White Paper

Oligo GEArrays®: The Pathway-Focused DNA Microarray System for Every Laboratory

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ABSTRACT

This paper describes the technology and performance characteristics of the Oligo GEArray® DNA microarray system. Oligo GEArrays® are an effective alternative to genome-wide microarrays, in that each array is designed to profile expression of a focused gene panel from a particular biological pathway or disease state. The data presented in this paper substantiates the sensitivity, reproducibility, and reliability of this innovative approach to microarray design. The Oligo GEArray's® flexible protocol options, ease-of-use, and adaptability to any laboratory setting provide researchers with a robust tool for routine use of microarray technology in everyday experiments.

Introduction to Microarray Technology and Oligo GEArrays®

Microarray technology utilizes RNA from cells, tissue, and other biological source materials to simultaneously determine the expression levels of many genes. Microarrays are composed of a set of distinct, gene-specific, nucleic acid probes immobilized on a solid support. During a microarray experiment, RNA is enzymatically converted to labeled cDNA (complementary DNA) or cRNA, and then hybridized to the immobilized nucleic acid probe. The labeled target bound at each gene-specific spot is typically detected using chemiluminescent, fluorescent, or radioactive methods. The signal produced at each spot is representative of the amount of message in the original RNA sample.

Advances in DNA microarray technology have facilitated genome-wide expression profiling with whole genome microarrays available from companies such as Affymetrix, Agilent or Illumina. While these microarrays provide a solution for discovery-based research endeavors, the large amount of data produced from genome-wide arrays requires the use of specialized software and daunting analysis procedures. SABiosciences pathway-focused, lower-density Oligo GEArray® microarray system provides a more economical and productive alternative for monitoring a focused panel of genes in a particular biological pathway or disease state. Oligo GEArrays® are comprised of a nylon membrane support spotted with gene-specific 60-mer oligos for up to 440 different genes. Oligo GEArrays® use as little as 10 pg of total RNA, with a chemiluminescence detection method. No special equipment is required for the Oligo GEArray® platform, facilitating its easy adoption into any biological research laboratory. These arrays offer comparable performance to high-density microarray formats while enabling hypothesis-driven experimental design, relevant data acquisition, and efficient data analysis.

Oligo GEArray Characteristics

The Oligo GEArray® product platform combines expertly designed pathway-specific microarrays and advanced oligonucleotide probe design with a streamlined labeling, hybridization, and detection protocol (Figure 1). The distinct advantages of the Oligo GEArray® system are outlined in Table 1. With the combined benefits of the membranes, reagents, uncomplicated protocol, and the GEArray® Analysis Suite Software, the Oligo GEArray® product line provides a complete and convenient microarray experiment package for any laboratory.

Table 1: Features and Benefits of the Oligo GEArray® System

Focused Array Design	Lower-density (113-440 genes) arrays designed by biological pathway or disease state for relevant data acquisition
Excellent Quality Manufacturing	Carefully-designed mRNA 3′ end biased 60-mer oligonucleotide probes (Median distance ~160 bases from 3′ end) on a positively-charged nylon membrane with non-contact printing method (10 nL deposition volume)
Easy Target Labeling	One-tube proprietary IVT-based linear RNA amplification & labeling kit using 0.1 to 3 µg of total RNA
Various Detection Method	Single color / channel chemiluminescent detection or X-ray film
Express Protocol	With the HybPlate Array format, you can easily process up to 32 arrays in one day
Straightforward Data Analysis Software	Web-based software available by subscription

