
GenHunter® Corporation
2015 Catalog
Products for Differential Display
and AP-TAG™ Technologies

Contact and Ordering Information

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orders@genhunter.com

Online Ordering: www.GenHunter.com
www.DifferentialDisplay.com

We need the following information to process an order by fax, phone, or email:

- ❑ Billing address
- ❑ Shipping address
- ❑ Purchase order / Credit card number
- ❑ Name and telephone number of persons placing the order
- ❑ Catalog number, description, and quantity ordered

For your convenience, GenHunter accepts these credit cards:

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Terms

Net 30 days from the date of invoice in U.S. dollars.

Conditions of Product Use

All products are for research use only. GenHunter disclaims any responsibility for injury or damage that may be caused by the failure of the buyer or any other person to use these products in accordance with the conditions outlined herein.

Pricing and Shipping

Prices listed are only for the U.S., Canada, & other countries without s and are subject to change without notice. Find distributors outside the U.S. and Canada on page 92 or www.GenHunter.com/distributors.

Products are shipped FOB Nashville, TN. Shipping charges are prepaid and added to your invoice. Orders requiring shipment in dry ice are delivered via Federal Express Priority Overnight, Monday through Thursday, unless otherwise requested.

Warranty

GenHunter warrants that the products will meet specifications listed. Free replacement of any non-conforming products will be made only if GenHunter is notified within 30 days of product receipt.

Patent Information

The mRNA Differential Display technology is covered by U.S. Patents 5,262,311; 5,599,672; 5,665,547; 5,965,409; Japanese Patent 2843675; and other issued or pending foreign patents exclusively licensed to GenHunter Corporation. Purchase of the RNAimage® and RNAspectra® kits from GenHunter Corporation comes with a sub-license to practice the differential display process.

The AP-TAG® Receptor/Ligand Interaction Detection System is covered by US patents 5,554,499 and 5,801,000 licensed to GenHunter Corporation which holds world-wide exclusive rights to the AP-TAG® technology in the field of research applications. Purchase of an AP-TAG® Kit or any pAPtag vector comes with a limited, single-user and non-transferable sublicense for use in research applications only. No part of the kit or pAPtag plasmid vectors shall be disseminated, propagated or distributed outside the user's own laboratory without written permission from GenHunter.

The PCR process is covered by patents owned by Hoffman-La Roche Inc. No license or immunity under any other patent is either granted or implied by the sale of any GenHunter product.

Cover photograph: Seashore of Bar Harbor, Maine (by Peng Liang). Catalog Design and Editing: David Turell, Jonathan Meade

To our valued customers

In 2012, we celebrated the twentieth year since the invention of the award-winning differential display technology. During this time, differential display (DD) has become the most widely used methodology to clone differentially expressed genes. In fact, the number of publications using differential display has exceeded the number of publications using all the other competitive methods. Despite the flurry of interest in DNA microarrays and next-gen sequencing, there have still been more scientists who have published the successful cloning of differentially expressed genes using DD than those who used other techniques. See the Technical Notes Section (page 64) which has more information on advantages of DD over microarray and other competing technologies. In 1998, in recognition of the great impact of DD technology, its inventors, Drs. Peng Liang and Arthur Pardee, were awarded the prestigious Molecular Bioanalytics prize by the German Society of Molecular Biology and Biological Chemistry (see page 6).

In addition to the sheer number of differentially expressed genes cloned by differential display, the biological functions of these genes are emerging. GenHunter Corporation is proud to have been the driving force in making this powerful technology within easy reach of biomedical researchers throughout the world (see the representative references from our customers starting on page 71).

Since 1992, the year when differential display was invented, GenHunter Corporation has continued to improve, perfect and streamline the technology. Our complete line of innovative products centered around differential display technology has made GenHunter synonymous with differential display. These kits include the **RNApure**® RNA isolation system (page 11), our well known **MessageClean**® kit for the removal of chromosomal DNA (page 12), the **RNAimage**® kit featuring the third generation of differential display technology (page 16), our magic **PCR-TRAP**® cloning system for cloning the PCR amplified cDNA (page 30), the **HotPrime**® DNA probe labeling kit for optimized Northern blot analysis (page 35) and the **ReversePrime**® cDNA labeling kit for reverse Northern blot analysis (page 37) of cDNA fragments identified by differential display. We continue to expand our world-wide market as the leader in differential display technology.

The **RNAspectra**® **Fluorescent Differential Display Kit** (page 20-24) is the only non-radioactive differential display system with similar sensitivity to that of isotope labeling. By translating the great success of our customers using our reagent kits, GenHunter is also proud to offer our state-of-the-art **Fluorescent Differential Display Service** (page 26-29) with complete computerized automation in liquid dispensing and digital fluorescent data capturing with unsurpassed data reproducibility. With our years of

GenHunter Corporation, Nashville, TN, USA

experience and relentless pursuit of perfection in differential display, we are confident that you will be able to obtain more valuable information on gene expression profiling from us than from any other methodology.

Our **AP-TAG**® **ligand/receptor interaction** detection technology (page 43-59) has revolutionized the way cell surface receptors and ligands are detected and cloned. This technology may allow one to further functionally characterize secreted proteins or cell surface molecules cloned by DD or other methods.

Our **PerfectWestern**® **Western blotting** line of products (page 56-57; download more info at www.PerfectWestern.com) are designed to save money while producing superior western blots. The specially designed **PerfectWestern containers** are available in 37 sizes and perfectly accommodate western blots of any size: whole blots, half blots, and strips. They significantly reduce the volume of expensive antibodies required while still producing beautiful western blots! We also offer pre-cut **PerfectMembranes™** in 3 sizes & 4 materials, **PerfectFilm™** ECL film, the **PerfectRocker™**, **The Belly Dancer**® shaker, **The Belly Button**® shaker, and **PerfectPipettors™**.

The completion of the human genome was undoubtedly a great achievement, however, a much more daunting task is trying to interpret the genomic information in the context of biology. Because of you, our valued customers, differential display has been and will continue to be the leading technology for deciphering genomic information for the benefit of scientific knowledge and human health.

Best wishes with gene hunting!

From all of us at GenHunter Corporation

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AP-TAG® Ligand/Receptor Detection & Cloning Products, cont.

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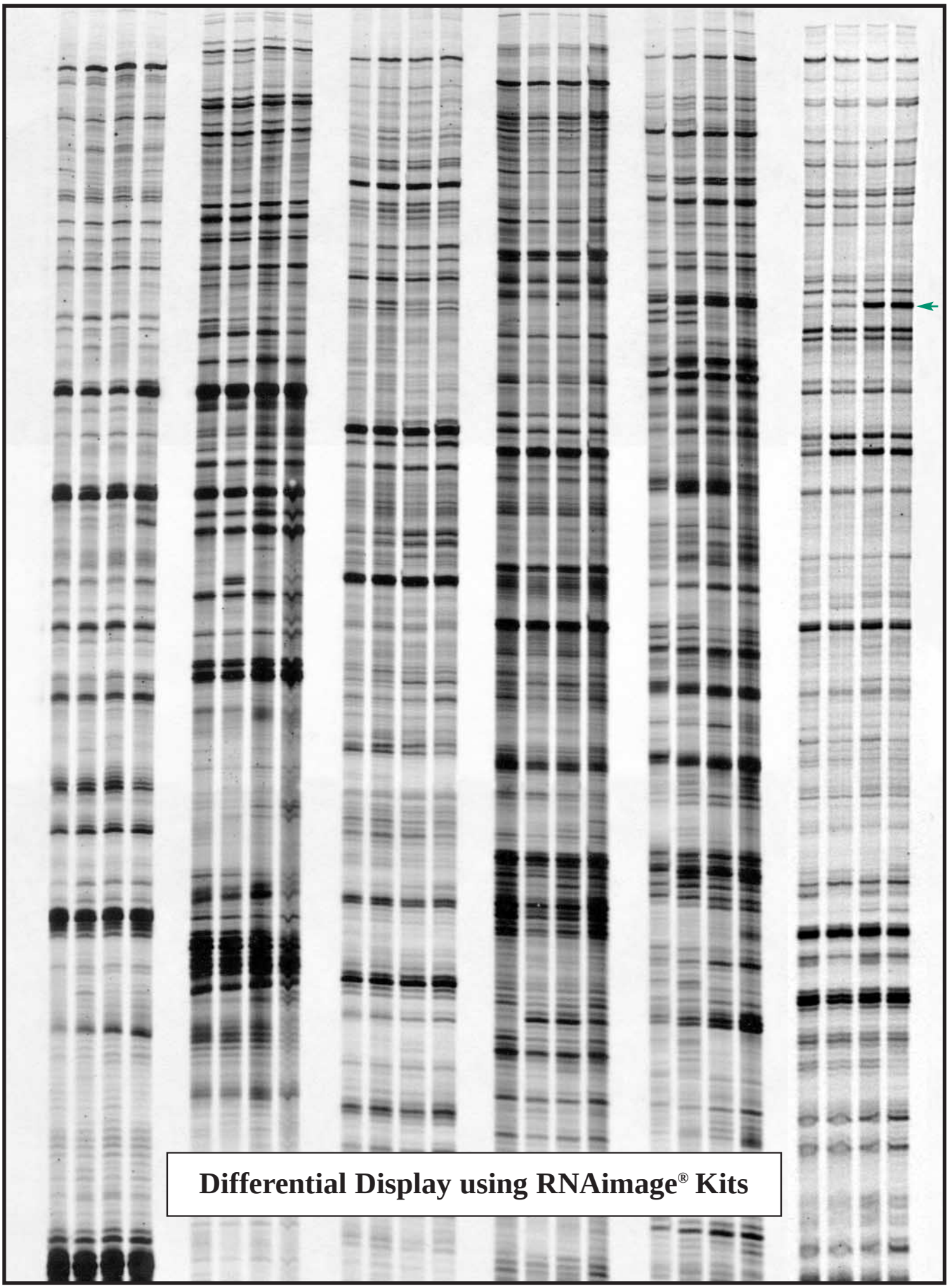
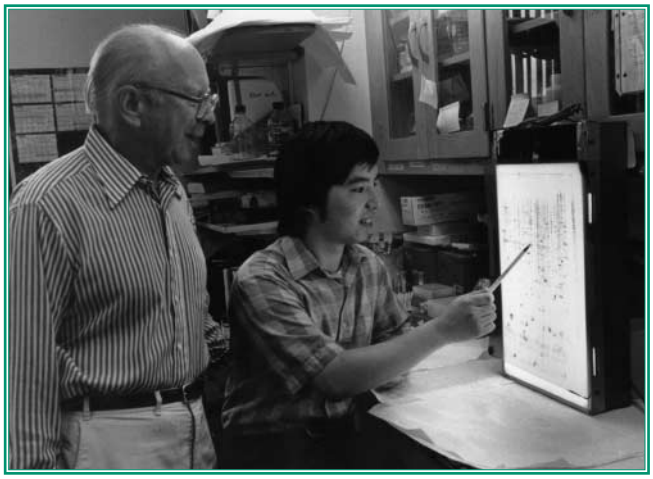


Differential Display using RNAimage® Kits

About GenHunter Corporation

GenHunter Corporation was founded in 1992, the year when differential display was invented, to provide the biomedical research community easy access to the technology. With expertise from the inventor, GenHunter Corporation continues to improve, streamline and perfect the mRNA Differential Display technology (covered by U.S. Patents 5,262,311; 5,599,672; 5,665,547; 5,965,409; Japanese Patent 2843675 and other pending patents). Our complete line of innovative products centered around differential display technology has made GenHunter synonymous with differential display. We have been the exclusive licensee for the manufacturing and marketing of differential display reagent business since its inception. Our intensive research and development, highest quality assurance, and expert technical support has established GenHunter as the world leader in differential display technology. In fact, GenHunter's customer base includes researchers from most major universities, research institutions, pharmaceutical and biotech companies in the U.S. and throughout the world.

In addition to our differential display line, our AP-TAG® ligand/receptor interaction detection technology has revolutionized the way cell surface receptors and ligands are detected and cloned. This technology may allow one to further functionally characterize secreted proteins or cell surface molecules cloned by DD or other methods. We carry a complete line of products centered around this revolutionary approach to receptor/ligand studies.



In 1992, Drs. Arthur Pardee and Peng Liang invented Differential Display technology

"The real challenge for Differential Display begins once the genes of interest are found."

Dr. Peng Liang
Associate professor at Vanderbilt University, co-inventor of Differential Display and founder of GenHunter Corporation.

About the 1998 Molecular Bioanalytics Award

In April of 1998, Drs. Peng Liang and Arthur Pardee were honored for their invention of the differential display process with the 1998 Molecular Bioanalytics Prize, a prize given by the German Society of Molecular Biology and Biological Chemistry for outstanding work in molecular bioanalytics. The two gentlemen shared the prize of 100,000 German Marks, endowed by Roche Diagnostics (formerly Boehringer Mannheim). The prize is awarded biannually in association with the Analytica conference in Germany. Past winners of this prize include Frederick Sanger, Walter Gilbert, Cesar Milstein, George Kohler and Kary Mullis, all of whom went on to win the Nobel Prize for their work.

Drs. Liang and Pardee in Munich, Germany receiving the 1998 Molecular Bioanalytics Prize for their invention of Differential Display

1998 Molecular Bioanalytics Prize Press Release

"Boehringer Mannheim GmbH: This year's Prize Molecular Bioanalytics goes to Arthur Pardee and Peng Liang. It is awarded for the development of the Differential Display technique, a novel and powerful tool for the identification and isolation of genes, which once again revolutionized genome analysis. The award of Molecular Bioanalytics Prize pays tribute to this contribution."

"I...feel very proud to be in the company of Sanger and Milstein. Receiving a major scientific prize is always a big thrill...The genome is like a piece of piano music with right and wrong notes. With differential display, it is possible to tell which notes are right and which are wrong."

— Arthur Pardee

"Prizes are always awarded as a surprise...I was completely taken aback, and I was even more pleased when I saw what famous scientists had won the Molecular Bioanalytics Prize in the past...I am proud to receive this prize in recognition of my scientific work."

— Peng Liang

Past Winners of the Molecular Bioanalytics Prize

Year	Winners	Inventions
1980	Maxam, Gilbert*, Sanger* and Coulson	DNA sequencing
1982	Milstein* and Kohler*	Monoclonal Antibody
1984	Southern	Southern Blot
1986	Wittmann-Liebold, Hood and Hunkapillar	Protein Microsequencing
1988	Canter and Schwartz	Pulsed Field Electrophoresis
1990	Mullis* and Erlich	Polymerase Chain Reaction
1992	J. Lawrence and D. Ward	In situ Hybridization
1994	G. Winter	High Affinity Synthetic Antibodies
1996	M. Capecchi and R. Jaenisch	Gene Knock-Out
1998	P. Liang and A. Pardee	Differential Display
2000	M. Karas and F. Hillenkamp	MALDI Mass Spectrometry
2002	R. Haugland	Fluorescence Resonance Energy Transfer (FRET)
2004	S. Fodor, E. Southern, A. Mirzabekov	Microarray technology
2006	T. Tuschl	RNAi
2008	Name changed to "analytica Research Award" and given only to young scientists in Germany	

* Also won the Nobel prize

About mRNA Differential Display

All living organisms have thousands to tens of thousands of unique genes encoded in their genome, of which perhaps only 15% are expressed in any individual cell. Therefore, it is the temporal and spatial regulation in gene expression that determines life processes. The course of normal cellular development as well as pathological changes that arise in diseases such as cancer are all believed to be driven by changes in gene expression.

A pressing problem is to identify and characterize those genes that are differentially expressed in order to understand the molecular nature of disease state and subsequently, to devise rational therapies. Differential Display was invented in 1992 by Drs. Arthur Pardee and Peng Liang to allow rapid, accurate and sensitive detection of altered gene expression (***Science.*** 1992, 257:967; U.S. Patents 5,262,311; 5,599,672; 5,665,547; 5,965,409; Japanese Patent 2843675 and other pending patents).

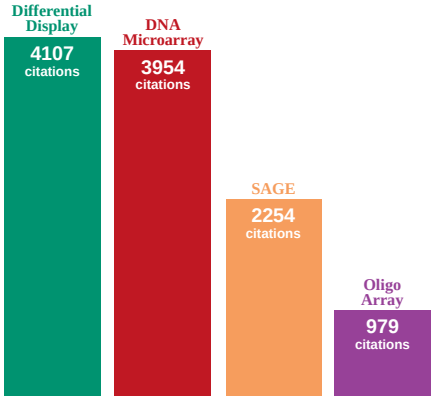
The mRNA Differential Display technology works by systematic amplification of the 3' terminal portions of mRNAs and resolution of those fragments on a DNA sequencing gel. Using anchored primers designed to bind to the 5' boundary of the poly-A tails for reverse transcription, followed by PCR amplification with additional upstream primers of arbitrary sequences, mRNA sub-populations are visualized by denaturing polyacrylamide electrophoresis (see Figures on pages 5 and 17). This allows direct side-by-side comparison of most of the mRNAs between or among related cells.

The differential display method is thus far unique in its potential to visualize all the expressed genes in a eukaryotic cell in a systematic, non-biased and sequence-dependent manner by using multiple primer combinations. More importantly, the method enables the recovery of sequence information and the development of probes to isolate their cDNA and genomic DNA for further molecular and functional characterizations. Because of its simplicity, sensitivity, and reproducibility, the mRNA Differential Display method is finding wide-ranging and rapid applications in developmental biology, cancer research, neuroscience, pathology, endocrinology, plant physiology, and many other fields.

Technical advantages of DD over other competing methods:

1. **Simplicity:** Technically, it is based on PCR and DNA sequencing gel electrophoresis.

2. **Sensitivity:** Five micrograms of total RNA is enough to cover all the anchored oligo-dT primers used in all combinations with 80 arbitrary 13mers. Statistically, this would cover the majority of mRNAs in a eukaryotic cell. Also, differential display is sequence-dependent. Therefore, if the sequences of the arbitrary primer match the target mRNA, whether abundant or rare, it will be amplified and detected. Other methodologies are mostly prevalence-dependent. Therefore, abundant messages are preferentially detected.



Method	Year invented	Total	Pubs / Year	Original Reference
Differential Display	1992	4107	242	Science 1992, 257:967-971.
DNA Microarray	1995	3954	282	Science 1995, 270:467-470.
SAGE	1995	2254	161	Science 1995, 270:484-487.
Oligo Array	1996	979	75	Science 1996, 274:610-614.

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

The Citation searches were performed January 8, 2009 on the ISI Web of Knowledge Citation Search (http://apps.isiknowledge.com). Number of Citations is the number of times the original publication has been cited by other papers, which reflects the number of times each technique has been used for publications.

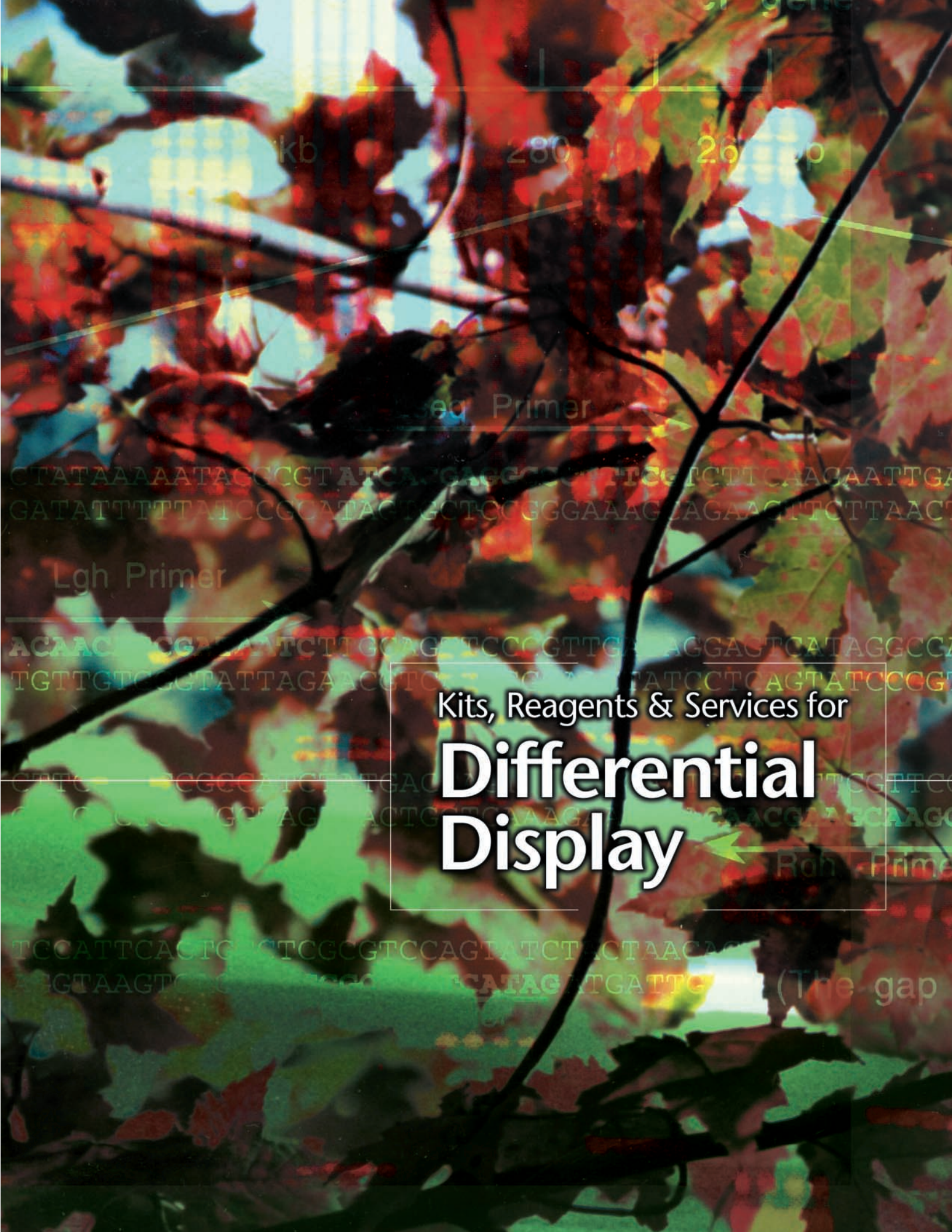
3. **Reproducibility:** Up to 99% of the bands on a mRNA display are reproducible. With our Automated FDD Service, the accuracy can be even higher.

4. **Versatility:** More than two RNA samples can be compared at one time, revealing genes unique to a process, such as certain types of cancer, instead of cell type specific genes; therefore characterization of the gene is built in. In addition, both up- and down-regulated genes can be detected simultaneously.

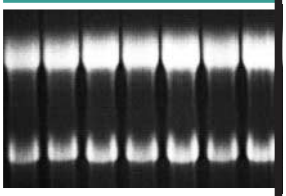
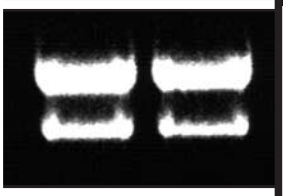
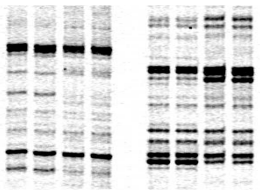
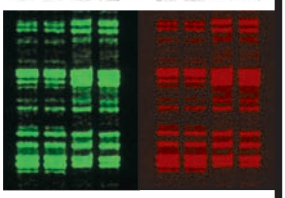
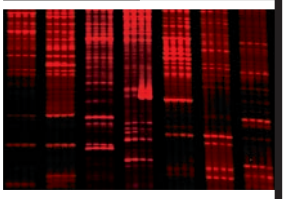
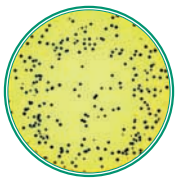
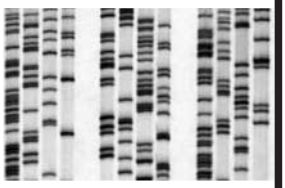
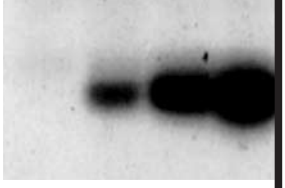
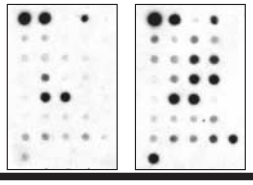
5. **Speed and Expense:** Within two days, the pattern of mRNA display can be obtained. Reamplification of probes and Northern blot confirmation take another week. Most importantly, since the assay may be checked at each step, it is no longer necessary, as it is with other methods, to wait until the end of the procedure to determine if it worked. Compared to DD, microarray is not only prohibitively expensive, but technically challenging for most labs.

6. **Detecting Novel Genes:** Unlike microarray, DD does not require prior knowledge of the mRNA sequence to be detected.

Visit the Technical Notes Section (beginning page 64) for further discussion on Differential Display compared to other methodologies, primer design, calculation of coverage provided, false positives, and much more.


Kits, Reagents & Services for
Differential
Display

GenHunter's Complete System for Differential Display

Step of DD	Product with Description	Page	Image
RNA Isolation, Cleaning, and Quantification	**RNApure® Reagent** For convenient extraction of total RNA from tissues or cultured cells.	11	
	RNA Loading Mix For one-step denaturation and gel loading of RNA.	11	
	MessageClean® Kit For complete cleaning of DNA contamination from RNA.	12	
Differential Display	**RNAimage® Kit** For radioactive Differential Display with three one-base anchored oligo-dT primers and rationally designed arbitrary 13mers.	16	
	RNAspectra® Kit For fluorescent Differential Display with the same primer design as the RNAimage® Kit, but using fluorescently labeled primers.	20	
	Differential Display Service GenHunter now offers an automated fluorescent Differential Display service. You send in your RNA samples and we do the rest!	26	
PCR Product Cloning and Sequencing	**PCR-TRAP® Cloning System** For positive-selection cloning of PCR products through blunt-end ligation. Allows direct cloning of PCR products without purification.	30	
	AidSeq™ Primer Sets For sequencing DNA products cloned into the PCR-TRAP® Cloning Vector.	34	
Confirmation of Differential Gene Expression	**HotPrime® DNA Labeling Kit** For optimized labeling of differential display fragments for use in Northern blots.	35	
	ReversePrime® cDNA Labeling Kit For labeling cDNA probes for use in "reverse Northern" blots.	37	

RNApure® Reagent

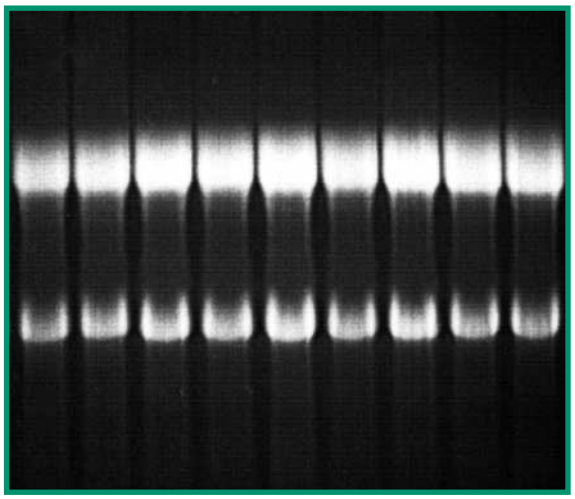
For convenient extraction of total RNA from tissues, cultured cells or blood.

