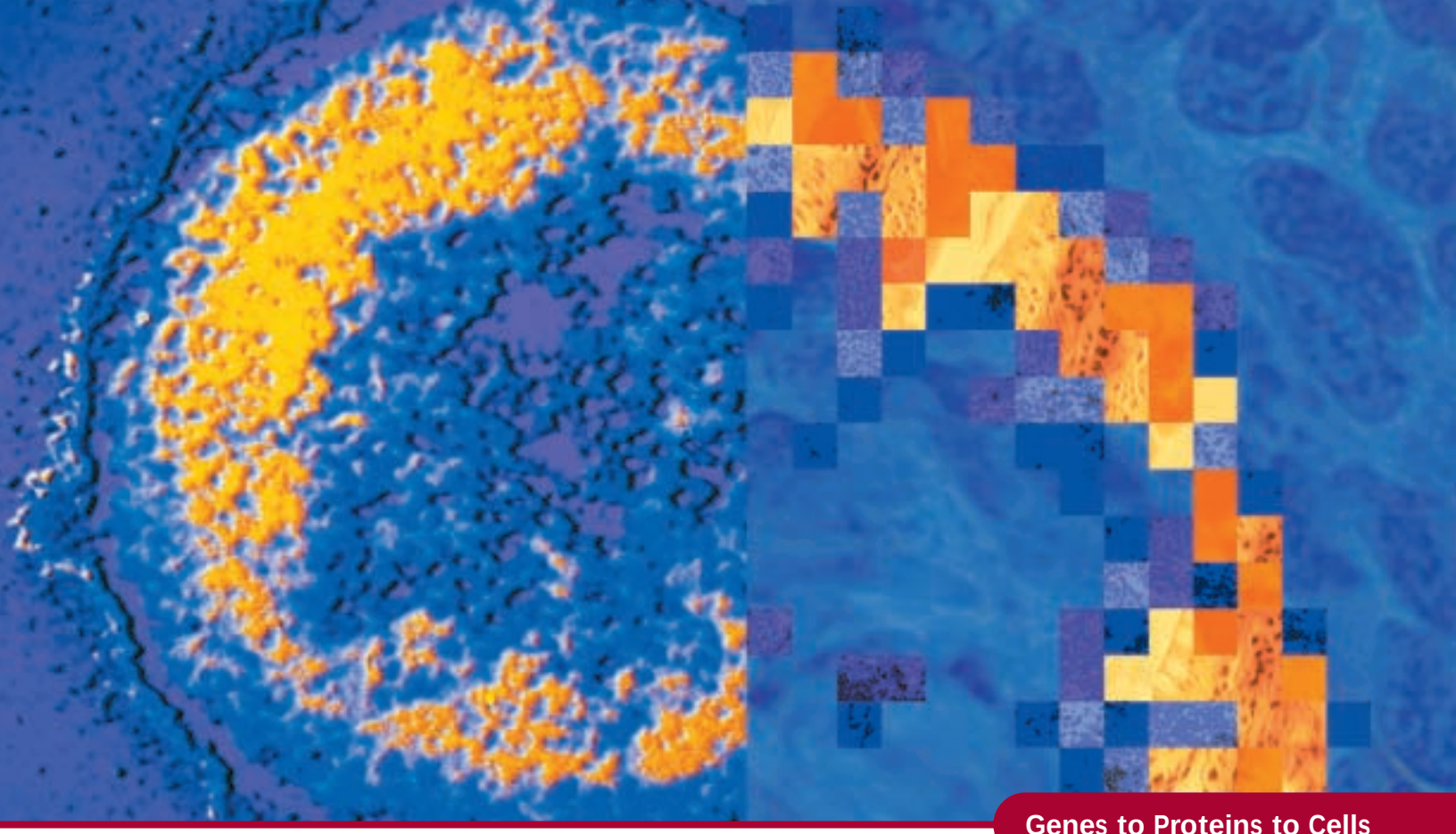
Clontechniques

July 2002

Highlights

BD Biosciences
Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen

BD



Genes to Proteins to Cells

BD Biosciences - Your Life Sciences Partner of Choice

BD Biosciences is a business segment of BD (Becton, Dickinson and Company) and is one of the largest businesses supporting the life sciences worldwide, providing biotechnology tools that leverage its expertise in molecular biology, cell biology, immunology, and cell analysis.

BD Biosciences Clontech is a leader in molecular biology, providing reagents and kits for genomics, functional genomics, and proteomics. Major products include BD Atlas™ Arrays, BD Living Colors™ Fluorescent Proteins, BD Creator™ Expression Systems, and BD Clontech™ Disease Profiling Arrays.

BD Biosciences Discovery Labware is a leader in engineered labware and cell growth and screening, providing products for drug discovery, ADME/Tox, cell and tissue culture, and fluid handling. Major products include BD Falcon™ Cultureware, BD BioCoat™ Assay Systems, and BD Gentest™ Reagents and Kits.

BD Biosciences Immunocytometry Systems is a leader in immunology and cell analysis, providing flow cytometry systems for immune function, vaccine research, infectious disease, and oncology. Major product brands include BD FACSCalibur™ System, BD TruCOUNT™ Tubes, and BD Oncomark™ Reagents.

BD Biosciences Pharmingen is a leader in research immunology, providing biomedical reagents and kits for cell biology, immunology, oncology, proteomics, and signal transduction. Major products include BD PowerBlot™ Screening Service, BD™ Cytometric Bead Array, BD Pharmingen™ Antibodies.

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BD

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IN THIS ISSUE

About the Cover

The cover shows an illustration designed for our BD Sprint™ technology. Illustration inspired by the art of Pablo Picasso (1882–1973).

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Trademarks

BD Sprint™ Advantage™ 96 Plate

Versatile PCR enzyme mix in a high-throughput format

- **96-well PCR in a fraction of the time**
- **High sensitivity, fidelity & yields for all high-throughput PCR applications**
- **Worry-free, room temperature storage—ideal for field applications**
- **Flexible format—plates separate into four sets of 24 wells**
- **Compatible with PCR blocks and robots from all major manufacturers**

Imagine that the acronym "PCR" was synonymous with "Performance, Convenience, and Reproducibility" in addition to "Polymerase Chain Reaction". Imagine also that you were able to simply take a prealiquoted 96-well plate off the shelf, add template and primers, and proceed directly to "PCR". Dispense with liquid-handling bottlenecks, excessive aliquoting, unending optimization, and proceed directly to *results*.

Use your imagination for more important things and leave the plate preparation to us. Introducing the revolutionary new **BD Sprint™ Advantage™ 96 Plate**—high-performance, high-throughput PCR in a lyophilized 96-well format. Each well of the BD Sprint Advantage 96 Plate contains a complete lyophilized master mix of BD TITANIUM™ *Taq*, BD TaqStart™ Antibody, a proofreading enzyme for increased fidelity, dNTPs and an optimized PCR buffer. To use, simply dissolve the mixture using your diluted primers and DNA and go directly to PCR (Figures 1 & 3).

