

Differential Display News

Volume 4, Number 1

April 1999

GenHunter Corporation Acquires Rights to AP-TAG™ Technology

GenHunter Corporation has acquired the exclusive rights to AP-TAG™ Ligand-Receptor Interaction Detection technology developed at Harvard University by Drs. John Flanagan and Philip Leder (US patents: 5,554,499 & 5,801,000 and pending foreign patents). Since its inception, this technology has revolutionized the way cell surface receptors and ligands are detected and cloned. Among the receptors and ligands recently cloned by the AP-TAG™ technology are the receptors for **leptin** (3) and **Semaphorin III** (4), and the cell surface ligands for **Kit**, **Mek4**, and **Sek receptor tyrosine kinases** (1, 2).

GenHunter is extremely pleased to be able to add this innovative method into its product line as a powerful tool for applications downstream of differential display (DD). If you are working with a secreted protein or cell surface molecule cloned by DD, AP-TAG™ technology will allow you to functionally characterize these genes further.

The essence of this invention is to allow a cDNA sequence encoding any secreted polypeptide ligand or extracellular domain of a receptor to be in-frame fused to human placental secreted alkaline phosphatase (AP) in pAPtag cloning vectors. The

resulting ligand-AP fusion protein, when expressed in 293T cells, can be secreted at high levels into the culture medium and thus easily detected by either the AP activity assay or Western blot analysis using antibodies to AP. The ligand-AP or soluble receptor-AP fusion protein thus can serve as an affinity agent, which allows the most convenient and sensitive detection and cloning of their corresponding cell surface receptors or ligands. Unlike the conventional radioactive ¹²⁵I labeling method, AP-TAG™ is safe and does not require ligand/soluble receptor purification.

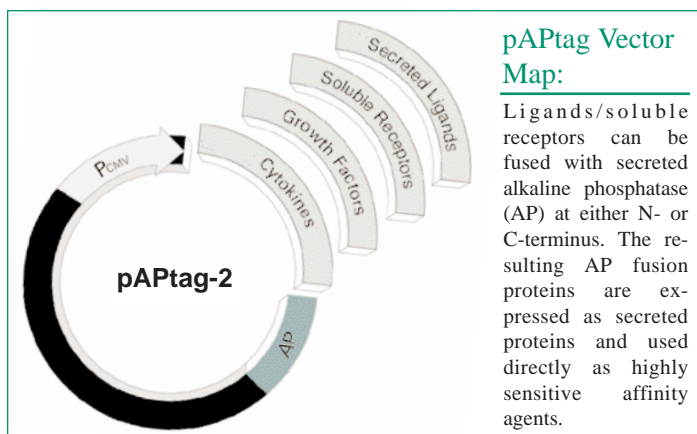
GenHunter offers a complete line of AP-TAG™ products:

- **AP-TAG™ Kit A (Cat. # Q201)**
pAPtag-2 and pAPtag-4 vectors, L-APtag and R-APtag primers
- **GH2/P3 Supercompetent cells (Cat. # T601)**
- **AP (human placenta) Antibody, (Cat. # Q301)**
Rabbit Polyclonal, (for Western Blot analysis)
- **293T Cells (Cat. # Q401)**
- **293T/pAPtag-4 Stable Cell Line (Cat. # Q402)**
(for producing AP alone control medium)
- **AP Assay Reagent A** (for AP Activity assay) (Cat. # Q501)
- **AP Assay Reagent S** (for Cell staining) (Cat. # Q502)
- **HBHA Wash Buffer (Cat. # Q503S, Q503L)**
(for receptor binding assay)
- **Cell Lysis Buffer** (for receptor binding assay) (Cat. # Q504)
- **AT Antibiotics Mix** (1000X, for LB-agar) (Cat. # Q601)

Please contact GenHunter or the distributor nearest you for a complete list of products with information on pricing and availability.

References for AP-TAG™ technology:

1. Flanagan, J. G. and Leder, P.: The kit Ligand: A cell surface molecule altered in steel mutant fibroblasts. *Cell*. 1990, 63:185-194.
2. Cheng, H.J., and Flanagan, J.G.: Identification and cloning of ELF-1, a developmentally expressed ligand for Mek4 and Sek receptor tyrosine kinases. *Cell*. 1994, 79:157-168.
3. Tartaglia, L.A. *et al.*: Identification and expression cloning of a leptin receptor, OB-R. *Cell*. 1995, 83:1263-1271.
4. He, Z. and Tessier-Lavigne, M.: Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. *Cell*. 1997, 90:739-751.