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 can be used for any application where total RNA is required including differential display, RT-PCR, DNA microarray, Northern and reverse Northern blot analysis.

Cheaper than RNAzol®, TRIzol®, and TRI Reagent® !

Detailed protocol included.

	Cat. No.	Volume	Price
RNApure®	P501	50 mL	$60
RNApure®	P502	100 mL	$96
RNApure®	P503	200 mL	$171
RNApure®	P504	500 mL	$423



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

This clear, mono-phasic reagent provides a one-step isolation of total RNA from cells with unsurpassed integrity. GenHunter RNA loading mix (see below) was used for the RNA sample denaturation and loadings.

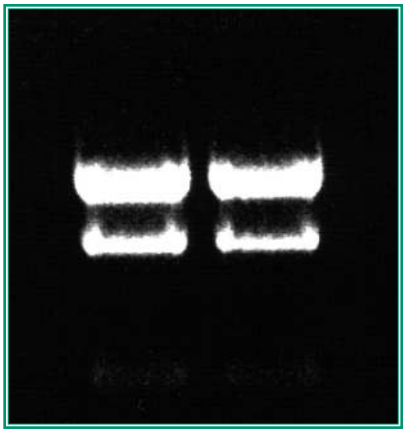
RNA Loading Mix

Cat. No.: R104 **Size: 1 mL** **Price: $27**

For denaturation of RNA before gel analysis.

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

Just mix 1 - 10 µL (2 - 50µg) of RNA with 20 µL RNA loading mix, heat denature for 10 min at 65°C and load on the gel without adding additional ethidium bromide in the gel or buffer.



Gel analysis of total RNA using GenHunter's RNA loading Mix.

No more guessing or worrying about RNA degradation. Get perfect RNA gel for optimal Northern blot analysis.

MessageClean® Kit

CAT. NO.: M601 **PRICE: $160**

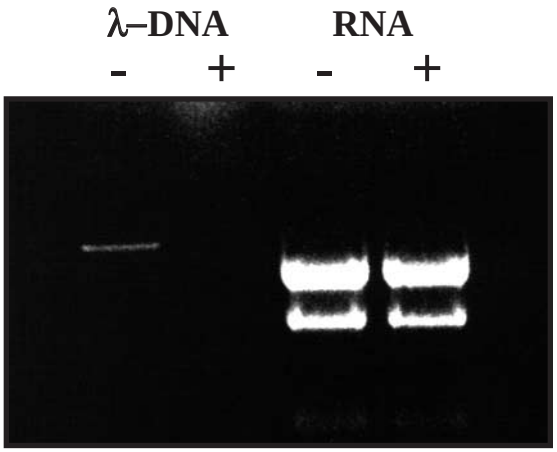
For complete cleaning of DNA contamination from RNA.

This very popular kit contains everything required for the complete removal of trace amounts of chromosomal DNA contamination from RNA isolated by any method, including the simple one-step acid phenol extraction and GIT-CsCl gradient centrifugation. **This step is absolutely essential for successful Differential Display**. GenHunter's GH-DNase I is specifically designed for the complete digestion of single- and double-stranded DNA with absolute integrity of the RNA before differential display or other applications. DNase I from other vendors are used mostly for DNase footprinting, thus may be contaminated with RNase.

Components (for 20 RNA sample cleanings):

1. 10X Reaction Buffer	140 µL
2. GH DNase I (RNase free, 10 u/µL)	20 µL
3. 3M NaOAc	140 µL
4. H_2O (DEPC treated)	1 mL
5. RNA Loading Mix	400 µL

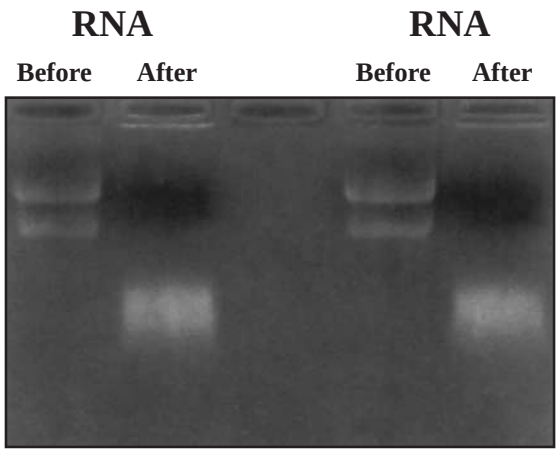
Detailed protocol included.
Shipped on dry ice via overnight delivery.



A: GenHunter's DNase I

Removal of chromosomal DNA from RNA using GenHunter's MessageClean® Kit.

Figure A shows the result of GenHunter's stringent QC with the MessageClean® Kit. λ-DNA (lanes 1 and 2) or total RNA isolated with GenHunter's RNApure® reagent (lanes 3 and 4) were incubated without (-) or with (+) 10 units of GH-DNase I for 30 minutes and then analyzed by gel electrophoresis. As expected, only the λ-DNA, not the RNA, was degraded.



B: Other vendors' DNase I

Removal of chromosomal DNA from RNA using other vendors' DNase I.

Figure B shows total cellular RNA before and after treating with 10 units of DNase I from two major vendors. Both RNA samples were completely degraded by the DNase after treatment.

Individual components for MessageClean® Kit sold separately:

DESCRIPTION	CAT. NO.	VOLUME	PRICE
10X Reaction buffer	R102	140 µL	$30
3M NaOAc	R103	140 µL	$30
RNA Loading Mix	R104	1 mL	$27
H_2O (DEPC treated)	R105	1 mL	$22
DNase I (10 u/µL)	R106	20 µL	$157

MatureMessage™ cRNA Isolation Reagent

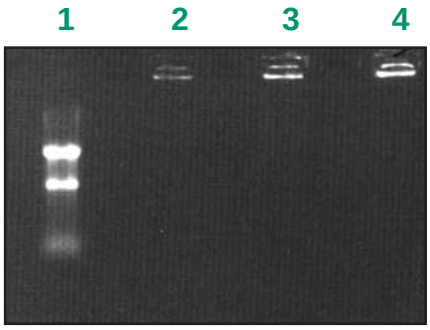
For purification of cytoplasmic RNA (cRNA)

Unlike unspliced mRNAs, mature mRNAs are transported to the cytoplasm where they are used as templates for protein synthesis. RNA isolated by traditional methods invariably are contaminated with both unspliced mRNAs as well as chromosomal DNA from the cell nucleus. While chromosomal DNA can be easily removed by GenHunter's popular MessageClean® kit, unspliced mRNA can complicate the gene expression analysis by differential display and other methods such as DNA microarray.

GenHunter's MatureMessage™ cRNA isolation reagent is specially designed to purify only the intact mRNAs from the cytoplasm*. The MatureMessage™ reagent works by lysing the cells without breaking the cell nucleus, where the unspliced mRNA and chromosomal DNA reside. The cell nucleus can be easily removed by centrifugation and the cRNA is protected by our proprietary RNA inhibitors. The cRNA is then purified by phenol/chloroform extraction (not included). It is still recommended, as a preventative measure, that any potential chromosomal DNA contamination be removed with the MessageClean® kit before cRNA is used for any gene expression analysis such as DD or DNA microarray. cRNA may also be useful for studying RNA splicing.

For cultured cells or cell suspension only.

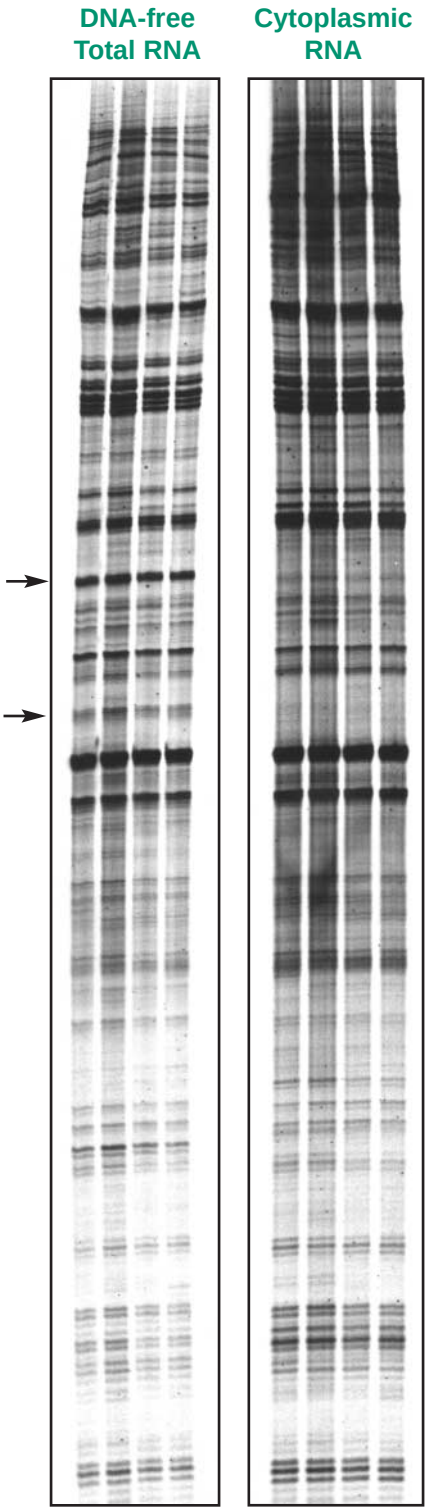
*Patent pending.



cRNA isolated with the MatureMessage™ reagent

Lanes 2-4 show cRNA from an increasing number of cells. Note that the ribosomal RNAs are still bound to the the ribosome and therefore the sample does not migrate through the gel. lane 1 shows cRNA from lane 4 after Phenol/Chloroform extraction.

	Cat. No.	Volume	Price
MatureMessage™ cRNA Isolation Reagent	M901	100 mL	$228



Comparison of RNA by Differential Display:

Arrows indicate potential unspliced mRNAs detected.

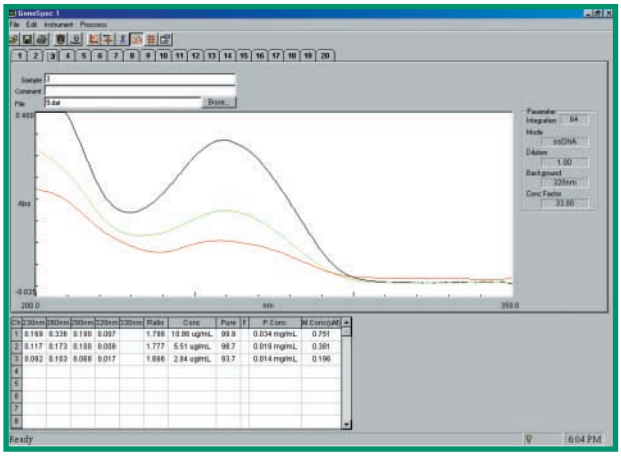
Gene Spec™ Micro-Spectrophotometers

For quantification of 1 - 50µL samples of RNA/DNA

The Gene Spec™ I and III micro-spectrophotometers are among the most sensitive and versatile spectrophotometers available. In less than eight seconds, you can obtain results from samples as small as 1 µL. From the sample's absorbance, the RNA/DNA concentration, 260/280nm ratio, purity, and protein concentration can all be determined instantly. Operations are controlled through a PC, allowing easy data storage and analysis.

The Gene Spec™ has a very small footprint (~9.5 x 13.4 in.)

The Gene Spec™ can be hooked up to an existing PC. The computer control allows easy data storage and analysis.



A full UV spectrum can be obtained calculating RNA/DNA concentration, 260/280 nm ratio, purity, and protein concentration within 8 seconds.

Features:

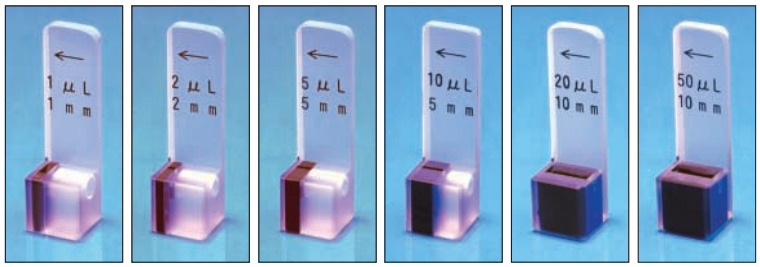
- A few nanograms can be accurately quantitated
- Very small sample size can be measured reducing sample waste (sample volume: 1 - 50µL)
- Six cuvette sizes available
- Full UV spectra within eight seconds
- Determination of RNA/ssDNA/dsDNA concentration, 260/280nm ratio, purity, and protein concentration
- No moving parts housed in a chemical-resistant metal casing
- Wavelength accuracy of ± 1.0 nm and photometric accuracy of ± 0.001 Abs
- Baseline correction
- Continuous display of measuring conditions
- Wavelength calibration and input

Specifications:

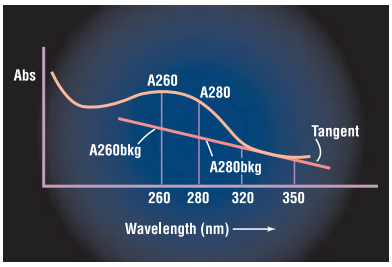
- **Wavelength range:**
  - Gene Spec™ I 190 to 350nm
  - Gene Spec™ III 190 to 1100nm
- **Wavelength accuracy:** ± 1.0 nm
- **Wavelength scan speed:** 0.05s (entire wavelength range)
- **Measuring time:** Approximately 8s, from start of measurement up to result display (includes auto zero correction time)
- **Spectral bandpass:**
  - Gene Spec™ I 5nm
  - Gene Spec™ III 3nm
- **Detector:**
  - Gene Spec™ I 512-element photodiode array
  - Gene Spec™ III 1024-element photodiode array
- **Light source:**
  - Gene Spec™ I Deuterium lamp
  - Gene Spec™ III Deuterium and tungsten-iodine lamps
- **Instrument dimensions** (W x D x H):
  - Gene Spec™ I 9.5" x 13.4" x 4.7"
  - Gene Spec™ III 11.6" x 16.5" x 4.7"
- **Sample compartment** (W x D x H)**:** 2.4" x 5.3" x 2.4"
- **Power requirement:** AC 100, 120, 230, 240V, 50/60 Hz, 200 VA

Pricing of the Gene Spec™ Micro-Spectrophotometers and Accessories

Catalog #	Product	Price
GS101	**Gene Spec™ I** includes general software, cables, fuses, manual, calibration filter; does not include cuvette	N/A
GS102	**Gene Spec™ I with PC System**	N/A
GS201	**Gene Spec™ III** includes DNA/RNA and general software, cables, fuses, manual; does not include cuvette or holmium oxide calibration filter	N/A
GS202	**Gene Spec™ III with PC System**	N/A
GS301-1	**1 uL cuvette**	$550
GS301-2	**2 uL cuvette**	$550
GS301-5	**5 uL cuvette**	$550
GS301-10	**10 uL cuvette**	$470
GS301-20	**20 uL cuvette**	$470
GS301-50	**50 uL cuvette**	$470
GS301-S	**Set of 6 cuvettes**	$2800
GS401	**Gene Spec™ I Deuterium lamp replacement**	$562
GS402	**Gene Spec™ I/III Holmium oxide calibration filter**	N/A
GS403	**Gene Spec™ III Deuterium lamp replacement**	$646
GS404	**Gene Spec™ III Tungsten-Iodine lamp replacement**	$677



The Gene Spec's quartz cuvettes are available in 6 different sizes.



The Gene Spec™ software allows the user to choose the type of baseline correction to apply to the data.

Warranty: Gene Spec™ I and III are warranted to be free from defect (under normal wear and tear) for a period of one (1) year. *Note: Cuvettes, lamps, computers or other third party peripheral devices are not covered under this warranty.

Note: All prices are subject to change without prior notice.
The Gene Spec™ line of products are only available in the U.S. and Canada.

RNAimage® Kits

For isotopic Differential Display with three one-base anchored oligo-dT primers and rationally designed arbitrary 13mers.

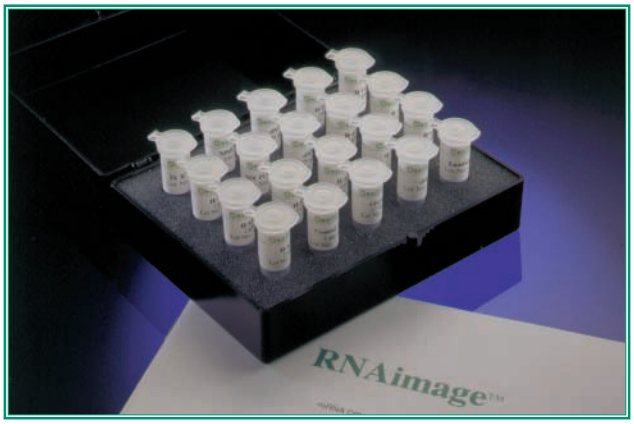
The industry standard for differential display!

These kits feature the latest generation of isotopic mRNA Differential Display technology (U.S. Patents 5,262,311; 5,599,672; 5,665,547; 5,965,409; Japanese Patent 2843675 and pending patents). Each kit contains all the reagents needed for differential display except Taq DNA polymerase and α-[^{33}P] dATP. The MessageClean® is highly recommended to remove trace amounts of DNA contamination from RNA samples prior to DD.

Components of RNAimage® Kit 1

(for displaying 10 RNA samples):

1.	5X Reverse Transcriptase buffer	200 µL
2.	MMLV Reverse Transcriptase (100 units/µL)	40 µL
3.	dNTP (250 µM)	200 µL
4.	H-T_{11}G Anchor Primer	300 µL
5.	H-T_{11}A Anchor Primer	300 µL
6.	H-T_{11}C Anchor Primer	300 µL
7.	10X PCR buffer	500 µL
8.	dNTP (25 µM)	500 µL
9.	H-AP1 Arbitrary Primer	150 µL
10.	H-AP2 Arbitrary Primer	150 µL
11.	H-AP3 Arbitrary Primer	150 µL
12.	H-AP4 Arbitrary Primer	150 µL
13.	H-AP5 Arbitrary Primer	150 µL
14.	H-AP6 Arbitrary Primer	150 µL
15.	H-AP7 Arbitrary Primer	150 µL
16.	H-AP8 Arbitrary Primer	150 µL
17.	Control RNA (1 µg/µL)	1 µL
18.	Glycogen (10 mg/mL)	200 µL
19.	dH_2O	1.2 mL
20.	Loading Dye	500 µL



The major improvements over the previous RNAmap™ technology are: (1) Optimized primer length ensures excellent reproducibility and maximized coverage of expressed genes. (2) One-base anchored primers minimize the redundancy of the displayed cDNA and simplifies the reverse transcription reactions. (3) Attached *Hind* III site to the primers facilitates the manipulation of the cDNA after cloning.

See Technical Notes Section (beginning page 64) for further discussion on primer design, calculation of coverage, comparison of DD to other methodologies, and false positives.

All kits are shipped on dry ice via overnight delivery.
A detailed step-by step protocol is included.

	Cat. No.	Price
RNAimage® Kit 1	G501	$540/kit
RNAimage® Kit 2	G502	$540/kit
RNAimage® Kit 3	G503	$540/kit
RNAimage® Kit 4	G504	$540/kit
RNAimage® Kit 5	G505	$540/kit
RNAimage® Kit 6	G506	$540/kit
RNAimage® Kit 7	G507	$540/kit
RNAimage® Kit 8	G508	$540/kit
RNAimage® Kit 9	G509	$540/kit
RNAimage® Kit 10	G510	$540/kit
5 or more RNAimage® Kits		$486/kit
10 or more RNAimage® Kits		$432/kit

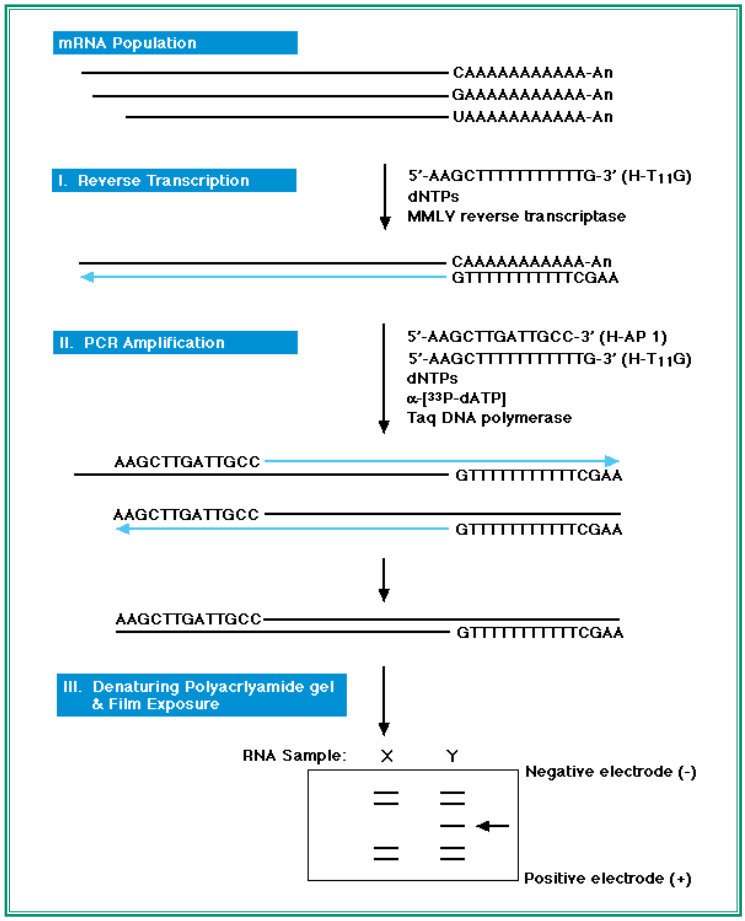
RNAimage® Kits 2-10 contain all the components of Kit 1, but each has 8 different arbitrary 13mers (H-AP9 to H-AP80).

One could start with any RNAimage® Kit since the sequences are random and designed to be maximally different from one another. But to ensure a maximum coverage, multiple kits may be needed (see Technical Notes on page 64).

References:

1. Liang, P. et al.: Differential display using one-base anchored oligo-dT primers. **Nucleic Acids Res.** 1994, 22:5763-5764.
2. Ikeda, S. et al.: An aquaporin-like gene required for the Brassica self-incompatibility response. **Science.** 1997, 276:1564-1566.
3. Blau, J. and Young, M.W.: Cycling *vrille* expression is required for a functional *Drosophila* clock. **Cell.** 1999, 99:661-671.
4. Schmidt, J.V. et al: The Dlk1 and Gtl2 genes are linked and reciprocally imprinted. **Genes & Development.** 2000, 14:1997-2002.

See the Representative DD Reference List starting page 71 for an extensive list of additional publications using our kits.

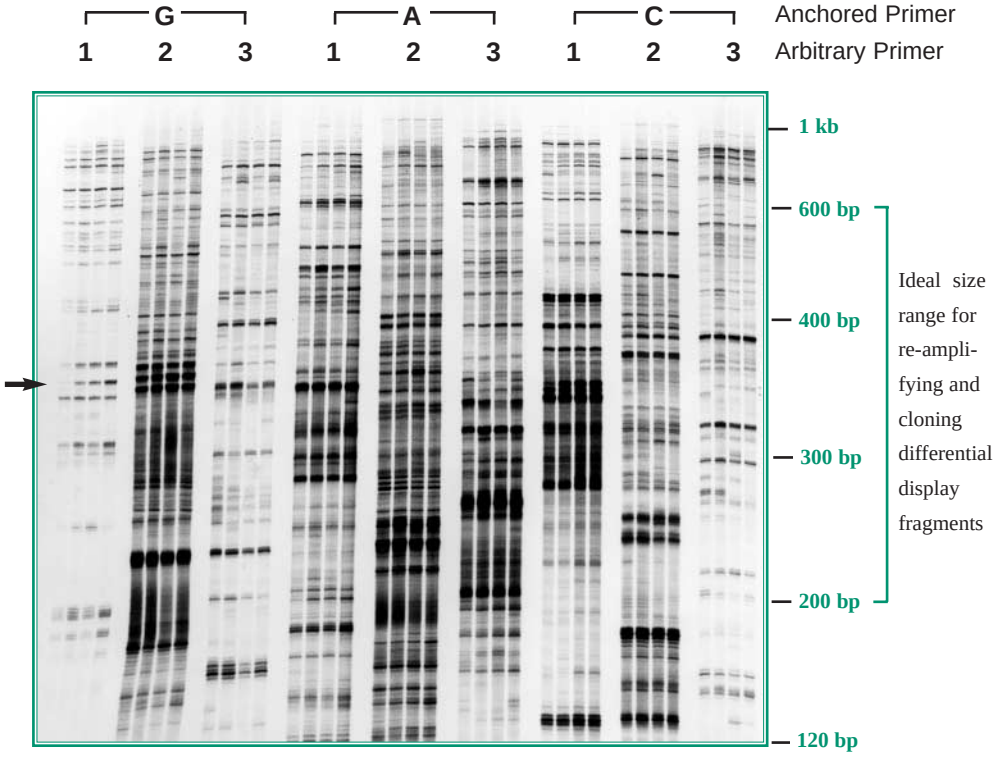


Schematic representation of mRNA Differential Display using RNAimage® Kits.

Three one-base anchored oligo-dT primers with 5' HindIII sites are used in combination with a series of arbitrary 13mers (also containing 5' HindIII sites) to reverse transcribe and amplify the mRNAs from a cell. Note the primer selectivity is provided by the 3' bases. See Technical Notes for more detailed information on the optimal primer designs for differential display.

Differential Display using an RNAimage® Kit.

Four RNA samples from rodent fibroblast (One normal, three tumorigenic) were compared by differential display using three different one-base anchored oligo-dT primers in combination with three arbitrary 13mers. Note the excellent reproducibility and primer selectivity. The arrow indicates a candidate for tumor-specific gene expression. The right side of the gel shows typical sizes of DD bands and the green bracket indicates the ideal size range for re-amplifying and cloning differential display fragments.