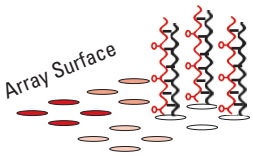
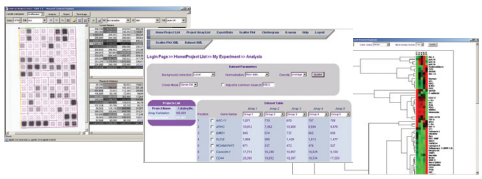
How It Works	HybPlate Express Protocol (Single Day Experiment)	Standard HybTube Protocol (Two Day Experiment)
Preparation of Labeled cRNA with TrueLabelingAMP™ 2.0 Start with as little as 0.1 - 3.0 µg of Total RNA Amplify Labeled cRNA Target 	3 hours	3 hours
Hybridization and Washing Simply add cRNA target to the GEArray and follow the easy hybridization and wash steps. 	3.5 hours in Thermoshaker	Overnight in Hyb Oven
Signal Development Detection is as simple as developing a Western Blot using chemiluminescence. 	0.75 hours	1.5 hours
Image Aquisition Use a CCD-camera system to capture chemiluminescence. You can also use X-ray film and a standard desktop scanner. 	0.5 hours	0.5 hours
Analysis with GEArray® Expression Analysis Suite Auto-align the gene table for your arrays to the captured image, select your analysis parameters, and go! Graphical and tabulated differential gene expression data is at your fingertips. 		

Figure 1: Outline of the Oligo GEArray® experimental procedure

Oligo GEArrays are composed of 113-440 gene-specific 60-mer oligos that have been rigorously evaluated for specificity, sequence complexity, secondary structure, melting temperature, GC content, and distance to 3' end of transcript. The oligo probes are immobilized on a positively charged, three-dimensional nylon membrane. The nylon membrane matrix offers distinct advantages over impermeable surfaces such as chips and glass slides, including larger probe immobilization and increased hybridization and detection area. When combined with an optimally formulated hybridization solution and sensitive chemiluminescent detection, the Oligo GEArray membrane maximizes both hybridization efficiency and signal generation.

The choice of two different target labeling kits is an integral part of the Oligo GEArray platform. The TrueLabeling-AMP™ 2.0 linear amplification and labeling kit is a universal target labeling method specifically designed to convert total RNA to amplified and labeled cRNA (Step 1, Figure 1). Based on standard ***in vitro*** transcription based methods, the TrueLabeling-AMP™ 2.0 procedure requires fewer enzymatic reactions, only one purification step, and a significantly shorter protocol as compared to traditional procedures. The biotinylated cRNA target contains the same 3' biased sequences as the gene-specific oligonucleotides printed on the array to

enhance efficient hybridization and maximize signal. Only 0.1 to 3 µg of total RNA starting material is required for the TrueLabeling-AMP 2.0 kit to produce sufficient cRNA for hybridization. When using a consistent amount of cRNA in the hybridization step, differing amounts of total RNA can be utilized to produce similar microarray results (Figure 2). The TrueLabeling-PicoAMP™ two-round amplification and labeling kit is designed for very small samples with as little as 100 picograms of RNA (10 cells), and provides comparable results to the standard one-round True Labeling-AMP 2.0. In Figure 3, a comparison is shown between microarray results with varying amounts of input RNA in the two-round TrueLabeling-PicoAMP™ kit, and results obtained with the one-round True Labeling-AMP kit. The TrueLabeling-PicoAMP™ kit offers an excellent solution for laboratories interested in performing microarray studies on sources of limited RNA, such as laser capture microdissection (LCM), Formalin-Fixed Paraffin Embedded (FFPE), or Fine Needle Aspiration Biopsy (FNAB) samples.

