

Differential Display News

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Differential Display Inventors Honored

In April of this year, Drs. Peng Liang - founder of GenHunter Corporation - and Arthur Pardee were honored with the 1998 Molecular Bioanalytics Prize for their co-invention of the differential display process. The two gentlemen shared the prize of one hundred thousand deutsch marks given during the Analytica Conference in Munich, Germany.

Liang and Pardee developed the technique in 1992 while Dr. Liang was a postdoctoral fellow in Dr. Pardee's lab at Harvard University. They reported the technique in the journal *Science* that year and patented the methodology the following year.

Differential display allows researchers to identify and study a small fraction of genes that cause one cell to be different from another. Since its development, differential display has become the overwhelmingly favored method for cloning differentially expressed genes, and has been cited in more than 1,500 scientific publications in many different fields (see comparison of different methodologies in current GenHunter catalog). The method has been used successfully in clinical studies to demonstrate gene changes during pregnancy or during pathological processes such as the development of tumors, diabetes, neurodegenerative disease or ischaemic attacks, or the consequences of alcohol consumption. It is also a valuable tool for investigating the effects of growth factors, hormones, pharmaceuticals, or toxins on their target cells.

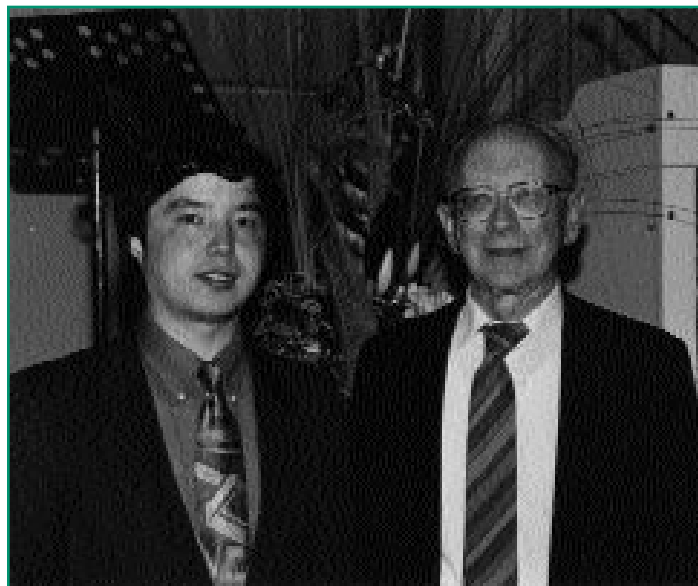
Dr. Liang, currently an assistant professor of Cell Biology at Vanderbilt University, is using the differential display method to research genes regulated by two of the cellular "master switches" involved in tumor development. He and his colleagues are studying the oncogene RAS and the tumor suppressor gene p53, which are mutated in a wide number of cancers.

Dr. Pardee is presently Professor of Biological Chemistry and Molecular Pharmacology at the Harvard Medical School and Chief of the Cell Growth and Regulation Division at

the Dana-Farber Cancer Institute at Harvard. His work has included more than 400 scientific publications, many of them discussing differential display.

The Molecular Bioanalytics prize is given by the German Society of Biochemistry and Molecular Biology and is endowed by Boehringer-Mannheim for outstanding work in molecular bioanalytics. Past winners, several of whom eventually received the Nobel Prize, include the developers of the Southern blot technique, the developers of monoclonal antibodies, and the developers of the PCR technique.

With their invention, Drs. Liang and Pardee have given gene research a unique tool which can be applied both to basic questions of molecular biology and to practical aspects of molecular medicine. (story adapted from the *VUMC Reporter*)



Drs. Peng Liang (L) and Arthur Pardee at the Analytica Conference in Munich, Germany

November 1998 is Customer Appreciation Month!!!

Special Offer #1: Get a FREE COPY of *Differential Display Methods and Protocols*, edited by Drs. Liang and Pardee (Humana Press, 306pp), if you order any combination of 2 or more of the following kits - RNAimage®, RNAmapping™, MessageClean®, and PCR-TRAP® - at one time. This book is normally a \$65 value, and was named the **1997 Best Health Science Book**. (Quote Reference # N981).

Special Offer #2: Get 50% Off Arbitrary Primer Sets! H-AP Primer Sets (*except* for H-AP Set 1) will be \$110 (normally \$220 each) and AP Primer Sets will be \$52.50 (normally \$105). (Quote Reference # N982).

