If You've Got the Genes, They've Got the Pathways

Combining Illumina genotyping and gene expression analysis products, proven isolation expertise, and its own real-time PCR arrays, SABiosciences (a QIAGEN company) has become the leader in pathway-focused biology solutions.

Introduction

While genotyping can define the genetic differences between cells, gene expression analysis can identify changes in the expression of proteins those genes encode. Together these genomic tools can provide valuable information about an organism's disease state and whether it will respond to certain pharmaceutical therapies. Yet, these data cannot account for what is occurring at all levels within an organism. To gain a more global perspective requires a systems biology approach that focuses on analyzing molecular interactions in biological pathways. Such studies yield more in-depth information about how genes change and work together under normal conditions, and as a disease progresses. This more comprehensive pathway-focused approach is facilitating the discovery of new therapeutics and diagnostics.

To enable these studies, SABiosciences (a QIAGEN company) has developed pathway analysis tools that simultaneously measure the expression of gene sets and proteins associated with particular biological and disease pathways. Now a subsidiary of QIAGEN, SABiosciences has grown to become the leader in pathway-focused biology and expression analysis products. Its Service Core became an Illumina Certified Service Provider (CSPro®) in 2009, offering Illumina genotyping and gene expression analysis, along with its own RT² Profiler PCR Arrays. The experienced Service Core team supports the needs of a global customer base, including researchers at drug companies, academic institutes, and private research centers.

QIAGEN's Brian McNally, Ph.D., Product Manager for the SABiosciences product portfolio tells us more about its PCR array products and how they can be used for discovery and research.

Brian McNally, Ph.D. is a Product Manager for SABiosciences, a QIAGEN company.

Q. What was the rationale behind the development of your real-time PCR arrays?

Brian McNally (BM): We realized that when researchers identify a gene, they often don't fully know what it does within a biological system. That is valuable information, but seven years ago there wasn't an easy way to determine what was happening at the pathway level—how genes interact, or how changes in expression or epigenetic mechanisms could have downstream repercussions that impact the pathway as a whole. So we began to design real-time PCR-based arrays that could analyze how a gene and all of its interacting neighbors behave in certain biological pathways or disease conditions with the aim of helping to contextualize the results of genotyping and gene expression studies.

Q. How do your products use real-time PCR to perform pathway analyses?

BM: Each preformatted PCR array combines the robust, quantitative nature of real-time PCR with the multi-gene profiling capability of a microarray. The RT² Profiler PCR Arrays simultaneously measure the expression of a set of genes for a specific biological pathway or disease state. Our experimentally verified design algorithm yields gene specific real-time PCR assays characterized by high, uniform PCR efficiencies and standardized amplification conditions. These arrays provide an accurate and scalable solution from single to multiple gene expression analyses for complete genome coverage. Each ready-to-use array includes experimentally tested and validated real-time PCR primer sets and instrument-specific PCR master mixes.

Soon after introducing our first arrays, researchers began requesting that we create arrays that incorporated the specific genes they had identified, often with Illumina products. We have streamlined the process and now can create custom real-time PCR arrays containing 24 to 384 genes.

Q. Are your products focused on analyzing gene expression within a biological pathway?

BM: We recognized that performing comprehensive pathway analysis involves more than just studying gene expression. As a result, we developed real-time PCR arrays and individual assays that analyze expression modulators such as microRNAs, and epigenetic mechanisms that influence gene function such as DNA methylation, and histone modifications. For stem cell research, we created a complete PCR array solution that incorporates a highly predictive gene set to aid in reprogramming and validation of different cell fates from induced pluripotent stem cells. We now have cell-based assays to measure signal transduction using transcription factor reporters available in DNA-based or lentiviral delivery formats. We also created protein

AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
TCAACGTACCGTAACGAACGTATCATTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGACGAAAAGAATGATAACAGTAACACACTTCTGTTAACCTT
CGACGAAAAGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACC
AACGTACCATTAAGAGCTACCGTGCAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGACGAAAAGAATGATAA
AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
GATTACTTGATCCACTGATTCAACGTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAGCAACGAGC
CGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGAAAAGAATGATAACAGTA

analysis arrays including multi-analyte ELISA kits to monitor multiple cytokines and chemokines simultaneously, and single-analyte quantitative ELISA kits to measure the amount of an individual cytokine or chemokine in a biological sample.

