
10	XDH	0.000896	2.2707
11	IGF2R	0.000967	2.1461
12	ABCC6	0.001346	-2.4395
13	SLC2A1	0.001552	-1.9195
14	TNFRSF10A	0.001718	2.1947
15	LTA	0.001998	1.8904
16	CTNNB1	0.004284	-1.5992
17	CYP46A1	0.005057	3.5484
18	TDG	0.005679	1.6220
19	CYP27A1	0.005712	-1.6677
20	CYP2B6	0.006508	3.4141
21	TRADD	0.007074	2.3028
22	CYP2F1	0.007913	1.6927
23	HSF1	0.007989	-2.1288
24	FANCA	0.009453	1.5597
25	POLI	0.010434	-1.4573
26	CCND1	0.010734	1.9355
27	BNIP3L	0.011573	1.7450
28	BAD	0.012439	1.8321
29	NDUFV1	0.013065	-1.4036
30	IGFBP3	0.013439	1.6556
31	ABCD1	0.015049	1.5840
32	WT1	0.016766	1.9785
33	DDIT3	0.017442	1.3742
34	ORM1	0.017586	-1.4208
35	HSGenomic	0.017737	-1.6592
36	EGR1	0.017809	1.6905
37	FGF2	0.019810	2.1166
38	ALOX5	0.023047	-1.4151
39	FADD	0.025898	-1.5276
40	CSK	0.026779	1.5934
41	RAD52	0.027359	1.9732
42	APOA1	0.028267	1.3382
43	CPT1A	0.030919	-1.3579
44	TNFRSF11B	0.031880	-2.4179
45	SULT1A1	0.032340	1.3962
46	HSPB1	0.033456	1.3157
47	FMO1	0.033576	1.9110
48	VDR	0.034058	1.9021
49	HGF	0.035274	-1.2740
50	IL6	0.036004	6.7460
51	PKMYT1	0.036333	1.3992
52	COL1A1	0.036926	1.3176
53	TH	0.037702	-1.3223
54	RB1	0.039865	1.3646
55	BNIP3	0.041356	1.5907
56	RARA	0.041602	-1.3193
57	CDH1	0.042138	-2.0772
58	AHR	0.042339	-1.2777
59	SRD5A2	0.042905	1.8402
60	CDK4	0.043001	2.1048
61	HSPD1	0.044228	2.3646

Table 1. Toxicology – GPR results derived from Acetaminophen treated Clonetics® Ready Heps™ Primary Human Hepatocytes. Results shown represent genes whose expression levels are statistically different between the treated and untreated groups and fold change values are displayed with respect to the treated group.

Results

Toxicology

In concert with Clonetics® Ready Heps™ Fresh Primary Human Hepatocytes, we evaluated the GPR-specific expression profile of the effect of exposure to Acetaminophen (APAP). This classic gene expression profiling example involves the demonstration of Acetaminophen-derived hepatotoxic effects.

GPR analysis yielded 61 significantly changing genes ('hitters') from 383 potential targets (see Table 1, Figure 5) using the Human General Toxicology 384 StellARray™ qPCR Array (Lonza Cat. No. 00188331). Ingenuity® Pathway Analysis (Ingenuity® Systems) (Figure 5a) identified the following top 5 canonical pathways associated with the GPR hitters:

1. **NRF2-mediated Oxidative Stress Response**
2. **IL10 Signaling**
3. **Hepatic Fibrosis / Hepatic Stellate Cell (HSC) Activation**
4. **Aryl Hydrocarbon Receptor Signaling**
5. **PXR/RXR Activation**