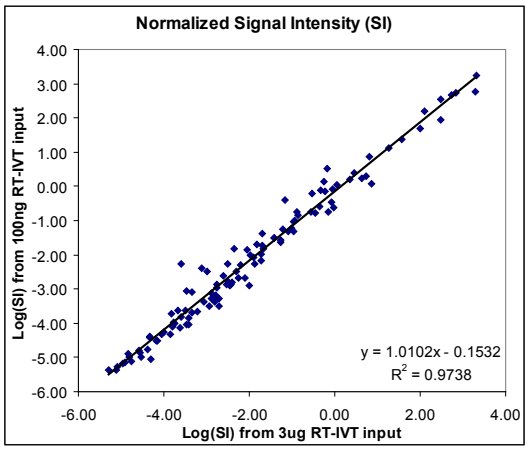


Figure 2: Biotinylated cRNA synthesized from different amounts of total RNA produce similar array results. A constant amount (2 µg) of cRNA generated from differing amounts of total RNA (listed above) was hybridized to separate Oligo GEArray Human Tumor Metastasis Microarrays (HybTube format, catalog number OHS-028) for 18 hours at 60°C. Hybridization, washing, and chemiluminescent detection were performed as outlined in the User Manual. A good correlation was observed between the normalized signal intensities obtained with different total RNA inputs.

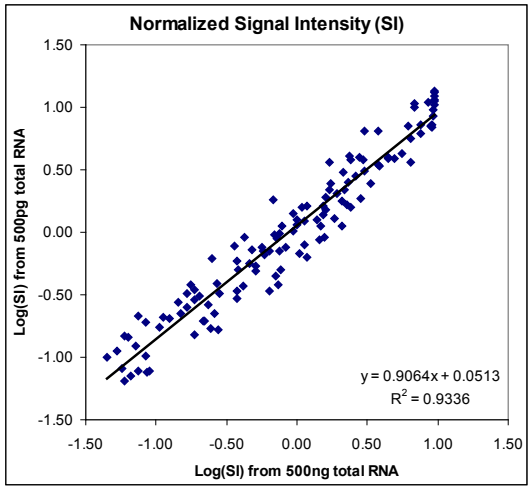


Figure 3: Similar microarray performance is observed with 500 pg total RNA starting material with the TrueLabeling-PicoAMP™ kit as compared to 500 ng total RNA starting material with the TrueLabeling-AMP™ 2.0 kit. Human XpressRef™ Total RNA (500 pg) was used in the TrueLabeling-PicoAMP™ procedure as outlined in the User Manual. The 1-round TrueLabeling-AMP™ 2.0 amplification and labeling procedure was performed as outlined in the User Manual with 500 ng of the Human XpressRef™ Total RNA. For the hybridization step, 2 µg of each cRNA target was hybridized to separate Oligo GEArray® Human Tumor Metastasis Microarrays (HybTube format, catalog number OHS-028). Hybridization with 2-round cRNA produced a similar pattern and comparable percent present call to the 1-round cRNA. A good correlation in normalized signal intensity was observed between the two labeling methods.

The Oligo GEArray® hybridization protocol, array formats, and detection methods also offer flexibility and processing options for every laboratory. The traditional HybTube array format is designed for use with the standard rotisserie-style hybridization ovens. The HybPlate format is ideal for high-throughput applications, allowing the processing of up to 32 arrays in one day with the use of SABiosciences' specialized GEArray® Express Thermoshaker. The GEArray® chemiluminescent detection system, optimized for use with the nylon membrane matrix, offers excellent sensitivity, wide dynamic range (up to 4 orders of magnitude), and versatile image acquisition options. Chemiluminescent detection provides comparable or better sensitivity and signal as compared to radioactive detection methods, without the worry of radioactive waste disposal and precautionary laboratory measures. The array images can be captured using a cooled CCD camera (for best results) or simply with X-ray film and the use of a desktop scanner. When used with autoradiography film, chemiluminescent detection methods are generally more sensitive than radioactivity. Array data analysis is facilitated through the use of SABiosciences' GEArray® Expression Analysis Suite web-based software. This subscription-based software offers automated, array-specific analytical functions as well as various analysis tools including scatter plots and hierarchical clustering.