Individual components for the RNAimage® Kits sold separately:

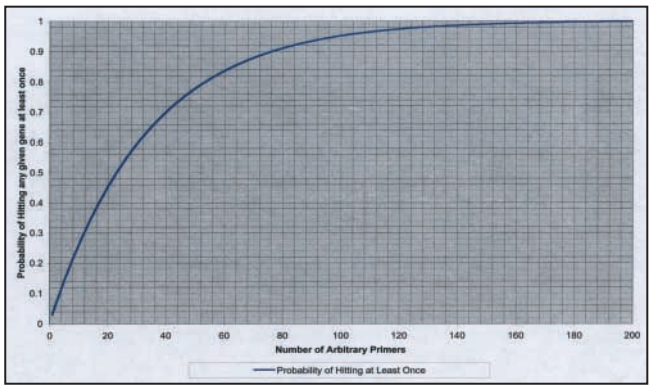
Description	Cat. No.	Volume	Price
H-T_{11}G Anchor Primer	H101-G	300 µL	$78
H-T_{11}A Anchor Primer	H101-A	300 µL	$78
H-T_{11}C Anchor Primer	H101-C	300 µL	$78
H-T_{11}N Anchor Primer Set	H101-S	300 µL each	$211
H-AP Arbitrary Primers (individually)	H-AP1 to H-AP80	150 µL each	$58 each
H-AP primer set 1 (H-AP1 to H-AP8)	H-AP-A	150 µL each	$372/set
H-AP primer set 2 (H-AP9 to H-AP16)	H-AP-B	150 µL each	$372/set
H-AP primer set 3 (H-AP17 to H-AP24)	H-AP-C	150 µL each	$372/set
H-AP primer set 4 (H-AP25 to H-AP32)	H-AP-D	150 µL each	$372/set
H-AP primer set 5 (H-AP33 to H-AP40)	H-AP-E	150 µL each	$372/set
H-AP primer set 6 (H-AP41 to H-AP48)	H-AP-F	150 µL each	$372/set
H-AP primer set 7 (H-AP49 to H-AP56)	H-AP-G	150 µL each	$372/set
H-AP primer set 8 (H-AP57 to H-AP64)	H-AP-H	150 µL each	$372/set
H-AP primer set 9 (H-AP65 to H-AP72)	H-AP-I	150 µL each	$372/set
H-AP primer set 10 (H-AP73 to H-AP80)	H-AP-J	150 µL each	$372/set
10X PCR buffer	S201	500 µL	$38
Glycogen (10 mg/mL)	S301	500 µL	$75
5X RT buffer	S401	500 µL	$38
MMLV Reverse Transcriptase (100 units/µL)	S402	40 µL	$154
DNA Loading dye	S403	1 mL	$38
dNTP (250 µM)	S501	200 µL	$55
dNTP (25 µM)	S502	500 µL	$51
Positive Control RNA pair for DD	R100	1 µg each	$133
Control RNA(DNA-free)	R101	1 µg	$30
dH_20	DH20	1.2 mL	$5

Our primers are synthesized in house on ABI 392 DNA synthesizers using reagents of highest purity and quality. The primers are then purified by reverse-phase chromatography to ensure only the full-length primers are obtained and then quality tested for differential display analysis.

Frequently asked questions about the RNAimage®/RNAspectra® Kits

How many kits do I need to purchase?

The answer to this question really depends on the design of your experiment and how much coverage is desired for genes expressed in a cell. Many researchers already know that they want to get a complete profile of all the differentially expressed genes and therefore choose to purchase all 10 kits at once which has an estimated coverage of up to 96%. This is also more cost effective because you get a 20% quantity discount on 10 or more kits. Other researchers just want to try the kit with their RNA samples before doing a complete screening and therefore start with one or two kits. The graph shows a representation of the estimated coverage based on number of arbitrary primers used. See page 66 for more details on gene coverage.



Which kit number should I start with?

The only difference between the RNAimage®/RNAspectra® Kits 1 through 10 are the sequences of the 8 arbitrary primers included in each kit. Each kit has a completely different set of 8 arbitrary primers. All primers are designed to be maximally different from each other to provide the maximum amount of coverage with each kit. Therefore, it does not matter which kit you start with because you will get the same amount of coverage with any of the kits. In addition, the kits were not designed for a specific eukaryotic organism. Therefore, any of the kits will work successfully, regardless of the species you are studying from yeast, worms, plants, insects, fish, to mammals. Most researchers start with kit #1, because it is #1 and numerically is first, but this kit is not any better than the others, so you can choose a lucky number if you have one in the range of 1 to 10. There is luck in science too, right?

What are the advantages of the RNAimage® and RNAspectra® Kits?

First, a little history. The RNAimage® Kit has been around since 1994 and is based on P^{33} radioactive labeling of bands. It is the most commonly used DD kit because of its excellent sensitivity, reproducibility, coverage, and overall reputation. In addition, before the RNAspectra® Kit was released, there was no way to do DD with similar sensitivity without using radioactivity. The RNAspectra® Kit was released in late 1999 and is based on fluorescent labeling of bands and has equivalent sensitivity to radioactive DD. Both kits have the same arbitrary primers and anchor primers, except that the anchor primers in the RNAspectra® Kit are labeled with a fluorophore. Both kits have the same excellent sensitivity, reproducibility, and coverage. However, each kit has a few advantages over the other.

The RNAspectra® Kit has the advantage of eliminating the need for radioactivity, a major priority for some labs. It also allows digital FDD images to be analyzed and stored. Finally, it allows one to run a gel, scan it, print out a real-size image, cut bands, and re-amplify those bands all in the same day, a process which takes at least two days with the RNAimage® Kit. Use of the RNAspectra® Kit does however require a fluorescent laser scanner.

The RNAimage® Kit has been used in more publications than the RNAspectra® Kit. In addition, use of this kit does not require a fluorescent laser scanner, often a significant expense for a lab.

RNAspectra® Green Kits

For Fluorescent Differential Display with three fluorescein labeled one-base anchored oligo-dT primers and rationally designed arbitrary 13mers.

The RNAspectra® Kits feature a new generation of Differential Display technology based on fluorescent detection of PCR products instead of radioactivity. It is the first non-radioactive differential display system with similar sensitivity to that of ^{33}P isotopic labeling (See figures on opposite page).

Each kit contains all the reagents needed for fluorescent differential display except Taq DNA polymerase and a fluorescent scanner. The MessageClean® is highly recommended to remove trace amounts of DNA contamination from RNA samples prior to DD.

Components of RNAspectra® Green Kit 1
(for displaying 10 RNA samples):

1.	5X Reverse Transcription Buffer	200 µL
2.	MMLV Reverse Transcriptase (100 u / µL)	40 µL
3.	FH-T_{11}G Anchor Primer	200 µL
4.	FH-T_{11}A Anchor Primer	200 µL
5.	FH-T_{11}C Anchor Primer	200 µL
6.	dNTP Mix (FDD)	500 µL
7.	10X PCR Buffer	500 µL
8.	H-AP1 Arbitrary Primer	150 µL
9.	H-AP2 Arbitrary Primer	150 µL
10.	H-AP3 Arbitrary Primer	150 µL
11.	H-AP4 Arbitrary Primer	150 µL
12.	H-AP5 Arbitrary Primer	150 µL
13.	H-AP6 Arbitrary Primer	150 µL
14.	H-AP7 Arbitrary Primer	150 µL
15.	H-AP8 Arbitrary Primer	150 µL
16.	Control RNA (1 µg / µL)	1 µL
17.	FDD Loading Dye	0.5 mL
18.	H-T_{11}G Anchor Primer	300 µL
19.	H-T_{11}A Anchor Primer	300 µL
20.	H-T_{11}C Anchor Primer	300 µL

As in the RNAimage® Kit, the RNAspectra® kit contains three one-base-anchored oligo-dT primers to subdivide the mRNA population, except they are labeled with 5' Fluorescein. The use of three one-base-anchored primers reduces the redundancy and potential under-representation of the sub-population of mRNAs encountered by using either twelve individual or four degenerate two-base-anchored oligo-dT primers. With built-in restriction sites at the 5' ends of both anchored and arbitrary 13mers, the longer primer pairs produce highly selective and reproducible cDNA patterns. These improvements ensure that differentially expressed genes are more readily identified, cloned, and manipulated.

See Technical Notes Section (beginning page 64) for further discussion on primer design, calculation of coverage, comparison of DD to other methodologies, and false positives.

All kits are shipped on dry ice via overnight delivery.
A detailed step-by step protocol is included.

	Cat. No.	Price
RNAspectra® Green Kit 1	F501	$790/kit
RNAspectra® Green Kit 2	F502	$790/kit
RNAspectra® Green Kit 3	F503	$790/kit
RNAspectra® Green Kit 4	F504	$790/kit
RNAspectra® Green Kit 5	F505	$790/kit
RNAspectra® Green Kit 6	F506	$790/kit
RNAspectra® Green Kit 7	F507	$790/kit
RNAspectra® Green Kit 8	F508	$790/kit
RNAspectra® Green Kit 9	F509	$790/kit
RNAspectra® Green Kit 10	F510	$790/kit

5 or more RNAspectra® Green Kits	$711/kit
10 or more RNAspectra® Green Kits	$632/kit

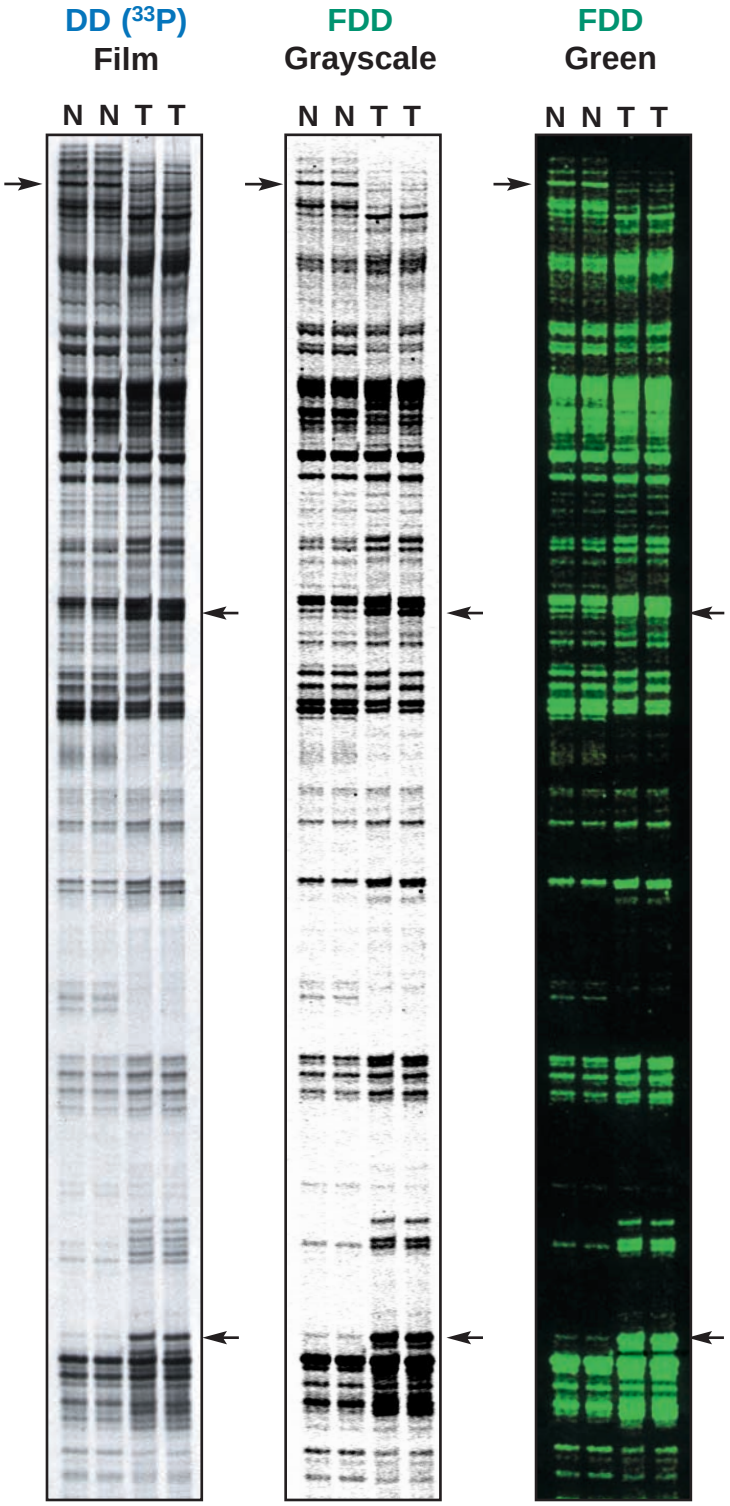
RNAspectra® Kits 2-10 contain all the components of Kit 1, but each has 8 different arbitrary 13mers (H-AP9 to H-AP80).

One could start with any RNAspectra® Kit, since the sequences are random and designed to be maximally different from one another, but to ensure a maximum coverage, multiple kits may be needed. See Technical Notes on page 64.

The RNAspectra® kits have been optimized for use with the Hitachi FMBIO® II or III Fluorescence Imaging System. For more information contact us at 800-311-8260 or info@genhunter.com

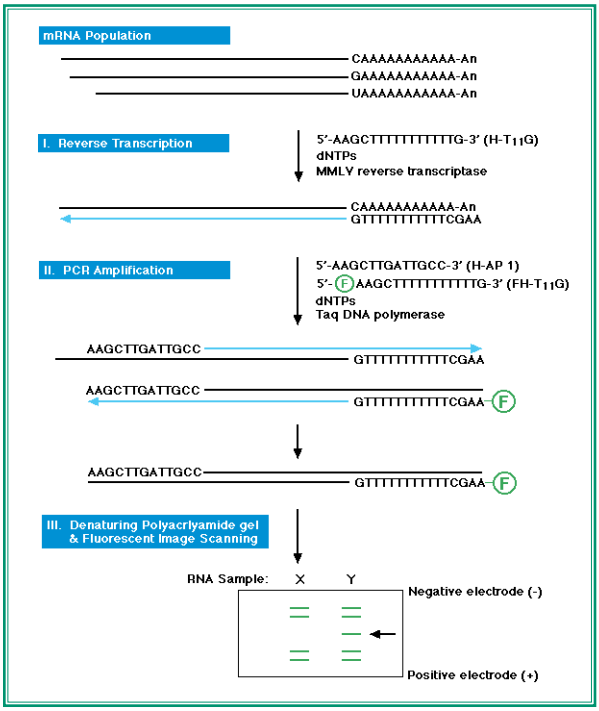
<u>Note</u>: An RNAspectra® Yellow Kit, using NED-labeled primers, is now available as well. Catalog numbers are Y501 to Y510.

FDD has Equivalent Sensitivity To ^{33}P Radioactive DD!



Comparison of Fluorescent DD (FDD) and radioactive DD.

RNA samples from normal (N) and tumor (T) specimens were compared using isotopic DD and FDD. Each image uses a one-base anchored oligo-dT primer, H-T_{11}G, in duplicate with one arbitrary primer. Note the excellent reproducibility and that the same differences are seen in both the radioactive and fluorescent versions.



Schematic representation of Fluorescent mRNA Differential Display using RNAspectra® Kits.

Three one-base anchored oligo-dT primers with 5' HindIII sites are used in combination with a series of arbitrary 13mers (also containing 5' HindIII sites) to reverse transcribe and amplify the mRNAs from a cell. Fluorescently labeled anchor primers are used in FDD-PCR as indicated above by the (F).

See Technical Notes for more detailed information on the optimal primer designs for differential display.

The 2 FDD images pictured were scanned using the FMBIO® II Fluorescence Laser Scanner from Hitachi. Contact us at 800-311-8260 or info@genhunter.com for more information.

RNAspectra® Red Kits

For Fluorescent Differential Display with three Rhodamine labeled one-base anchored oligo-dT primers and rationally designed arbitrary 13mers.

The RNAspectra® Kits feature a new generation of Differential Display technology based on fluorescent detection of PCR products instead of radioactivity. It is the first non-radioactive differential display system with similar sensitivity to that of ^{33}P isotopic labeling (See figures previous page).

Each kit contains all the reagents needed for fluorescent differential display except RNA samples, Taq DNA polymerase, and a fluorescent scanner. The MessageClean® is also highly recommended to remove trace amounts of DNA contamination from RNA samples.

Components of RNAspectra® Red Kit 1
(for displaying 10 RNA samples):

1.	5X Reverse Transcription Buffer	200 µL
2.	MMLV Reverse Transcriptase (100 u / µL)	40 µL
3.	RH-T_{11}G Anchor Primer	200 µL
4.	RH-T_{11}A Anchor Primer	200 µL
5.	RH-T_{11}C Anchor Primer	200 µL
6.	dNTP Mix (FDD)	500 µL
7.	10X PCR Buffer	500 µL
8.	H-AP1 Arbitrary Primer	150 µL
9.	H-AP2 Arbitrary Primer	150 µL
10.	H-AP3 Arbitrary Primer	150 µL
11.	H-AP4 Arbitrary Primer	150 µL
12.	H-AP5 Arbitrary Primer	150 µL
13.	H-AP6 Arbitrary Primer	150 µL
14.	H-AP7 Arbitrary Primer	150 µL
15.	H-AP8 Arbitrary Primer	150 µL
16.	Control RNA (1 µg / µL)	1 µL
17.	FDD Loading Dye	0.5 mL
18.	H-T_{11}G Anchor Primer	300 µL
19.	H-T_{11}A Anchor Primer	300 µL
20.	H-T_{11}C Anchor Primer	300 µL

As in the RNAimage® Kit, the RNAspectra® Red kit contains three one-base-anchored oligo-dT primers to subdivide the mRNA population, except they are labeled with 5' Rhodamine. The use of three one-base-anchored primers reduces the redundancy and potential under-representation of the sub-population of mRNAs encountered by using either twelve individual or four degenerate two-base-anchored oligo-dT primers. With built-in restriction sites at the 5' ends of both anchored and arbitrary 13mers, the longer primer pairs produce highly selective and reproducible cDNA patterns. These improvements ensure that differentially expressed genes are more readily identified, cloned, and manipulated.

See Technical Notes Section (beginning page 64) for further discussion on primer design, calculation of coverage, comparison of DD to other methodologies, and false positives.

All kits are shipped on dry ice via overnight delivery.
A detailed step-by step protocol is included.

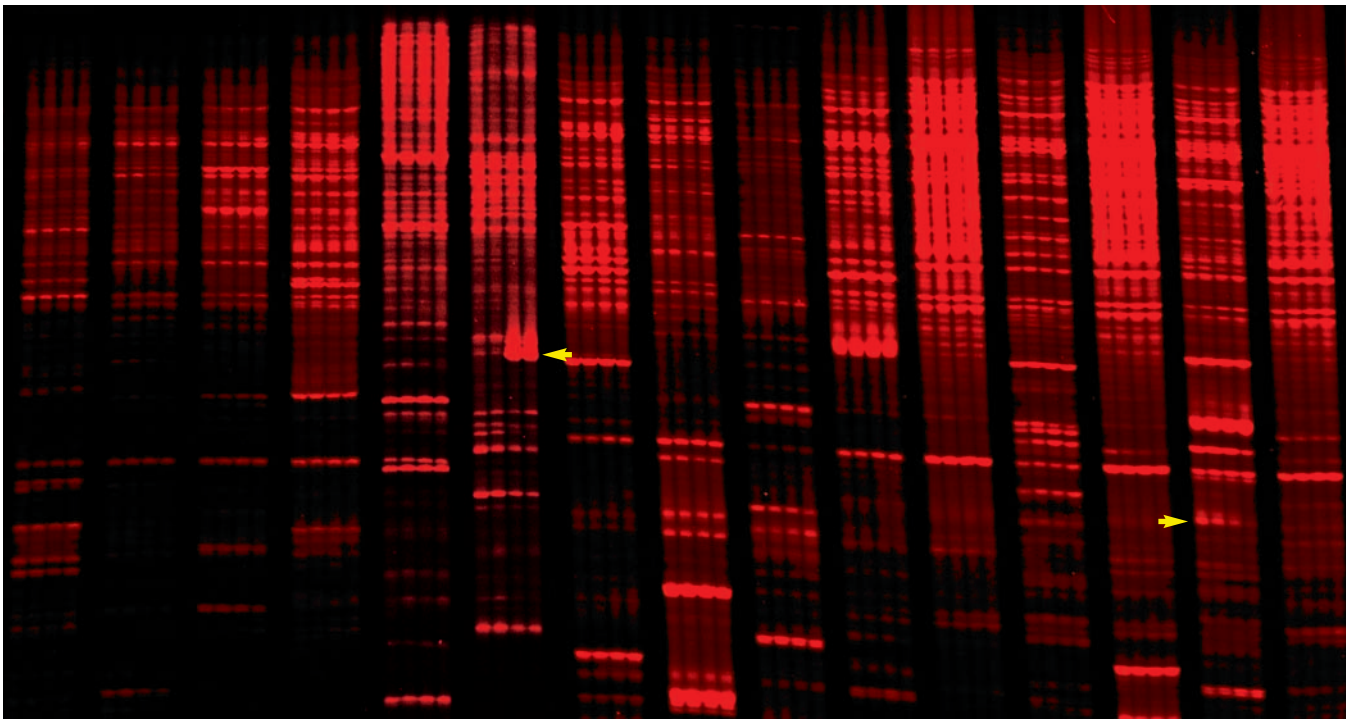
	Cat. No.	Price
RNAspectra® Red Kit 1	R501	$790/kit
RNAspectra® Red Kit 2	R502	$790/kit
RNAspectra® Red Kit 3	R503	$790/kit
RNAspectra® Red Kit 4	R504	$790/kit
RNAspectra® Red Kit 5	R505	$790/kit
RNAspectra® Red Kit 6	R506	$790/kit
RNAspectra® Red Kit 7	R507	$790/kit
RNAspectra® Red Kit 8	R508	$790/kit
RNAspectra® Red Kit 9	R509	$790/kit
RNAspectra® Red Kit 10	R510	$790/kit
5 or more RNAspectra® Red Kits		$711/kit
10 or more RNAspectra® Red Kits		$632/kit

RNAspectra® Kits 2-10 contain all the components of Kit 1, but each has 8 different arbitrary 13mers (H-AP9 to H-AP80).

One could start with any RNAspectra® Kit, since the sequences are random and designed to be maximally different from one another, but to ensure a maximum coverage, multiple kits may be needed. See Technical Notes on page 64.

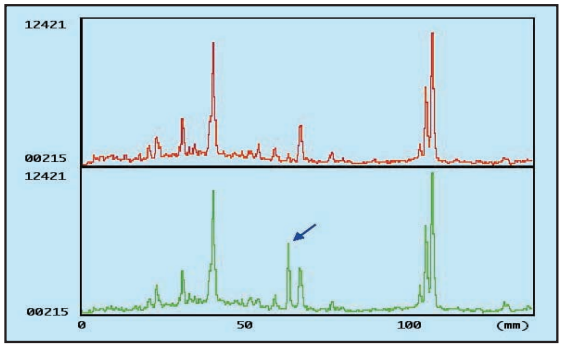
The RNAspectra® kits have been optimized for use with the Hitachi FMBIO® II or III Fluorescence Imaging System. For more information contact us at 800-311-8260 or info@genhunter.com

Note: An RNAspectra® Yellow Kit, using NED-labeled primers, is now available as well. Catalog numbers are Y501 to Y510.



Fluorescent DD Image using the RNAspectra® Red Kit

Comparison of 4 RNA samples (before and 6, 9, and 12 hours after a drug treatment) with automated reaction setup.

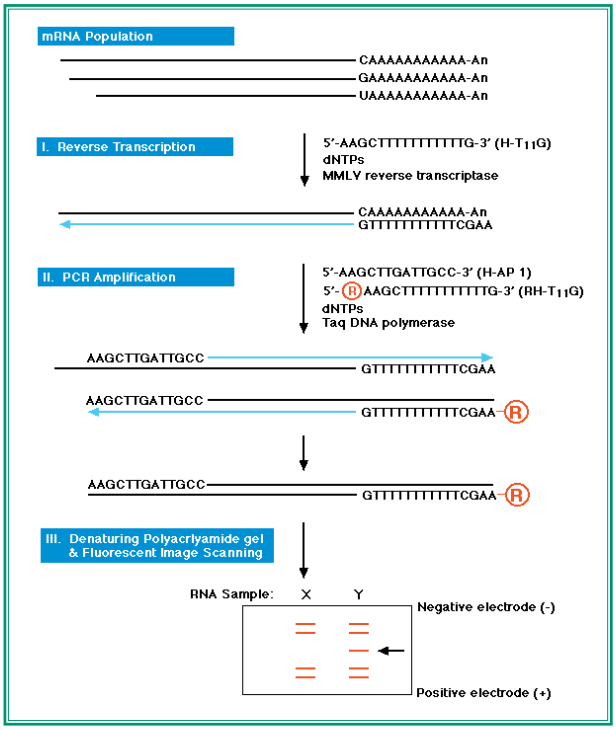


Digital FDD spectra analysis

Digital quantatative analysis of an FDD gel spectra accurately reveals a drug inducible gene (blue arrow). The red and green spectra represent different RNA samples being compared.

Image Scanning with a Fluorescent Imager

The Digital FDD images above were produced using the FMBIO® II Fluorescence Laser Scanner from Hitachi. Contact us at 800-311-8260 or info@genhunter.com for more information.



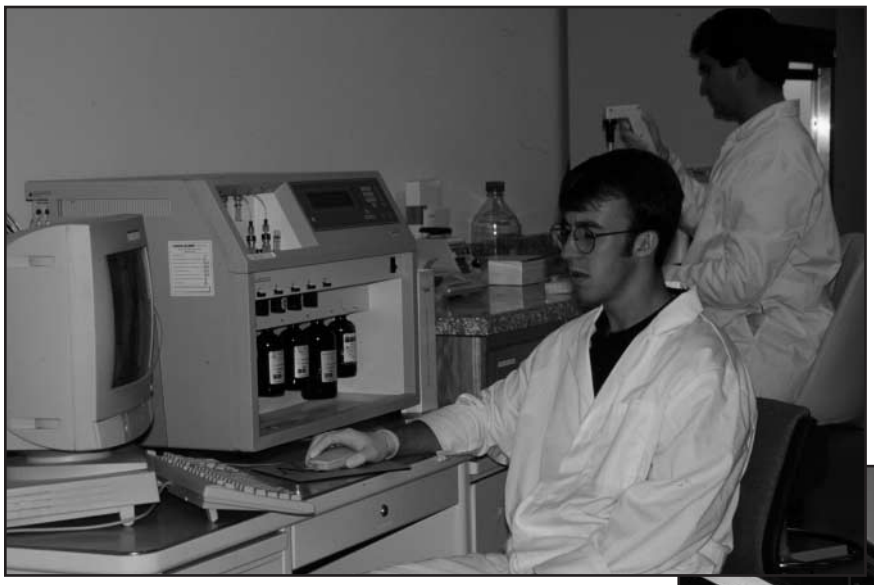
Schematic representation of Fluorescent mRNA Differential Display using RNAspectra® Kits.

Three one-base anchored oligo-dT primers with 5' HindIII sites are used in combination with a series of arbitrary 13mers (also containing 5' HindIII sites) to reverse transcribe and amplify the mRNAs from a cell. Fluorescently labeled anchor primers are used in FDD-PCR, as indicated by the ®.

See Technical Notes for more detailed information on the optimal primer designs for differential display.