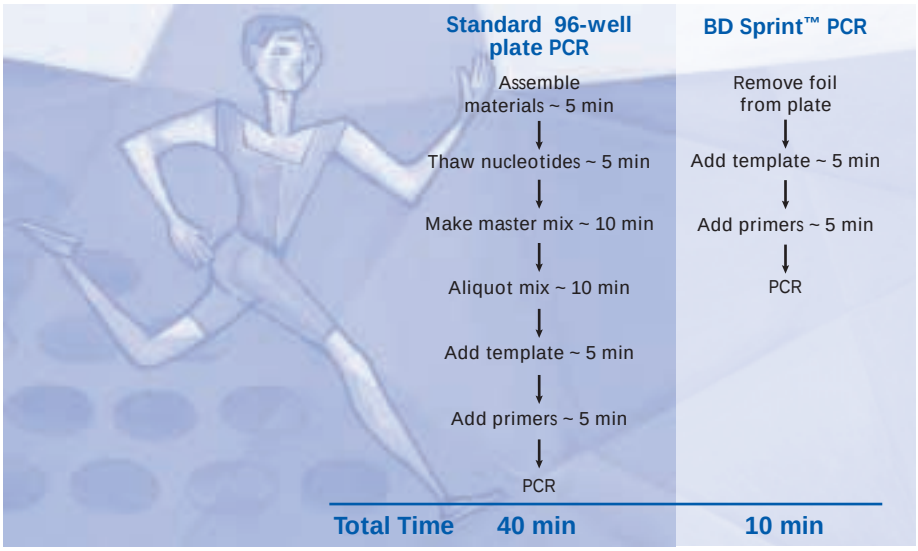


Figure 1. BD Sprint™ technology speeds up PCR preparation.

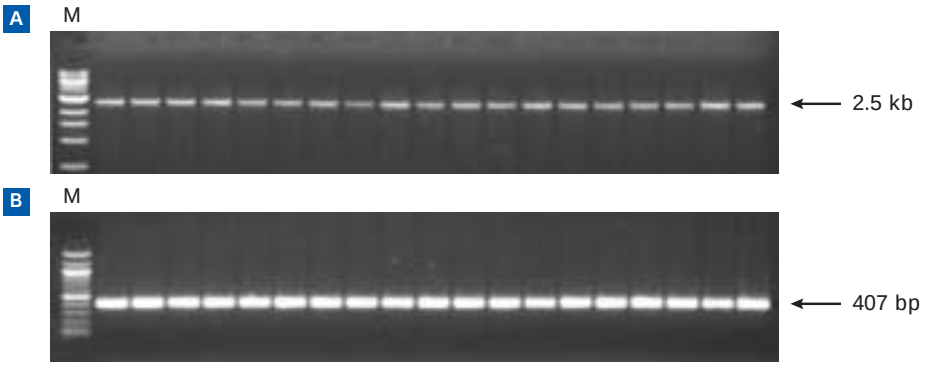


Figure 2. The BD Sprint™ Advantage™ 96 Plate produces consistent results when performing high-throughput PCR. Each well of the BD Sprint Advantage 96 Plate was reconstituted with 25 µl of PCR-grade water containing 10 µM Control BPTI primers targeted to different portions of the BPTI gene (Panels A and B) and 50 ng of Control Calf Thymus DNA. PCR was carried out under the following conditions: 95°C for 1 min, followed by 30 cycles of 95°C for 30 sec and 68°C for 3 min; then 68°C for a final 3 min. 5 µl of each PCR reaction was electrophoresed on a 1.2% agarose/TAE gel, alongside the appropriate size markers. Representative reactions are shown. **Panel A.** Lane M: 1-kb DNA size markers. **Panel B.** Lane M: 100-bp DNA size markers.

High-throughput PCR is now easier... and faster!

The new BD Sprint Advantage 96 Plate provides our robust BD Advantage Polymerase Mix, in a revolutionary 96-well format. Since their introduction, our BD Advantage Polymerase Mixes have given you all of the advantages of high fidelity, sensitivity, and yield. Now the new BD Sprint format gives you these same advantages in a matter of minutes (Figure 2). With such time-consuming and demanding applications as high-throughput cloning, long and accurate PCR, RACE (Figure 4), cDNA library construction, and preparative PCR, can you really afford to wait any longer?

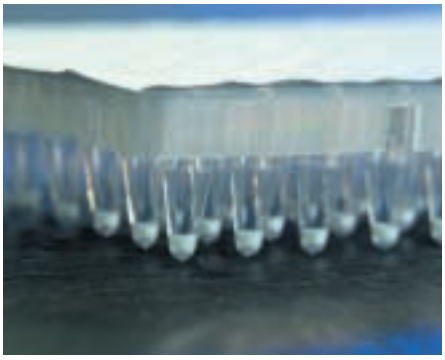
Increased sensitivity, greater flexibility

The BD Sprint Advantage 96 Plate is ideal for use in PCR applications that require both high fidelity and performance. The BD Sprint Advantage plate contains BD TITANIUM *Taq* DNA Polymerase and a proofreading enzyme, which together generate high yields of PCR products and longer, more accurate reads of DNA, with a fidelity that is up to five times higher than that of wild-type Taq polymerase. The polymerase also contains BD TaqStart™ Antibody for a convenient, automatic hot start. The lyophilized format conveys unparalleled stability—plates can be stored at room temperature for months at a time while still providing all of the advantages of the most sensitive polymerase available, BD TITANIUM *Taq*. And for researchers performing smaller scale PCR, the flexible pre-scored design lets you separate the plate into four individual sets of 24 wells, when working with fewer samples.

Sprint into high-throughput PCR

The BD Sprint Advantage 96 Plate provides simplified PCR preparation—an

BD Sprint™ Advantage™ 96 Plate...continued



Each well includes:

- BD TITANIUM™ *Taq* (with BD TaqStart™ Antibody)
- Proofreading polymerase
- dNTPs
- Buffer

Figure 3. The BD Sprint™ Advantage™ 96 Plate contains all the necessary components for fast, accurate PCR.

invaluable asset for large-scale, high-throughput PCR. While our new BD Sprint format yields results comparable to the traditional polymerase mixes on which it was based, the entire PCR set-up procedure requires just a fraction of the time.

Get on the inside track and get more experiments processed faster with our new BD Sprint Advantage 96 Plates.