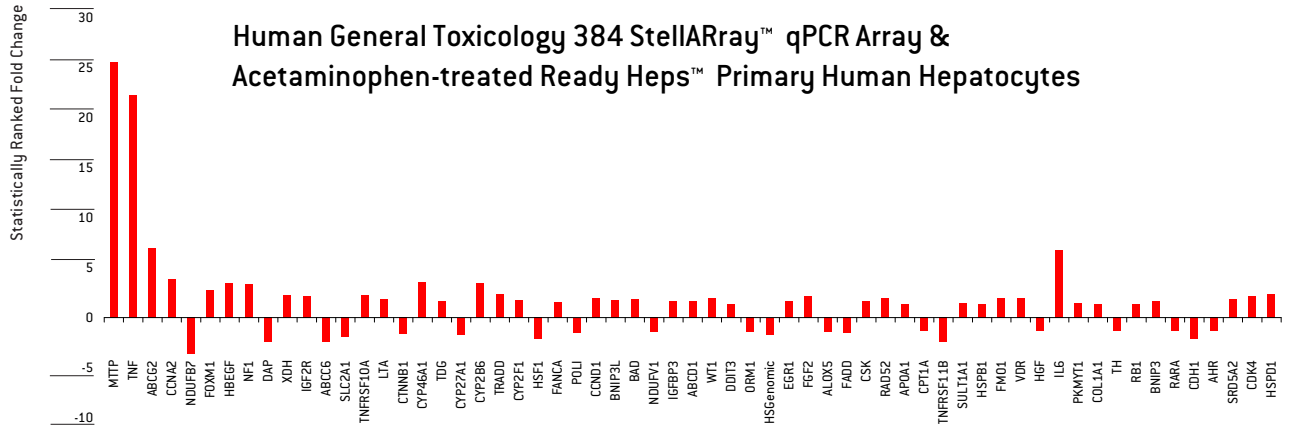

Figure 5. Graphical representation of Table 1. Toxicology GPR results.