GenHunter Special Spring Deals

Special Offer #1: Get a **FREE COPY** of *Differential Display Methods and Protocols*, edited by Drs. Liang and Pardee (Humana Press, 306pp), when you order an RNAimage® and a MessageClean® Kit in one order. (Quote Reference #A991).

Special Offer #2: Get **50% Off All Arbitrary Primer Sets**, except H-AP Set 1 (Quote Ref. #A992).

Special Offer #3: Get a **FREE PCR-TRAP® Cloning System Sample Kit** (for 5 Clonings) when you purchase 2 or more RNAimage® Kits in one order (Quote Ref. #A993).

Cloning has never been easier! PCR-TRAP® is the most efficient, easiest to use PCR-product cloning system available! Specifically designed for DD product cloning, but great for any PCR application, **efficiency of 90-95% is typically achieved**. See 98/99 GenHunter Catalog for more details.

These special offers expire June 30th, 1999...Orders received after this date will not be accepted...Only orders quoting above ref. #'s will be accepted. Offers not combinable with any other discounts...subject to availability...**International customers**, please contact local distributor or GenHunter for details.

Literature Reviews

Barry Johnson, GenHunter Corp.

"Cap43, a Novel Gene Specifically Induced by Ni²⁺ compounds." Daoji Zhou, Konstantin Salnikow, & Max Costa. *Cancer Research* 1998, 58:2182-2189.

The widespread use of nickel compounds in everyday industry has been believed to contribute to increased rates of cancer by both occupational and non-occupational exposure. The molecular mechanisms of the compounds' toxicity has been the focus of several studies, the results of which seem to indicate that the majority of cellular damage caused by exposure may result in altered gene expression, rather than direct DNA damage. In order to study this proposal, this study used differential display to isolate genes that were induced by nickel compounds in human bronchoalveolar epithelial cells.

Costa's group exposed the cells to Ni₃S₂ at either 0, 0.1, or 0.3 μg per cm² for 24 hours. They then used GenHunter's RNAimage® Kits 1 and 2 to perform the differential display analysis. From the resulting DD gel, a differentially expressed cDNA of approximately 300bp in length (identified as *Cap43*) was recovered. The band was then cloned into GenHunter's PCR-TRAP® Cloning Vector, and used as a probe for Northern blot analysis and library screening. The Northern blot confirmed the beautiful induction of the *Cap43* gene by the metal compound. In comparison, the A549 cells were exposed to other water-soluble chloride salt compounds such as zinc, cobalt, magnesium, etc. None of these compounds induced the expression of the *Cap43* gene.

Other studies performed showed that exposure to nickel elevated the levels of free intracellular Ca²⁺, and that this elevation was the direct signal for *Cap43* induction. Zhou *et al.* report the fact that the *Cap43* gene was also cloned independently by a group in the Netherlands, and that their findings indicated that *Cap43* was a differentiation marker for colon epithelium; further, its expression was lost or decreased in 17 colon adenocarcinomas.

GenHunter selected this study as one of the classical successful applications of differential display, and recommends this paper for a better understanding of experimental design.

"Differential Display Method and Application" Editor: Peng Liang. *Methods: A Companion to Methods in Enzymology*. 1998, Vol. 16, Number 4. Academic Press.

This new issue of *Methods* edited by Dr. Peng Liang addresses some of the key aspects of Differential Display, and compiles some of the most successful applications of the method in systems from yeast, plants, and humans. A must-read for beginners or veterans alike using Differential Display.

Table of Contents:

1. **Factors Ensuring Successful Use of Differential Display.** Editorial: By Peng Liang.
2. **Cloning Oncogenic Ras Regulated Genes by Differential Display.** By Hakryul Jo, Hong Zhang, Rong Zhang, Peng Liang.
3. **Identification of a v-Rel Oncogene Induced Inhibitor of Apoptosis by Differential Display.** By Mingjian You, Henry R. Bose, Jr.
4. **Differential Display Cloning of Genes Induced in Regenerating Neurons.** By F. J. Livesey, S. P. Hunt.
5. **Identification of genes involved in Innate Responsiveness to Bacterial Products by Differential Display.** By Fenyu Jin, Carl Nathan, Aihao Ding
6. **Use of Differential Display in Conjunction with Bulked Segregants to Target Specific Genomic Loci.** By Amy L. Casselman, Seishi Ikeda, June B. Nasrallah, Mikhail E. Nasrallah.
7. **Analysis of Selective Gene Activation in Yeast by Differential Display.** By Wu-Cheng Shen, Michael R. Green
8. **Identification of Yeast Meiotic-specific Genes by Differential Display.** By Dilip K. Nag, Jed Axelrod
9. **Non-Radioactive Differential Display Cloning of Genes Induced by Homocysteine in Vascular Endothelial Cells.** By Koichi Kokame, Hisao Kato, Toshiyuki Miyata.
10. **Post-Differential display: Parallel Processing of Candidates Using Small Amount of RNA.** By Ghislaine M.-C. Poirier, Mark G. Erlander.