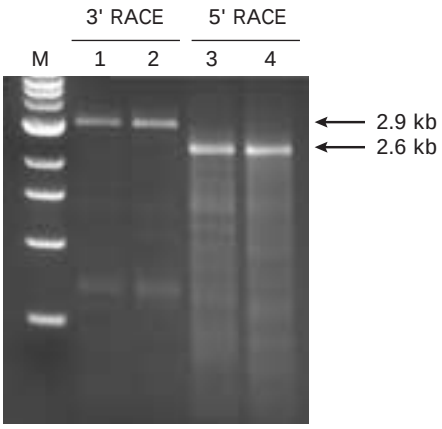


Figure 4. Performing RACE with the BD Sprint™ Advantage™ 96 Plate. 3'- and 5'-RACE PCR were performed with 5 µl of PCR control cDNA template and primers from the BD™ SMART RACE cDNA Amplification Kit (#K1911-1), in a final volume of 25 µl. PCR was carried out under the following conditions: five cycles of 94°C for 5 sec and 72°C for 4 min, five cycles of 94°C for 5 sec and 70°C for 4 min, and 25 cycles of 94°C for 5 sec and 68°C for 4 min. Samples were electrophoresed on a 1.2% agarose/TAE gel. Lane M: DNA size markers. The products of 3'-RACE (Lanes 1-2) and 5'-RACE (Lanes 3–4) were 2.9 kb and 2.6 kb, respectively.

Applications for BD Sprint™ Advantage™ 96 Plates

- High-throughput PCR
- Long & Accurate PCR
- RACE (Rapid Amplification of cDNA Ends)
- cDNA library construction
- Preparative PCR

Product	Size	Cat. #
BD Sprint Advantage 96 Plate	96 rxns	K1950-1

NEW!

Components

- BD Sprint™ Advantage™ 96 Plate
- Optically Clear PCR Cap Strips
- Microseal A Film
- Control DNA Template
- Control Primer Mix
- User Manual (PT3588-1)

Related Products

- BD Advantage™ 2 Polymerase Mix (#8430-1, -2)
- BD Advantage™ 2 PCR Kit (#K1910-1, -y)
- BD TITANIUM™ *Taq* DNA Polymerase (#8434-1, -2)
- BD TITANIUM™ *Taq* PCR Kit (#K1915-1, -y)
- BD Advantage™ Genomic Polymerase Mix (#8418-1)
- BD Advantage™ Genomic PCR Kit (#K1906-1, -y)
- BD Advantage™-GC 2 Polymerase Mix (#8433-1)
- BD Advantage™-GC 2 PCR Kit (#K1913-1, -y)
- BD Advantage™-GC Genomic Polymerase Mix (#8420-1)
- BD Advantage™-HF Genomic PCR Kit (#K1909-1, -y)
- BD Advantage™-HF 2 PCR Kit (#K1914-1, -y)
- BD Advantage™ UltraPure dNTPs (many)
- BD NucleoFast® 96 PCR Plates (#K3100-1, -2)

Notice to Purchaser

BD Advantage™ products are covered by U.S. Patent #5,436,149. The PCR process is covered by patents owned by Hoffmann-La Roche, Inc. and F. Hoffmann-La Roche, Ltd.

TaqStart® Antibody is licensed under U.S. Patent #5,338,671.

A license under U.S. patents #4,683,202, 4,683,195, and 4,965,188 or their foreign counterparts, owned by Hoffmann-La Roche, Inc. and F. Hoffmann-La Roche, Ltd. ("Roche"), has an up-front fee component and a running-royalty component. The purchase price of Advantage products includes limited, non-transferable rights under the running-royalty component to use only this amount of the product to practice the Polymerase Chain Reaction ("PCR") and related products described in said patents solely for the research and development activities of the purchaser when this product is used in conjunction with a thermal cycler whose use is covered by the up-front fee component. Rights to the up-front fee component must be obtained by the end-user in order to have a complete license. These rights under the up-front fee component may be purchased from Perkin-Elmer or obtained by purchasing an authorized thermal cycler. No right to perform or offer commercial services of any kind using PCR, including without limitation reporting the results of purchaser's activity for a fee or other commercial consideration, is hereby granted by implication or estoppel. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at the Perkin-Elmer Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404 or Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, CA 94501.These products are sold under licensing arrangements with F. Hoffmann-La Roche Ltd., Roche Molecular Systems, Inc., and The Perkin-Elmer Corporation.

BD Atlas™ SMART™ Fluorescent Probe Amplification Kit

Sensitive fluorescent microarray probes from limited starting material

- **Generate microarray hybridization probes starting with just 10 ng of total RNA**
- **Indirect labeling for highest efficiency and consistency**
- **Compatible with any glass array**

Now you can generate fluorescently-labeled probes for microarrays, even if you have limited starting material. Our new **BD Atlas™ SMART™ Fluorescent Probe Amplification Kit** combines our PCR-based BD SMART™ technology (**S**witching **M**echanism **A**t the 5' end of **R**NA **T**ranscript) with a reliable two-step labeling procedure, allowing you to generate fluorescent probes from as little as 10 ng of total RNA. Probes created with this kit can be used with our BD Atlas™ Glass Microarrays as well as other oligonucleotide or cDNA-based microarray platforms.

From cDNA amplification to probe labeling

A major limitation of array gene expression profiling has been the substantial amount of RNA required for standard probe labeling techniques—as much as 5–50 µg of total RNA. BD SMART amplification bypasses this obstacle by allowing accurate cDNA amplification from nanogram quantities of total RNA. Figure 1 outlines the process of probe synthesis. The cDNA amplification step uses a modification of the procedure in our BD Super SMART™ PCR cDNA Synthesis Kit (#K1054-1) to produce full-length double-stranded cDNA from limited starting material.

Following cDNA synthesis, aminoallyl-modified dUTP is incorporated into the cDNA molecule during several rounds of primer extension. Because the modified nucleotide is not significantly larger than unmodified dUTP, it is incorporated with the same efficiency. Next, in the coupling step, N-hydroxysuccinimide-activated fluorescent dyes react specifically with the modified dUTPs in the cDNA, producing evenly labeled probes. Our labeling kit is optimized for uniform incorporation of any dye, so your data is more consistent.