Q. How many PCR arrays have you developed?

BM: To date, we have developed PCR arrays in four species—human, rat, mouse, and rhesus macaque—for more than 100 biological and disease pathways, ranging from the AHR pathway that controls a variety of developmental and physiological events, including neurogenesis and toxin metabolism, to cancer pathways such as apoptosis. On our website[1], we have organized the pathways by biological process and disease state, and researchers can use our Pathway Central or Gene Network Central search engines to easily find all the pathways their genes of interest may impact.

Q. Most of your PCR arrays simultaneously analyze multiple components in a pathway. How do you handle all of the data produced?

BM: We have worked hard to make this data accessible through a combination of robust assay design and bioinformatics expertise. Controls are incorporated into each product that allow users to perform PCR analysis simultaneously across multiple plates (96- and 384-well plates or 100-well discs) and control all the variables associated with different types of samples, real-time reactions, and PCR steps. These controls enable researchers to reliably produce data and support easy data analysis no matter what the size of the sample set.

Still, there are customers who need to analyze hundreds of samples. That's not only a lot for them to process on a single machine, but potentially more data analysis—dozens of genes over dozens of different samples—than they may be willing to tackle. In that case, it is easier for them to give the project to our Service Core, where our team is used to analyzing large data sets and can look into all the permutations that could be relevant for that customer's research.

Q. Does your Service Core perform analyses using only SABiosciences PCR arrays and assays?

BM: Our Service Core brings together the best platforms on all fronts. We have the proven sample purification expertise of QIAGEN, which brings the ability to isolate nucleic acids from a diverse set of samples. Customers feel confident that we can take a difficult sample and recover enough material for validation and analysis. We offer the best in whole-genome genotyping, focused panel genotyping, copy number analysis, and whole-genome gene expression profiling with Illumina products and systems that deliver high-quality data that customers trust. And we offer the best in pathway analysis with SABiosciences' real-time PCR arrays.

The beauty of the Service Core is that we are bringing sample isolation confidence, real-time PCR pathway arrays, and Illumina genotyping and gene expression products together to solve the pathway puzzle. But, not all of our customers need this level of service. Many have used Illumina products to identify a gene set and all they want is a custom PCR array so they can perform the real-time PCR analysis in their own laboratory. Our custom products allow researchers to capitalize on their Illumina discovery experiments to find out what is happening at the pathway level.

Q.How do Illumina products fit into your Service Core's pathway analysis workflows?

BM: We are using several Illumina arrays and assays, including Infinium® Whole-Genome DNA Analysis[2] and Methylation BeadChips[3], Whole-Genome Expression BeadChips[4], and DASL® Gene Expression Profiling[5] assays. All are being performed on Illumina's industry-leading iScan system[6] and we have been able to leverage them in several ways.

We can perform whole-genome expression profiling with an Illumina array and discover a set of interesting genes. We can then identify the associated pathways and determine their impact on those pathways using the appropriate real-time PCR arrays.

We can also confirm the results of an Illumina expression array by using the industry gold-standard—real-time PCR—to validate the expression results. In this case, we would create a custom real-time PCR array with just the genes of interest identified by the Illumina array.

"We offer the best in whole-genome genotyping, focused panel genotyping, copy number analysis, and whole-genome gene expression profiling with Illumina products and systems that customers trust."

Some of the most interesting work we perform involves studying the downstream repercussions of the SNPs and methylation patterns identified using Illumina whole-genome genotyping and methylation analysis BeadChips. These studies can go in either direction. We can perform gene expression pathway analysis with our real-time PCR arrays, identify genes that are changing, and then use Illumina BeadChips to determine if the changes are caused by a SNP or methylation.

We could also move in the other direction and conduct whole-genome genotyping with an Illumina BeadChip, identify SNPs or methylation changes, and use one of our gene expression PCR arrays to see whether these changes are modifying gene expression. For example, an Illumina whole-genome BeadChip could identify a SNP in a known inflammatory cytokine. With one of our real-time PCR arrays, we could look at the entire inflammatory cytokine's pathway to understand how that one SNP impacts the rest of the pathway on an expression level.

