

Affymetrix and Genpathway Collaborate to Provide ChIP-on-chip and Methylated DNA Assays and Services

Read the interview with Marry Warren, Chief Scientific Officer at Genpathway

By Bronwyn Barnett

SANTA CLARA, Calif., and SAN DIEGO, Oct. 13, 2006 — Affymetrix recently signed an agreement with Genpathway that establishes Genpathway as a service provider for chromatin immunoprecipitation (ChIP) and Methylated DNA assays using Affymetrix GeneChip® Tiling Arrays. The agreement allows researchers to combine Affymetrix Tiling Arrays with Genpathway's full-service ChIP and Methylated DNA assays, services and analysis to gain a better understanding of cellular events involving interactions between protein and DNA such as transcription, as well as epigenetic regulatory mechanisms such as DNA methylation.

To use these services, customers order

Affymetrix Tiling Arrays and have them shipped to Genpathway. In parallel, they send their cell or tissue samples and assay-related information directly to Genpathway, which handles the rest—from initial validation of samples and qualification of the antibody for each factor of interest, to running the assays and delivering fully analyzed results.

“Genpathway provides what we call ‘ChIP/DNA IP services from A to Z,’” said Mary Warren, Chief Scientific Officer at Genpathway. “Because Affymetrix is foremost in the world in whole-genome tiling arrays and Genpathway is the expert in ChIP assays, we have combined two very powerful technologies that together offer the

total ChIP/Methylated DNA solution.”

Genpathway's customers include pharmaceutical and biotechnology companies, government agencies and academic laboratories that deal with a variety of different genomes.

“We use optimized ChIP/DNA IP procedures that give maximum sensitivity and minimal background binding,” said Warren. “In addition, because we carry out ChIP and DNA IP assays day in and day out, we know how to recognize and troubleshoot unexpected results. We can see problems when they arise that other researchers may not notice.”

Warren recently spoke to *UserForum* editor



Mary Warren

Rachel Shreter about the agreement with Affymetrix, and how Genpathway can help researchers take advantage of its powerful new ChIP-on-chip and Methylated DNA assays and services.

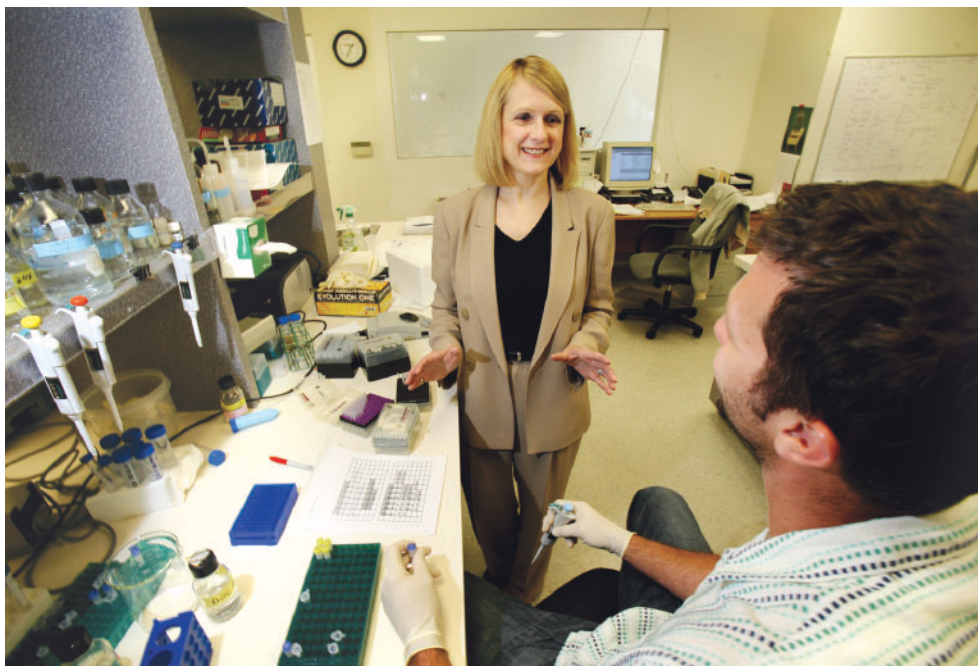
Shreter: How exactly will Genpathway work with Affymetrix to provide ChIP-on-chip and Methylated DNA services?