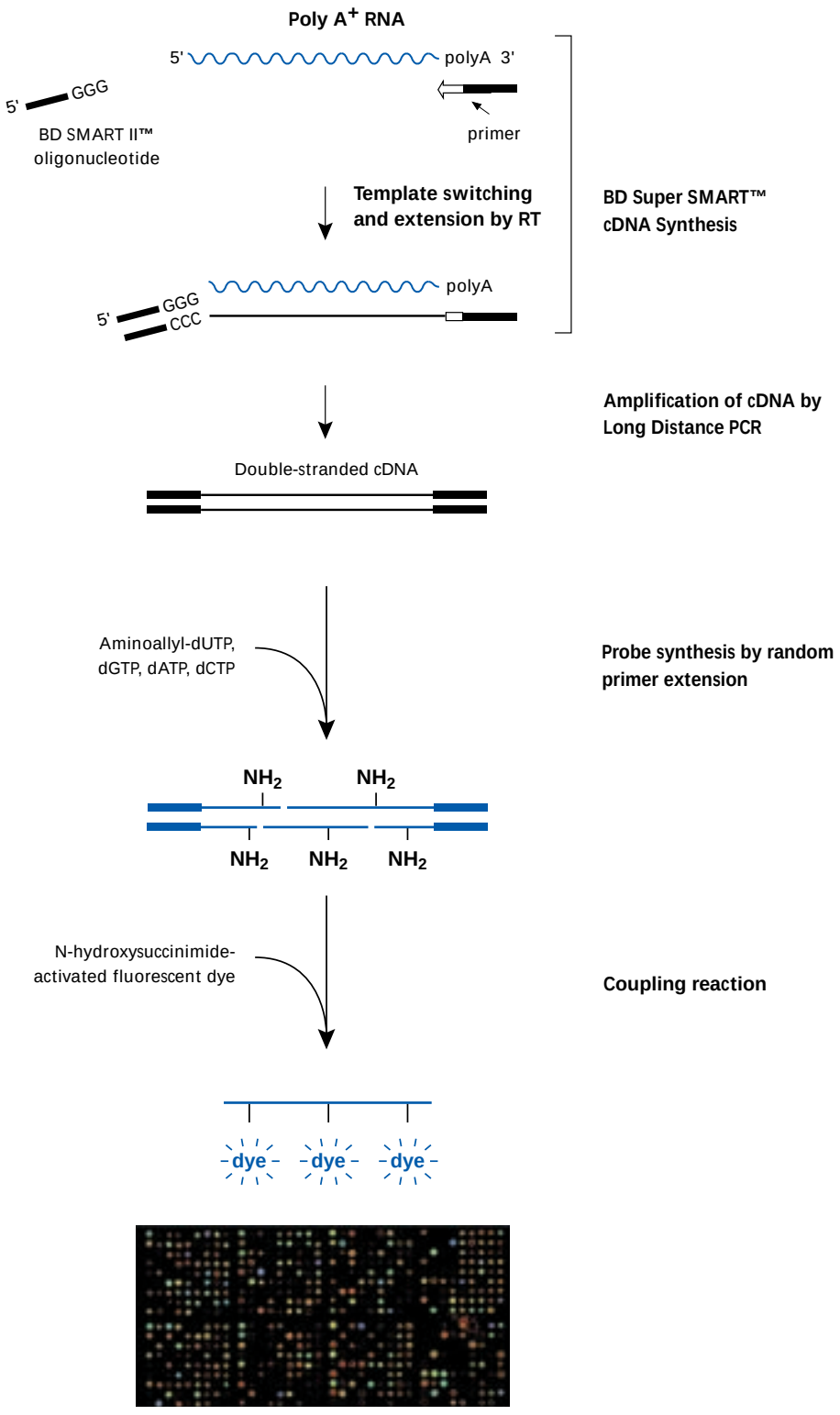


Figure 1. Overview of the Fluorescent Probe Amplification procedure.

BD Atlas™ SMART™ Fluorescent Probe Amplification Kit...continued

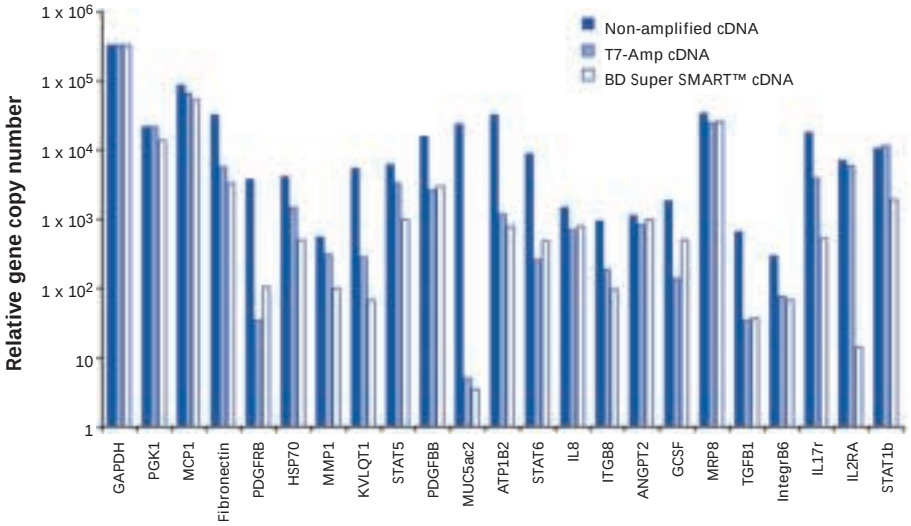


Figure 2. BD Super SMART™ cDNA representation confirmed by real-time PCR. The BD Super SMART method maintains gene representation as well as T7 amplification. Aliquots of BD Super SMART and T7 amplified cDNA were subjected to real-time PCR analysis using primers for the indicated genes. Data is normalized to GAPDH. Data provided by Dr. G. Dolganov, University of California San Francisco, CA.

Accurate representation of genes with BD SMART™ cDNA

A common concern in any amplification procedure is that genes expressed at low levels will be lost during the amplification process. With the BD SMART protocol, we recommend starting with a minimum of 10 ng of total RNA, which corresponds to about 1,000 cells or 100 µg of tissue. This amount of RNA ensures that BD SMART amplification yields cDNA that reflects the sample's original complexity (Figure 2; references 1, 2).

When using PCR for array probe generation, there is often the worry that transcripts might be amplified to saturation, known as the "plateau effect." If a sample is overcycled during PCR, many cDNAs could reach a concentration plateau and therefore be present at the same level in the amplified product, regardless of their original abundance. We have found that by limiting the number of SMART amplification cycles, the plateau effect does not occur in the amplification of complex cDNA pools (2).

An additional concern with the use of amplified cDNA for gene expression analyses is that different templates amplify with different efficiencies, based on their sequences. However, in array experiments comparing the relative abundance of the same target genes in multiple samples, the amplification efficiency of any given gene is equivalent in the two RNA samples under comparison—if they are amplified in the same manner. For this reason, it is important to generate both probes using the same technique. Comparing data from amplified probes to data from non-amplified probes will not yield accurate results.

The intelligent way to make array probes

The BD Atlas SMART Fluorescent Probe Amplification Kit contains reagents for performing at least 8 labeling reactions. You need only provide the appropriate fluorescent dye. The kit is compatible with all fluorescent dyes that are available in N-hydroxysuccinimide reactive form, including fluorescein, rhodamine, Alexa dyes, Cy3, and Cy5, so you can create just the right probe for screening your microarrays.

Product	Size	Cat. #
BD Atlas SMART Fluorescent Probe Amplification Kit	8 rxns	K1861-1

NEW!