The Oligo GEArray® is a user-friendly, efficient, and focused DNA microarray tool that offers superior pathway-focused solutions for gene expression studies. The straightforward protocol includes a cRNA amplification and labeling portion that can be completed in as little as 2.5 hours. In addition, the protocol provides flexible hybridization options that include a 1-day express procedure (HybPlate format only), and a simple and safe chemiluminescent detection. Oligo GEArrays® provide a convenient, cost-effective, sensitive, and pathway-focused alternative to genome-wide microarrays.

Oligo GEArray Performance: Sensitivity, Dynamic Range, Reproducibility, and Reliability

Typical performance parameters for microarray validation include sensitivity and dynamic range, reproducibility, and reliability (compatibility with real-time PCR results). The Oligo GEArray® system affords excellent sensitivity, a wide dynamic range (up to 4 orders of magnitude), robust reproducibility, and good correlation with real-time PCR experiments. Oligo GEArrays® offer similar or better performance as compared to popular genome-wide array systems (Table 2).

Table 2: Typical System Performance (*based on published Affymetrix technical notes)

	Oligo GEArray®	Affymetrix™ GeneChip®*
Sensitivity	As low as 10 fM synthetic target	125 fM
Linear Dynamic Range	> 3 orders of magnitude	> 3 orders of magnitude
Specificity	60-mers provide superior specificity as compared to shorter probes	25-mer probes provide less sensitivity that 60-mer or 70-mer probes
Reproducibility	~ 10% CV	10-15% CV
Sample Input	10 pg – 3 µg total RNA	At least 100 ng RNA

To address the qualities of sensitivity and dynamic range, known quantities of labeled synthetic RNA target were spiked into a labeled human RNA sample for the performance of a series of hybridization experiments (Figure 4). The Oligo GEArray® exhibits a low-limit of detection in the 10 fM range, as demonstrated by detection of the target at a spiking ratio of 1:20,000,000. This performance level indicates that the Oligo GEArray® is an excellent tool for studying low abundance transcripts (less than 10 copies per cell); however, the wide linear dynamic range allows for the simultaneous, accurate detection of both high and low abundance messages.

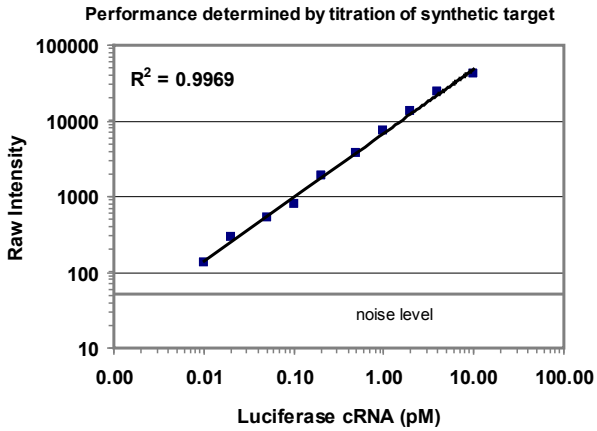


Figure 4: The Oligo GEArray exhibits a high level of sensitivity and wide linear dynamic range. A firefly luciferase in vitro transcript was converted to labeled cRNA target using the TrueLabeling-AMP method. Different known amounts of the target (10 fM to 10 pM) were mixed with a constant amount of labeled cRNA target generated from XpressRef™ Human Universal Reference Total RNA (GA-004). Each mixture of labeled cRNA target was hybridized to replicate Oligo GEArray® Human Microarrays. The luciferase gene raw signal intensity was plotted versus the quantity of labeled luciferase cRNA. These results demonstrate that the Oligo GEArray® can detect as little as 10 fM labeled target with a linear dynamic range of four orders of magnitude (from 0.01 pM to 10 pM).

Oligo GEArrays® are manufactured utilizing an advanced, non-contact printing technology that ensures excellent array print quality and reproducibility. Technical replicates within an experiment generally yield coefficient of variation values (CVs) of less than 10%. Figure 5 illustrates a representative comparison between the results of duplicate Oligo GEArrays®. The results demonstrate a high degree of correlation between the data sets; thus indicating that the Oligo GEArray® system is a robust, reproducible method to reliably detect small, yet biologically significant changes in gene expression.