Warren: The ChIP-on-chip and Methylated DNA assays we offer combine two technologies that are unique to the Genpathway-Affymetrix relationship: Affymetrix' GeneChip Tiling Arrays and Genpathway's highly optimized ChIP/DNA IP assays. Upon receiving a customer's samples, we isolate the chromatin (or DNA, in the case of Methylated DNA), carry out the immunoprecipitations and amplify and label the resulting ChIP or IP DNAs. We then hybridize the labeled DNAs to the Affymetrix arrays—primarily human and mouse whole-genome tiling arrays and, in some cases, promoter arrays or arrays from other species.

Genpathway brings extensive ChIP expertise to our assays, beginning with procedures for isolating chromatin from a wide range of cells and tissues, carrying out high-quality immunoprecipitation and finishing with the delivery of comprehensive data packages consisting of fully analyzed results. These capabilities, as well as our reputation for accuracy, specificity and reproducibility, have led to our recognition as the "ChIP Assay Experts."

Genpathway begins all assays with several important validation steps, including the qualification of every antibody to be used in immunoprecipitating the customer's chromatin, or the use of a previously qualified antibody. In addition, we validate the customer's chromatin or DNA using our PCR-based analysis mode called "Query." No one else does this up-front validation nor the intermediate quality control along the way, which gives customers confidence that they are going to receive high-quality results.

After the data are compiled, Genpathway provides a complete analysis package, including genome annotation of the assay results generated by Genpathway's proprietary ChIP Analysis Software (ChAS). Genpathway provides the results in a form that is easy to view and adapt using standard programs such as Excel.



Customers can further view the results using several genome browsers such as Affymetrix' Integrated Genome Browser (IGB), which is freely available from Affymetrix. Customers can also use links in the analysis package or additional data files provided to view the results on the UCSC Genome Browser.

Shreter: How do you differentiate your specific products and services?

Warren: Genpathway provides three unique assays and several unique analysis modes. The three assays are FactorPath™, which allows us to identify and quantify the binding sites of transcription factors across the genome, TranscriptionPath™, which directly analyzes the process of transcription and Methylated DNA, which identifies and quantifies methylated DNA regions across the genome. First, with regard to FactorPath, our assays can discover locations and associations not only for transcription factors, but also for co-regulating proteins such as co-activators and co-repressors as well as proteins involved in chromatin modification such as acetylated and methylated histones and modifying enzymes.

With TranscriptionPath, the immunoprecipitation is done using an antibody against a protein in the transcription machinery. We make use of a variety of different targets, but the protein target we use most often is RNA polymerase II, the enzyme present on every region of the genome undergoing active transcription (except genes for specialized RNAs such as

rRNA and tRNA). This assay allows us to pull out the transcriptome for any cell, but at the DNA level, and we can quantify differential transcription with unparalleled reproducibility.

Particularly striking is our application of TranscriptionPath to the whole-genome tiling arrays. This process clearly identifies not only all known genes undergoing transcription, but also novel genes as well as differential transcription between different samples. All these results can be visualized on one whole-genome set of arrays, which makes the combination of our TranscriptionPath and the Affymetrix whole-genome tiling arrays particularly powerful.

Our newest assay, which identifies regions of methylated DNA across the entire genome, makes use of an optimized procedure for immunoprecipitating methylated cytosine. Because this approach does not discriminate against any type of sequence (unlike methods that use restriction enzymes), is high-throughput (unlike bisulfite sequencing) and provides information across the entire genome, this assay is the only total solution for assaying these important epigenetic changes. Our assays are able to detect methylation changes across the genome with high sensitivity, accuracy and reproducibility. With the fast-growing body of evidence linking aberrant DNA methylation with cancer and other diseases, we are excited about the potential of these Genpathway-Affymetrix assays to open up promising new avenues for therapeutic and diagnostic research.

Shreter: How exactly are pharmaceutical companies using ChIP-on-chip analysis?