Other Successful Gene Hunters - Selected Recent Publications from our Customers:

1. Arakawa, H. *et al.*: Identification and characterization of the ARP1 gene, a target for the human acute leukemia ALL1 gene. *Proc. Natl. Acad. Sci. USA*. 1998, 95:4573-4578.
2. Wu, M.X. *et al.*: IEX-1L, an apoptosis inhibitor involved in NF-κB-mediated cell survival. *Science*. 1998, 281:998-1001.
3. Lawlor, E.R. *et al.*: The Ewing tumor family of peripheral primitive neuroectodermal tumors expresses human gastrin-releasing peptide. *Cancer Research*. 1998, 58:2469-2476.
4. Furumura, M. *et al.*: Characterization of genes modulated during pheomelanogenesis using differential display. *Proc. Natl. Acad. Sci. USA*. 1998, 95:7374-7378.
5. Takeda, Kohsuke *et al.*: Identification of a novel bone morphogenetic protein-responsive gene that may function as a noncoding RNA. *J. Biol. Chem.* 1998, 273:17079-17085.
6. Tabor, D.E. *et al.*: Transcriptional activation of the Stearoyl-CoA desaturase 2 gene by sterol regulatory element-binding protein/adipocyte determination and differentiation Factor 1. *J. Biol. Chem.* 1998, 273:22052-22058.
7. Dandoy-Dron, F. *et al.*: Gene expression in Scrapie. Cloning of a new Scrapie-responsive gene and the identification of increased levels of seven other mRNA transcripts. *J. Biol. Chem.* 1998, 273:7691-7697.
8. Kemppainen, R.J. and Behrend, E.N.: Dexamethasone rapidly induces a novel Ras superfamily member-related gene in AtT-20 cells. *J. Biol. Chem.* 1998, 273:3129-3131.
9. Zhang, R. *et al.*: Identification of rCop-1, a new member of the CCN protein family, as a negative regulator for cell transformation. *Mol. Cell. Biol.* 1998, 18:6131-6141.
10. Castagnino, P. *et al.*: Induction of tissue inhibitor of metalloproteinases-3 is a delayed early cellular response to hepatocyte growth factor. *Oncogene*. 1998, 17:481-492.
11. Gao, L. *et al.*: A novel response to Dioxin. Induction of Ecto-ATPase Gene Expression. *J. Biol. Chem.* 1998, 273:15358-15365.
12. Fang, Z. *et al.*: Identification of novel factors that regulate GnRH gene expression and neuronal migration. *Endocrinology*. 1998, 39:3654-3657.
13. Ivanova, A.V. *et al.*: The chromo and SET domains of the Clr4 protein are essential for silencing in fission yeast. *Nature Genetics*. 1998, 19:192-195.
14. Sun, H. B. *et al.*: MRG1, the product of a melanocyte-specific gene related gene, is a cytokine-inducible transcription factor with transformation activity. *Proc. Natl. Acad. Sci.* 1998, 95:13555-13560.

GenHunter Forms Partnership with Hitachi Genetic Systems for Fluorescent Differential Display Technology

A common lament among researchers considering using differential display is that they cannot work with radioisotopes in their laboratory. GenHunter, the recognized world leader in differential display technology, has embarked on a joint venture with Hitachi Genetic Systems to develop a cutting-edge non-isotopic differential display technology! This fluorescent DD technology will be optimized with the Hitachi FM-BIO® II ultrasensitive fluorescence Imaging System.

Jonathan Meade, GenHunter's product manager, responded to the agreement by saying "we are very excited about the challenge of trying to fill this gap in the research community. Hopefully the combination of our resources will result in fluorescent DD products which offer not only safety and sensitivity, but also the possibility for high-throughput and automation in anticipation of the post-genome era of research."



Hitachi's FM-BIO® II Imaging System

FROM THE EXPERTS...

THIS ISSUE'S TIP: REAMPLIFYING LARGER BANDS

It is known and accepted that Differential Display is one of the best methods for finding differentially expressed genes (see page 35 of GenHunter's 1998/1999 catalog.) But sometimes problems can occur...many times with attempting to reamplify larger-size bands that have been found and cut out of a sequencing gel. The optimum size for re-amplifying is approximately 200 to 600 bp. However, larger bands can be re-amplified. If you find yourself having trouble, the following suggestions may help!