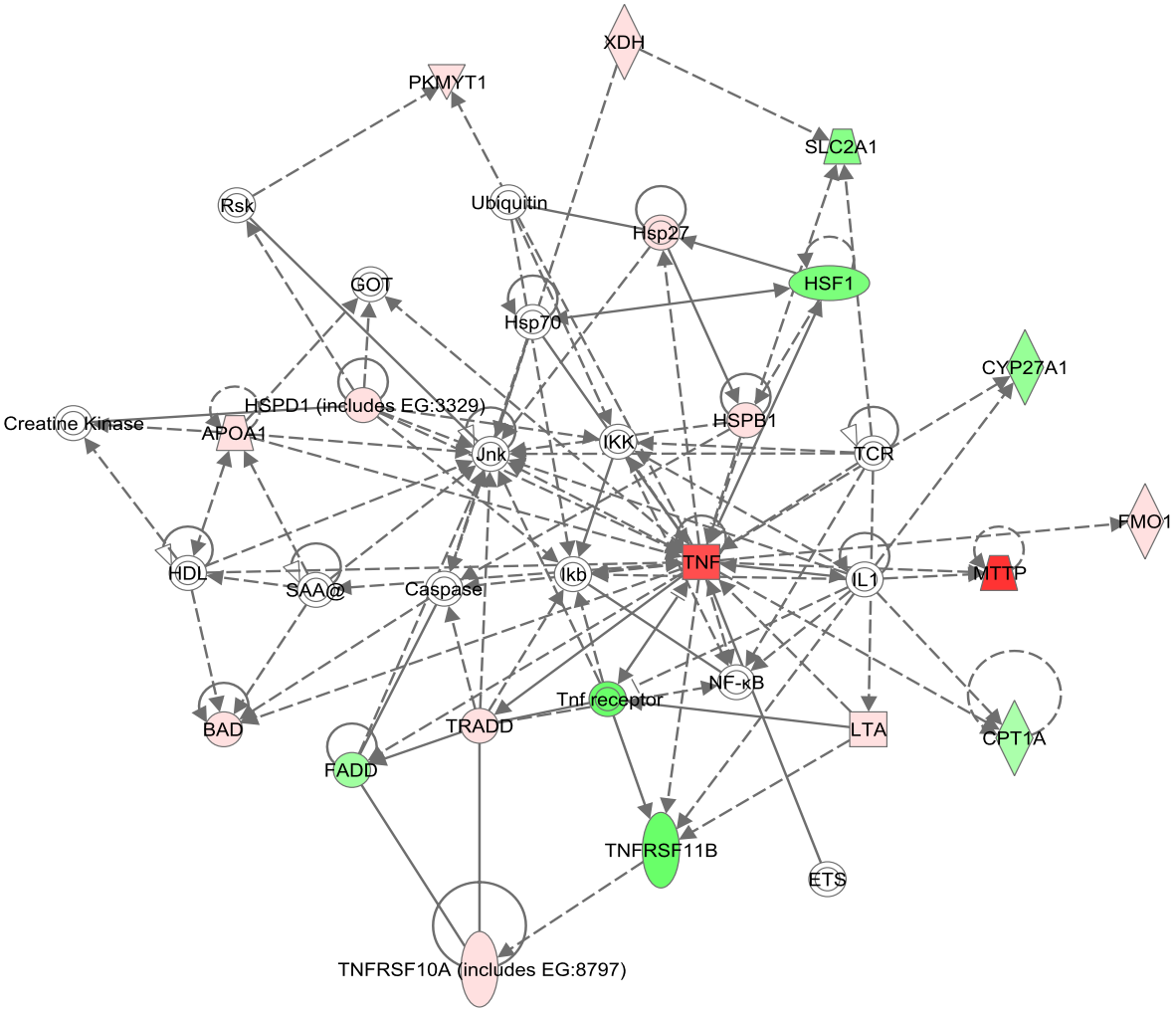

Figure 5a. Ingenuity® Pathway Analysis generated pathway associated with expression levels of MTTP and TNF, the two highest statistically ranked genes, and other associated genes.

Rank	Gene Name	p-value	Fold Change
1	CD83	0.0016	59.8957
2	IGHA1	0.0039	-88.5932
3	BLK	0.0041	57.9644
4	CD79A	0.0048	30.0239
5	LRMP	0.0049	34.5381
6	IL21R	0.0053	20.2479
7	IGHD	0.0054	65.0405
8	CD80	0.0061	15.2789
9	CD79B	0.0064	41.1093
10	FOSB	0.0069	-28.0115
11	FOS	0.0070	-37.4616
12	BCL11A	0.0074	16.0076
13	REL	0.0083	22.4958
14	LMO2	0.0083	15.0837
15	CD40	0.0099	16.0065
16	POU2AF1	0.0109	11.0425
17	PTPRC	0.0113	11.5661
18	TNFRSF13C	0.0119	9.7202
19	EZH2	0.0121	9.6434
20	DTX1	0.0121	10.2096
21	CD19	0.0125	19.4257
22	LEF1	0.0141	8.3117
23	RAD51	0.0147	12.8059
24	IKZF1	0.0148	11.0648
25	IL8	0.0150	8.3743
26	CD86	0.0153	9.2918
27	IL2RA	0.0161	13.9822
28	TRAF4	0.0166	9.9143
29	PAX5	0.0175	13.3893
30	LTB	0.0184	6.9180
31	BCL6	0.0192	6.6047
32	BLR1	0.0197	24.9634
33	CDC20	0.0203	9.6912
34	POU2F2	0.0211	7.9444
35	SYK	0.0217	5.3916
36	KIT	0.0224	-8.6915
37	IGF2	0.0238	-9.4461
38	FANCA	0.0243	6.1093
39	CDKN1C	0.0243	-10.9654
40	LCK	0.0245	8.5314
41	CXCL9	0.0247	7.0802
42	CXCR4	0.0258	6.3874
43	MAF	0.0263	-9.3062
44	IRF8	0.0275	4.7797
45	CHEK1	0.0276	6.7642
46	RUNX3	0.0283	5.7920
47	IGF1R	0.0289	-9.0576
48	IKZF3	0.0289	6.2917
49	CCNB2	0.0291	5.6233
50	ID2	0.0302	-7.8832
51	H2AFX	0.0304	5.2044
52	HOXA1	0.0321	4.1611
53	MKI67	0.0321	4.7219
54	LMO1	0.0330	-25.5439
55	BLM	0.0349	4.7992
56	IRF6	0.0349	-7.7860
57	CCND2	0.0355	-9.2957
58	SHH	0.0356	-16.5320
59	PTGS1	0.0361	-5.8911
60	SOCS2	0.0381	-5.9421
61	IRF4	0.0387	4.5656
62	DKK3	0.0387	-5.2665
63	CDKN1A	0.0395	-6.4348
64	IgL	0.0399	-5.7854
65	CCND1	0.0408	-5.3136
66	BRCA1	0.0423	4.4661
67	MAFK	0.0427	-9.9578
68	BIRC3	0.0429	3.6171
69	CNTN1	0.0432	-10.6860
70	AKT1	0.0441	-6.2361
71	WRN	0.0451	3.6743
72	NOTCH3	0.0453	-5.7461
73	DNMT1	0.0453	4.0170
74	PDGFRB	0.0486	-5.9342
75	IL1B	0.0496	4.0665