Warren: The range of applications is extensive, from basic research to discovery research, drug screening, preclinical studies and clinical testing in patient material. Our ChIP assays are used to understand disease mechanisms and develop new models; identify and validate targets; test compounds for efficacy and side effects; identify drug mechanisms of action; provide information for preclinical research in pharmacology, toxicology and pharmacogenomics; identify biomarkers; and test clinical samples.

Shreter: Are there any examples that you can talk about?

Warren: We have carried out a number of discovery-type assays involving transcription factors, co-regulators and proteins important in chromatin modification to locate the associated genomic sites and the genes being regulated. In assays carried out for one of our pharmaceutical customers, the transcription factor is important in liver toxicity, and we identified thousands of binding sites for the factor (and the genes regulated) in liver from treated animals. Of particular interest are those genes not previously identified by standard RNA profiling. Our results have provided critical information for better understanding this type of liver toxicity and narrowing down the most important genes and markers to track when testing their compounds. Other discovery projects for pharma and biotech companies have involved nuclear receptors and their co-regulators, and in all cases have revealed important downstream genes not previously known that are now being used in testing their compounds. In another case, a gene determined by RNA profiling to not be differential but believed to be important in a drug's mechanism of action was indeed found to be differentially regulated in

Genpathway's FactorPath assay.

Combinations of our assays are also highly informative and provide data not obtainable with any other method. In the case of another pharmaceutical company, we used our FactorPath and TranscriptionPath Query (Q-PCR) assays to determine the mechanism of action for an important prototype compound. We accomplished this by determining how a set of genes was being regulated by certain transcription factors in cells treated with the compound under a variety of conditions. As a result of our assays, the company obtained mechanistic information that exceeded what they had generated using other methods over the previous year.

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Mary Warren, Chief Scientific Officer at Genpathway.

Shreter: In terms of sample requirements, what do customers need to send you?

Warren: Genpathway carries out ChIP and Methylated DNA IP assays on cultured cells, primary cells or solid tissues. If the samples are cultured or primary cells, the customer carries out a simple fixation procedure using a brief protocol available on our website and sends them to us on dry ice. Solid tissues may be fresh or frozen, including tissue that has been archived in a frozen state for extended periods of time. We can also test whole blood, either fresh or frozen. Along with their samples, customers

send the information needed to carry out the assays, such as the transcription factor to be tested and any genes or genomic regions to be queried. We source all antibodies and qualify the antibodies and Q-PCR primers before using them on the customer's sample material.

Shreter: How often do you speak to customers throughout the process?

Warren: We maintain contact with the customer throughout the assay as needed. In some cases, customers send us multiple samples, have us test part of the samples first, review the data and then order more testing with the remaining samples. We can also do follow-on assays with

other factors or other genes, or using one or more of our other assays with any remaining material. Alternatively, we can test additional samples, and then combine the results. Because we work with DNA, the complexes we analyze are very stable in cells and tissue. This gives our customers a variety of choices and the flexibility needed to obtain the maximum gene expression and regulatory information. It is also why our customers regularly say that we are helping them to obtain critical new information, have more confidence in their other data and truly accelerate their R&D programs.

USERFORUM RESOURCES

Companies:

- Affymetrix, Inc. - <http://www.affymetrix.com>
- Genpathway, Inc. <http://www.genpathway.com>

Further Reading:

- **UserForum:** Affymetrix Offers Two-for-One Promotion on Tiling Arrays for ChIP-on-chip and Transcript Mapping Experiments <http://www.affymetrix.com/userForum/news/newProducts/burt.uf>

- **Affymetrix Microarray Bulletin:** Novel Combination of Chromatin Modifications Governs Embryonic Stem Cell Maintenance <http://www.microarraybulletin.com/community/article.php?p=234>

- **Affymetrix Microarray Bulletin:** Thousands of Previously Unknown Transcription Factor Binding Sites Mapped Using Tiling Microarrays <http://www.microarraybulletin.com/community/article.php?p=44>

- **Affymetrix Microarray Bulletin:** Highest Resolution Study of Genome Replication Surprises Scientists with Unexpected Timing in S-phase <http://www.microarraybulletin.com/community/article.php?p=43>