Q. Do you find researchers are increasingly interested in the more global view your pathway analysis tools provide?

BM: Yes, and there is especially interest in whole-genome experiments. Researchers often feel like they are on a fishing expedition. Illumina GenomeStudio® data analysis software does a great job of mining the whole-genome BeadChip data generated on the iScan system and narrowing it down to a digestible list of genes. We help researchers make sense of what it all means. With our products, we can introduce researchers to the appropriate pathways and help them contextualize and verify those results. We help make it all accessible.

Q. How has your product offering changed over the years?

BM: We originally focused on pathways associated with inflammation and stress, whether exogenous (resulting from drug treatments) or endogenous (caused by reactive oxygen species), and later we moved into signal transduction and cancer pathways such as apoptosis. We just released several new arrays in the neuroscience area for Alzheimer's, Parkinson's, and Huntington's disease.

We also want to be proactive in supporting new research methods. For induced pluripotent stem cells (iPSCs) research, we developed PCR arrays for iPSC induction analyses, including colony screening, pluripotency validation, Yamanaka and Thomson factor validation, and reprogramming factors expression. These, together with our PCR arrays for iPSC differentiation analyses, enable the quick and accurate reprogramming and validation of different iPSC cell fates, supporting new research in drug development and disease modeling.

As our product offering has grown, it has become increasingly granular. For example, we are now looking at cell surface markers and have arrays for different tissue responses to toxicity, such as cardiotoxicity, neurotoxicity, hepatotoxicity, etc. that are useful in testing new drugs.

Q. In addition to expanding the number of pathway tools for existing product lines, what new research areas have you targeted for new product development?

BM: We have moved quickly to develop tools to support new biomedical research fields. MicroRNA (miRNA) research has really exploded in the last few years, impacting studies in immune response, neural development, DNA repair, oxidative stress, and apoptosis. We now offer 10 miRNA PCR arrays for human, mouse, and rat samples that will help expedite research in this area.

Epigenetics is an exciting area for SABiosciences. We have launched a technology that uses real-time PCR to profile DNA methylation at targeted sets of CpG islands. This allows scientists to focus their epigenetic research on specific pathways or genes that are important to them. Of course, this technology could be used to validate the results obtained from using Illumina whole-genome methylation BeadChips. We have seen a high demand for our DNA methylation PCR arrays to support this research area and continue to add new epigenetic products.

Since 2003, SABiosciences has helped academic and industry researchers with validation studies, identification of molecular mechanisms, and discovery experiments. With these new products we hope to deliver unique and tailored research solutions for our colleagues in the life sciences.

References

1. http://www.sabiosciences.com/
2. www.illumina.com/applications/gwas.ilmn
3. www.illumina.com/applications/detail/gene_regulation_and_epigenetic_analysis/Array_based_methylation_analysis.ilmn
4. www.illumina.com/applications/detail/gene_expression_analysis/whole_genome_gene_expression.ilmn
5. www.illumina.com/applications/detail/gene_expression_analysis/ffpe_sample_analysis.ilmn
6. www.illumina.com/systems/iscan.ilmn

Illumina, Inc. • 9885 Towne Centre Drive, San Diego, CA 92121 USA • 1.800.809.4566 toll-free • 1.858.202.4566 tel • techsupport@illumina.com • illumina.com

FOR RESEARCH USE ONLY

© 2011 Illumina, Inc. All rights reserved.
Illumina, illumina*Dx*, BeadArray, BeadXpress, cBot, CSPro, DASL, Eco, Genetic Energy, GAIIx, Genome Analyzer, GenomeStudio, GoldenGate, HiScan, HiSeq, Infinium, iSelect, MiSeq, Nextera, Sentrix, Solexa, TruSeq, the pumpkin orange color, and the Genetic Energy streaming bases design are trademarks or registered trademarks of Illumina, Inc. All other brands and names contained herein are the property of their respective owners.

Pub. No. 070-2011-013 Current as of 27 June 2011

illumina®