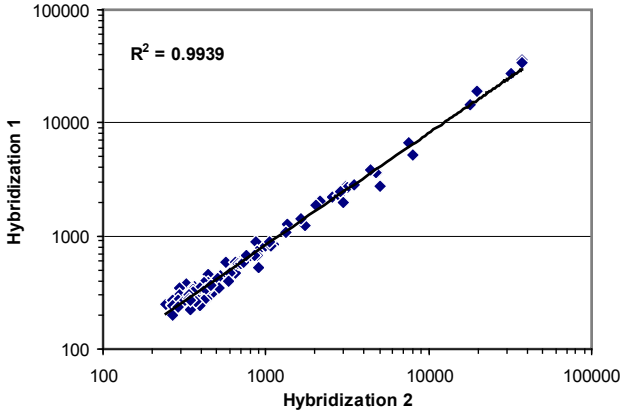


Figure 5: The Oligo GEArray® exhibits excellent array-to-array reproducibility. XpressRef™ Human Universal Reference RNA (GA-004) was converted to labeled cRNA target using the TrueLabeling-AMP™ Linear RNA Amplification Kit (GA-010). Equal amounts (2 µg) of amplified product were hybridized to replicate Oligo GEArray® Human Microarrays. The Chemiluminescent Detection Kit (D-01) was used to detect the presence of labeled target on the arrays. The raw intensity values for each gene from one array were plotted against those values from the second array, and the data were fit to a straight line having a correlation factor (R^2) of 0.9939.

A complete gene expression profiling experiment requires the validation of microarray results by real-time PCR (or an alternative method). Verification by real-time PCR should yield a closely matched gene expression profile (70-90% agreement) to that observed with the microarray in order to regard the data as reliable. Figure 6 illustrates the results of a gene expression profiling experiment in which the cancer-related gene expression was compared between samples from two different breast cancer cell lines. The results obtained with the Oligo GEArray® were confirmed by real-time PCR analysis of 84 genes using SABiosciences' RT² Real-Time™ Primer Sets. Using a fold-change threshold of 1.3, the relative gene expression changes for the cell samples using the Oligo GEArray® and real-time PCR exhibited greater than 70% agreement, indicating a high degree of reliability in the Oligo GEArray® results.