Components

- 3' SMART CDS Primer II A
- BD PowerScript™ Reverse Transcriptase*
- First-Strand Buffer
- 5' PCR Primer II A
- cDNA Synthesis dNTP Mix
- DTT
- Fluorescent Labeling Buffer
- Labeling dNTP Mix
- Random Primer Mix
- Sodium Acetate
- Fluorescent BD SMART™ Blocking Solution
- BD SMART II A™ Oligonucleotide
- Control Human Placenta Total RNA
- DMSO
- EDTA
- QuickClean Purification Resin
- 0.22-µm Spin Filters/Collection Tubes
- BD Advantage™ 2 PCR Kit (trial size; #K1910-y)*
- User Manual (PT3676-1)

* Free with each purchase of the BD Atlas SMART Fluorescent Probe Amplification Kit.

References

1. Chenchik, A., *et al.* (1998) In Gene Cloning and Analysis by RT-PCR, Eds. Siebert, P. & Larrick, J. (BioTechniques Books, MA), pp. 305–319.
2. Diatchenko, L., *et al.* (1998) In Gene Cloning and Analysis by RT-PCR, Eds. Siebert, P. & Larr.

BD Atlas™ Antisense Oligo Mixes and Universal Reference RNA

Improve your microarray standardization and quality control

- **Universal standards for microarray comparison**
- **Perform quality control for microarray printing**
- **Ideal for database generation**

Bring a new level of quality to microarray production at your Microarray Core Facility. Our **BD Atlas™ Antisense Oligo Mixes** provide the ultimate calibration and quality control standard. Each species-specific mix contains an exact and complete antisense complement to the genes represented in our BD Atlas™ Ready-to-Print Long Oligos and Glass Microarrays, resulting in the most precise and reliable microarray control available. If you require a control that can be used with any type of microarray, use our **BD Clontech™ Human Universal Reference Total RNA**. Our Reference RNA is derived from a mixture of whole tissue sources, providing a reliable reference for the widest range of genes—more than 92% gene representation. Whether you choose Antisense Mixes or Reference RNA, you'll enhance your research with the ability to compare data sets from different experiments performed at different times.

Antisense oligos—the superior reference standard

The consistency of our BD Atlas Antisense Oligo Mixes allows you to accurately standardize your microarray experiments. These mixes are the ultimate reference standard for normalizing data within and between microarray hybridization experiments performed with BD Atlas Glass Microarrays or arrays printed with our Long Oligo sets. Data normalization with our Antisense Mixes gives you confidence that changes in hybridization intensity reflect genuine shifts in gene expression (1).

Antisense oligos—for superior quality control

Our Antisense Oligo Mixes—available for Human, Mouse and Rat—ensure that arrays printed in your facility are of the highest quality. By hybridizing these mixes to your arrays, you can confirm that each spot on your array produces a clear and strong hybridization signal without worrying about expression variation. In fact, over 98% of the spots on an array should display signal intensities of more than 3-fold over background when the microarray is printed correctly (Figure 1). By using one of our Antisense Oligo Mixes as a printing control, you can be certain that all missing spots will be detected, ensuring the highest quality control of your microarray printing.

Each Antisense Oligo Mix has been generated in very large lots, encompassing tens of thousands of kits. Therefore, you can be sure that whether you are testing your first or your 100th print lot, the Antisense Oligo Mix will perform consistently.

Universal RNA—the most comprehensive RNA reference standard

If your arrays contain sequences not represented in our Long Oligo sets, we recommend using our Human Universal Reference Total RNA as a normalization control. Our Reference RNA is comprised of RNA extracted from a range of different whole tissue sources and purified using our BD™ Premium RNA method. The RNA from each tissue is pooled, creating one master stock of high-quality, ultra-pure Reference RNA that has a more even gene distribution than any individual tissue tested (2). We have found that RNA from whole tissues shows higher overall expression with less variation than RNA from cell lines (2). This provides a homogenous signal intensity across the majority of genes during hybridization analysis.

Figure 2 compares gene expression in total RNA isolated from a single tissue and our Universal Reference RNA. All genes expressed in the individual tissue show comparable or higher levels of expression in the Reference RNA. Real-time PCR shows the higher, more-consistent level of gene representation in our Universal Reference RNA compared to a competitor's product (Figure 3). Our Reference RNA consistently outperforms the competition.

Which product is right for you?

It depends on your application. Antisense Oligo Mixes are ideal since they provide a uniform signal intensity for nearly all spots on BD Atlas Arrays, whereas our Reference RNA provides the greatest flexibility. Our Antisense Oligo Mixes ensure maximum accuracy in microarray standardization, calibration and quality testing, but can be used only with glass microarrays printed with the genes represented in our Long Oligo collections (including our premade and custom Atlas Glass Microarrays). Our Reference RNA can be used for data normalization and validation with any array and any labeling method.

Figure 1. Uniform hybridization with BD Atlas™ Antisense Oligo Mixes. A BD Atlas™ Glass Human 3.8 Microarray was hybridized with Cy3-labeled Human 8K Antisense Oligo Mix.

BD Atlas™ Antisense Oligo Mixes and Universal Reference RNA...continued

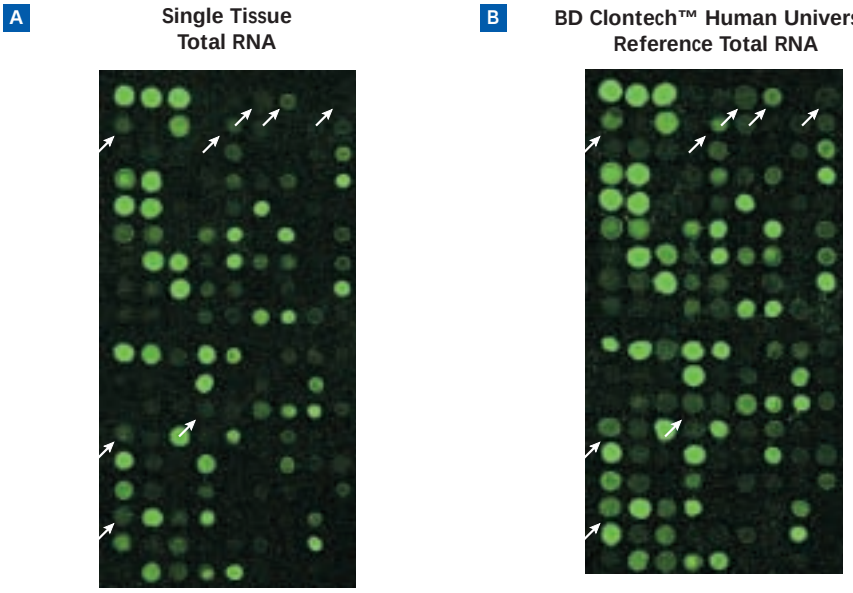


Figure 2. BD Clontech™ Human Universal Reference Total RNA shows comparable or stronger gene expression than tissue-specific RNA in array hybridization. Total RNA isolated from a single tissue and Universal Reference RNA were reverse transcribed with Cy3-modified dUTP. The labeled cDNA probes were hybridized to individual BD Atlas™ Glass Human 3.8 Microarrays. The microarrays were analyzed using an Axon scanner and GenePix Pro 3.0 software. Images represent a portion of the arrays. Arrows indicate some of the genes showing higher expression in the Universal Reference RNA.