Table 2. Cancer – GPR results derived from FirstChoice® Human Tumor and Normal Adjacent Tissue RNA. The tumor RNA sample is derived from a large B cell type lymphoma, which arose in the small intestine of a 61-year old female.

Cancer

A lymphoma is a type of solid neoplasm that originates from lymphocytes. The FirstChoice® Human Tumor RNA sample is derived from a large B-cell type lymphoma, which arose in the small intestine of a 61 year old female. We analyzed a tumor and matched normal sample from a single individual. As expected from the tumor type, there is a significant enrichment for genes associated with a B-cell lineage in the GPR results.

GPR analysis yielded 75 significantly changing genes ('hitters') from 383 potential targets (see Table 2, Figure 6) using the Human Lymphoma & Leukemia 384 StellARray™ qPCR Array (Lonza Cat. No. 00188333). Ingenuity® Pathway Analysis (Ingenuity® Systems) (Figure 6a) identified the following top 6 canonical pathways associated with the GPR hitters:

1. **B Cell Receptor Signaling**
2. **IL2 Signaling p53 Signaling Cell**
3. **Cycle: G2/M**
4. **DNA Damage Checkpoint Regulation**
5. **Aryl Hydrocarbon Receptor Signaling**
6. **Glucocorticoid Receptor Signaling**

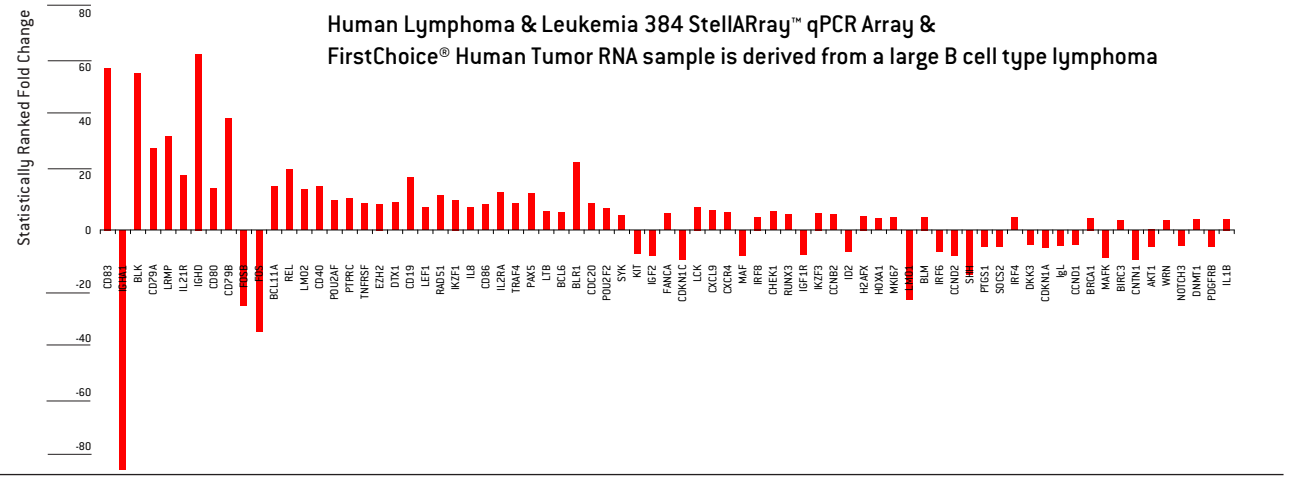

Figure 6. Graphical representation of Table 2. Cancer GPR results.