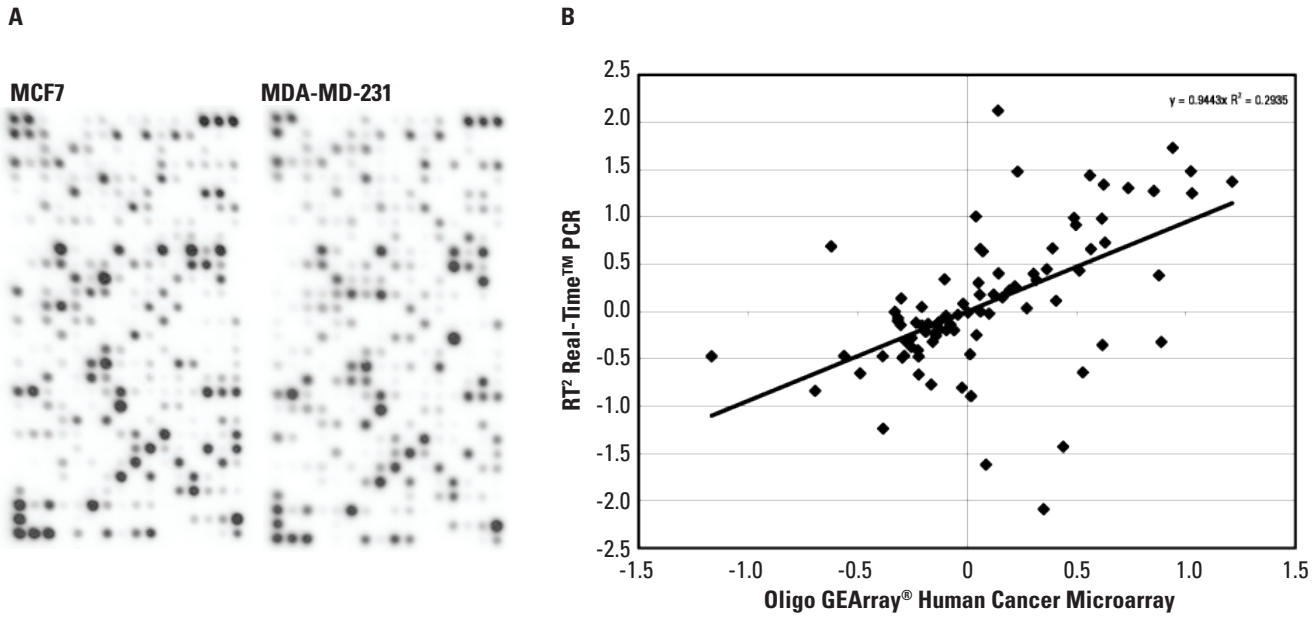


Figure 6: The Oligo GEArray® demonstrates good correlation with real-time PCR results. RNA (3 µg) from two different of human breast cancer cell lines, MCF7 and MDA-231, was converted to labeled cRNA target with the TrueLabeling-AMP™ RNA Linear RNA Amplification Kit (GA-010). Triplicate samples of labeled target (6 µg) were hybridized to separate Human Oligo GEArray® Cancer Microarrays (OHS-802). Sample array images are shown in Panel A. RNA (7 ng) from each cell line was also converted into first strand cDNA. Each cDNA sample was used in multiple PCR reactions (n=5) of 84 genes represented by the microarray using RT² Real-Time™ PCR Primer Sets. A ratio of relative gene expression between the two cell lines was calculated for each gene. The results determined with the two different experimental methods are plotted against one another in Panel B. This result demonstrates a good agreement between the data sets indicating that the GEArray® results are sufficiently reliable when compared to real-time PCR.

Discussion and Product Information

The Oligo GEArray® DNA microarray system offers an exceptional research solution for investigators interested in studying gene expression profiles related to specific pathways or disease states. The pathway-focused approach to array design, combined with the excellent performance characteristics described above, affords researchers an effective and reliable tool for human, mouse, or rat gene expression discovery and screening applications. Coupled with the straightforward procedure, the web-based data analysis software and flexible protocol options allow the easy integration of the Oligo GEArray® platform into any laboratory. SABiosciences offers a variety of products complimentary to the Oligo GEArray® line, including an ArrayGrade™ Total RNA Isolation Kit, RT² Real-Time™ Primer Sets, instrument-specific master mixes, and other real-time PCR reagents. A list of available microarrays is shown on the next page. Visit www.SABiosciences.com for more information and a complete product catalog.

Pathway-Focused Oligo GEArray® Microarrays.