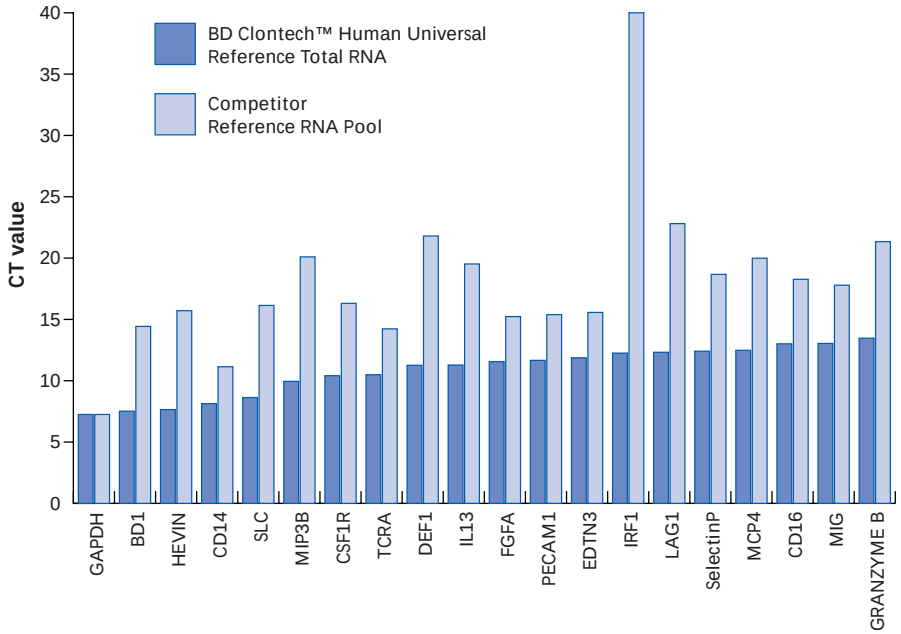


Figure 3. Our BD Clontech™ Human Universal Reference Total RNA outperforms the competition. Real-time PCR was used to measure the expression level of 150 genes in our Universal Reference RNA and a competitor's product. Data from 20 representative genes are shown. Overall, the genes display a more homogeneous signal distribution and a higher level of expression in our Universal Reference RNA. This result is indicated by the lower cycle threshold (CT) values, which are inversely related to the amount of PCR product generated, and thus, to the amount of RNA in the original pool.

Product	Size	Cat. #
BD Atlas Human 8K Antisense Oligo Mix	50 µl	7913-1
BD Atlas Mouse 5K Antisense Oligo Mix	50 µl	7914-1
BD Atlas Rat 4K Antisense Oligo Mix	50 µl	7915-1
BD Clontech Human Universal Total Reference RNA	2 x 200 µg	64115-1
BD AtlasNavigator 2.0*	each	V1221-1

* BD AtlasNavigator system minimum requirements:
- Windows 95/98/NT 4.0
- Pentium II processor or higher
- 128 MB RAM (256 recommended)

Antisense Oligo Mix Components

- Antisense Oligo Mix
- Fluorescent Labeling Buffer
- DMSO
- User Manual (PT3681-1)

Reference

1. Dudley, A. M. *et al.* (2002) *Proc. Natl. Acad. Sci. USA* **99**:7554–7559.
2. Control RNA for Microarray Experiments (April 2002) *Clontechniques* **XVII**(2):6.

Notice to Purchaser

BD AtlasNavigator is licensed for use on only one computer. For information on licenses for multiple installations, contact BD Biosciences Clontech at 800-662-2566, ext. 1140.

Coming Soon!

BD Atlas™ Plastic Human 12K Microarray

Get expression data from over 12,000 human genes in a single experiment with our new BD Atlas™ Plastic Human 12K Microarray. Our plastic microarrays combine the best features of macro and microarrays—they can be stripped and reprobed and require no special equipment for imaging, just a standard phosphorimager. In addition, each spot on the plastic surface is uniform and discrete, allowing high-density printing. All these factors add up to easy, accurate analysis of human gene expression.

BD AtlasNavigator™ 2.0 Software

High-quality data mining made affordable

- **Organize your data using functional classes and cluster analysis**
- **View your data using graphs, histograms, scatter plots & more**
- **Now compatible with BD Atlas™ Plastic, Glass & Nylon Arrays**

The challenge of interpreting thousands of array data points just got easier with **BD AtlasNavigator™ 2.0**. With sophisticated options for data analysis and display, this update to our feature-rich software is the ideal data-mining tool for nylon, plastic, or glass arrays.

Import data directly from BD AtlasImage™ reports

Importing data into BD AtlasNavigator is a snap when you're using BD AtlasImage to quantify array expression data. The new Autoloader feature is customized to read BD AtlasImage reports directly. The original Experiment Import Wizard is still available to customize imports.

Organize and display data using a variety of visual formats

After importing your data and defining the experimental parameters of interest, you can perform a number of different analyses such as k-Means Clustering and statistical treatment of replicates. You can also use a Drawn Gene to define an expression pattern of interest and then find genes that match it. Regardless of the analysis performed, the expression data for the entire experiment—as many arrays as you wish—can be viewed in a variety of formats including line graphs, histograms (Figure 1), and scatter plots. BD AtlasNavigator also makes it easy to focus on just the genes of interest by allowing you to restrict the display to user-determined gene sets. When you evaluate data using the pre-defined functional classifications, which have been carefully selected by our Bioinformatics team, you can quickly see the biological relevance of your data. And with the new Global Error Model, you can verify that your conclusions are statistically significant—even when you're working with a small number of samples.

See ordering information on page 7.