Individual components for the RNAspectra® Kits sold separately:

Description	Cat. No.	Volume	Price
FH-T_{11}G Green Anchor Primer	F101-G	200 µL	$161
FH-T_{11}A Green Anchor Primer	F101-A	200 µL	$161
FH-T_{11}C Green Anchor Primer	F101-C	200 µL	$161
FH-T_{11}N Green Anchor Primer Set	F101-S	200 µL each	$415
RH-T_{11}G Red Anchor Primer	R201-G	200 µL	$161
RH-T_{11}A Red Anchor Primer	R201-A	200 µL	$161
RH-T_{11}C Red Anchor Primer	R201-C	200 µL	$161
RH-T_{11}N Red Anchor Primer Set	R201-S	200 µL each	$415
NH-T_{11}G Yellow Anchor Primer	Y101-G	200 µL	$161
NH-T_{11}A Yellow Anchor Primer	Y101-A	200 µL	$161
NH-T_{11}C Yellow Anchor Primer	Y101-C	200 µL	$161
NH-T_{11}N Yellow Anchor Primer Set	Y101-S	200 µL each	$415
H-AP Arbitrary Primers (individually)	H-AP1 to H-AP80	150 µL each	$58 each
H-AP primer set 1 (H-AP1 to H-AP8)	H-AP-A	150 µL each	$372/set
H-AP primer set 2 (H-AP9 to H-AP16)	H-AP-B	150 µL each	$372/set
H-AP primer set 3 (H-AP17 to H-AP24)	H-AP-C	150 µL each	$372/set
H-AP primer set 4 (H-AP25 to H-AP32)	H-AP-D	150 µL each	$372/set
H-AP primer set 5 (H-AP33 to H-AP40)	H-AP-E	150 µL each	$372/set
H-AP primer set 6 (H-AP41 to H-AP48)	H-AP-F	150 µL each	$372/set
H-AP primer set 7 (H-AP49 to H-AP56)	H-AP-G	150 µL each	$372/set
H-AP primer set 8 (H-AP57 to H-AP64)	H-AP-H	150 µL each	$372/set
H-AP primer set 9 (H-AP65 to H-AP72)	H-AP-I	150 µL each	$372/set
H-AP primer set 10 (H-AP73 to H-AP80)	H-AP-J	150 µL each	$372/set
dNTP Mix (FDD)	S505	500 µL	$159
10X PCR buffer	S201	500 µL	$38
Glycogen (10 mg/mL)	S301	500 µL	$75
5X RT buffer	S401	500 µL	$38
MMLV Reverse Transcriptase (100 units/µL)	S402	40 µL	$154
FDD Loading dye	F201	1 mL	$38
Fluorescein Locator Dye	F202	100 µL	$55
Rhodamine Locator Dye	R202	100 µL	$55
Yellow Locator Dye	Y202	100 µL	$55
H-T_{11}G Anchor Primer	H-101-G	300 µL	$78
H-T_{11}A Anchor Primer	H-101-A	300 µL	$78
H-T_{11}C Anchor Primer	H-101-C	300 µL	$78
H-T_{11}N Anchor Primer Set	H-101-S	300 µL each	$211
Positive Control RNA pair for DD	R100	1 µg each	$133
Control RNA(DNA-free)	R101	1 µg	$30
dH_20	DH20	1.2 mL	$5

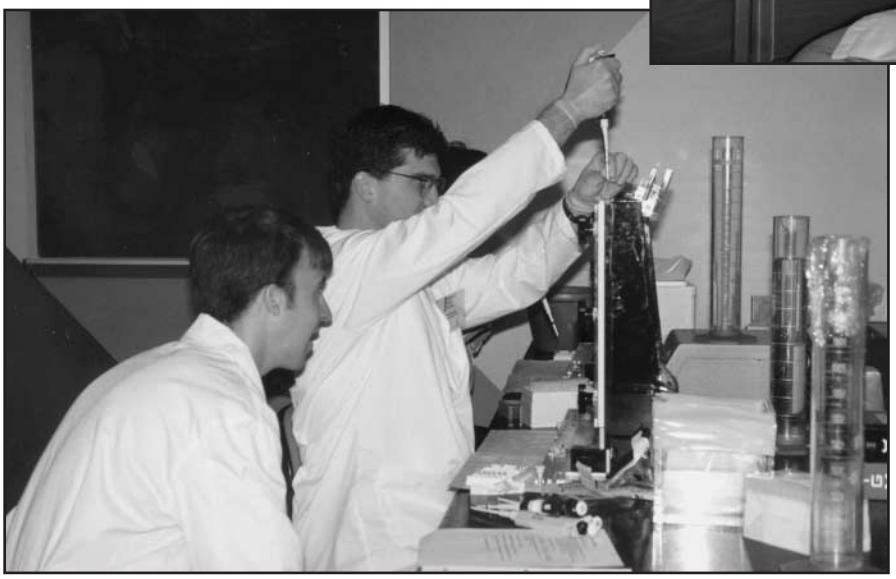


1

All of our primers are synthesized in-house on ABI 392 DNA synthesizers using reagents of highest purity and quality.

2

The primers are then purified by reverse-phase chromatography to ensure only the full-length primers are obtained.



3

Each primer is then individually tested for quality with differential display analysis.

Fluorescent Differential Display Service

Discover the art of differential display with the experts who invented the proven technology. Since 1992, GenHunter has helped thousands of its customers in successfully identifying differentially expressed genes with our kits. We are proud to extend our unsurpassed expertise in the state-of-the-art differential display technology by providing you with the most reliable gene expression profiling service, using completely automated, fluorescent differential display technology.

Just provide us with your RNA samples and we can do the rest - from removal of DNA contamination; to automated, reproducible, fluorescent differential display; and finally subcloning and sequencing your genes of interest.

We have many service options available:

24, 72, 144, and 216 primer combinations can be performed; 70-89% coverage (with 216 primer combination option).

There are many reasons to take advantage of our FDD services including:

- a quick, accurate, and systematic way to visualize gene expression differences in a system with a proven technology and the experience of DD experts
- an evaluation of the DD method itself or a comparison to other competitive techniques such as microarray, subtractive hybridization, or SAGE
- data and images that can be used in grant applications
- more reliable and cost-effective than hiring a technician to work out the method.

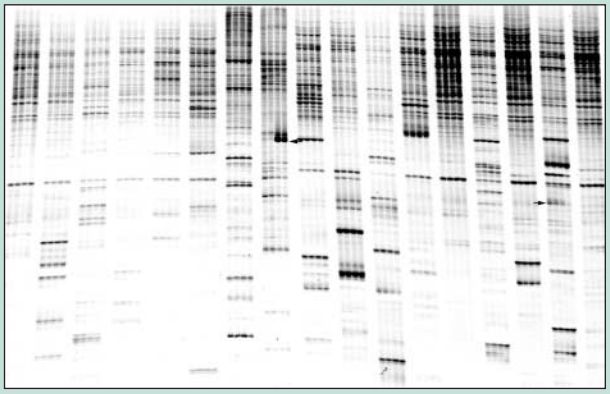
We are confident your investment in our FDD services will be rewarded with the discovery of genes of real interest to your project, as opposed to the possibly misleading information generated by DNA microarrays with which it is often hard to make sense of the data.

Differential display has already been proven to be the best method to discover new genes that are differentially expressed by the overwhelming number of publications using the technology. In fact, the number of publications using DD exceed the number of publications using any of the other competitive methods (See Technical Notes). In essence, researchers are able to find something significant enough to publish much more often with DD than with other techniques. We believe this is due to DD's simplicity, reproducibility, sensitivity, versatility, ability to detect novel genes, and cost-effectiveness.

Our service allows you to focus on the biology of the system under investigation by simply providing GenHunter with your RNA samples and letting us do the rest, with peace of mind!

Complete automation in liquid dispensing using Beckman Biomek 2000 ensures high-throughput and reproducibility.

Digital fluorescence laser scanning allows computerized data acquisition, analysis and storage.



Reproducible and sensitive gene expression profiling by fluorescent differential display.

GenHunter FDD Screening Services

Experience the power of differential display in analyzing your RNA samples by the experts who invented the technology. All we need is your RNA and we do the rest.

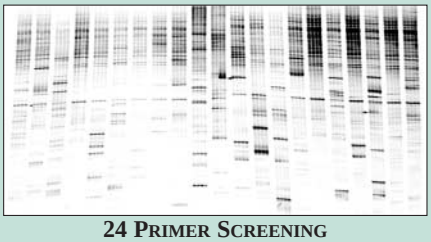
We can do up to 216 primer combinations with 70-89% coverage of both known and unknown genes expressed in a cell!

Service includes:

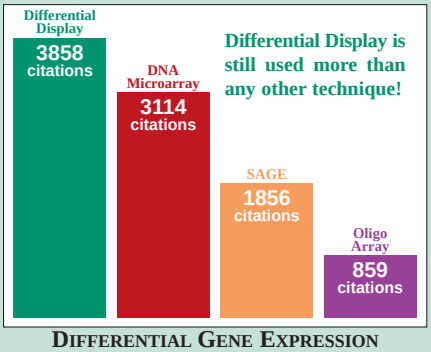
1. Removal of DNA contamination from RNA (20-50 µg of total RNA requested).
2. Check for RNA integrity and quantity.
3. Reverse transcription of mRNA with one-base anchored oligo-dT primer(s).
4. Automated FDD reaction set-up in duplicate with one-base anchored primer(s) in combination with selected number of arbitrary 13mers (by Biomek® 2000 robotic liquid dispensing workstation).
5. 6% denaturing gel electrophoresis.
6. Digital fluorescent imaging with Hitachi FMBIO® fluorescent laser scanner.
7. Digital FDD images and complete service report shipped to client.
8. GenHunter consultation with client on bands to select for further pursuit.

We can then excise, re-amplify, clone, and sequence any bands of interest based on pricing at the bottom of the page.

Only selected projects with proven integrity of the RNA samples will be accepted for any FDD service. Pre-application is required.



24 Primer Screening
21 of 24 primer combinations shown.
Reproducible differences indicated with arrows.



Differential Gene Expression Technologies Publications
Search done January 2007 ISI Web of Knowledge

Service options:

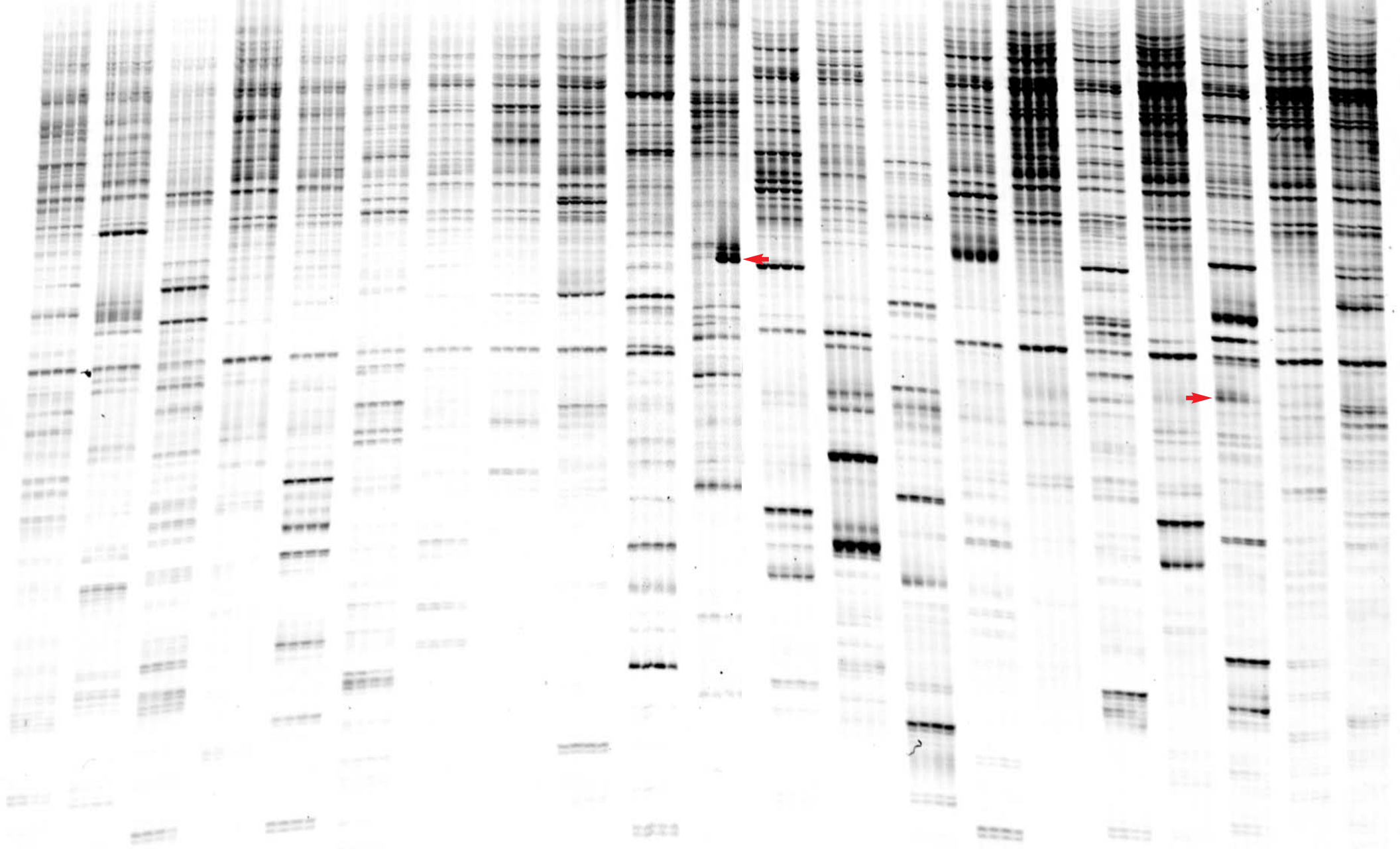
Cat. No.	Primer combinations / RNA	Coverage*	Academic Cost / RNA sample	Industry Cost / RNA sample
J102	**24** (1 anchor, 24 arbitrary)	11% - 17%	Inquire	Inquire
J103	**72** (3 anchors, 24 arbitrary)	33% - 52%	Inquire	Inquire
J104	**144** (3 anchors, 48 arbitrary)	55% - 77%	Inquire	Inquire
J105	**216** (3 anchors, 72 arbitrary)	70% - 89%	Inquire	Inquire

*Estimated coverage of gene expression based on 2 published calculations:
First number is based on Yang and Liang (2004) **Molecular Biotechnology**, 27:197-208.
Second number based on Liang, Averboukh, and Pardee (1994) **Methods in Molecular Genetics.** 5:3-16.

GenHunter requires a deposit of 50% of the service cost for any service before the project begins and the remaining balance paid in full 30 days after project ends.

Additional Downstream Options for Screening Services:

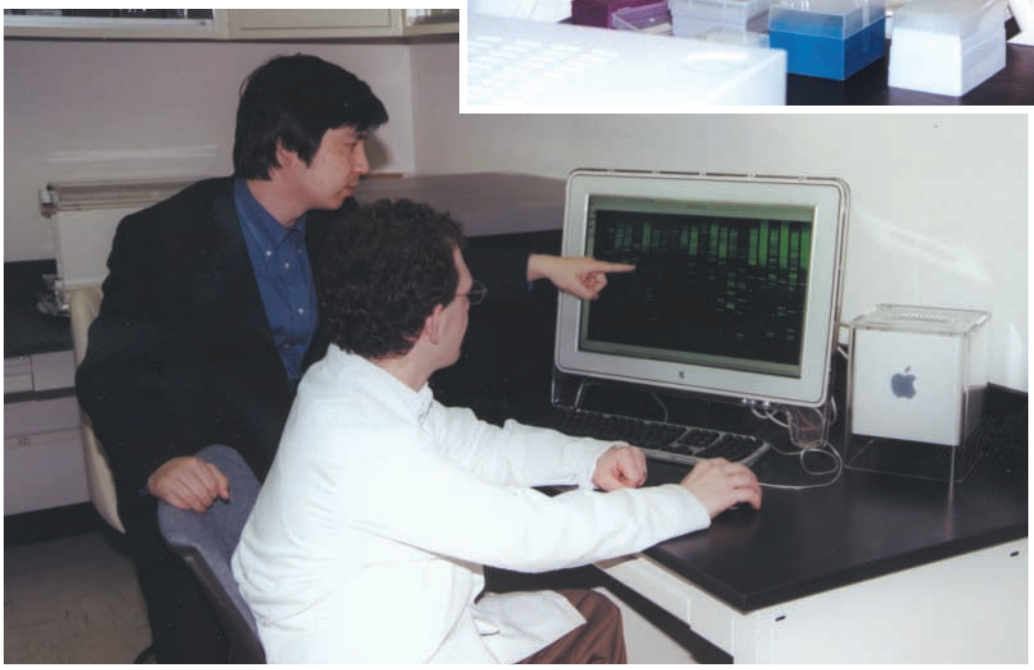
Option	Cat. #.	1-5	6-10	>10	Cost per
• cDNA re-amplification	J201	$150	$125	$100	band
• cDNA cloning	J202	$150	$125	$100	cDNA
• cDNA sequencing	J203	$150	$125	$100	primer



Automated FDD Comprehensive Screening Service (Digital image in grayscale)

Four RNA samples (before, and 6, 9, and 12 hours after a drug treatment) were compared with 1 anchor primer in combination with 24 arbitrary primers (only 21 shown here). These samples were then run on the Horizontal FDD Electrophoresis System (See page 40).

At GenHunter, our mission is to assist you in finding the true differences in gene expression using differential display.



PCR-TRAP® Cloning System

CAT. NO.: P404

For positive-selection cloning of PCR products through blunt-end ligation. Allows direct cloning of PCR products without purification.

GenHunter's PCR-TRAP® Cloning System is the most efficient method for cloning PCR products by blunt-end ligation. This system uses a third generation cloning vector that features positive selection for cloning PCR products (see figures next page). Only recombinant plasmids confer antibiotic resistance, making PCR-TRAP® extremely efficient. There is no need for any post-PCR manipulation before cloning, since a significant fraction of PCR products do not contain the 3' overhanging A (Clark, 1988, Nucleic Acids Res. 16:9677).

The mechanism of this unique cloning system involves the phage Lambda repressor gene, cI, which has been incorporated into the PCR-TRAP® Vector. When transcribed, the gene codes for a repressor protein which binds to the Lambda right operators Or1 to Or3 of the cro gene, turning off the promoter which drives the Tet^R gene on the plasmid. Therefore, cloning the PCR product directly into the cI gene leads to the inactivation of the repressor gene and thus the expression of the Tet^R gene. **So the PCR-TRAP® Cloning System is indeed a cloning TRAP!**

The cloned PCR product can be quickly and easily retrieved by colony-PCR for the purpose of insert confirmation or probe generation using primers which flank the cloning site (provided in the kit). If cDNA amplified by an RNAimage® or RNAspectra® Kit is cloned into a PCR-TRAP® System, the insert can also be excised by HindIII digestion. The flanking primers also allow the cloned PCR product to be readily sequenced.

The kit contains GH Competent cells as well as a positive control, the lacZ gene, which after PCR and cloning will confer blue color upon X-gal staining. This kit is also ideal for conducting reverse Northern blot screening for positive clones during differential display (Ref. 2 and 3).

See Page 32 for a Partial Map of the PCR-TRAP® Vector and other uses for the vector.

Components (for 30 clonings):

1.	Insert-ready PCR-TRAP® Cloning Vector (150 ng/µL)	60 µL
2.	T4 DNA Ligase (200 units/µL)	30 µL
3.	10X Ligase buffer	70 µL
4.	dH_2O	1.2 mL
5.	Colony Lysis Buffer (5 vials)	5 mL
6.	10X PCR Buffer	500 µL
7.	Lgh Primer (2 µM)	200 µL
8.	Rgh Primer (2 µM)	200 µL
9.	dNTP (250 µM)	200 µL
10.	pUC18 control template	10 µL
11.	Llac Primer	20 µL
12.	Rlac Primer	20 µL
13.	Tetracycline	1 mL
14.	Competent cells	3 mL

Detailed protocol for cloning included.
Shipped on dry ice via overnight delivery.

PCR-TRAP® Cloning System Pricing:

1 - 2 kits: $360/kit
3 - 4 kits: $324/kit
5 or more kits: $288/kit

Trial-Size PCR-TRAP® Cloning System

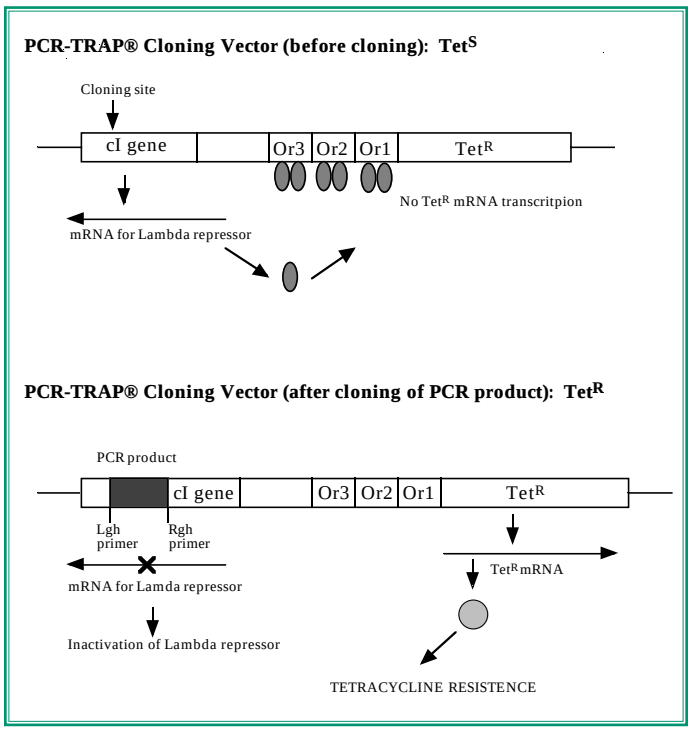
Cat. # P404S (for 5 Clonings): $80

Experience the power of PCR-TRAP® with minimal cost and risk. We guarantee the highest cloning efficiency for PCR products (100-2000 bp) or your money back! This trial size version contains all the components of the standard PCR-TRAP® Cloning System, except 6X less volume.

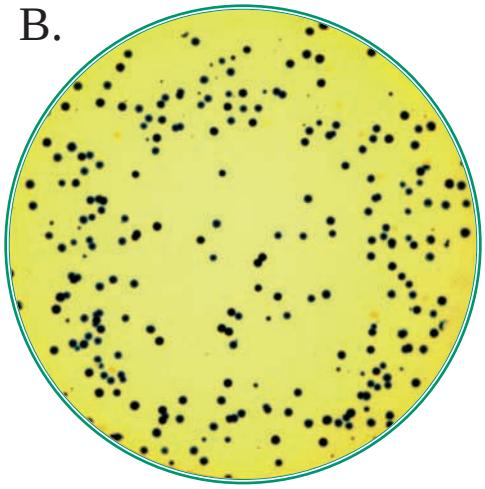
References:

1. Liang, P.: Analysis of messenger RNA by Differential Display. **A laboratory guide to RNA.** 1996, pp223-236.
2. Vogeli-Lange, R. et al.: Rapid selection and classification of positive clones generated by mRNA differential display. **Nucleic Acid Res.** 1996, 24:1385-1386.
3. Zhang, H. et al.: Differential screening of gene expression difference enriched by differential display. **Nucleic Acid Res.** 1996, 24:2454-2455.

A.

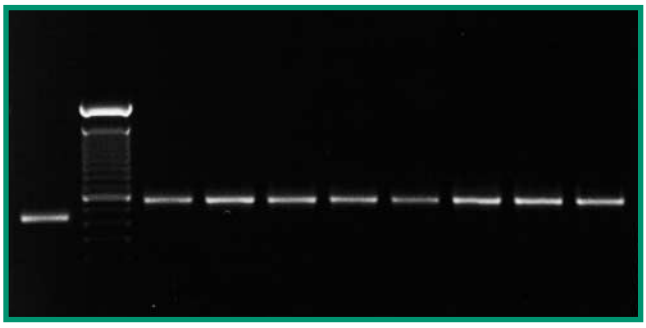


B.



>90% Tet^R colonies are positive for LacZ insert (blue colonies)

C.



Positive Clones

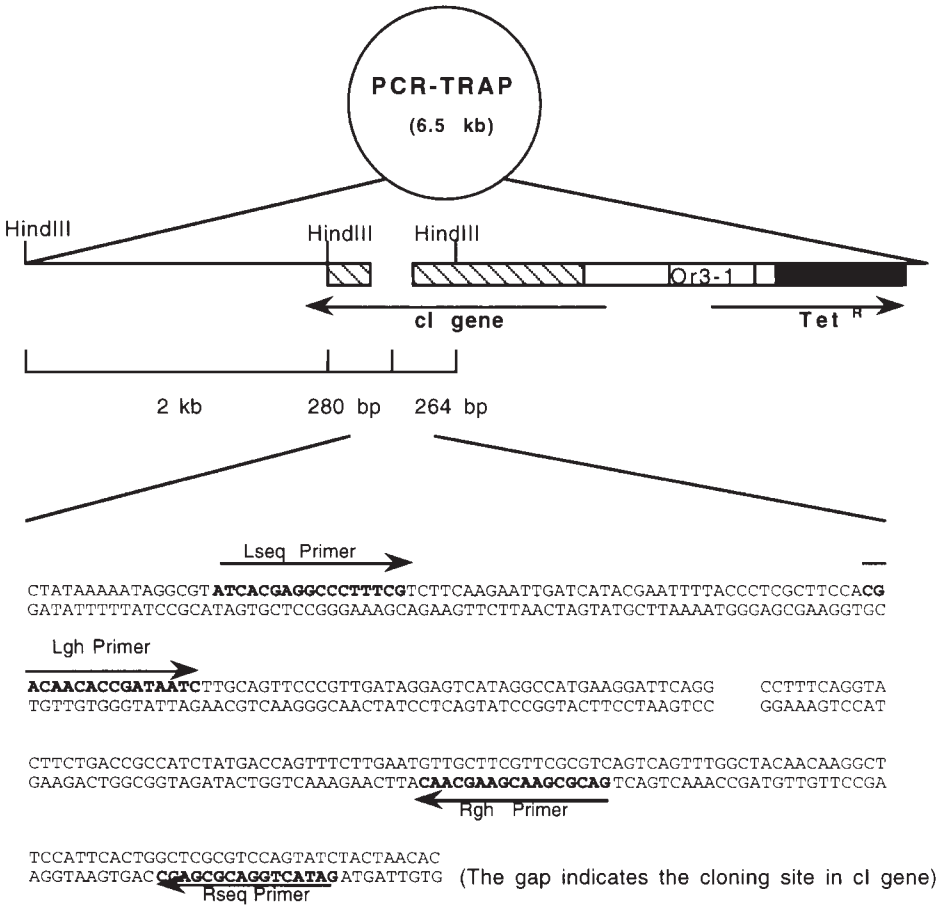
PCR-TRAP® cloning system.

(A) *A schematic diagram of the PCR-TRAP® cloning vector before and after cloning of a PCR product.*

(B) *PCR-TRAP® Cloning Vector ligated with a 450 bp PCR amplified LacZ gene was transformed into E. coli and plated on a tetracycline plate. Blue colonies are positive for insert, while white colonies indicate background colonies.*

(C) *Colony PCR analysis of the 450 bp LacZ inserts cloned into the PCR-TRAP® vector. Lane one is a 450 bp LacZ PCR fragment before cloning. Lane 2 is a 100 bp DNA ladder as a MW standard. Lanes 3-10 are randomly picked blue Tet^R colonies that were checked for insert by colony PCR using a pair of primers located 60 bp away from either side of the cloning site. All positive clones will give a PCR product 120 bp bigger than the actual insert before cloning. The flanking primers can then be used for sequencing. Note all clones checked contain the expected insert.*

Partial Restriction Map Flanking PCR-TRAP® Cloning Site



Other uses for the PCR-TRAP® Cloning System

Another popular application of this efficient cloning vector is to clone the PCR product with desired " built-in" restriction sites at the 5' ends of the PCR primers (no flanking nucleotides necessary). Upon successful cloning, the PCR inserts can be easily cut out by the appropriate enzyme(s) and then subcloned into the target vectors (eg. expression vectors). For example, if a PCR product needs to be cloned into the Bgl II site of an expression vector, one could design primers with built-in Bgl II sites. Please note that Bgl II ends are compatible with BamHI ends for ligation, so the cloned PCR product can also be subcloned easily into a BamHI site of another expression vector. Below is list of restriction sites not found in PCR-TRAP® Cloning Vector that can be used for this purpose.