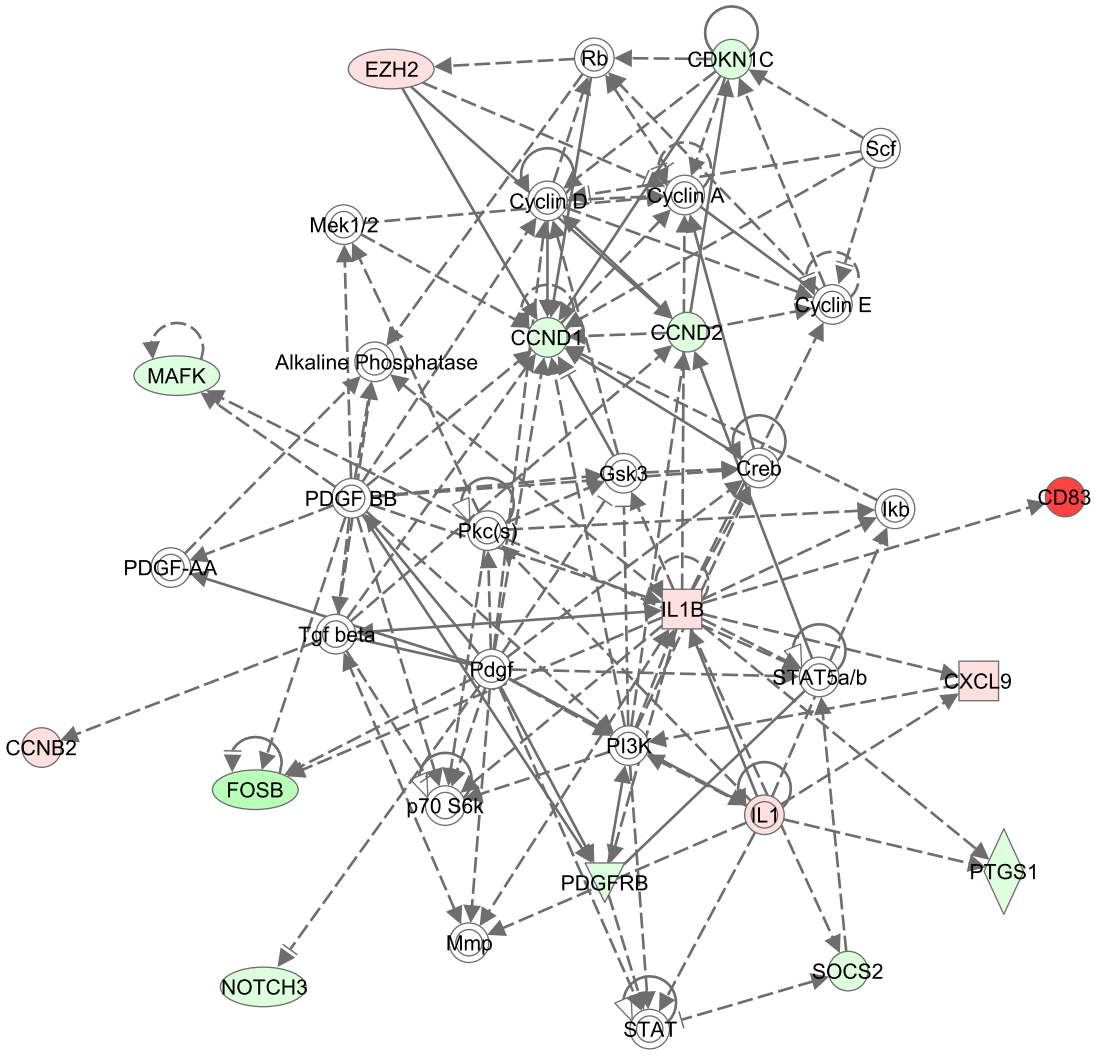

Figure 6a. Ingenuity® Pathway Analysis generated pathway associated with expression levels of CD83, the highest statistically ranked gene, and other associated genes.

