

Differential Display News

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Differential Display Workshops

During two different weeks in June, 1997, 48 people from 12 countries gathered at Vanderbilt University in Nashville, Tennessee for the first and second Differential Display lab workshops offered by the Vanderbilt Cancer Center. The corporate sponsors for the workshops included GenHunter Corporation, Qiagen, New England Nuclear-DuPont, Perkin-Elmer Corporation, National Diagnostics and Owl Scientific.



The first differential display workshop

The attendees worked intensively in the classroom and laboratory through the entire differential display process, starting with total RNA and finishing with cloning and reverse Northern dot blot confirmation of differentially expressed genes. The course was led by Dr. Peng Liang, an assistant professor of Vanderbilt University and the organizer of the workshops. For some students, this was their first time to use differential display. For others, it was an opportunity to hone their differential display skills and learn to deal with potential problems that can arise with the method. There were also a few people who came to the workshop with either suspicion of the technique or bad past experience with differential display. The success of the workshops is a testimony that differential display can be a very dependable method for identifying and cloning differentially expressed genes if done correctly. The following are comments from some of those who attended the workshops:

"I found [the workshop] very helpful and would definitely recommend it to others. The technique was made clear and the instructors were very helpful"- Janine McMillan, Deakin University, Australia.

"Evaluating [DD data] with the experts was a valuable learning tool for me. Hearing the routes others took to troubleshoot problems was also very valuable. I have a great deal of confidence in the GenHunter products, so I won't waste time looking at other kits" - Kristin Douglas, UM Medical School, Michigan.

"I think that for any workshop such as this, the key is to have a

well organized set of experiments to work through so that people understand the methodology - this workshop was a good example of that. I feel that in my personal research this workshop will make a big difference in terms of the quality of my results" - David Silva, Boston University, Massachusetts.

"We really appreciated the Differential Display workshop and the kits like MessageClean, RNAimage, PCR-TRAP, and ReversePrime from GenHunter Corporation. After we learned to follow their protocol precisely, we are no longer nervous during experiments" - Dr. Toshio Nikaido, Shinsu University School of Medicine, Japan.

"I thought the workshop was a great success; it was very well organized and the experiments had obviously been planned by an expert!" - Rachel Ibbotson, Bournemouth General Hospital, U.K.

"[GenHunter's] primers work by far better than any others tested by us. I am convinced of the feasibility and reliability of Drs. Liang and Pardee's Differential Display technique. I would recommend that all researchers who are interested in Differential Display analysis participate in the workshop" - Tibor Gorogh, University of Kiel, Germany.

"[The workshop] was excellent! I had been attempting Differential Display with other companies' kits and was struggling. After attending the workshop and ordering GenHunter products everything is falling into place. I would highly recommend the workshop to anybody!" - Marie Lacy, University of Arkansas.



The second differential display workshop

Obviously, the workshops were a resounding success! 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 applications. During the workshop, the attendees also toured the beautiful Vanderbilt University and Music City USA, Nashville. If you are interested in joining the 1998 Summer Differential Display Workshop, please send your name and contact information to us by fax or email (genhunt@telalink.net).

Literature Reviews

Barry Johnson, GenHunter Corp.

“An Aquaporin-Like Gene Required for the *Brassica* Self-Incompatibility Response”. Seishi Ikeda, June Nasrallah, Ram Dixit, Susanne Preiss, and Mikhail Nasrallah. *Science* 1997, 276:1564-1566.

Self-fertilization of many flowering plants is prevented or reduced by the mechanism known as self-incompatibility, which is controlled by haplotypes of the S locus. These gene clusters encode for the plasma-membrane localized receptor kinase SRK which, upon detection of self-pollen, initiates an intracellular phosphorylation cascade that blocks pollen development. Dr. Nasrallah and his associates investigated another protein not linked to the S locus, the MOD protein of *Brassica campestris*. By cross-fertilizing the self-incompatible (SI) S8 / S8 homozygote and the self-compatible (SC) USDA C634 strain; after which self-fertilizing one resulting SI plant, Dr. Nasrallah's group found the self-compatibility gene to be recessive. They then used all 10 GenHunter RNAimage™ kits to cover and compare approximately 96% of mRNA isolated from the stigmas of 10 SI plants (MOD/mod or MOD/MOD) and 10 SC plants (mod/mod). By saturation screening like this, they identified 29 differentially expressed messages. To find out which of these genes is directly responsible for the phenotype, they performed Southern blot hybridization to determine which gene has chromosomal abnormality in the SC plants. They discovered that one of these genes, designated DD33, was missing from the SC, but not SI plants. The 119 bp DD33 fragment isolated by differential display was then used as a probe to screen a cDNA library, which detected a 1.1 kb transcript in MOD/MOD plants that contains a reading frame similar in sequence to a subfamily of major intrinsic proteins, whose products transport water. Knowing that hydration is very important for pollen germination and subsequent pollen tube development, Dr. Nasrallah and his associates propose that the MOD protein function concerns the regulation of water availability to the papillar cell surface. As a result, the activation of SRK upon self-pollination results in MOD activation and the diverting of water away from the pollen, preventing adequate hydration.