This offer is good for Domestic and Canadian customers only...offer only good for orders received in the month of November...offer not combinable with any other discounts...subject to availability...These special offers end November 30th, 1998.

Literature Reviews

Barry Johnson, GenHunter Corp.

“Complete genome expression monitoring: The human race”. Arthur B. Pardee. *Nature Biotechnology* 1997, 15:1343-1344.

For as long as science has known of the existence of genes, we've wondered how those genes are expressed by an organism in response to its surroundings. In this paper, Dr. Arthur Pardee comments on the work done in 1997 by David Lockhart's group at Affymetrix. Lockhart and Company were able, using an application of DNA array technology, to provide a glimpse of the entire complement of genes from the yeast *Saccharomyces cerevisiae* all at once.

In their groundbreaking research, Lockhart's group used light-directed, solid-phase combinatorial chemistry to synthesize a high-density matrix oligonucleotide array *in situ* on glass wafer supports. After scanning the recently determined entire genomic sequence of the yeast, 260,000 DNA sequences were chosen via computer, mainly on the basis of their uniqueness. The sequences were arranged on the glass in the order in which they appear on the chromosome arms in yeast. This allowed not only immediate identification of chromosomal location, but the total sequence of each identified gene as well. To this array, a pool of biotin-labeled total mRNA was hybridized. Bound mRNAs were then detected via scanning microscopy. Arrays were performed on yeast grown in rich versus minimal media.

Dr. Pardee praises the research, calling it “uniquely capable of determining complete gene expression patterns at the genome level.” He also mentions the advantage of quantitative gene expression measurement (90% of the mRNAs were shown to be present in similar amounts in both yeast populations; around 3% of the remainder differed by more than fivefold). Dr. Pardee sees the array technique as a valuable tool in human gene expression research, but not without its challenges. Among these are the 95% still-unfinished human genome sequence (needed for array completeness), the problem of complexity (humans have around 80,000 genes, as compared to the 6,000 of yeast), and the sequence differences between not only gene copies, but individual humans. The array technique is also, at present, very expensive to perform; hence it is not readily accessible to indi-

vidual laboratories. Finally, the sensitivity is not optimal; as it is difficult to interpret a blank lane as being either a gene not EXPRESSED, or just not DETECTED.

These problems, Dr. Pardee believes, may eventually be solved. But until then, he says we will see “a tortoise and hare race...will cottage industry, using individual identification methods, such as differential display, discover the majority of the useful genes before parallel arrays get up to speed?” Maybe, maybe not. The array process has awesome potential, to be sure. But for now, differential display is the best method for isolating differentially expressed genes, as seen by the exponentially growing number of scientific papers using the method.

“A Schwann cell mitogen accompanying regeneration of motor neurons”. Frederick J. Livesey, John A. O'Brien, Meng Li, Austin G. Smith, Liam J. Murphy, & Stephen P. Hunt. *Nature* 1997, 390:614-618.

In the adult mammalian central nervous system, only the motor neurons are able to regenerate following injury. In this paper, Livesey et al., knowing that this ability is not only intrinsic, but based on the environment surrounding the peripheral nerve, attempt to identify the genes involved in this regeneration process.

By using differential display, Livesey's group identified Reg-2 as a gene expressed in regenerating motor and sensory neurons. Gene expression of resting dorsal root ganglia (DRGs) were compared to that in regenerating DRGs. A 600bp cDNA that encodes Reg-2 was expressed solely in the regenerating ganglia.

The role of the protein in nerve regeneration, however, was still unclear. To this end, Livesey et al. performed experiments both *in vivo* and *in vitro* to try and make the role of Reg-2 clearer. *In vivo*, the group performed, on mice, a sciatic nerve crush, followed by a polyclonal antiserum injection that blocked the mitogenic effects of the protein. Following this, the group observed that the number of Reg-2 containing axons close to the injury was reduced to approximately one-third.

In vitro, the group examined the effect of Reg-2 on Schwann cell proliferation (stimulated by axons; essential for neural regeneration in the adult PNS), and observed that in the presence of adenylyl cyclase, Reg-2 is strongly mitogenic for Schwann cells.

The classic Schwann cell mitogens are constitutively expressed by motor and sensory neurons, but are not upregulated during regeneration. In contrast, Reg-2 was shown to only be produced during periods of Schwann cell proliferation.