Stem Cell Differentiation

Cell differentiation is a transition of a cell from one cell type to another and involves a switch from one pattern of gene expression to another. Mesenchymal stem cells, derived from the bone marrow, can give rise to stromal cells, fat cells, and types of bone cells. In this application, differentiation conditions were selected to drive the hMSC cells to an osteogenic phenotype.

GPR analysis yielded 87 significantly changing genes ('hitters') from 383 potential targets (see Table 3, Figure 7) using the Human Stem Cell 384 StellARray™ qPCR Array (Lonza Cat. No. 00188335). Ingenuity® Pathway Analysis (Ingenuity® Systems) (Figure 7a) identified the following top 8 canonical pathways associated with the GPR hitters:

1. Hepatic Fibrosis / Hepatic Stellate Cell Activation
2. Wnt/ß-catenin Signaling
3. IL6 Signaling
4. Aryl Hydrocarbon Receptor Signaling
5. Acute Phase Response Signaling
6. Ephrin Receptor Signaling
7. Cell Cycle: G1/S Checkpoint Regulation
8. JAK/STAT Signaling

Rank	Gene Name	p-value	Fold Change
1	ANPEP	0.0001	171.8273
2	ELOVL6	0.0004	28.3697
3	IGF1	0.0005	-39.6098
4	SHH	0.0006	21.3665
5	PTCH1	0.0006	-28.8467
6	MCFD2	0.0007	15.9657
7	PROM2	0.0008	23.8314
8	ALPL	0.0009	13.5332
9	FGF13	0.0011	-23.5143
10	CD34	0.0019	-29.6974
11	FN1	0.0019	10.6660
12	VEGFA	0.0020	-10.4183
13	MAT2A	0.0037	5.9807
14	TNC	0.0037	7.0222
15	FZD7	0.0041	7.5943
16	SLC22A18	0.0043	-5.6540
17	GATA2	0.0045	6.9530
18	SC4MOL	0.0048	5.6275
19	E2F1	0.0050	-5.8964
20	IL11	0.0057	-7.2315
21	ADIPOQ	0.0060	4.8878
22	EPHA2	0.0069	4.8783
23	CADM1	0.0073	-8.1671
24	ITGA2B	0.0079	4.8379
25	EMP1	0.0083	4.7432
26	SOCS3	0.0087	-5.9710
27	TFAP2C	0.0096	-4.2680
28	HDAC1	0.0100	-6.3748
29	PSCA	0.0100	-4.0750
30	GLI1	0.0102	4.5207
31	CSF1	0.0106	4.2384
32	DPPA2	0.0125	4.1976
33	HIF1A	0.0128	3.3618
34	ITGA1	0.0129	3.8579
35	ABCB1	0.0135	-12.0501
36	GYPA	0.0159	-3.3431
37	COL2A1	0.0160	3.4108
38	PHC1	0.0180	3.5785
39	RNF2	0.0184	2.9688
40	NR6A1	0.0192	3.4012
41	GBX2	0.0197	3.4574
42	ZFP42	0.0216	3.3794
43	BCL2	0.0222	3.1717
44	WWTR1	0.0225	3.3775
45	CXCR4	0.0228	3.3432
46	LIF	0.0229	-7.7641
47	BMP4	0.0250	-2.9390
48	IL6ST	0.0250	3.1186
49	PPIC	0.0256	-9.2678
50	SELE	0.0261	3.2129
51	KDR	0.0263	-3.2824
52	EPAS1	0.0269	-2.9272
53	HSPG2	0.0279	2.9689
54	EZH2	0.0280	-2.9007
55	FGFR1	0.0289	3.1448
56	CDKN1B	0.0294	2.9627
57	GABPA	0.0303	-3.7474
58	SHMT1	0.0311	-3.5336
59	CTNNB1	0.0317	2.9508
60	PARP4	0.0359	2.7453
61	DAB2	0.0365	2.7914
62	HOXA10	0.0368	-8.2912
63	TRIM14	0.0373	-2.7718
64	NANOG	0.0373	-3.7979
65	SALL1	0.0381	2.6980
66	NANOS1	0.0387	-2.5449
67	TCEA3	0.0387	-3.0997
68	ZFX	0.0388	2.5808
69	CDKN2A	0.0389	2.6394
70	SOX6	0.0403	2.5409
71	SLAMF1	0.0404	2.5655
72	TP53	0.0405	-3.3174
73	SPP1	0.0417	-3.1436
74	EPO	0.0417	2.5623
75	SIPA1	0.0420	-3.2578
76	CD4	0.0424	2.5278
77	MSH2	0.0437	2.5176
78	IL9	0.0455	2.9102
79	RPS6KA5	0.0463	2.5442
80	MAML3	0.0468	2.5161
81	GSK3B	0.0475	2.4918
82	KITLG	0.0482	2.3202
83	CD9	0.0486	-2.9131
84	CLDN7	0.0488	2.4815
85	PROM1	0.0490	2.4050
86	GCM1	0.0491	2.5171
87	IL5	0.0494	-3.0107