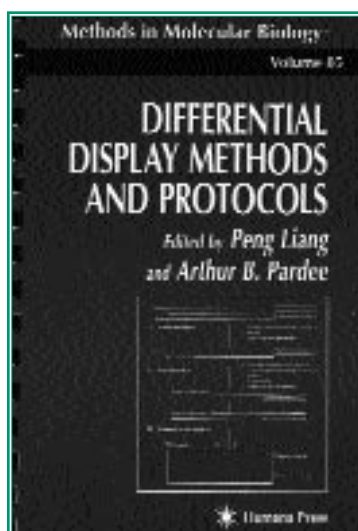
“Wip1, a Novel Human Protein Phosphatase that is Induced in Response to Ionizing Radiation in a p53-dependent manner”. Michele Fiscella, HongLiang Zhang, Saijin Fan, Kazuyasu Sakaguchi, Songfa Shen, Edward Mercer, George Vande Woude, Patrick O' Connor, and Ettore Appella. *Proc. Natl. Acad. Sci. USA* 1997, 94:6048-6053.

It has long been known that exposure of mammalian cells to ionizing radiation (IR) will induce a complex array of cellular responses, including apoptosis and/or cell cycle arrest. Both of these responses are at least partially dependent on the presence of p53, a tumor suppressor gene. Dr. Fiscella and colleagues identified in this paper the presence of *wip1*, a p53-dependent transcript induced in response to IR. The group used the GenHunter RNAimage™ kit on WMN Burkitt Lymphoma cells (containing a wild type-p53 gene) that were exposed to IR. Analysis of RNA

from both irradiated cells and non-irradiated controls showed two cDNA fragments that were enriched after IR. One of these fragments was identified as *mdm2*, a known gene activated by p53. The other gene, homologous to the serine/threonine PP2C phosphatases, was named *wip1*. This protein was observed to have the same IR-induced response pattern as *waf1*, another product known to be involved in the G1 cell cycle checkpoint. They also found that by observing cells expressing the human papilloma virus E6 protein, which disrupts p53 function, they were able to see that *wip1* was not induced. Although their experiments showed that *wip1* may play a role in the inhibition of tumor growth, it is still unclear exactly where in the cell cycle its effects occur.

“Differential Display Methods and Protocols”. Peng Liang and Arthur B. Pardee, ed. *Methods in Mol. Biology* Vol 85., 1997, 306 pp. Humana Press.

Drs. Peng Liang and Arthur B. Pardee have, in *Differential Display Methods and Protocols*, assembled for the first time a comprehensive review of the state of the art of their powerful new methodology and its practical applications. The book's pioneering contributors describe all the major elements of this novel technology, including optimal primer designs, DD using fluorescence detection, cloning family-specific genes and major causes and solutions for false positives. Also provided are numerous examples - along with detailed experimental procedures in which differentially expressed genes have been successfully identified in diverse biological systems ranging from plants to songbirds to humans.



Comprehensive and on the cutting-edge, *Differential Display Methods and Protocols* provides readers with precise new tools for studying how gene expression is regulated throughout the development of a living organism, and how the failure of this intricate control mechanism leads to pathological complications. This novel and powerful methodology, fully detailed here is already playing a major role in the development of selective antagonists and inhibitors for treating cancer, cardiovascular disease, CNS disorders, and inflammation and tissue repair. This long-awaited guide for differential display edited by its inventors provides a much needed roadmap for those who are about to or have already started using the method.

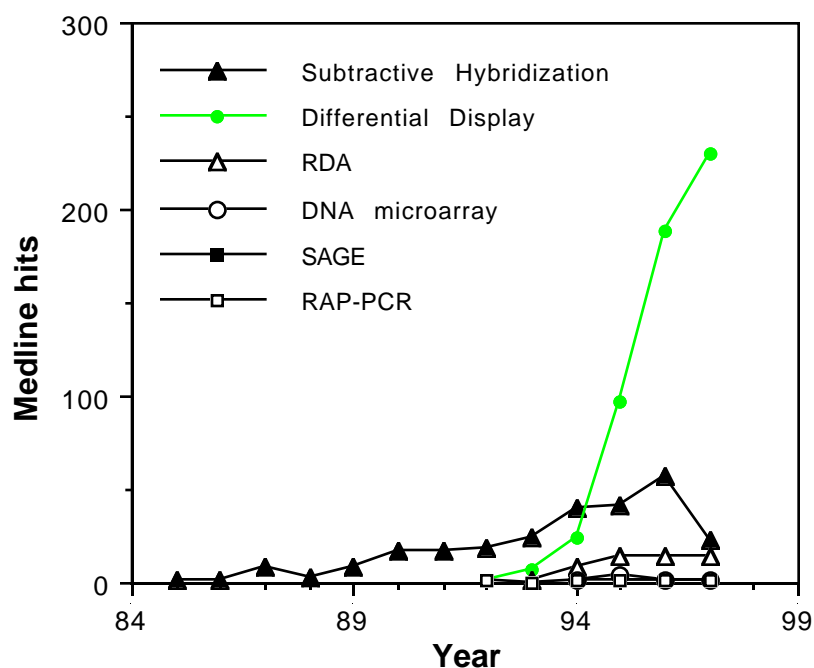
This book is now available from GenHunter Corporation. Cat. No.: HP1, Price: \$64.50. For a limited time only to our valued customers, you can also receive this book absolutely free if you place an order of GenHunter's reserch products over \$500 by Dec. 31, 1997 (Quote No.: 71231). Act now while supplies last!