For a complete listing, visit www.SABiosciences.com/ArrayList.php

Pathway / Topic Focus	HybPlate Format			HybTube Format		
	Human	Mouse	Rat	Human	Mouse	Rat
Alzheimer's Disease	EHS-057			OHS-057		
Angiogenesis	EHS-024	EMM-024	ERN-024.2	OHS-024	OMM-024	ORN-024.2
Apoptosis	EHS-012	EMM-012	ERN-012.2	OHS-012	OMM-012	ORN-012.2
Atherosclerosis				OHS-038	OMM-038	
Autoimmune and Inflammatory Response	EHS-803	EMM-803		OHS-803	OMM-803	
Breast Cancer Biomarkers	EHS-402			OHS-402		
Cancer	EHS-802			OHS-802		
Cancer PathwayFinder™	EHS-033	EMM-033		OHS-033	OMM-033	
Cardiovascular Disease Biomarkers	EHS-037			OHS-037	OMM-037	
Cell Cycle	EHS-020		ERN-020	OHS-020	OMM-020	ORN-020
Cell Surface Markers				OHS-055	OMM-055	
Chemokines and Receptors		EMM-022		OHS-022	OMM-022	ORN-022
Common Cytokines	EHS-021	EMM-021		OHS-021	OMM-021	ORN-021
Diabetes					OMM-023	
DNA Damage Signaling Pathway				OHS-029	OMM-029	ORN-029
Endothelial Cell Biology	EHS-015	EMM-015	ERN-015.2	OHS-015	OMM-015	ORN-015.2
Extracellular Matrix and Adhesion Molecules	EHS-013	EMM-013		OHS-013	OMM-013	ORN-013
Genome Stability / DNA Repair	EHS-042			OHS-042	OMM-042	
Hematology/Immunology	EHS-801			OHS-801		
Hematopoietic Stem Cells and Hematopoiesis				OHS-054	OMM-054	
HIV Infection and Host Response				OHS-051		
Hypoxia Signaling Pathway	EHS-032	EMM-032	ERN-032	OHS-032	OMM-032	ORN-032
Inflammatory Cytokines and Receptors	EHS-011	EMM-011.2	ERN-011.2	OHS-011	OMM-011	ORN-011.2
Innate and Adaptive Immune Responses		EMM-052		OHS-052	OMM-052	
Insulin Signaling Pathway				OHS-030	OMM-030	
Interferon α,β Response					OMM-016	
JAK / STAT Signaling Pathway	EHS-039	EMM-039		OHS-039	OMM-039	
Neurogenesis and Neural Stem Cells	EHS-404	EMM-404	ERN-404	OHS-404	OMM-404	ORN-404
Neuroscience-1 Ion Channels and Transporters				OHS-036	OMM-036	
Neurotransmitter Receptors and Regulators	EHS-060	EMM-060	ERN-060	OHS-060	OMM-060	ORN-060
Neurotrophins and Receptors			ERN-031	OHS-031	OMM-031	ORN-031
NFκB Signaling Pathway	EHS-025			OHS-025	OMM-025	ORN-025
Notch Signaling Pathway	EHS-059	EMM-059		OHS-059	OMM-059	
Nuclear Receptors & Coregulators	EHS-056			OHS-056	OMM-056	
Obesity					OMM-017	
Osteogenesis	EHS-026	EMM-026	ERN-026	OHS-026	OMM-026	ORN-026
p53 Signaling Pathway	EHS-027			OHS-027	OMM-027	ORN-027
PI3K-AKT Signaling Pathway	EHS-058	EMM-058	ERN-058	OHS-058	OMM-058	ORN-058
Prostate Cancer Biomarkers	EHS-403			OHS-403		
Signal Transduction PathwayFinder™	EHS-014	EMM-014	ERN-014.2	OHS-014	OMM-014	ORN-014.2
Stem Cell	EHS-405	EMM-405		OHS-405	OMM-405	
Stress Response to Cellular Damage					OMM-019	
T-cell and B-cell Activation				OHS-053	OMM-053	
TGFβ / BMP Signaling Pathway	EHS-035			OHS-035	OMM-035	ORN-035
Th1-Th2-Th3	EHS-034	EMM-034		OHS-034	OMM-034	ORN-034
Toll-Like Receptor Signaling Pathway	EHS-018.2	EMM-018.2		OHS-018.2	OMM-018.2	ORN-018
Toxicology and Drug Resistance	EHS-401	EMM-401	ERN-401	OHS-401	OMM-401	ORN-401
Tumor Metastasis	EHS-028		ERN-029	OHS-028	OMM-028	ORN-028
WNT Signaling Pathway	EHS-043	EMM-043		OHS-043	OMM-043	ORN-043

Contact us for more information on Oligo GEArrays®:

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