Table 3. Stem Cell Differentiation – GPR results derived from Poietics® hMSC Human Mesenchymal Stem Cells that have been cultured under conditions to stimulate osteogenic differentiation. Results shown represent genes whose expression levels are statistically different between the differentiated and undifferentiated groups. Fold Change values are displayed with respect to the differentiated group.

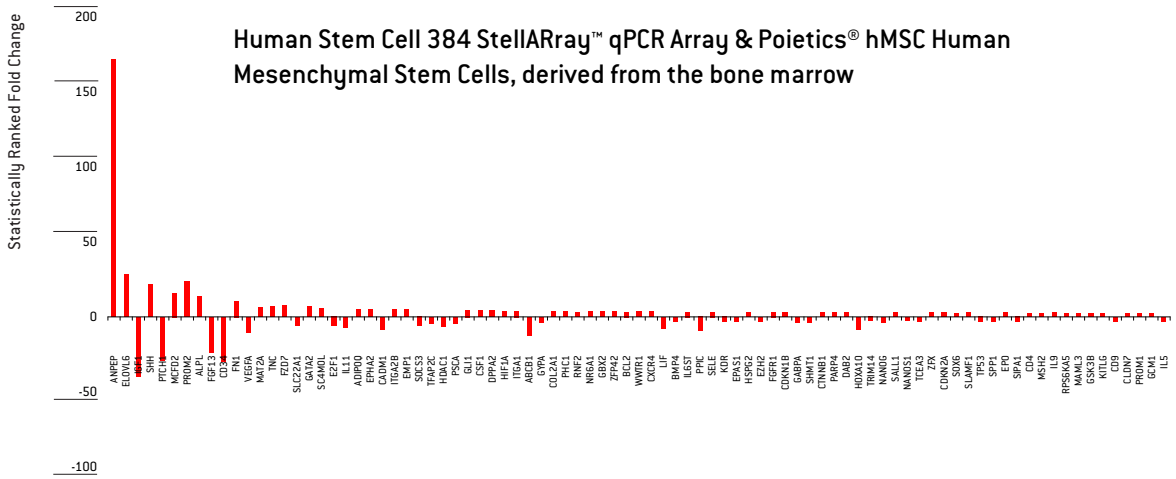

Figure 7. Graphical representation of Table 3. Cancer GPR results.