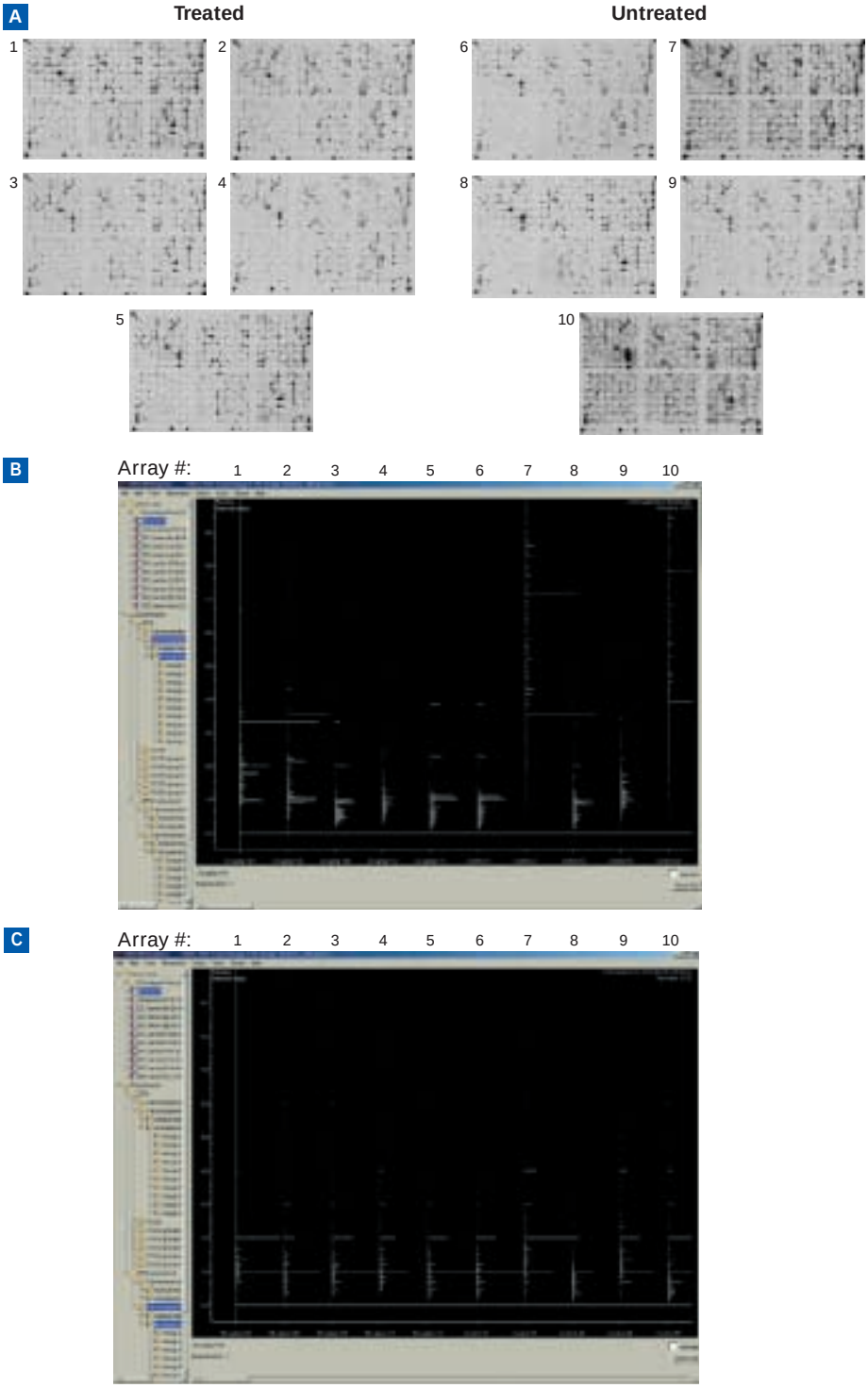


Figure 1. BD AtlasNavigator™ 2.0 software facilitates the management of data from multiple experiments. Panel A. Samples from 5 rats treated with 50 mg/kg of a potential carcinogen (arrays 1–5) and 5 untreated rats (arrays 6–10) were hybridized to the BD Atlas™ Rat Toxicology 1.2 Array (#7860-1). The variation in signal intensities from array to array is common and may be attributed to many factors, including animal-to-animal differences. **Panel B.** A graphical representation of array-to-array signal intensity differences. Comparing such arrays requires normalization. **Panel C.** The signal intensities after arrays are normalized to the average combined array signal.

BD Adeno-X™ Virus Purification Kits

A practical alternative to cesium chloride gradient centrifugation

- **Purify recombinant adenovirus in just 3 hours**
- **No CsCl gradients or ultracentrifugations required**
- **Obtain consistent yields of active adenovirus**

Now there is a safe, easy, and rapid method for purifying and concentrating recombinant adenovirus: The **BD Adeno-X™ Virus Purification Kit**, a complete chromatography-based system that enables you to purify adenovirus in just 3 hours.

These kits provide a superior alternative to cesium chloride (CsCl) density gradient centrifugation—an effective, but technically demanding and time-consuming procedure. The ultracentrifugation steps, which require expensive equipment and meticulous preparation, may take 3 days to complete, and the strict volume limits discourage any attempts to adjust the purification scale.

The BD Adeno-X Purification Kit, on the other hand, offers no such challenges. Once your stocks have been amplified, they can be processed to purity in less than 3 hours. Both small and large-scale kits are available, and each kit has a wide range of viral capacities, so you can scale up or down without difficulty. And because our method lets you harvest adenovirus from both the growth medium and the cell pellet, you obtain consistently high yields of adenovirus without having to worry about when to pull the culture from the incubator; simply remove cells when the cytopathic effect is complete.

Simple chromatographic procedure

There are no ultracentrifugation steps in our protocol. Instead, adenovirus is purified chromatographically using a unique membrane adsorbent that selectively binds adenoviral particles based on their distinctive surface properties. The membrane, developed by VIRAPUR, is housed in a small, single-use cartridge—the BD Adeno-X Purification Filter—which fits on disposable syringes or house vacuum lines. As virus-containing medium is drawn through the cartridge, adenoviral particles are trapped and effectively removed from the solution. The bound particles can then be eluted with a small volume of Elution Buffer. The entire protocol, from harvest to purification, takes 2–3 hours to complete (Figure 1).