Acc I	Afl II	Age I	Asc I	Avr II	Bgl II	BseR I	Kpn I	Nco I	Not I	Nsi I	Pac I
Pme I	Pml I	Rsr II	Sac II	SexA I	Sfi I	Sma I	Spe I	Srf I	Swa I	Xba I	Xmn I

Problems for traditional Blunt-end Ligation Cloning Kits

Before PCR-TRAP®, cloning PCR amplified DNA fragments directly by blunt-end ligation was difficult. This difficulty has been attributed to several possible factors:

(1) The unexpected nature of Taq polymerase which has some template independent nucleotidyl transfer activity, resulting in a 3' overhanging A on some of the amplified DNA products.

(2) High dNTP concentration in the PCR reaction which inhibits DNA ligase activity, due to competitive inhibition of dATP against ATP which is a co-substrate for DNA ligase.

(3) Inefficient method of selection for cloned PCR products.

PCR-TRAP® Cloning Vector-Primer Kit

Cat. No.: P405 **Price: $275**

Components (for 30 clonings):

1. Insert-ready PCR-TRAP® Cloning Vector (150 ng/µL)	60 µL
2. Colony Lysis Buffer (3 vials)	3 mL
3. Lgh Primer (2 µM)	200 µL
4. Rgh Primer (2 µM)	200 µL

This kit contains some of the essential components of the PCR-TRAP® Cloning System. Other components such as T4 DNA Ligase, Competent cells, and Tetracycline are not provided.

Individual components for the PCR-TRAP® Cloning System sold separately:

Description	Cat. No.	Volume	Price
PCR-TRAP® Cloning Vector	V101	60 µL	$199
T4 DNA Ligase (200 units/µL)	L101	30 µL	$93
Colony Lysis Buffer	L102	5 mL	$59
10X Ligase Buffer	L103	70 µL	$36
Lgh Primer (2 µM)	L201	200 µL	$67
Rgh Primer (2 µM)	L202	200 µL	$67
GH Competent Cells (6 vials)	L301	3 mL	$210
Tetracycline (20 mg/mL)	L401	1 mL	$33
Lseq Primer (2 µM)	L402	100 µL	$58
Rseq Primer (2 µM)	L403	100 µL	$58
dH_20	DH20	1.2 mL	$5

Riboprobe Primer Set

Cat. No.: P406 **Price: $255**

For PCR amplification of cDNAs cloned into the PCR-TRAP® system for riboprobe preparation.

This reaction creates a template for riboprobe synthesis by placing the T7 and SP6 promoter sites adjacent to the insert.

Components (for 100 reactions):

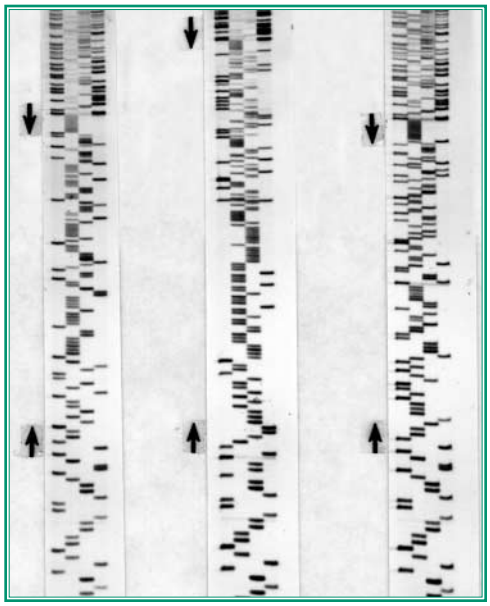
1. T7-Lgh primer (2 µM)	200 µL
2. SP6-Rgh primer (2 µM)	200 µL

AidSeq™ Primer Set A

Cat. No.: P201 **For 50 reactions** **Price: $116**

Primer pair for sequencing DNA cloned into vectors such as TA cloning vector (Invitrogen) and pGEM (Promega) that contain SP6 and T7 promoter binding sites.

Components	
1. SP6 Sequencing primer (1 µM)	100 µL
2. T7 Sequencing primer (1 µM)	100 µL



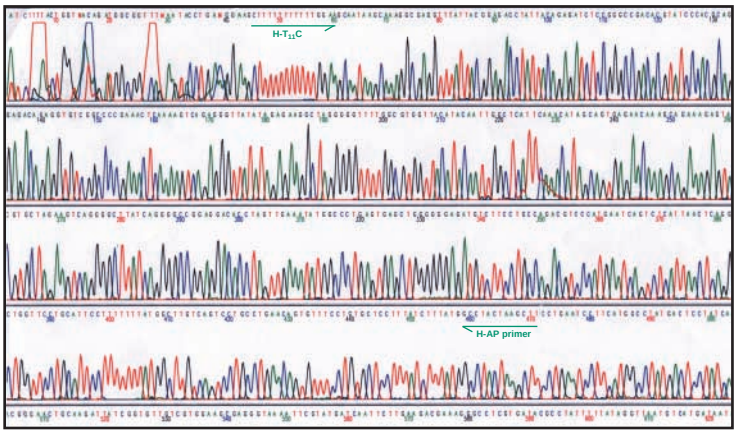
cDNA from Differential Display sequenced with AidSeq™ Primer Set A

AidSeq™ Primer Set C

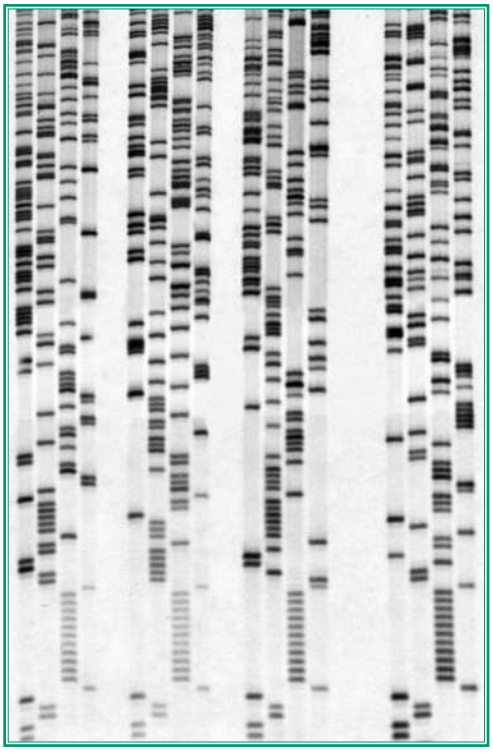
Cat. No.: P203 **For 50 reactions** **Price: $116**

Primer pairs for Sequencing DNA cloned into PCR-TRAP® cloning vector.

Components	
1. Lseq Sequencing primer (1 µM)	100 µL
2. Rseq Sequencing primer (1 µM)	100 µL



cDNA from Differential Display sequenced with AidSeq™ Primer Set C using an automated sequencer. The green arrows indicate the anchor and arbitrary primers.



cDNA from Differential Display sequenced with AidSeq™ Primer Set C using standard di-deoxy terminator sequencing procedure.

HotPrime® DNA Labeling Kit

CAT. NO.: H501 **PRICE: $255**

For optimized labeling of DNA fragments for Northern/Southern blotting.

The HotPrime® DNA labeling kit features three unique improvements over the traditional "random priming" method which allow for radioactive labeling of **DNA probes with at least 5-10X more specific radioactivity**. It is designed to efficiently label cDNA probes isolated from differential display for Northern or Southern blot analysis as well as library screening. However, the HotPrime® Kit can also label DNA probes for any other application to a similar high specific activity.

The first improvement over traditional random-prime kits involves using random decamers instead of hexamers. This ensures more efficient priming of DNA probes of any sequence based on the observation that primers shorter than 9 bases are inefficient in initiating DNA synthesis (Williams et al., 1991, Nucleic Acids Res., 18:6531-6535).

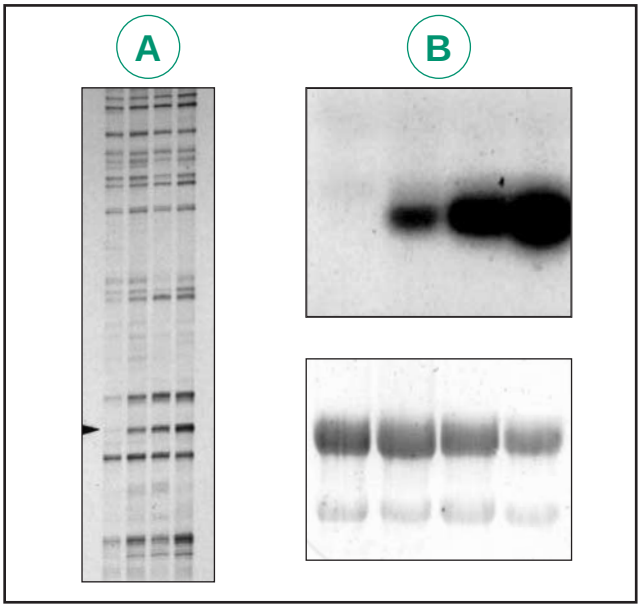
Components (for 20 labelings):

1.	Klenow DNA Polymerase	20 µL
2.	10X labeling buffer	40 µL
3.	dNTP(-dATP) (500 µM)	80 µL
4.	Stop buffer	150 µL
5.	dH_2O	1.2 mL
6.	Control DNA	1 µg

Second, because the cDNA fragments from differential display are relatively short (150-700 bp), the incorporation of anchored oligo-dT primers into the labeling buffer ensures the "full length" anti-sense cDNA probes to be labeled, which greatly increases the chance for signal detection on the Northern blot or library screening.

Third, the use of radioactive dATP instead of dCTP allows labeling of DNA to the highest specific activity by taking advantage of the AT rich nature of the 3' untranslated regions of mRNA detected by differential display.

The HotPrime® DNA Labeling Kit is also available with dNTP (-dCTP) for use with α-[^{32}P] dCTP (Cat. # H501C).

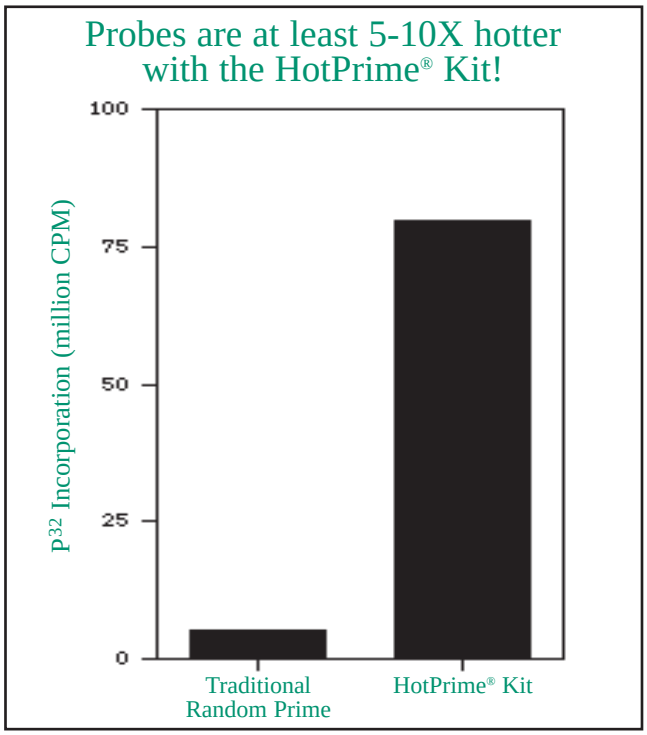


Left figure: Northern blot analysis of differential expression of cDNA probe isolated by differential display.

The band of interest from differential display was excised (A, arrowhead), reamplified and labeled with the Hotprime® DNA labeling kit as a probe for Northern blot confirmation (B).

Right figure: Typical CPM labeling of probes with the HotPrime® Kit as compared with traditional random prime kits.

This shows typical CPM labeling figures using traditional random prime kits and our HotPrime® Kit on the same probe. In this experiment, traditional random prime labeled at 7 million CPM. The HotPrime® labeled the same probe at 78 million CPM.



Individual components for the HotPrime® Kit sold separately:

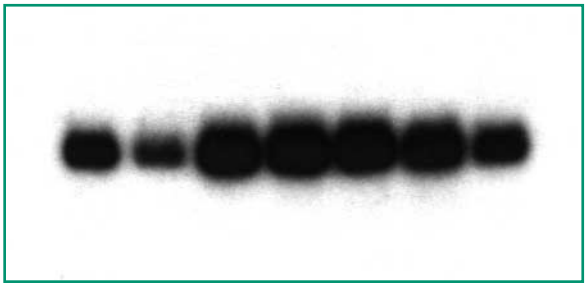
Description	Cat. No.	Volume	Price
dNTP (-dATP) (500µM)	S504	80 µL	$78
dNTP (-dCTP) (500µM)	S503	80 µL	$78
Klenow DNA Polymerase (20 units)	H601	20 µL	$117
10X Labeling Buffer	H602	60 µL	$99
Stop Buffer	H603	150 µL	$26
Control DNA	H604	20 µL	$33
dH_20	DH20	1.2 mL	$5

Prehybridization Solution

Cat. No.: ML1 **Size: 50 mL** **Price: $117**

For convenient and reproducible Northern hybridization.

GenHunter's Prehybridization Solution is based on the traditional formula with 50% formamide. Works extremely well and is cheaper and easier than trying to make it yourself.



Excellent signal to noise ratio for Northern blot using GenHunter's Prehybridization Solution

Detailed protocol for Northern blot included.
Shipped on dry ice via overnight delivery.

Salmon Sperm DNA

Cat. No.: ML2 **Size: 1 mL** **Price: $55**

For preventing non-specific hybridization during blotting.

Our salmon sperm DNA is already diluted to 10mg/mL and is ready to use.

ReversePrime® cDNA Labeling Kit

Cat. No.: R701 **Price: $255**

For labeling cDNA probes for use in "Reverse Northern" blots.

GenHunter's ReversePrime® Kit allows one to label cDNA for use in "Reverse Northern" blots to conduct differential screening of positive clones generated by differential display. This kit is ideal for either dot blot or colony hybridization screening of differential display PCR products cloned into the PCR-TRAP® Cloning System (Cat. No. P404) or other commonly used PCR cloning vectors. This significantly reduces the amount of labor required for screening by Northern Blots or Ribonuclease Protection Assays (RPA). However, like microarrays, reverse Northerns may only reliably detect more abundant mRNAs. For rare messages, we still advise doing a Northern blot using our HotPrime® Kit (page 35).

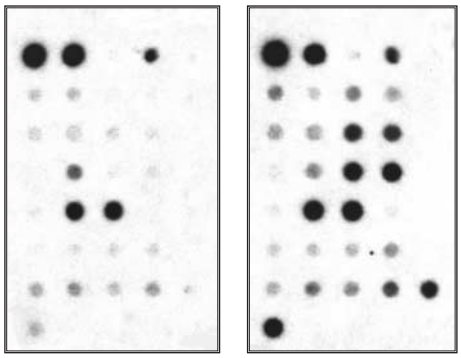
Components (for 10 labeling reactions):

1. 5X RT buffer	200 µL
2. MMLV Reverse Transcriptase	40 µL
3. T20 Primer	60 µL
4. dNTP (-dCTP) (500 µM)	80 µL
5. dH_2O	1.2 mL

References:

1. Zhang, H. et al.: Differential screening of gene expression difference enriched by differential display. **Nucleic Acid Res.** 1996, 24:2454-2455.

The ReversePrime® cDNA Labeling Kit is also available with dNTP (-dATP) for use with α-[^{32}P] dATP (Cat. # R701A).



Normal **Tumor**

Reverse Northern dot blot screening of cDNA fragments.

cDNA fragments identified by differential display to be expressed only in tumor cells were reamplified, cloned into the PCR-TRAP® cloning vector, and dot-blotted onto duplicate nylon filters. RNA from normal and tumor cells were then labeled with ^{32}P with the ReversePrime® cDNA labeling kit and hybridized differentially to the duplicate filters. Note many positives were identified on the blot probed with labeled tumor cell RNA.

Individual components for the ReversePrime® Kit sold separately:

Description	Cat. No.	Volume	Price
5X RT buffer	S401	500 µL	$38
MMLV RT (100 units/µL)	S402	40 µL	$154
dNTP (-dCTP) (500µM)	S503	80 µL	$78
dNTP (-dATP) (500µM)	S504	80 µL	$78
T20 Primer (2 µM)	T20	60 µL	$58
dH_20	DH20	1.2 mL	$5


Supplies & Equipment for
Differential
Display

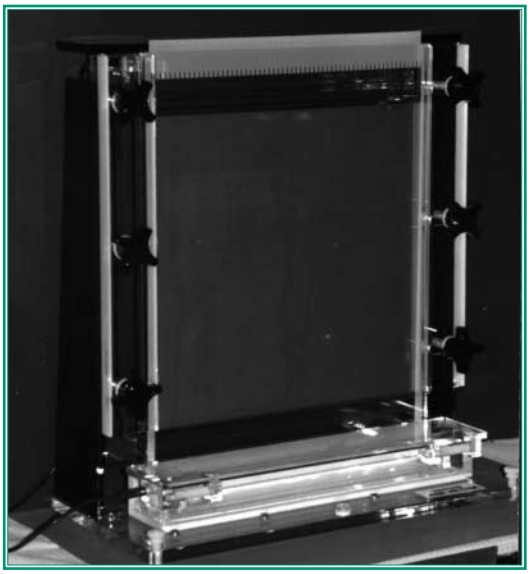
Sequencing Gel Apparatus for Differential Display

Cat. No.: SA1 **Price: $1400**

This apparatus has a glass size measuring 35 cm in width and 45 cm in length with an estimated buffer requirement of 800 mL. The total dimensions of the apparatus are 44 cm wide by 50 cm high by 25 cm deep. It comes with a 60-well comb ideally designed for Differential Display.

Components:

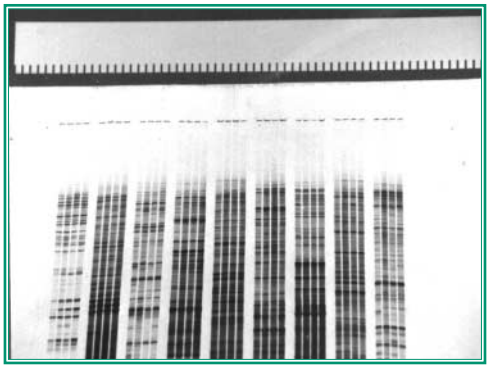
1. Anodized, floating aluminum plate heat sink
2. Upper buffer chamber with removable drain bottle
3. Removable lower buffer chamber
4. Safety lids with attached power cords
5. Built-in sliding side clamps with easy-to-turn knobs
6. Notched glass plate
7. Blank glass plate
8. 60-well comb and spacer set (0.4 mm thick)
9. Adhesive temperature indicator



Sequencing gel apparatus for differential display

Individual components for the Sequencing Gel Apparatus sold separately

	Cat. No.	Price
Comb: 60-well, 0.4 mm thick, 33cm wide approximate well volume: 9 µL, well dimensions: 4mm x 6mm	CM1	$67
Spacers: 1 cm wide, 0.4 mm thick 45 cm long	SP1	$35
Glass Plate set: 35 cm x 45 cm	GL303	$89



Convert your Sequencing Apparatus into a "98 well Microtrough System"

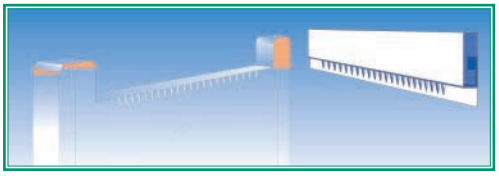
With this 98-well Microtrough Conversion Set, you can convert the above Sequencing Gel Apparatus (Cat. # SA1) into a microtrough system. In the patented microtrough system (pictured at right), the plates have grooves ground into the glass surface. A casting comb is used during polymerization to form a flat interface at the top of the gel into which the sharktooth comb rests and the small rubber plugs occupy the microtrough grooves to prevent unwanted accumulation of unpolymerized acrylamide.

	Cat. No.	Price
98-well Microtrough Conversion Set	SA2	$582

Includes:

- Sharktooth Comb (98-well, 0.4 mm)
- Spacer Set (1 cm wide, 0.4 mm thick,45 cm long)
- 98-well Microtrough Standard Glass Plate Set (35 cm x 45 cm {W x H})
- Microtrough Casting comb (0.4 mm x 98 wells)

Note: These microtrough plates are standard sodalime glass, not the low-fluorescent borosilicate glass included in the FDD Electrophoresis Systems.



The Microtrough System:

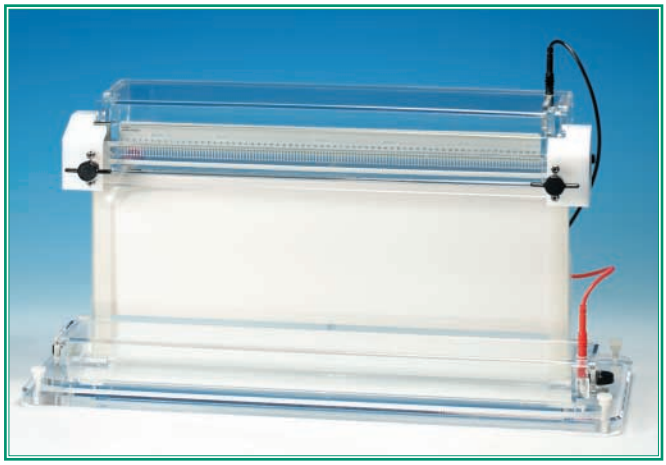
This process significantly improves sharktooth gel systems. It simplifies and expedites loading of sequencing gels. Regular pipette tips can be easily placed into the desired groove. Each groove corresponds to a sample application site of the sharktooth comb allowing the sample to be gently funneled into place. Hand position during loading is more stable and relaxed.

Horizontal FDD Electrophoresis System

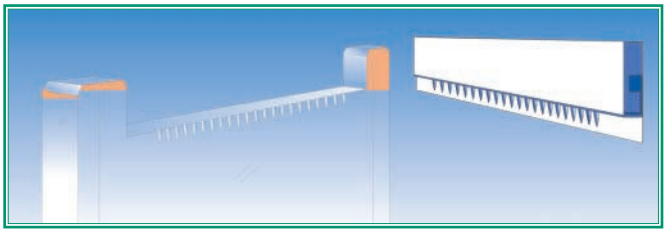
Cat. No.: SA101 **Price: $1970**

Our new Horizontal FDD Gel Electrophoresis System is designed for high-throughput fluorescent differential display (FDD). With a glass size measuring 45cm in width and 28 cm in length, and 132 lanes, one can process at least one 96-well plate per gel. This apparatus uses the patented microtrough system (pictured at right) in which the plates have grooves ground into the glass surface which simplifies and expedites loading of sequencing gels. Regular pipette tips can be easily placed into the desired groove and sample expelled without concern that sample will leak into adjacent well. Each groove corresponds to a sample application site of the sharktooth comb allowing the sample to be gently funneled into place. Hand position during loading is more stable and relaxed. **See FDD gel image on page 28**.

The total dimensions of the apparatus are 56 cm wide by 43 cm high by 23 cm deep.



Horizontal Fluorescent DD Electrophoresis System



The Microtrough System: Grooved glass plates with a microtrough casting comb

Components:

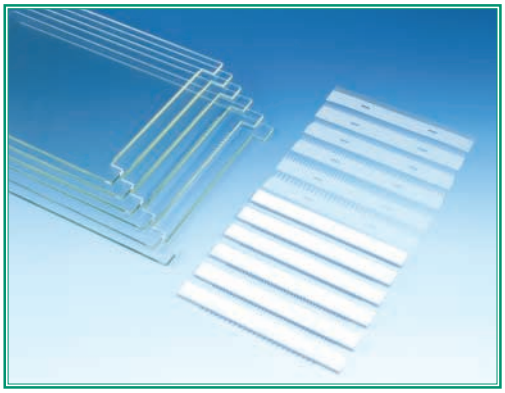
1. Epoxy-coated, anodized, and spring loaded aluminum heat dispersion plate
2. Removable upper and lower reservoirs for easy cleaning
3. Leak-proof silicone gasket on upper reservoir
4. Safety flip-lids with attached power cords
5. Safety-tip power leads
6. Bar clamp
7. 4 molded white spring clamps
8. Leveling base and bubble level
9. Buffer drainage valve
10. Notched microtrough low-fluorescent borosilicate glass plate
11. Blank low-fluorescent borosilicate glass plate
12. 132 lane sharktooth comb and Mylar spacer set (0.4 mm thick)
13. Microtrough casting comb

When using a sharktooth comb a specially designed microtrough plug comb is an essential accessory and performs two functions:

1. It forms a flat interface at the top of the gel into which the sharktooth comb rests.
2. Each microtrough casting comb is equipped with small rubber plugs which occupy the microtrough grooves during gel casting to prevent unwanted accumulation of unpolymerized acrylamide.

Individual components for the Horizontal FDD Electrophoresis System sold separately

	Cat. No.	Price
Sharktooth Comb 132-well, 0.4 mm thick	SA102	$106
Spacer Set for 132-well system 1 cm wide, 0.4 mm thick, 28 cm long	SA103	$43
132-well Microtrough Low-Fluorescent Glass Plate Set 45 cm x 28 cm (W x H)	SA104	$389
Bar Clamp for Horizontal System 45 cm wide	SA105	$139
Microtrough Casting comb 0.4 mm x 132 wells	SA106	$82
132-well Microtrough Standard Sodalime Glass Plate Set (not included in System) 45 cm x 28 cm (W x H)	SA107	$203



Vertical FDD Electrophoresis System

Cat. No.: SA201 **Price: $1700**

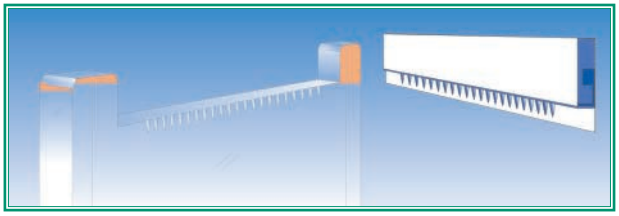
Our new Vertical FDD Gel Electrophoresis System is designed for fluorescent differential display (FDD). It has glass size measuring 25cm in width and 45 cm in length, and 66 lanes. This apparatus uses the patented microtrough system (pictured at right) in which the plates have grooves ground into the glass surface which simplifies and expedites loading of sequencing gels. Regular pipette tips can be easily placed into the desired groove and sample expelled without concern that sample will leak into adjacent well. Each groove corresponds to a sample application site of the sharktooth comb allowing the sample to be gently funneled into place. Hand position during loading is more stable and relaxed.

The total dimensions of the apparatus are 36 cm wide by 60 cm high by 23 cm deep.