Comparison of Differential Display with other methodologies for cloning differentially expressed genes

Since its invention in 1992, differential display has quickly overtaken subtractive hybridization to become the method of choice for cloning differentially expressed genes. In fact, the number of publications using differential display has far surpassed the combined publications using other competitive methodologies based on a recent Medline search. In addition to

the sheer number of genes cloned by differential display, the biological functions of these genes are emerging. GenHunter Corporation is very proud to be the major driving force for making this powerful method within easy reach of the biomedical research community world wide (see literature review).

For those who are unsure of which method is better for cloning differentially expressed genes, the Medline search would most objectively confirm that differential display is without doubt the method of choice.



Two major biotech companies obtained site licenses for Differential Display

Chiron and Millennium Pharmaceutical have obtained site licenses for differential display technology from the patent holder, Dana-Farber Cancer Institute, for their in-house use of differential display to identify disease related genes.

Most of the biotech and pharmaceutical companies as well as academic institutions can purchase GenHunter's Differential Display kits which automatically come with a license for the use of the patented technology.

GenHunter Corp. holds an exclusive license for the differential display reagent business.

Method	Year invented	Medline entry/yr.	Total entry
Subtractive Hybridization	1985	20	249
Differential Display	1992	87	434
RAP-PCR	1992	2	9
RDA	1993	12	47
DNA Microarray	1995	1	2
SAGE	1995	2	4

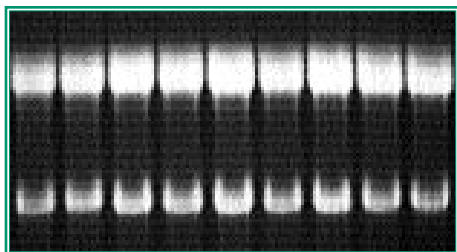
The Medline searches were performed in Sept., 1997 on OvidWEB using key words depicting the corresponding technologies.

New Research Products for Differential Display

RNApure™ Reagent

For convenient extraction of intact total RNA from tissues or cells.

The extraction of total RNA from tissues or cells is an important step in the Differential Display process. GenHunter has developed a simple mono-phasic solution for rapid isolation of intact total RNA. The RNA isolated is optimal for differential display, Northern and reverse Northern blot analysis.



Gel analysis of intact total RNA isolated from 9 different tissues of rat using RNApure™ .

This yellow colored, clear, mono-phasic reagent provides a one-step isolation of total RNA from cells or tissues with unsurpassed integrity and purity.

Cat. No.	Size	Price
P501	50 mL	\$46
P502	100 mL	\$69

RNApure™ is cheaper than RNazol and Trizol!

Visit the GenHunter Booth at the following upcoming conferences:

Oct. 25-29, 1997. Neuroscience Meeting, New Orleans. Booth 1939.

Dec. 13-17, 1997. Cell Biology Meeting, Washington, DC. Booth 844.

The new GenHunter 98/99 Catalog will be released in Nov., 1997.

This catalog contains many new research products and supplies for differential display. The Technical Notes section of the catalog compiles valuable information on differential display technology. Don't miss it!

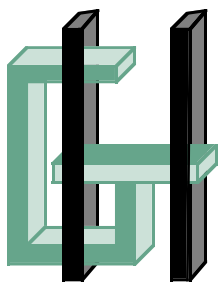
Differential Display News is published Quarterly by GenHunter Corporation. Comments and contributions of articles are welcome and should be sent to:

GenHunter Corporation, 624 Grassmere Park Rd. Ste. 17, Nashville, TN 37211.

**Toll free: 800-311-8260 or 615-833-0665
Fax: 615-832-9461**

email: genhunt@telalink.net

Internet: <http://www.nashville.net/~genhunt>



GenHunter Corporation

624 Grassmere Park Rd. Ste.17
Nashville, TN 37211
USA

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