BEFORE PCR:

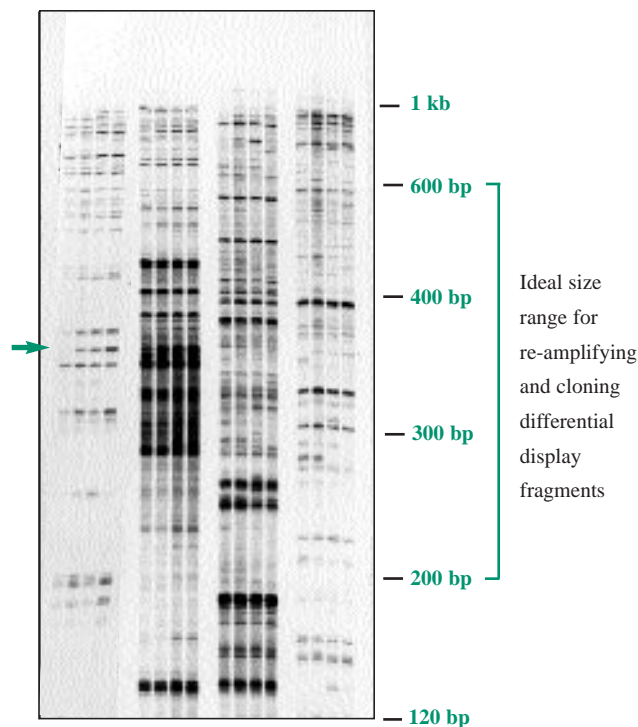
- 1) Make sure the film is aligned correctly with your dried gel.
- 2) Make sure you are using the correct primers (the same ones used for DD) and dNTP mix (250µM).
- 3) Make sure you are using at least 85% ethanol to wash your DNA pellet.
- 4) Older primers and/or dNTP mix can be less effective for re-amplifying. If your primers are more than two years old, or your dNTP mix is over a year old, you may need new ones.

FOR PCR:

- 5) You can increase the final concentration of dNTPs in your reamp. mix (add extra dNTP, but decrease the amount of dH₂O in order to keep a constant volume.)
- 6) The Taq Polymerase also makes a huge difference. GenHunter recommends either Qiagen or Perkin-Elmer Taq Polymerase.
- 7) Try increasing the extension time (72°C) for PCR from 30 seconds to 1-2 minutes per cycle.
- 8) Make sure you are using the correct PCR program for your thermocycler.
- 9) An older thermocycler can give inconsistent results. GenHunter recommends either the Perkin-Elmer 9600 or the Eppendorf MasterCycler™ for best results.

AND ABOVE ALL:

- 10) It is difficult to tell anything from one attempt. If you do not re-amplify your band after one attempt, try again! And don't get discouraged!



A typical differential display gel (four samples from rodent fibroblasts--one normal, three tumorigenic) using GenHunter's RNAImage® kit. Arrowhead indicates differentially expressed band to be reamplified and cloned. (Sizing indicated is approximate)

What's New at GenHunter?

4th Annual International Differential Display Summer Workshop

WHEN: July 12-16, 1999

WHERE: Vanderbilt University in Nashville, Tennessee

As in the past, the workshop will be taught by Dr. Peng Liang, co-inventor of differential display. The workshop is in part sponsored by Vanderbilt Cancer Center, GenHunter Corporation, Qiagen, NEN, and National Diagnostics.

TUITION (excluding room and board):

US \$950 for Academic/Government institutions

US \$1800 for private industry.

The week-long, intensive lab course will focus on all aspects of the most up-to-date differential display techniques in identifying and cloning differentially expressed genes and discuss advantages of differential display to other existing methods. A few participants will be selected to bring their own RNA samples to be compared.

As in previous workshops, this year space for the workshop will be limited to 25-30 participants, and judging by the number of inquires we have had so far, space will probably fill quickly. **So please apply early!**

CONTACT CELESTE RILEY at Vanderbilt if you are interested to receive further information for application and registration for the workshop:

Phone: (615)-343-7328

Fax: (615)-343-7534

email: CRT@toxicology.mc.vanderbilt.edu

New Distributors...

GenHunter is pleased to announce that it has added two new international distributors! If you have friends or colleagues in these areas, please let them know!

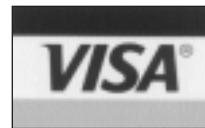
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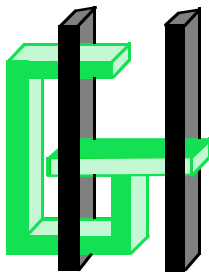
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