Vertical Fluorescent DD Electrophoresis System

Components:

1. Epoxy-coated, anodized, and spring loaded aluminum heat dispersion plate
2. Removable upper and lower reservoirs for easy cleaning
3. Leak-proof silicone gasket on upper reservoir
4. Safety flip-lids with attached power cords
5. Safety-tip power leads
6. Bar clamp
7. 4 molded white spring clamps
8. Leveling base and bubble level
9. Buffer drainage valve
10. Notched microtrough low-fluorescent borosilicate glass plate
11. Blank low-fluorescent borosilicate glass plate
12. 66 lane sharktooth comb and Mylar spacer set (0.4 mm thick)
13. Microtrough casting comb

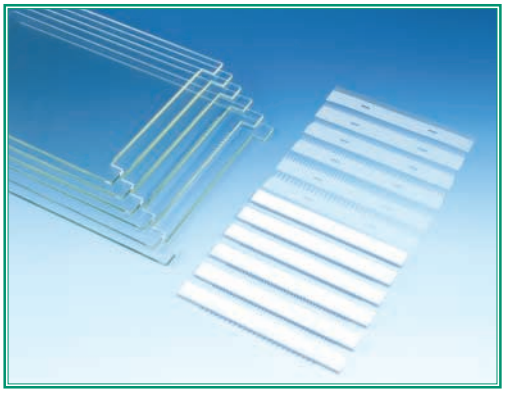


The Microtrough System: Grooved glass plates with a microtrough casting comb

See previous page for description on the microtrough system.

Individual components for the Vertical FDD Electrophoresis System sold separately

	Cat. No.	Price
Sharktooth Comb 66-well, 0.4 mm thick	SA202	$55
Spacer Set for 66-well system 1 cm wide, 0.4 mm thick, 45 cm long	SA203	$43
66-well Microtrough Low-Fluorescent Glass Plate Set 25 cm x 45 cm (W x H)	SA204	$357
Bar Clamp for Vertical System 25 cm wide	SA205	$135
Microtrough Casting comb 0.4 mm x 66 wells	SA206	$82
66-well Microtrough Standard Sodalime Glass Plate Set (not included in System) 25 cm x 45 cm (W x H)	SA207	$175



EasyTransfer™ Replica Plating Device

CAT. NO.: R702 **PRICE: $176**

GenHunter's EasyTransfer™ Replica-plating device is ideal for efficient and uniform colony transfer for colony hybridization during reverse Northern screening of positives in differential display. This item features a locking ring which secures sterile velveteen squares onto the black cylinder base prior to replica plating. Twelve re-usable velveteen squares are included.

Diameter of top: 80cm
Base Material: PVC
Velveteen Square Size: 152 x 152mm, 6 x 6 inches

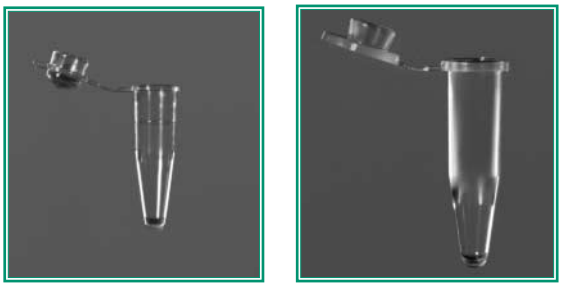
	QTY.	CAT. NO.	PRICE
Additional Velveteen Squares	12	R703	$65

Thin-walled PCR Reaction Tubes

Differential Display is particularly sensitive to variations in the type and thickness of reaction tubes. (Chen et al. *BioTechniques*. 1994. 16:1003-1006.). This may be one of the most common causes for false positives in differential display analysis.

GenHunter polypropylene reaction tubes feature thin-walls to ensure uniform and efficient heat transfer during thermal cycling. This ensures the excellent reproducibility and sensitivity of differential display. The following tubes have been carefully tested and can be used successfully for differential display.

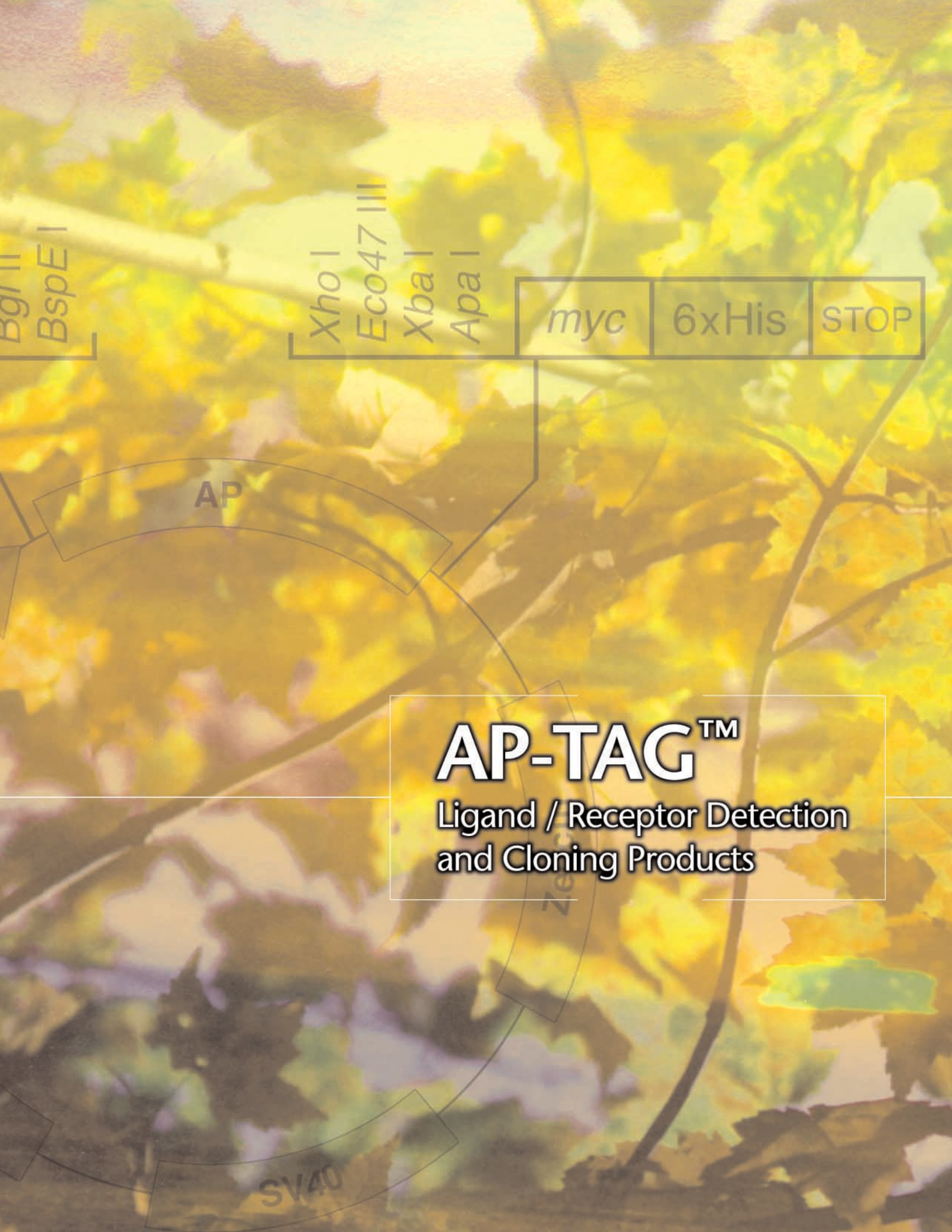
Our tubes are free of DNase, RNase, DNA, PCR inbitors, & pyrogens.



0.2 mL 0.5 mL

Thin-walled PCR Reaction Tubes

	QTY.	CAT. NO.	PRICE
0.2 mL thin-walled micro reaction tubes with cap	1000/bag	T101	$86
0.5 mL thin-walled micro reaction tubes with cap	1000/bag	T102	$77



AP-TAG™

Ligand / Receptor Detection and Cloning Products

AP-TAG® Introduction

AP-TAG® technology (US patents 5,554,499 and 5,801,000; ref. 1), invented by Drs. J. Flanagan and P. Leder at Harvard Medical School, has revolutionized the way cell surface receptors and ligands are detected and cloned. GenHunter is proud to be the exclusive licensee of this powerful method. Purchase of an AP-TAG® Kit or any pAPtag vector comes with a limited, single-user and non-transferable sublicense for use in research applications only. No part of the kit or pAPtag plasmid vectors shall be disseminated, propagated or distributed outside the user's own laboratory without written permission from GenHunter. A separate license is required for drug screening or other commercial applications. Contact GenHunter for details.

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 AP fusion protein, designated as an AP-body™, 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 antibody against AP. The ligand-AP or soluble receptor-AP fusion proteins thus can serve as affinity agents much like antibodies, which allow the most convenient, safe, and sensitive detection and cloning of their corresponding cell surface receptors or ligands. Unlike the conventional radioactive ^{125}I labeling method, AP-TAG® is safe and does not require ligand/soluble receptor purification.

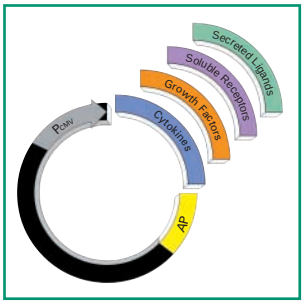
Since its invention, many important cell surface receptors and ligands have been cloned by AP-TAG™ technology including receptors for **Leptin**, **Semaphorin III**, **Nogo-66**, **IL-24**, **Jelly Belly**, and ligands for **Kit**, **Mek4** and **Sek receptor tyrosine kinases** (see references on page 46). A more extensive list of publications using AP-TAG® technology can be found on page 59 or on our website.

GenHunter was extremely pleased to be able to add this innovative method into our 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 or other methods, AP-TAG® technology may allow you to functionally characterize these genes further.

Comparison of AP-TAG® technology and the conventional radioactive ^{125}I ligand-labeling method:

	AP-TAG®	^{125}I labeling
Ligand purification	Not required	Required
Labeling Reaction	Not required	Required
Hazardous	No	Yes
Detection	Colorimetric	Scintillation counting
Sensitivity	High	High
Cell Staining	Yes	No
Expression Cloning	Yes	Yes
Ligand-Receptor Binding Kinetics/Affinity	Yes	Yes

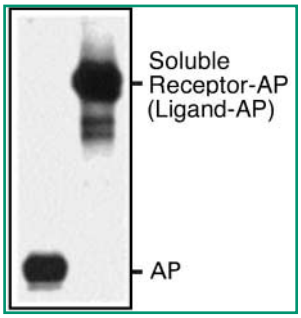
Schematic Illustration of AP-TAG® technology and its major applications



AP fusion construct

Create an in-frame fusion of your cDNA encoding a secreted ligand or soluble receptor with either the N- or C-terminus of secreted alkaline phosphatase (AP) in pAPtag expression vectors.

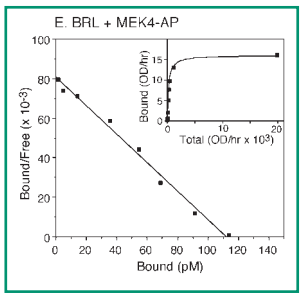
GenHunter Products: AP-TAG® Kit A
AP-TAG® Kit B



AP fusion protein expression

After transfecting the above AP fusion plasmid construct into 293T or NIH 3T3 cells, the expression of the secreted AP fusion protein (AP-body™) can be measured by either colorimetric AP activity assay or immunoblotting (or IP) with antibody to AP.

GenHunter Products: 293T Cells
AP Antibody (Polyclonal and Monoclonal)
AP Assay Reagent A
Monoclonal AP Antibody Sepharose Beads



Receptor/ligand binding assay

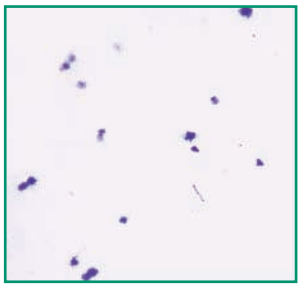
The culture medium containing the secreted AP fusion protein can be used directly to measure the presence or absence of a cell surface receptor (or ligand) of interest by assaying the AP activity bound to the cells. The secreted AP alone is used as a negative control.

GenHunter Products: AP Assay Reagent A
293T/pAPtag-4 stable cell line
AP control AP-body™

in situ staining of receptor/ligand

The secreted AP fusion protein can be used much like an antibody to detect the tissue distribution of a cell surface receptor/ligand of interest. An expression cDNA library thus can be made with mRNA isolated from tissues that express the highest level of the receptor/ligand for subsequent expression cloning.

GenHunter Products: AP Assay Reagent S



Expression cloning of receptor/ligand

The secreted AP fusion protein can be used as a probe to clone a cell surface receptor or ligand of interest by traditional expression cloning strategy (panning).

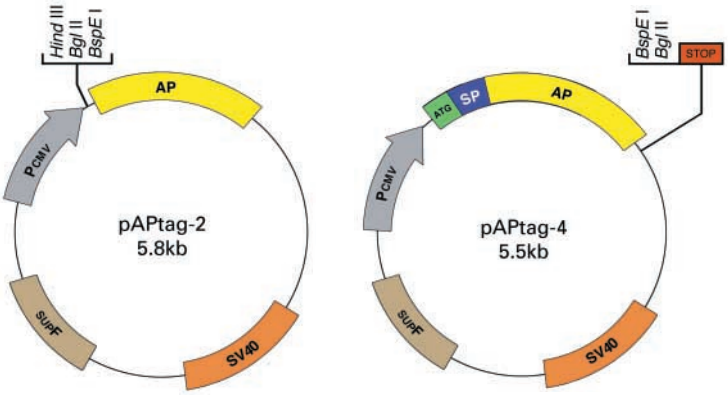
GenHunter Products: Expression Cloning Kit
AP Assay Reagent S
Kit-AP AP-body™
Kit Ligand Positive Control Vector
pMT21 Expression Vector

AP-TAG® Kit A

For Academic/Non-Profit: Cat. No.: Q201 Price: $980
For Industry: Cat. No.: Q201P Price: $3400

For non-radioactive detection of receptor/ligand interaction

This is the second generation of AP-TAG® technology. A secreted ligand or soluble receptor can be fused with secreted alkaline phosphatase (AP) at either its N- or C-terminus to produce an "AP-body™". The resulting AP fusion protein can be expressed as a secreted protein and used directly as highly sensitive affinity agents much like an antibody.



Features	pAPtag-2	pAPtag-4
Size (kb)	5.8	5.5
AP fusion Type	Ligand-AP (Receptor-AP)	AP-Ligand (AP-Receptor)
Cloning Sites	*Hind* III, *Bgl* II, *BspE* I	*Bgl* II, *BspE* I
Promoter	CMV	CMV
SV40 Ori.	Yes	Yes
E. coli Host	GH2/P3	GH2/P3
Vector selection	Tet/Amp	Tet/Amp
Secretion Signal	From insert	From AP
AP negative Control (secretes AP alone)	No	Yes

AP-TAG® Kit A

1. pAPtag-2 (10 µg)	40 µL
2. pAPtag-4 (10 µg)	40 µL
3. L-AP Primer (2 µM)	100 µL
4. R-AP Primer (2 µM)	100 µL
5. Colony lysis buffer	2 X 1 mL

NOTE: pAPtag-2 and pAPtag-4 plasmid vectors can only be transformed or propagated in E. coli host cells with P3 episome such as **GH2/P3 Supercompetent cells (Cat. No. T601)**.

The L(left)- and R(right)-AP primers flanking the cloning sites of pAPtag-2 are used in PCR to check for the presence and size of DNA insert cloned into the vector and for sequence verification of the Ligand-AP or soluble receptor-AP fusion constructs. The L-AP4 and R-AP4 primers (not included in kit) flank the cloning sites of pAPtag-4 and can be purchased separately (see below).

This kit is shipped on dry ice via overnight delivery.
A detailed step-by-step protocol is included.

References:

1. Flanagan, J. G. and Leder, P. (1990). The kit ligand: A cell surface molecule altered in steel mutant fibroblasts. ***Cell*** 63, 185-194.
2. Cheng, H.J., and Flanagan, J.G. (1994). Identification and cloning of ELF-1, a developmentally expressed ligand for Mek4 and Sek receptor tyrosine kinases. ***Cell*** 79, 157-168.
3. Tartaglia, L.A. et al. (1995). Identification and expression cloning of a leptin receptor, OB-R. ***Cell*** 83, 1263-1271.
4. He, Z. and Tessier-Lavigne, M. (1997). Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. ***Cell*** 90, 739-751.
5. Flanagan, J.G., et al. (2000). Alkaline phosphatase fusions of ligands or receptors as *in situ* probes for staining of cells, tissues and embryos. ***Methods in Enzymology*** 327, 17-35.
6. Flanagan, J.G., and Cheng, H.-J. (2000). Alkaline phosphatase fusion proteins for molecular characterization and cloning of ligands and receptors. ***Methods in Enzymology*** 327, 198-210.
7. US patents 5,554,499 and 5,801,000.

See page 59 for an extensive list of AP-TAG® References.

Individual components for the AP-TAG® Kit A sold separately:

Description	Volume	Cat. No. Academic	Cat. No. Industry	Price Academic	Price Industry
pAPtag-2 (10 µg)	40 µL	QV2	QV2P	$960	$3390
pAPtag-4 (10 µg)	40 µL	QV4	QV4P	$960	$3390
L-AP Primer (2 µM)	100 µL	Q210	Q210	$55	$55
R-AP Primer (2 µM)	100 µL	Q211	Q211	$55	$55
L-AP4 Primer (2 µM)	100 µL	Q213	Q213	$55	$55
R-AP4 Primer (2 µM)	100 µL	Q214	Q214	$55	$55
Colony Lysis Buffer	5 mL	L102	L102	$59	$59

AP-TAG® Kit B

For Academic/Non-Profit: Cat. No.: Q202 Price: $980
For Industry: Cat. No.: Q202P Price: $3400

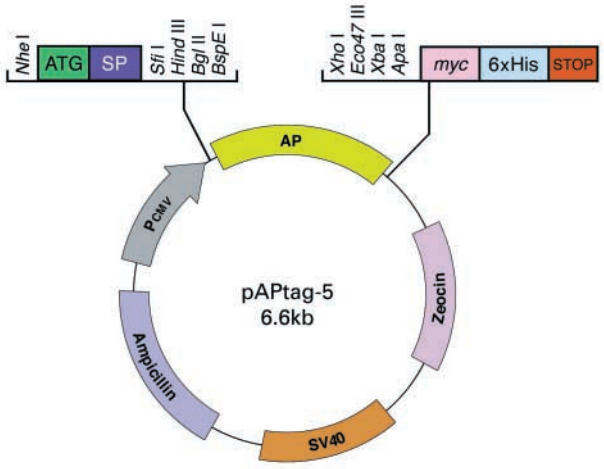
For non-radioactive detection of receptor/ligand interaction

This single vector system is the third generation AP-TAG® technology. A secreted ligand or soluble receptor can be fused with secreted alkaline phosphatase (AP) at either its N- or C-terminus to produce an "AP-body™". The resulting AP fusion protein can be expressed as a secreted protein and used directly as highly sensitive affinity agents much like an antibody. The epitope tags (6xHis and *myc*) allow easy purification, detection and interaction assays (IP) of the AP-fusion proteins. Other improved features are listed below.

Features	pAPtag-5
Size (kb)	6.6
AP fusion Type	Ligand-AP or AP-Ligand (Receptor-AP or AP-Receptor)
Cloning Sites	*Sfi* I, *Hind* III, *Bgl* II, *BspE* I or *Xho* I, *Eco47* III, *Xba* I *Apa* I
Promoter	CMV
SV40 Ori.	Yes
E. coli Host	GH (no need for P3)
Vector Selection	Ampicillin
Secretion Signal	From vector or insert
AP negative Control (secretes AP alone)	Yes
Affinity Tags	6xHis and *myc*
Transfection marker	Zeocin (Invitrogen)

AP-TAG® Kit B

1. pAPtag-5 (20 µg)	40 µL
2. L-AP5 Primer (2 µM)	100 µL
3. R-AP Primer (2 µM)	100 µL
4. Colony lysis buffer	2 X 1 mL



NOTE: pAPtag-5 plasmid vector can be transformed or propagated in **GH Competent cells (Cat. No. L301)**.

The L(left)-AP5 and R(right)-AP primers flank the N-terminal cloning site of pAPtag-5 and are used to check for the presence and size of DNA insert cloned into the vector and for sequence verification of the Ligand-AP or soluble receptor-AP fusion constructs (N-terminal of AP only). For C-terminal cloning, the L-AP5C and R-AP5C primers (not included in the kit) can be used (see below).

This kit is shipped on dry ice via overnight delivery.
A detailed step-by-step protocol is included.

References:

1. Flanagan, J.G., et al. (2000). Alkaline phosphatase fusions of ligands or receptors as *in situ* probes for staining of cells, tissues and embryos. ***Methods in Enzymology*** 327, 17-35.
2. Flanagan, J.G., and Cheng, H.-J. (2000). Alkaline phosphatase fusion proteins for molecular characterization and cloning of ligands and receptors. ***Methods in Enzymology*** 327, 198-210.
3. US patents 5,554,499 and 5,801,000.

See page 59 for an extensive list of AP-TAG® References.

Individual components for the AP-TAG® Kit B sold separately:

Description	Volume	Cat. No. Academic	Cat. No. Industry	Price Academic	Price Industry
pAPtag-5 (20 µg)	40 µL	QV5	QV5P	$960	$3390
L-AP5 Primer (2 µM)	100 µL	Q212	Q212	$55	$55
R-AP Primer (2 µM)	100 µL	Q211	Q211	$55	$55
L-AP5C Primer (2 µM)	100 µL	Q215	Q215	$55	$55
R-AP5C Primer (2 µM)	100 µL	Q216	Q216	$55	$55
Colony Lysis Buffer	5 mL	L102	L102	$59	$59

GH2/P3 Supercompetent Cells

Cat. No.: T601 **Price: $210**

For transformation of pAPtag-2 and pAPtag-4 vectors

The pAPtag-2 and pAPtag-4 AP-fusion cloning vectors contain the supF gene which confers both ampicillin and tetracycline resistance when transformed into the GH2/P3 Supercompetent Cells. pAPtag-2 and pAPtag-4 vectors will not confer antibiotic resistance in an *E. coli* host which does not contain the P3 episome. A tube of 1000X AT antibiotics mix is included for your convenience to prepare ampicillin (25 µg/mL) and tetracycline (10 µg/mL) plates for 1 L of LB-agar.

Detailed protocol included.

GH2/P3 Supercompetent Cells

1. GH2/P3 Supercompetent Cells	5 x 0.4 mL
2. 1000X AT Antibiotics Mix (Amp and Tet)	1 mL

AT Antibiotics Mix (1000X)

Cat. No.: Q601 **Size: 1 mL** **Price: $43**

For selection of pAPtag-2 and pAPtag-4 plasmids

Each tube of AT antibiotics mix is conveniently packaged to prepare ampicillin (25 µg/mL) and tetracycline (10 µg/mL) plates for 1 L of LB-agar.

GH Competent Cells

Cat. No.: L301 **Size: 6 x 0.5 mL** **Price: $210**

For transformation of pAPtag-5 vector

The pAPtag-5 AP fusion cloning vector contains the ampicillin resistance gene. It can be easily and efficiently transformed and propagated in GH Competent cells.

293T/pAPtag-4 Stable Cell Line

Cat. No.: Q402 **Size: 5 x 10^6 Cells / Vial** **Price: $309**

For production of high levels of AP alone

The 293T/pAPtag-4 stable cell line is used to produce high levels of secreted human placental alkaline phosphatase (AP) which can be used as a negative control for a ligand-AP or soluble receptor-AP fusion protein in cell surface binding assays or cell staining. High level production of secreted AP can be achieved with sub-confluent to confluent culture a few days after medium change. The secretion of AP can be monitored easily with the culture medium by AP activity assay using GenHunter AP Assay Reagent A (Cat. No. Q501).

Detailed protocol included.

293T Cells

Cat. No.: Q401 **Size: 5 x 10^6 Cells / Vial** **Price: $236**

For transfection with pAPtag vectors

293T is a human embryonic kidney (HEK) cell line commonly used for transfection assays. Due to the expression of the large T antigen in the cells, plasmids with SV40 origin of replication (such as pAPtag-2, pAPtag-4, and pAPtag-5) can be transiently transfected and give extremely high levels of expression of AP fusion proteins (e.g. ligand-AP fusion proteins). Thus, we strongly recommend using this cell line for your production of AP fusion proteins with pAPtag vectors. The fusion proteins can be easily monitored 2-3 days after transfection by alkaline phosphatase assay (See our AP Assay Reagent A, Cat. No. Q501) or by Western blot using AP Antibody. But for long term production of AP fusion proteins, we recommend that a stable cell line be cloned by co-transfecting with a puromycin or hygromycin-resistant plasmid (293T is G418 resistant). See below for information on the co-transfection vectors we offer. ***Detailed protocol included.***

293T-S Cells (for Serum Free)

Cat. No.: Q401-S **Size: 5 x 10^6 Cells / Vial** **Price: $318**

For transfection with pAPtag vectors for serum free production

The 293T-S cell line is a clone of the same HEK 293T cell line (above), but it has already been adapted for use in serum-free (SFM) production. They can be used with the pAPtag-2, pAPtag-4, and pAPtag-5 vectors as well. Just like the standard 293T cells, the fusion proteins can be easily monitored after transfection by alkaline phosphatase assay or by Western blot using AP Antibody. But for long term production of AP fusion proteins, we recommend that a stable cell line be cloned using a co-transfection vectors (see below). ***Detailed protocol included.***

GH-CHO (DHFR-) Cells

Cat. No.: Q420 **Size: 5 x 10^6 Cells / Vial** **Price: $247**

For transfection with DHFR vectors

GenHunter's Chinese Hamster Ovary (CHO) cells are dihydrofolate reductase deficient (DHFR-) and allow for high-level expression of recombinant proteins in suspension or attached cultures. The cell line is developed for rapid growth and ease for adaptation in serum free culture. ***Detailed protocol included.***

DHFR Vectors

For cloning, expression, & gene amplification of recombinant proteins

The pAPtag-2-DHFR and pDHFR vectors allow for high-level expression of recombinant proteins in suspension or attached cultures using the GH-CHO (DHFR-) cells (Cat. No. Q420 - above).

Vector	Cat. No.	Price
pAPtag-2-DHFR vector	QVD2	$2600
pDHFR vector (DHFR expression cassette)	Q430	$1059

Co-transfection Vectors

For use as a selectable marker for transfection of cultured mammalian cells.

The pSV2-Hygro or pBabe-Hygro vectors confer hygromycin resistance and the pBabe-Puro vector confers puromycin resistance when co-transfected into cells.

Vector	Selectable Marker	Cat. No.	Volume	Price
pSV2-Hygro co-transfection vector	Hygromycin	Q455	stab	$163
pBabe-Hygro co-transfection vector	Hygromycin	Q455-B	stab	$163
pBabe-Puro co-transfection vector	Puromycin	Q456	10 µg	$163

AP Western Blot Kit

Cat. No.: Q310 **Price: $414**

For immunoblotting of AP fusion proteins

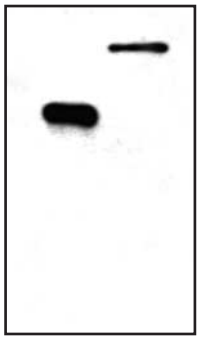
This kit contains the AP Antibody (rabbit polyclonal) which is specific to human placental secreted AP as well as two controls for AP Western blots. The antibody works optimally when used for Western blot analysis of secreted AP fusion proteins from culture media. It should be noted that although this antibody works extremely well for Western blot detection of AP fusion proteins, AP itself (with a MW of 67 KD) may not be detected directly from the culture media due to the amount of albumin which runs at a similar MW. Therefore, the purified AP from human placenta and a known soluble receptor AP fusion protein are provided as positive controls for the antibody. In addition, this antibody only recognizes the denatured form of AP. The Monoclonal AP Antibody (Cat. # Q320) can be used for applications where recognition of the native form is required.