Based on this observation, and the fact that that blockage of Reg-2 expression resulted in lower numbers of axons, Livesey and his associates propose that Reg-2 is an essential component of the neuron-glia interactions that are key to regeneration of mammalian motor neurons.

Visit the GenHunter Booth at the following upcoming conferences:

Oct. 8-9, 1998. *NIH Research Festival*, Bethesda, MD.

Nov. 8-11, 1998. *Society for Neuroscience Meeting*, Los Angeles, CA. Booth 841.

GenHunter employees will be available to answer questions and offer advice. 1998/1999 Catalogs will be available along with informative handouts.

1998 Differential Display Summer Workshop

In June, people from all around the world gathered at Vanderbilt University in Nashville, Tennessee for the third annual Differential Display Summer Workshop. The workshop was sponsored by: GenHunter Corporation; Qiagen, Inc.; New England Nuclear-DuPont; Perkin-Elmer Corporation; and National Diagnostics, Inc. Building on the success of the past two workshops, this year's actually had more applicants than spaces available! One can see how interest in the differential display technique has spread in just a few years!

In a weeklong intensive workshop, led by Dr. Peng Liang of Vanderbilt, the attendees worked through the entire differential display laboratory process, from DNase treatment of the RNA sample to confirmation of the differentially expressed band. For some, it was their first time to use the procedure. For others, it was an opportunity to hone their differential display skills and learn to deal with any problems. The attendees also learned the history of differential display and discussed its possible future applications.

The workshop, like last year's, was a tremendous success! But unlike previous workshops, many participants brought

their own RNA samples from yeast, plants, or animals. The attendees saw results ranging from fair to excellent, but all left with a better understanding of the differential display technique, and a greater appreciation of its many and varied uses. For more information on future Differential Display Summer Workshops, please send your name and contact information to GenHunter by fax or email (genhunt@telalink.net).



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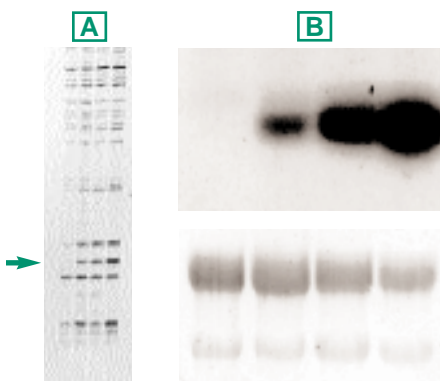
New Research Products for Differential Display

HotPrime™ DNA Labeling Kit

The HotPrime™ kit for radioactively labeling DNA fragments is now available from GenHunter. **This kit allows for labeling DNA probes with up to ten times more specific radioactivity than the traditional random priming method.**

The kit is designed to maximize the sensitivity of traditional Northern or Southern blot analysis by first using random decamers (10mers) instead of hexamers (6mers), which are very poor primers for DNA polymerase; second by incorporating anchored oligo-dT primers into the labeling buffer which ensures the "full-length" antisense cDNA probes to be labeled if necessary; and third by using radioactive α -dATP instead of α -dCTP to take advantage of the AT rich nature of 3' untranslated regions of mRNA. The kit is ideal for labeling probes generated by differential display but it can also label any other DNA probes to a similar high specific activity.

The labeled probes can then be used for the most sensitive analysis by Northern blotting, Southern blotting, or library screening.



The band of interest from differential display was excised (A, arrow-head), reamplified, and labeled with the HotPrime™ DNA Labeling Kit as a probe for Northern blot confirmation (B).

Catalog #: H501 Price: \$145
For 20 Labelings

RNA Loading Mix

Get perfect RNA gels every time! This pH buffered solution contains the denaturant, tracking dye, and ethidium bromide for convenient one-step preparation of an RNA sample before loading for gel or Northern blot analysis. This loading mix solves the oft-encountered problem of apparent RNA degradation.

Just mix 1-10 μ L (2 - 50 μ g) of RNA with 20 μ L of RNA Loading mix, heat denature for 10 minutes at 65°C, and load entire sample onto the gel without adding additional Ethidium bromide in the gel or buffer.

Catalog #: R104 Price: \$25
Volume: 1 mL

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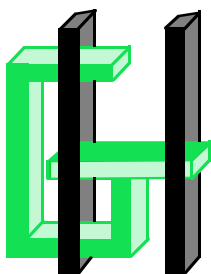
Differential Display News is published by GenHunter Corporation. Comments and contributions of articles are welcome and should be sent to:

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