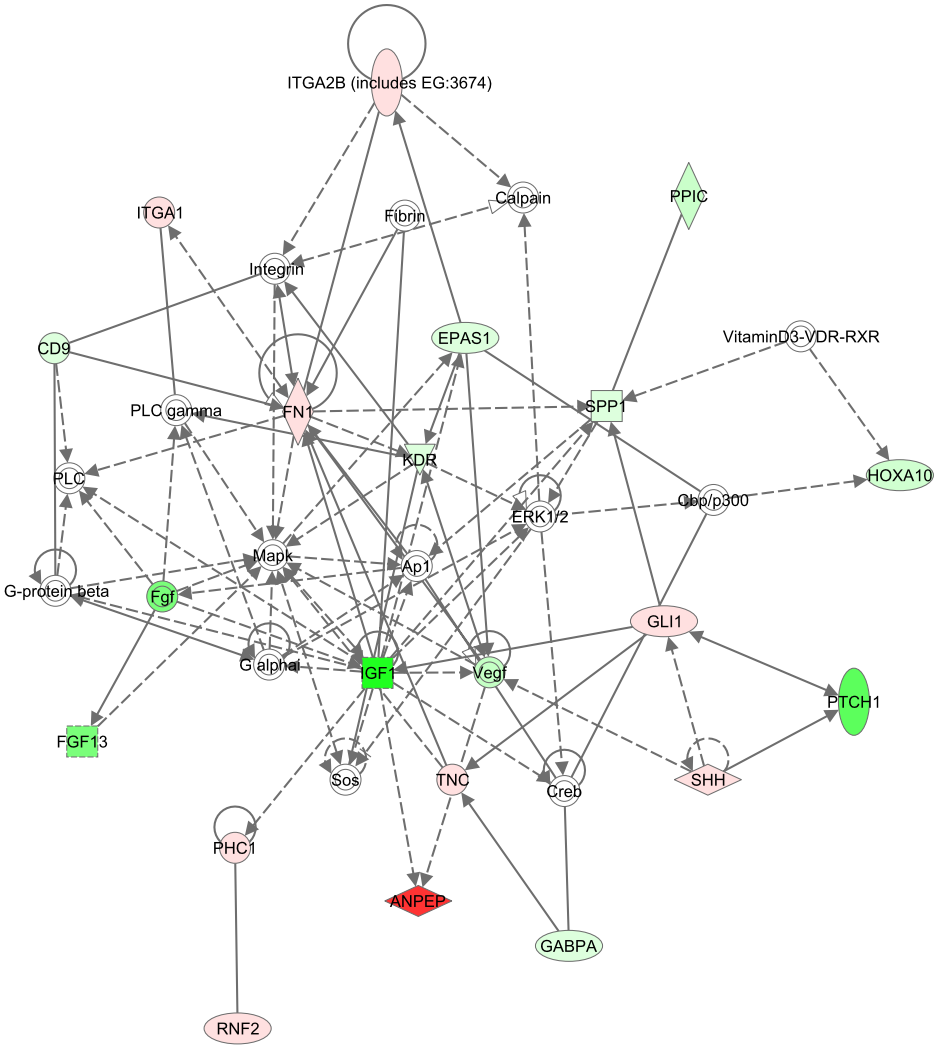

Figure 7a. Ingenuity® Pathway Analysis generated pathway associated with expression level of ANPEP, the highest statistically ranked gene, and other associated genes.

Summary

The StellARray™ Gene Expression System revealed gene expression level changes in three diverse biological contexts. Clonetics® and Poietics® Primary Human Cells from Lonza were challenged with various experimental conditions and the StellARray™ Gene Expression System revealed true changes in gene expression levels. The StellARray™ Gene Expression System shows obvious merit for the purpose of accurately measuring gene expression levels within *in vitro* models of human tissues as well as tumor samples. By utilizing objective designs of gene content within a StellARray™ qPCR Array and the novel Global Pattern Recognition™ Analysis Tool, a statistically significant gene expression profile was easily obtained.

Using existing qPCR platforms and standard chemistries, reliable information can be derived from relatively small sets of target genes without the error associated with *a priori* chosen normalizer genes and fold-change biases.

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