Detection Limit: 20 mU of AP

AP Western Blot Kit

1. AP Antibody (human placenta) - Polyclonal From rabbit	100 µL
2. Purified AP (Western blot control) human placenta, 1 unit/mL	100 µL
3. Kit-AP fusion protein control media 1unit/mL	200 µL

This kit is shipped on dry ice via overnight delivery.
A detailed step-by-step protocol is included.



— **Kit-AP (150 KD)**

— **AP (67 KD)**

Western blot analysis of AP fusion proteins (antibody dilution 1:2000)

Individual components for the AP Western Blot Kit sold separately:

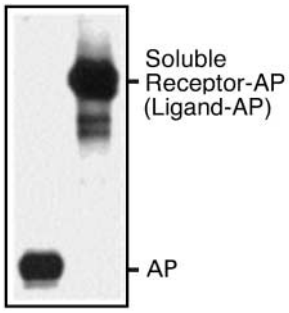
Description	Cat. No.	Volume	Price
AP Antibody (human placenta) - Polyclonal	Q301	100 µL	$295
Purified AP (Western blot control)	Q302	100 µL	$73
Kit-AP fusion protein media (positive control)	Q303	200 µL	$73

AP Antibody (human placenta) - Monoclonal

For ELISA Assay of Alkaline Phosphatase or IP (Immunoprecipitation)

This Monoclonal AP Antibody (human placental) is purified IgG 2a with a concentration of approximately 2.3 mg/mL. It is purified by DEAE chromatography and is in 15mM potassium phosphate buffer, 150mM sodium chloride, 0.1% sodium azide, pH 7.2. This antibody does not recognize denatured AP and therefore cannot be used for Western Blot directly.

	Cat. No.	Volume	Price
AP Antibody (human placenta) - Monoclonal	Q320	100 µg	$323
AP Antibody (human placenta) - Monoclonal	Q320-5	500 µg	$982
AP Antibody (human placenta) - Monoclonal	Q321	1 mg	$1596



IP with Monoclonal AP Antibody

Monoclonal AP Antibody-Sepharose Beads

For one-step purification of AP-fusion proteins and proteins interacting with AP-fusion proteins

Applications:

1) Concentrating AP fusion protein from the conditioned media
2) Purification of AP fusion proteins
3) Affinity column purification of protein(s) or molecules interacting with the ligand/receptor-AP fusion protein
4) IP Western analysis of the AP fusion proteins (see Polyclonal AP Antibody)

Technical parameters:

Coupling condition: 1 mg of pure IgG/mL of beads

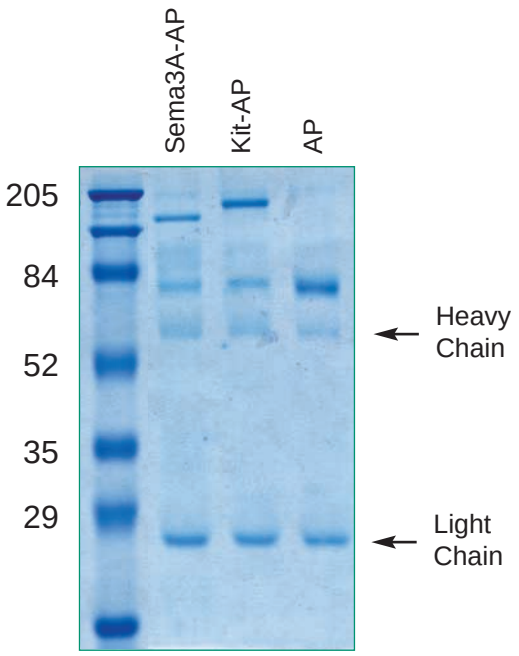
AP binding capacity: up to 200 Units/mL of beads

Specificity of the antibody: Native human placental AP

Interference of AP activity: The antibody binding does not interfere with the AP activity

Product Form:

The Monoclonal AP antibody-sepharose beads are supplied in a 50% suspension in 0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.4.



Coomassie blue stain of different secreted AP-fusion proteins purified with our Monoclonal AP Antibody-Sepharose Beads.

	Cat. No.	Total Volume	Price
AP Antibody-Sepharose Beads	Q330	100 µL	$323
AP Antibody-Sepharose Beads	Q331	500 µL	$1249
AP Antibody-Sepharose Beads	Q332	1 mL	$1828

Antigen Elution Solution

For eluting AP fusion protein from the Monoclonal AP Antibody-Sepharose Beads

The Antigen Elution Solution is specially formulated for eluting AP or AP fusion proteins from the monoclonal AP Antibody-sepharose beads. This solution breaks the extremely tight interaction between the antibody-antigen complex, allowing up to 80% recovery of the bound antigen.

It is available in both Acidic and Basic versions, depending on the pH sensitivity of your protein.

	Cat. No.	Volume	Price
Antigen Elution Solution (Acidic)	Q340A	10 mL	$45
Antigen Elution Solution (Acidic)	Q341A	50 mL	$111
Antigen Elution Solution (Basic)	Q340B	10 mL	$45
Antigen Elution Solution (Basic)	Q341B	50 mL	$111

Expression Cloning Kit

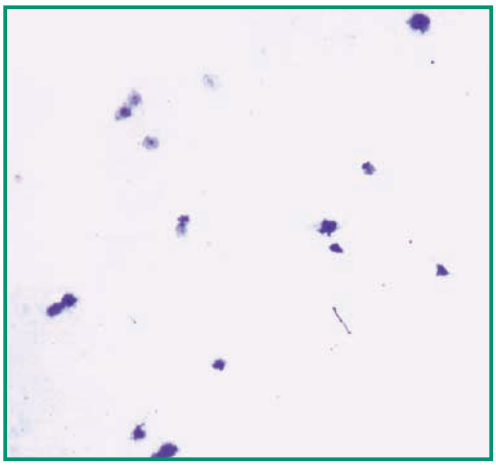
CAT. NO.: Q450 **PRICE: $433**

For expression cloning of cell surface receptor/ligand using AP fusion proteins (AP-bodies™)

This kit consists of a clonally purified cos-1 host cell line ideal for expression cloning by panning and a positive control receptor/ligand-AP pair.

An expression cDNA library potentially containing the receptor/ligand gene of interest can be transiently transfected into these cells. Positive cDNA pools can be identified by staining the transfected cells with your AP fusion protein. Cells over-expressing the corresponding cell surface receptor/ligand will be stained blue with GenHunter AP Activity Assay Reagent S (see figure below).

The Kit ligand positive control vector [containing a 1 kb cDNA encoding the transmembrane form of Kit ligand (stem cell factor), Ref. # 1 below] can be transfected into the cos-1 host cell line. Cells overexpressing cell surface Kit ligand will be stained blue by soluble receptor Kit-AP fusion protein using GenHunter AP Assay Reagent S.



Expression cloning of cell surface receptor/ligand by panning

Expression Cloning Kit

1. cos-1 Host Cell Line	1 x 10^6 cells / vial
2. Kit-AP fusion protein (media) 1 unit/mL	10 mL
3. Kit ligand (stem cell factor) positive control vector	10 µg

This kit is shipped on dry ice via overnight delivery.
A detailed step-by-step protocol is included.

References:

1. Flanagan, J.G. *et al*. (1991). Transmembrane Form of the *kit* Ligand Growth Factor is Determined by Alternative Splicing and is missing in the SI^d Mutant. ***Cell*** 64, 1025-1035.
2. Cheng, H.J., and Flanagan, J.G. (1994). Identification and cloning of ELF-1, a developmentally expressed ligand for Mek4 and Sek receptor tyrosine kinases. ***Cell*** 79, 157-168.
3. Tartaglia, L.A. *et al*. (1995). Identification and expression cloning of a leptin receptor, OB-R. ***Cell*** 83, 1263-1271.
4. He, Z. and Tessier-Lavigne, M. (1997). Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. ***Cell*** 90, 739-751.
5. Wang, M., Tan, Z., Zhang, R., Kotenko, S.V. and Liang, P.: Interleukin-24 (Mob-5/Mda-7) signals through two heterodimeric receptors, IL-22R1/IL-20R2 and IL-20R1/IL-20R2. ***J. Biol. Chem.*** 277, 7341-7347.

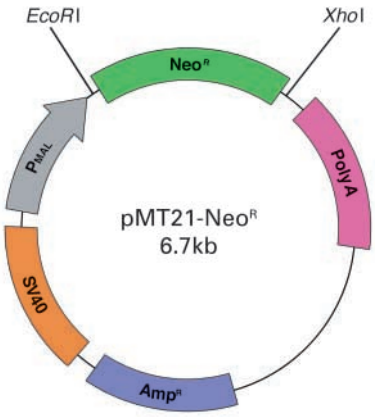
Individual components for the Expression Cloning Kit sold separately:

DESCRIPTION	CAT. NO.	VOLUME	PRICE
cos-1 Host Cell Line	Q451	1 x 10^6 cells/vial	$203
Kit-AP fusion protein (media), 1 unit/mL	Q452	10 mL	$107
Kit ligand (stem cell factor) positive control vector	Q453	10 µg	$201

pMT21-Neo Mammalian Expression Cloning Vector

CAT. NO.: Q454 **PRICE: $453**

For construction of expression cDNA libraries.



This cloning vector has been used extensively to construct mammalian expression cDNA libraries (see below references). The vector contains an SV40 origin of replication and the major adeno late promoter (P_{MAL}) in front of Neo resistance cDNA insert flanked by an *EcoR* I and *Xho* I site. Using the Stratagene cDNA Synthesis Kits generally results in cDNA ends with *EcoR* I and *Xho* I sites, which can be directionally cloned into the pMT21-Neo^R vector.

Amount:	20 µg
Cloning Sites:	*EcoR* I, *Xho* I (the excised Neo^R insert is 2kb)
Promoter:	Major Adeno Late Promoter (P_{MAL})
Antibiotic Resistance:	Ampicillin

References:

1. He, Z. and Tessier-Lavigne, M. (1997). Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. ***Cell*** 90, 739-751.

2. Kolodkin, A.L. *et al.* (1997). Neuropilin is a Semaphorin III receptor. ***Cell*** 90, 753-762.

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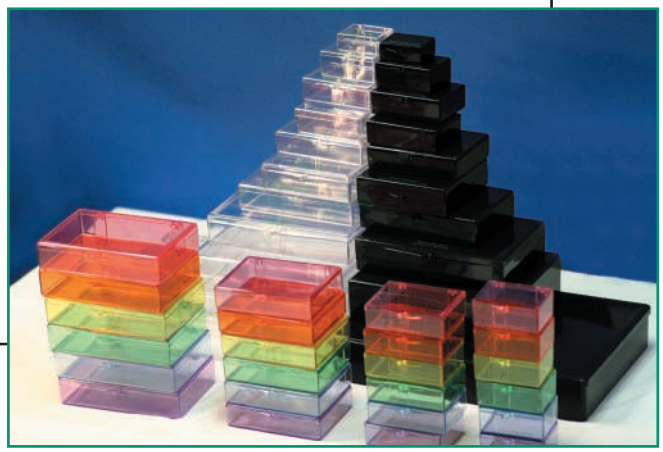
See page 56 for information on our PerfectWestern® Containers!!

These containers come in 37 different sizes to fit different size membranes. This allows you to save money on expensive antibodies because significantly less volume can be used.



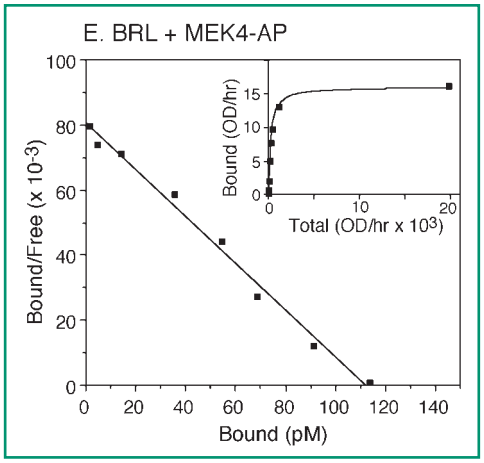
AP Assay Reagent A (For 200 Reactions)

Cat. No.: Q501 **Size: 10 mL** **Price: $128**

For AP Activity Assay

The AP assay reagent A is formulated specifically for measuring the enzymatic activity of the alkaline phosphatase (AP). The secretion of human placenta AP or a secreted ligand(receptor)-AP fusion protein can be easily monitored by this assay using the culture media in which the transfected cells are grown. The dephosphorylation of the p-Nitrophenyl phosphate by AP leads to the generation of yellow color which serves as both qualitative (by eye) and quantitative (Absorbance 405 nm) measurement of AP activity.

Detailed protocol included.



Receptor/Ligand Binding Assay using AP Assay Reagent A

Reference:

1. Flanagan, J. G. and Leder, P. (1990). The kit Ligand: A cell surface molecule altered in steel mutant fibroblasts. ***Cell*** 63, 185-194.

AP Assay Reagent S

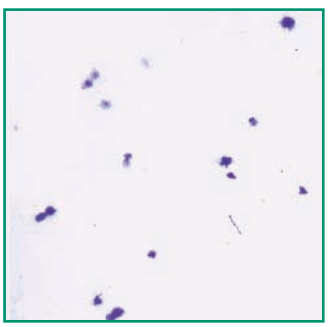
Cat. No.: Q502 **Size: 10 mL** **Price: $51**
Cat. No.: Q502L **Size: 100 mL** **Price: $359**

For Cell Staining

The AP Assay Reagent S is formulated specifically for cell staining or affinity blotting analysis of enzymatic activity of the alkaline phosphatase (AP). The AP substrate BCIP upon dephosphorylation forms an insoluble blue precipitate, thus it can be used for tissue or cell staining for the presence of receptors to which the ligand-AP fusion proteins bind. This assay kit can also be used during expression cloning of a receptor (Cheng and Flanagan, 1994, Cell 79:157-168).

Detailed protocol included.

***in situ* staining of receptor/ligand** (embryonic chick brain)



Expression cloning of receptor/ligand by panning

Reference:

1. Flanagan, J. G. and Leder, P. (1990). The kit Ligand: A cell surface molecule altered in steel mutant fibroblasts. ***Cell*** 63, 185-194.
2. Wang, M., Tan, Z., Zhang, R., Kotenko, S.V. and Liang, P. (2002). Interleukin-24 (Mob-5/Mda-7) signals through two heterodimeric receptors, IL-22R1/IL-20R2 and IL-20R1/IL-20R2. ***J. Biol. Chem.*** 277, 7341-7347.
3. He, M. and Liang, P. (2010) IL-24 Transgenic Mice: *in vivo* evidence of overlapping functions for IL-20, IL-22, and IL-24 in the epidermis. ***J. Immunol.*** 184, 1793-1798.

HBHA Wash Buffer

For Receptor Binding Assay

HBHA wash buffer consists of Hank's balanced salt solution with 0.5 mg/mL BSA and 20 mM HEPES, pH 7.0. This buffer has been used extensively for ligand-receptor binding assays.

Detailed protocol included.

	Cat. No.	Volume	Price
HBHA Wash Buffer	Q503S	100 mL	$56
HBHA Wash Buffer	Q503L	500 mL	$203

Reference:

1. Flanagan, J. G. and Leder, P. (1990). The kit Ligand: A cell surface molecule altered in steel mutant fibroblasts. ***Cell*** 63, 185-194.

Cell Lysis Buffer

Cat. No.: Q504 **Size: 100 mL** **Price: $56**

For Cell Lysis in Receptor Binding Assay

The cell lysis buffer is used in ligand-receptor binding assay. This buffer allows rapid lysis of the cells and removal of cell nuclei before bound AP activity is measured.

Detailed protocol included.

Reference:

1. Flanagan, J. G. and Leder, P. (1990). The kit Ligand: A cell surface molecule altered in steel mutant fibroblasts. ***Cell*** 63, 185-194.

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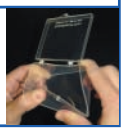
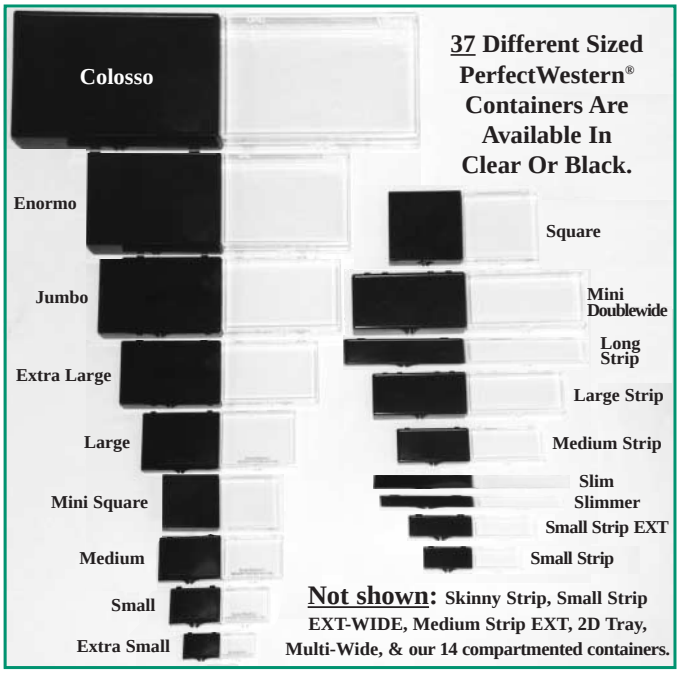


These specially designed containers perfectly accommodate Western blots of different size, including whole blots, half blots, and strips. They come in 37 different sizes in both clear and black plastic. Our PerfectWestern® containers significantly reduce the volume of expensive antibodies required while still producing beautiful Western blots!

These containers were conceived by our own frustration in searching for suitable containers for Western blots. The choices were often much too large and never flat enough, causing waste of antibody and concern about scratching the membrane. We decided to design our own containers offering many different sizes and options to truly achieve a PerfectWestern®. Our containers are made of durable polystyrene plastic, with ultra-smooth flat inner surfaces and lids to reduce evaporation. Their 90° edges create excellent membrane movement for more efficient incubation.

Most of the containers come in both clear and black versions. The clear container allows easy visualization of the blot, whereas the black version keeps light-sensitive reagents protected. In addition, 4 of our most popular sizes (Small, Medium, Large, and Extra Large) are available in 6 translucent colors: pink, orange, yellow, green, blue, and violet.

See next page for more information including dimensions, catalog numbers, and prices. **We highly recommend downloading or requesting the complete 2015 PerfectWestern brochure including a real-size layout of all 37 sizes at *www.PerfectWestern.com*.**



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Biometra (formerly from Invitrogen)		
Mini-V 8•10	Medium	B101
V15•17 Vert. Gel. Elect.	Jumbo/Enormo	B138/B111
V16 or V16-2	Jumbo/Enormo	B138/B111
Maxigel	Enormo/Colosso/2D Tray	B111/B140/B142
Bio-Rad Systems		
Mini-PROTEAN 3	Medium	B101
Criterion Precast	Extra Large	B109
PROTEAN II xi, 16cm	Jumbo/Enormo	B138/B111
PROTEAN II xi, 20cm	Enormo	B111
PROTEAN II XL, 20cm	Enormo/Colosso/2D Tray	B111/B140/B142
PROTEAN Plus	Colosso/2D Tray	B140/B142
GE Healthcare / Hoefer (formerly Amersham)		
Mighty Small SE250	Medium/Mini Square	B101/B144
Mighty Small SE260	Mini Square/Large	B144/B107
miniVE Vert. Gel Elect.(SE300)	Large/Square	B107/B119
SE600/SE400 (18 x 8)	Doublewide/Extra Large	B136/B109
SE600/SE400 (18 x 16)	Jumbo/Enormo	B138/B111
SE640 (18 x 8)	Mini Doublewide	B136
SE660 (24 x 18)	Enormo/Colosso/2D Tray	B111/B140/B142
Ettan Dalt II/six/twelve (2D gel)	Colosso/2D Tray	B140/B142
Multiphor II, Excel 2D 12.5	Multi-Wide	B148
Multiphor II, Excel XL 12-14	Colosso/2D Tray	B140/B142
Invitrogen / Novex		
Novex XCell SureLock Mini	Mini Square	B144
Novex XCell SureLock Midi	Extra Large	B109
E-PAGE Gels	Extra Large	B109
Lonza (formerly Cambrex/FMC/BMA)		
PAGEr Gold Precast (8.3 x 7.1)	Medium	B101
PAGEr Gold Precast (8.3 x 8.1)	Mini Square	B144
Owl Systems		
Puffin P81	Large/Square	B107/B119
Penguin P8DS	Large/Square	B107/B119
Penguin P9DS	Jumbo/Enormo	B138/B111
Penguin P10DS	Enormo/Colosso/2D Tray	B111/B140/B142

Important Notes: 1) All above catalog numbers are given for the clear version. If black or colored versions are desired, check on catalog number. **2)** The above recommendations are not guaranteed to be correct. Please check the exact size of gels from your apparatus and compare it to the PerfectWestern® container (inside dimensions are given) to be sure.

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  PVDF in 0.45 µm, 0.2 µm filter pore size, & fluorescent-optimized
  NC in standard 0.45 µm filter pore size
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- **Half the cost of competitors**

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hIzumo-AP	Unknown	Q707	vial	$453
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AP-Collagen (Human, C-terminal of alpha1, Type III)	Unknown	Q713	vial	$453
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AP-LRR2-hSlit1	Robo1 and Robo2 Receptors	Q715	vial	$453
AP-LRR2-hSlit2	Robo1 and Robo2 Receptors	Q716	vial	$453

Same receptors for both constructs

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International Symposium on Differential Display Technology

Organized by Dr. Peng Liang and Harold Robertson, the first Differential Display (DD) Symposium was successfully held in 1995 in Nova Scotia, Canada. The second DD Symposium was held in Cold Spring Harbor, New York in 1996 with a record turnout of over 400 scientists. The third symposium was held in 2002 in Nashville for the 10th anniversary of the invention of DD and had over 150 scientists.

1996 International DD Symposium, Cold Spring Harbor Laboratory

Resources & Technical Notes on

Differential Display

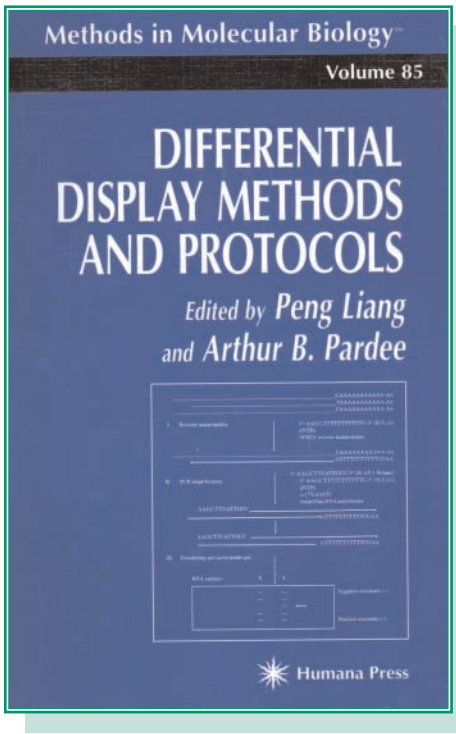
Differential Display Methods and Protocols, 1st edition CAT. NO.:HP1 PRICE: $103

Award-Winning Best Health Science Book 1997

In *Differential Display Methods and Protocols*, Drs. Peng Liang and Arthur B. Pardee have for the first time assembled 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 exactly 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 productive 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. Now scientists everywhere can successfully apply this powerful set of techniques using the step-by-step procedures of its innovators.

FEATURES



306 pp. 1997, Humana Press.
Edited by Peng Liang and Arthur B. Pardee
Best Health Science Book, 1997

- ★ **The first book dedicated to differential display technology**
- ★ **Contains numerous step-by-step protocols and improvements by experts in the field**
- ★ **Discusses major avoidable causes for false positives.**
- ★ **Includes applications from a wide range of biological systems from plants to animal brains.**
- ★ **Offers suggestions for the future development of the technology**
- ★ **Treats all aspects of differential display for identifying and cloning differentially expressed genes**
- ★ **Presents numerous examples of successfully identified differentially expressed genes**
- ★ **Truly a road map for all those who are interested in differential display technology**

Differential Display Methods and Protocols, 2nd edition CAT. NO.:HP2 PRICE: $103

Get the newest book dedicated to the most popular differential gene expression technology.*

Carrying on the high standards and building on the huge success of the first edition of Differential Display Methods and Protocols, Peng Liang et al. released a second edition of the book in October 2005. This edition includes a varying collection of 20 chapters highlighting both recent Differential Display (DD) methodological refinements (Chapters 1-8) and some of the most elegant examples of DD research applications (Chapters 9-20).

These well-versed authors explain and highlight all the latest DD refinements, including a new mathematical model, automation of fluorescent DD and digital data acquisition/analysis, capillary electrophoresis, a prototype computer program for automatic band identification, DD screening linking a band directly to a given gene, prokaryotic DD, and a technique combining Microarrays and DD.

The collection includes outstanding examples of applications of DD in the discovery of important genes involved in cancer, viral infection, prion disease, ovulation, circadian clock, floral color, transcription repression gene silencing, mRNA polymorphism, and protein-RNA interactions.

As with all "Methods in Molecular Biology" books, each author includes specific hints/notes to ensure success.

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* Based on search of publication numbers at ISI Web of Knowledge (*www.isinet.com*).

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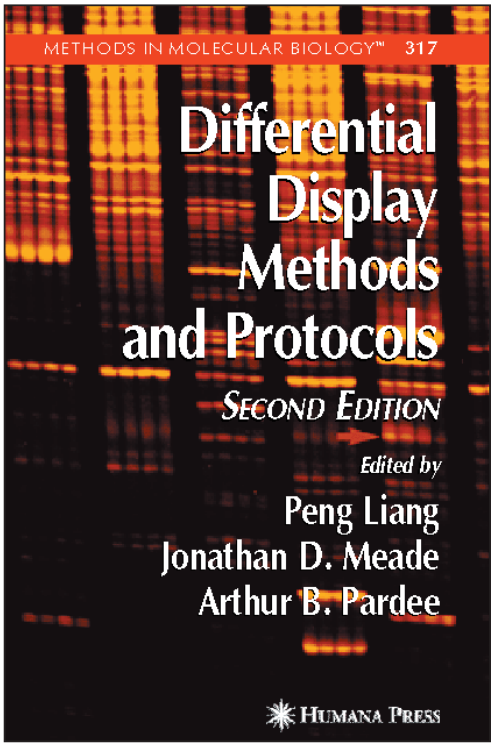

325 pp. 2005, Humana Press.
Edited by Peng Liang, Jonathan D. Meade, and Arthur B. Pardee

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- ★ **Discusses major avoidable causes for false positives.**
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Differential Display Technical Notes

Optimal arbitrary primer length for Differential Display

The concept of differential display is to use a limited number of short arbitrary primers in combination with the anchored oligo-dT primers to systematically amplify and visualize most of the mRNA in a cell. The optimal length of arbitrary primers is determined by statistical consideration that each primer will recognize 50-100 mRNA species. To do so, these primers have to hybridize as 6-8mers (Liang and Pardee, *Science*. 1992, 257:967.). In practice, however, primers shorter than 9 bases failed to be used for PCR amplification (Williams et al, 1991, *Nucleic Acids Res*. 18:6531), probably due to the minimum contact surface between Taq polymerase with the double-stranded DNA template. Initially, arbitrary decamers (10mers) were employed for differential display in combination with two-base anchored primers. Although the method worked well in displaying the expected number of mRNA per primer combination under optimal conditions, weak signals, fewer bands, and poorer reproducibility were encountered by people who might have assembled the method under sub-optimal conditions due to the variation in the quality of RNA samples, primers, MMLV reverse transcriptase, isotopes, Taq polymerase and PCR tubes among other things. It is important to point out that the analysis of priming kinetics of arbitrary 10mers under the PCR conditions used has been extremely predictable (Liang and Pardee, 1994, *Methods Mol. Gen.* 5:3). These primers, as expected, hybridize initially to the first strand cDNA as 6-9mers depending on the GC content, with most of the base-pairing occurred at the 3' end of the primers.

To determine the optimal length of arbitrary primers for differential display, we have kept in mind the following essential restrictions:

1) The theoretical consideration suggests that the optimal selectivity of the arbitrary primers should be provided by no more than 7 bases for each pair of primers to display 50-100 mRNAs.

2) The minimum primer length for PCR is 9 bases.

3) The arbitrary primers have to hybridize in a predictable way in order to rationally design a representative set of such primers to display most of mRNAs in a cell.

4) The arbitrary primers in practice require 8-9 base pair matches for optimal priming.

5) The redundancy (one mRNA species is represented as more than one band in each lane) should be kept to a minimum.

As a result, arbitrary primer length is increased from the sub-optimal value of 9-10 bases to 13 bases. The 13mers fulfill the above criteria for being optimal arbitrary primers (Liang et al., 1994, *Nucleic Acids Res.* 22:5763). First, 13mers are perfect PCR primers as compared to longer primers in terms of being able to specifically amplify a target DNA sequence. They are much more efficient primers than 10mers, producing highly reproducible and uniform band intensities in differential display. Secondly, since the first 6-8 3' bases of these short primers provide the specificity of sequence recognition of the mRNA sequences, the arbitrary 13mers can be rationally designed in such a

way that all primers are maximally different in their 7 3' base sequence (bases in bold, see table below), while the 6 5' bases are fixed and statistically can provide an additional 1.5 base pairing for each primer. So the arbitrary 13mers will hybridize as 8-9mers as required in the initial cycle of PCR amplification. Such rational primer design allows a limited number of arbitrary 13mers to be made to ensure that most of the mRNA can be represented (see figure next page). PCR conditions used for 10mers were preserved for 13mers to ensure the predictable nature of primer bindings. The table below shows the excellent agreement of the theory for arbitrary primer designs and the actual priming of the arbitrary 13mers in differential display based on the genes cloned.

Predictable Degeneracy of the Arbitrary 13mers in Differential Display

cDNA cloned	Arbitrary 13mer*	Mismatches (bp)
SP100	AAgCTt**GCACCAT**	2
Mob-1	AAgctt**CGACTGT**	4
Pai-2	aagCtt**GCACCAT**	5
Mob-5	AagctT**GATTGCC**	4
Osteopontin	aagctT**AGAGGCA**	5
Mob-40	aagcTT**TCATATG**	4
TK	aagCTT**GATTGCC**	3
No. of Mismatches	457443**0000000**	**4**

* The mismatches between the arbitrary primers and the target mRNAs amplified are denoted by lower cased bases.

Other companies have blindly increased the primers to 20 bases or longer in an attempt to increase the "reproducibility" of the cDNA pattern displayed. One has to remember that longer primers are used to amplify specific genes, while shorter primers are used to specifically amplify multiple genes. The longer primers under degenerate conditions will hybridize in a non-predictable way, making a rational design of a representative set of primers impossible. Although, longer primers can also generate a complex cDNA pattern which may appear reproducible, many of the bands may in fact represent the same mRNA due to the "Stickiness" of long primers when used under low stringency. In fact, A. Simpson and colleagues have used this low stringency PCR for long primers to detect sequence polymorphism of the same gene (Simpson et al., 1994, *PNAS*, 91:1946). To do this, they first specifically amplified a target gene of interest under high stringency conditions. Then, using the DNA sequence specifically amplified as a template and the same pair of long primers to perform PCR under low stringency (decreasing the annealing temperature), they were able to obtain a complex pattern of PCR products from a single template. Therefore the "more reproducible nature" of long primers may be misleading because of the redundancy of the PCR products amplified.

One-base versus two-base anchored primers

The initial choice of using two-base anchored oligo-dT primers (Liang and Pardee, 1992, Science 257:967; Liang et al., 1993, *Nucleic Acids Res.* 21:3269.) instead of one-base anchored primers (Liang et al., 1994, *Nucleic Acids Res.* 22:5763.) was due to historical rather than scientific reasons. The initial idea was to use one-base as an anchor, but the cloned murine thymidine kinase (TK) cDNA originally used as a control cDNA template had only 11 A's in its poly(A) tail. It was found that one-based anchored primer T_7C up to $T_{11}C$ failed to amplify the TK 3' terminus in combination with an upstream primer specific to TK. Extension of one more base from the 3' end instead of the 5' end of the anchored primer was a logical choice. Interestingly, $T_{11}CA$ started to work successfully in PCR to amplify the expected TK cDNA template. That was how two-base anchored primers were first described.

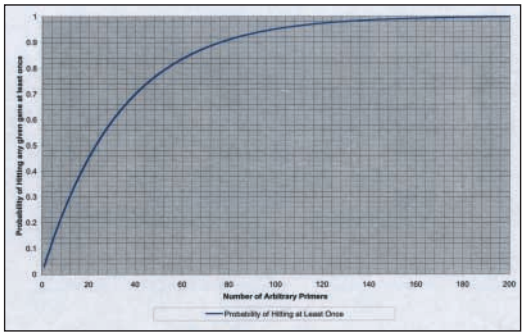
Later, longer one-base anchored primers were shown to be much more efficient for differential display in sub-dividing the mRNA populations into three groups. One-base anchored primers have significant advantages over the two-base anchored primers in that the former reduces the redundancy of priming, eliminates the high background smearing problem for two-base anchored primers ending with the 3' "T", and reduces the number of reverse transcription reactions from 12 to 3 per RNA sample.

Theoretical estimation of the number of arbitrary primers necessary to cover most of the genes expressed in a cell

Based on the experimental support that each arbitrary 13mer hybridizes with 7 bases to provide specificity, which represents 1/16,000 possible sequences, a theoretical estimation for the number of arbitrary primers needed to cover most of the expressed genes in a cell can be derived (Liang and Pardee, 1994, *Methods Mol. Gen.* 5:3). This is based on a given mRNA 3' end sequence of 600 bp resolved on a 6% denaturing polyacrylamide gel contains about 600 sites for recognition by all possible 16,000 7mers. Others have claimed that fewer primers may cover most of the genes expressed, but those calculations were seriously flawed.

Estimation of the number of arbitrary 13mers needed in combinations with all three one-base anchored primers to detect a given fraction of mRNA by differential display.

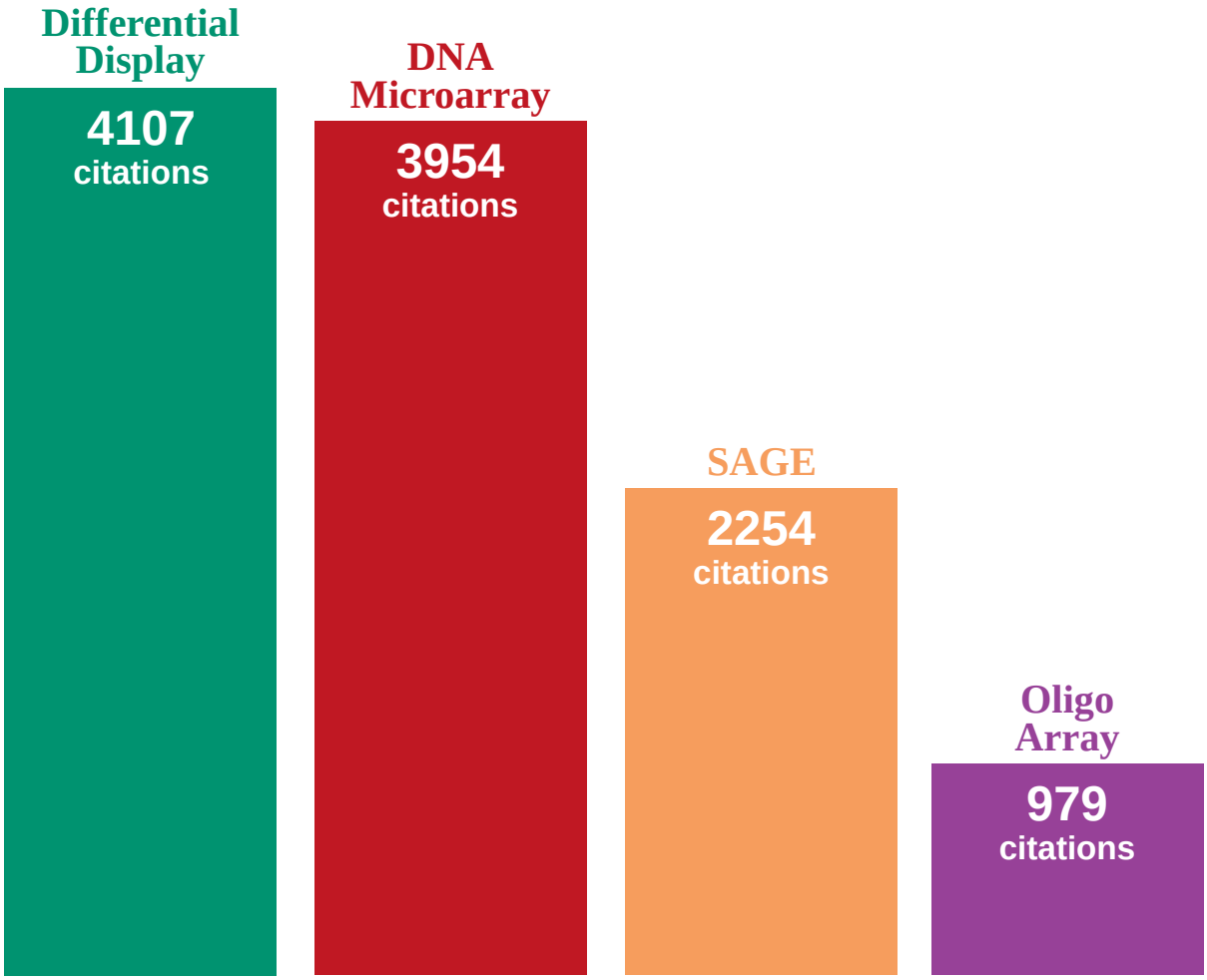
Number of arbitrary primers n	Reactions	Probability of Detection $P = 1 - (0.96)^n$
8	24	28%
16	48	48%
40	120	80%
80	240	96%



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 (See Figures below).

GenHunter is proud to be the driving force for making differential display accessible to the biomedical research community (see references, page 71).



Method	Year invented	Total	Pubs / Year	Original Reference
Differential Display	1992	4107	243	Science 1992, 257:967-971.
DNA Microarray	1995	3954	282	Science 1995, 270:467-470.
SAGE	1995	2254	161	Science 1995, 270:484-487.
Oligo Array	1996	979	75	Science 1996, 274:610-614.

*The Citation searches were performed **January 8, 2009** on ISI Web of Knowledge Citation Search (http://apps.isiknowledge.com). Number of Citations is the number of times the original publication has been cited by other papers, which reflects the number of times each technique has been used for publications.*

Fundamental Differences between DD and DNA Microarrays

Given the great publicity of DNA microarrays techniques in recent years, many researchers have put their blind trust into this methodology, only to be overwhelmed by the confusing data generated. Knowing the major differences between differential display and DNA microarrays, both in theory and in practice, may help you to find the genes of real interest and save your valuable resources and effort.

Both differential display (DD) and microarrays are conceptually simple, however, the two methods are principally different. The fundamental difference is that differential display visualizes the mRNAs in subsets directly without any data normalization after their amplification and labeling by either isotopes or fluorescent dyes. In contrast, DNA microarrays visualize the mRNAs indirectly after the hybridization of extremely complex mRNA probes as first-strand cDNAs with fluorescent labels to a set of cDNA templates spotted on a glass surface. In fact, a cDNA probe used for microarray can be so complex that it consists of as many as 10,000 different species ranging from only a few copies to thousands of copies per cell. Further compounding the problem in signal specificity has been the fact that eukaryotic genes often come in families with many conserved sequences among the family members.

Therefore, lack of sensitivity, nonspecific- and cross-hybridization are major problems for microarrays. Also with microarrays, one is going to be limited to the detection of whatever genes that are spotted on a slide, making it a "closed' system for gene discovery. In contrast, differential display is capable of detecting both known and novel genes with much higher gene coverage.

No matter which method is to be used, proper controls are absolutely critical before meaningful expression data can be obtained and analyzed. For example, cDNA pattern reproducibility is the key to reducing the rate of false positives for differential display. Likewise, labeling of each RNA sample being compared in microarrays with both red and green fluorescent dyes followed by reciprocal mixing them in color of fluorescence (e.g. RNA1-red/RNA2-green and RNA1-green/RNA2-red) before hybridization to duplicate slides is absolutely essential not only for data scoring, but also for data proof-reading. Although these important controls seem to be obvious for anyone serious about the quality of the microarray data, few published works actually have emphasized such controls.

One of the promising potentials of microarray is its capability of computerized data quantification and analysis. However, without knowing the actual quality of the data obtained, one could easily waste a lot of "clear" thinking on "dirty" data. The accuracy in gene expression analysis with microarrays is at best 3% or higher (3 out of 100 will be false positives), whereas GenHunter's automated FDD service is at least 10 time more accurate.

Such confusing massive data generated by microarray is starting to draw sharp criticisms:

- Brenner, Sydney: Sillycon valley fever. **Current Biology**. 1999, 9:R671.
- Liang, Peng.: Gene discovery using Differential Display. **Genetic Engineering News**. 2000, 20:37.
- Mir, Kalim. U.: The hypothesis is there is no hypothesis. **Trends in Genetics.** 2000, 16:63-64.
- Wooster, R.: Cancer classification with DNA microarrays, is less more? **Trends in Genetics.** 2000, 16:327-329.
- Gibbs, W. W.: Shrinking to enormity: DNA microarrays are reshaping basic biology - but scientists fear they may soon drown in the data. **Scientific American**. 2001, 284:33-34.
- Many more references critical of arrays can be found at: http://www.genhunter.com

Therefore, the choice is clear of whether you want to go to a "mine field" with hundreds of unconfirmed differences, or if you would like to find the few real "needles in the haystack".

Some of the major differences between differential display and microarrays are summarized in the following chart.

Comparison of Differential Display and DNA Microarrays

	Differential Display	DNA Microarray
Year Invented	1992	1995
No. of Publications* (Medline hits)	3965	3462
Publications/year	243	282
Detect novel genes	Yes	No
Compare more than 2 RNA samples	Yes	Mostly not
Signal detection	Directly (gel display)	Indirectly (by hybridization)
Total RNA required	5 µg / 240 PCR reactions	>20 µg / hybridization
Gene coverage#	~96% with 240 PCR reactions	~25% with a 10K gene "chip"
Sensitivity (Detecting rare mRNA)	High (PCR based)	Low-medium
Immediate knowledge of gene sequence	No	Yes
Cost	Low-medium	High
Do it yourself?	Yes	Mostly not
Commercial services	GenHunter	Many companies

Medline search was conducted in January 8, 2009 on ISI Web of Knowledge Citation Search *(http://apps.isiknowledge.com)*.

Method Enzymol., 254:304-320, 1995.

False positives in Differential Display

One of the most frequently asked questions for those who want to use differential display is what the false positive rate is. There is really no clear answer to this very complex question, because it depends on so many factors, both intrinsic and extrinsic, of the differential display method. The intrinsic factors include the quality of reagents and enzymes, type and purity of primers, integrity, concentration and purity of RNA used, etc. The extrinsic factors such as the systems being compared, experimental designs, appropriate internal controls, criteria for picking bands, reaction setup, type of PCR reaction tubes, type of thermal cyclers, and, of course, the training and experience of a researcher, all can greatly contribute to the rate of false positives.

Taking an extrinsic factor, the system being compared, as an example, if one is to compare rat liver versus rat brain where it is known that nearly 50% of the genes expressed are brain specific, the false positives are going to be very low since the difference between samples compared are very large. However, if one is to compare gene expression between the same type of cells with and without a 30 min. treatment of a drug or hormone in hopes of identifying the immediate early genes, the false positives are going to be much higher simply due to the fewer differences in gene expression. Differential display itself can be perfectly reproducible if there are no intrinsic and extrinsic problems. For example, if you set up a 100 µL PCR reaction that is thoroughly mixed and aliquot 20 µL into each of 5 thin-walled PCR reaction tubes, the pattern of cDNA displayed among the five tubes can be perfectly the same. But if instead, 5 individual reactions are set up independently, the cDNA pattern amplified may not be the same simply due to pipetting errors. However, in practice, there is always going to be background "noise" due to pipetting errors because you have to compare different samples. These few spurious bands or "noise" can be as low as 0.1-1% of the cDNA bands displayed, which can be translated to 10-100 bands if you perform all 240 PCR reactions consisting of all the primer combinations in GenHunter's RNAimage® or RNAspectra® kits. As you can see, given the system that you will be comparing, the false positive rate can be estimated simply as the signal (truly differentially expressed genes) to noise (spurious, non-reproducible bands) ratio. Unlike the comparison between liver and brain, even if you encounter much higher false positives but are able to find the few immediate early genes induced by your drug or hormone, it would be a great accomplishment.

The other often-ignored extrinsic factor is the appropriate internal controls. Assuming that you are looking for the immediate early genes induced by a drug or hormone, you must make sure that the treatment truly worked based on either the phenotype of the cells, or more importantly, the induction of a known gene as a control. If this is not done, the differential display comparison is going to be flawed, because there may simply be no difference to begin with if the treatment did not work. In this case, if any differences are found, they are bound to be false positives. For a more detailed review on factors contributing to false positives in differential display, please see Liang and Pardee, 1994. Methods in Mol. Gen. 5:3.; Liang and Pardee, ed., 1997. *Differential Display Methods and Protocols*, Humana Press (Cat. No.: HP1).

There is no doubt that Differential Display is a very dependable method given its enormous popularity and the success of hundreds of thousands of researchers, thanks in part to GenHunter's complete line of reagent systems and step-by-step protocols (see representative publications on facing page). Although you can count on GenHunter to solve the intrinsic problems of differential display, having a realistic understanding of the extrinsic problem areas will help you to minimize false positives and eliminate the fears and myths of differential display, one of the most powerful molecular biological tools ever invented.

Representative DD Reference List (Sorted by Fields of Application)

(Reference numbers underlined in green are from GenHunter® customers; numbers used are the same as in the chronological list)

Note: A more up-to-date version of this list can be found at: http://genhunter.com/support/references.html

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Note: A more up-to-date version of this list with hundreds of additional references can be found at:

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Frequently used conversion factors and other useful information

Spectrophotometric Conversions

Nucleic Acids	OD_{260}	µg
RNA	1	40
DNA (Single-stranded)	1	37
DNA (Double-stranded)	1	50

Dye Migration in Polyacrylamide Denaturing Gels

% Gel	Bromophenol Blue*	Xylene Cyanol*
5.0	35 bp	130 bp
6.0	26 bp	106 bp
8.0	19 bp	75 bp
10.0	12 bp	55 bp
20.0	8 bp	28 bp

* numbers represent the approximate size of DNA (in nucleotide pairs) with which the dyes would migrate.

Radioactivity Information and Half-Lives

Nuclide	Half-life	Emission	Energy, max (MeV)	Range of emission	Approx. specific activity at 100% enrichment	Atom resulting from decay
^{32}P	14.3 days	ß	1.71	610 cm (air)	285 Ci/mg	$^{33}_{16}S$
^{33}P	25.4 days	ß	0.249	49 cm (air)	156 Ci/mg	$^{33}_{16}S$
^{35}S	87.4 days	ß	0.167	24.4 cm (air)	43 Ci/mg	$^{35}_{17}Cl$

Metric Prefixes

M	=	mega	=	10^{6}
k	=	kilo	=	10^{3}
m	=	milli	=	10^{-3}
µ	=	micro	=	10^{-6}
n	=	nano	=	10^{-9}
p	=	pico	=	10^{-12}
f	=	femto	=	10^{-15}
a	=	atto	=	10^{-18}

Conversion Factors (DNA Na^{+})

1 kb dsDNA = 6.6 x 10^{5} Da
1 kb ssDNA = 3.3 x 10^{5} Da
1 kb ssRNA = 3.4 x 10^{5} Da
1 MDa dsDNA = 1.52 kb

Abbreviations

ds	double-stranded (as in dsDNA)
ss	single-stranded (as in ssDNA)
bp	basepair
kb	kilobase: 1000 bases or basepairs
Mb	megabase: 1,000,000 bp
Da	Dalton, the unit of molecular mass; kDa = 1000 Da MDa = 1,000,000 Da
M	molarity, moles of solute per liter of solution

Conversions

1 µg of 1kb DNA = 1.52 pmol
1 pmol of 1kb DNA = 0.66 µg
100 pmol of 100 kDa protein = 10 µg
1 kb of DNA = 333 amino acids
= 3.7 x 10^{4} dalton protein
10,000 dalton protein = 270 bp DNA
50,000 dalton protein = 1.35 kb DNA
100,000 dalton protein = 2.7 kb DNA
1 bp DNA = 37 dalton protein
1 amino acid = 111 dalton protein

Codon Usage Table

First Letter	Second Letter: U	C	A	G	Third Letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA STOP	UGA STOP	A
	UUG Leu	UCG Ser	UAG STOP	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Amino Acid Abbreviations and Molecular Weights

Amino Acid	Three-letter Abbrev.	One-letter Symbol	MW (g/mol)
Alanine	Ala	A	89.1
Arginine	Arg	R	174.2
Asparagine	Asn	N	132.1
Aspartate	Asp	D	133.1
Cysteine	Cys	C	121.2
Glutamate	Glu	E	147.1
Glutamine	Gln	Q	146.2
Glycine	Gly	G	75.1
Histidine	His	H	155.2
Isoleucine	Ile	I	131.2
Leucine	Leu	L	131.2
Lysine	Lys	K	146.2
Methionine	Met	M	149.2
Phenylalanine	Phe	F	165.2
Proline	Pro	P	115.1
Serine	Ser	S	105.1
Threonine	Thr	T	119.1
Tryptophan	Trp	W	204.2
Tyrosine	Tyr	Y	181.2
Valine	Val	V	117.1

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GenBank on line:	http://www.ncbi.nlm.nih.gov
EMBL:	http://www.ebi.ac.uk
SWISS-PROT:	http://www.expasy.ch/
DDBJ:	http://www.nig.ac.jp

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Isotopes	NEN	800-551-2121
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QIAquick® PCR purification kit	Qiagen	800-426-8157
Sequencing gel and buffers	National Diagnostics	800-526-3867
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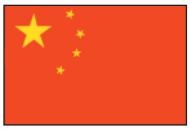

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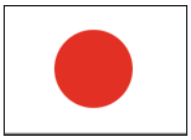

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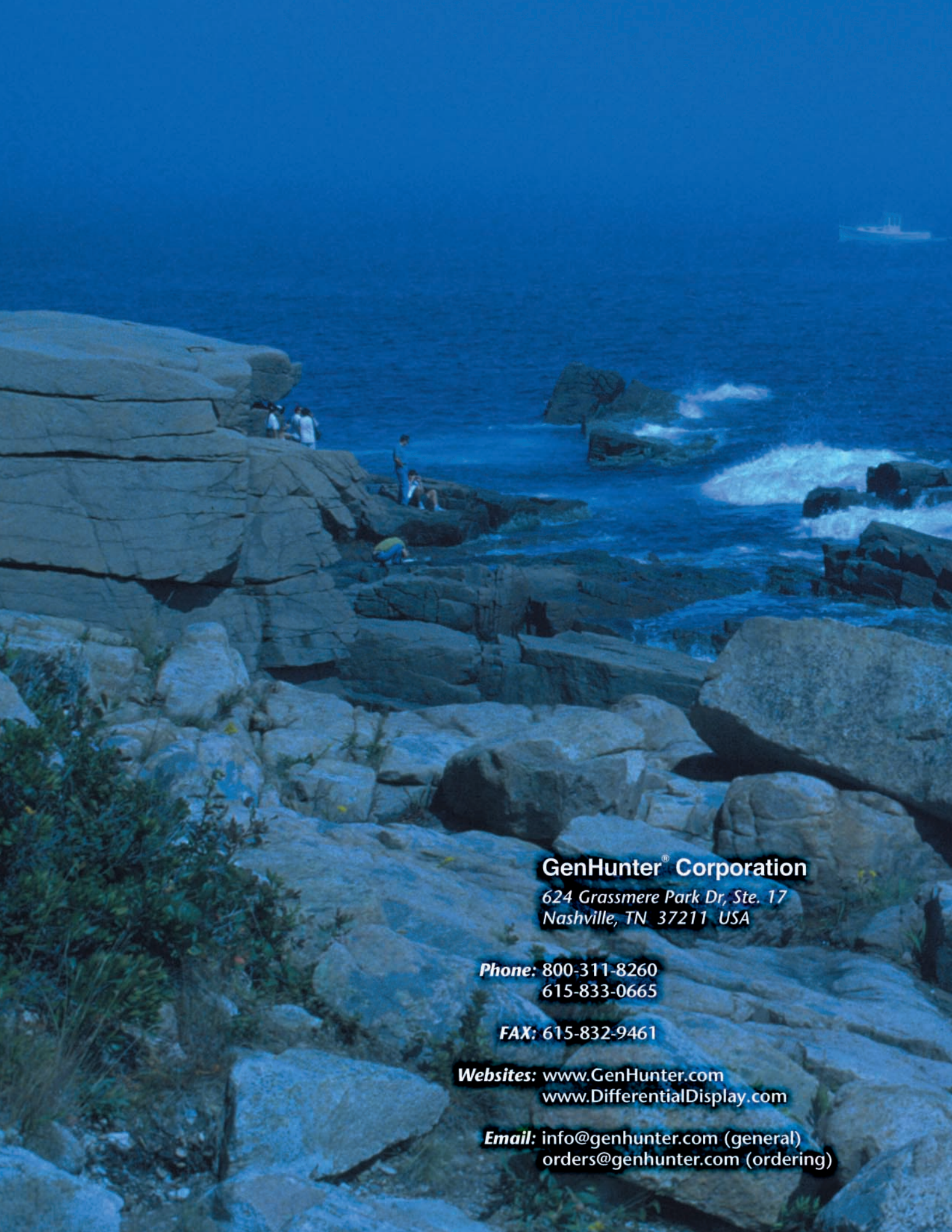
For all other countries not listed here, please contact GenHunter directly.



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