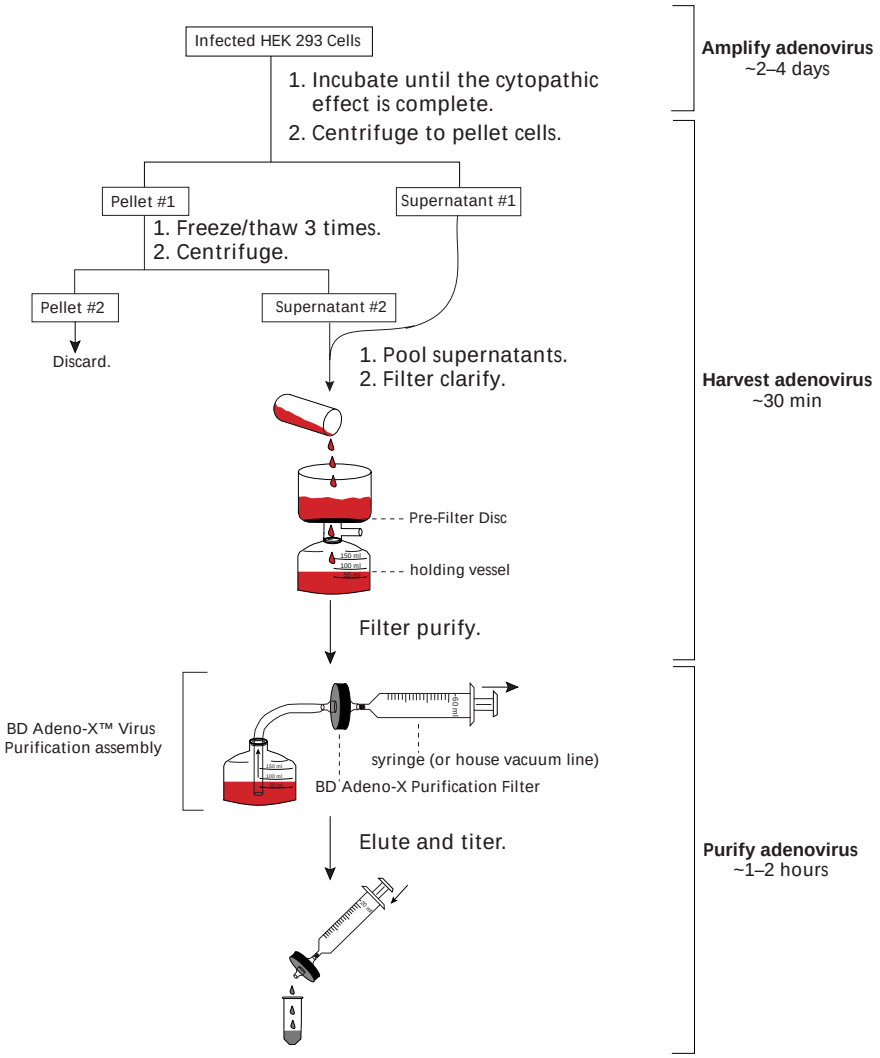


Figure 1. Overview of the BD Adeno-X™ Virus Purification protocol.

Easy set up

The BD Adeno-X Purification assembly consists of four main parts: the holding vessel, the tubing assembly, the Purification Filter, and the vacuum source (Figure 2). The BD Adeno-X

Table I: Purification of Recombinant Adenovirus

Purification Method	Final Volume	OD_{260} Titer (particles)	Infectious Titer[a] (infectious units)	Particle/ Infectious Virus[b]
BD Adeno-X	2.5 ml	3.9×10^{11}	1.7×10^{10}	23
CsCl	2.1 ml	2.6×10^{11}	6.8×10^{9}	38

[a] Measured with the Adeno-X Rapid Titer Kit (#K1653-1)
[b] This ratio may vary among viral strains.
[c] The adenovirus was amplified in a culture containing 2×10^8 cells.
Results shown are the means of three purifications. A 293 cell culture was infected with adenovirus and then split into equal portions. The adenovirus from each portion was harvested and purified by the indicated methods.

BD Adeno-X™ Virus Purification Kits...continued

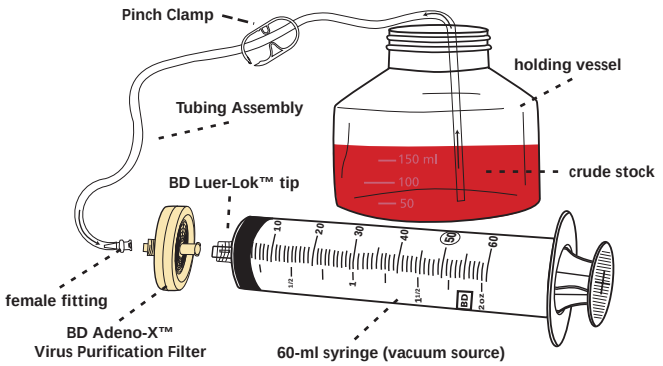


Figure 2. The BD Adeno-X™ Virus Purification Assembly. Syringe not included.

Purification Filter is the central part of the assembly. In Standard Kits, the filter connects directly to a BD Luer-Lok™ tip syringe—the vacuum source. Mega Kits provide additional tubing to connect the larger Mega Filter to a house vacuum line.

The vacuum is used to draw the crude adenoviral stock from the holding vessel through the tubing and into the Purification Filter. After rinsing with Wash Buffer, the filter is disconnected from the tubing, attached to a BD Luer-Lok™ tip syringe, and then flushed with a small volume of Elution Buffer to remove the bound adenovirus.

The titer and purity of the adenovirus is comparable to that achieved by CsCl density gradient centrifugation (Table I and Figure 3). The particle:infectious virus ratios typically range from 25:1 to 100:1. But unlike CsCl techniques, our method requires no advanced training. Only simple manipulations are required to produce consistently high yields of purified adenovirus (Figure 4).

Available in Standard and Mega sizes

Our Standard Kit (#K1654-1, -2) can deliver as many as 1 x 10^{12} adenoviral particles and yields between 1–5 x 10^{10} total infectious units (ifu). Yields from Mega Kits are generally ~10-fold higher (Figure 4). Both kits provide all the essential buffers and materials necessary for purification. You need only provide PBS, DNase, and syringes. Purifying recombinant adenovirus has never been easier.

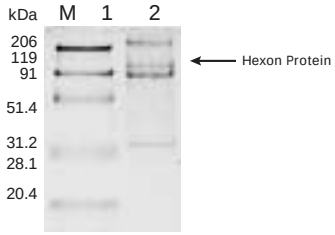


Figure 3. Obtain chromatographically pure adenovirus in 2–3 hours. Aliquots of purified adenovirus (equivalent to 14 µg total protein) were resolved on a Tris-Glycine; SDS/10% polyacrylamide gel, and then stained with Coomasie blue. Lane 1: BD Adeno-X; Lane 2: CsCl; Lane M: molecular weight markers.

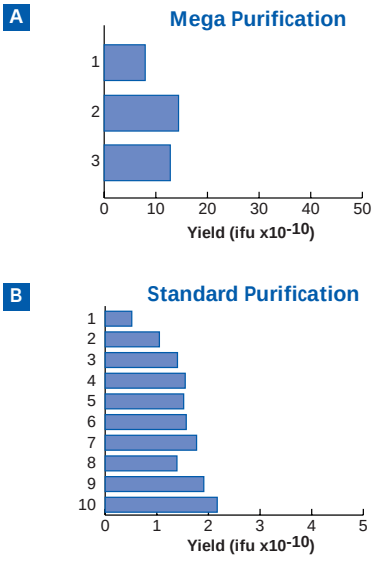


Figure 4. Obtain consistently high yields of adenovirus. The results of 3 Mega purifications (**Panel A**) and 10 Standard purifications (**Panel B**) are shown. Adenoviral yields were determined using the BD Adeno-X™ Rapid Titer Kit. Adenovirus was amplified in 1–2 x 10^8 cells (Standard) or 5–10 x 10^8 cells (Mega).

Product	Size	Cat. #
BD Adeno-X Virus Purification Kits		
	1 purification	K1654-1
	5 purifications	K1654-2
BD Adeno-X Virus Purification Mega Kit		
	1 purification	K1655-1

Components

- Dilution Buffer
- Wash Buffer
- Elution Buffer
- Formulation Buffer
- Tubing Assembly
- Bottle-Top Filters and Pre-Filter Discs
- BD Adeno-X™ Purification Filter

Related Products

- BD Adeno-X™ Expression System (#K1650-1)
- BD Adeno-X™ Tet-Off™ Expression System (#K1651-1)
- BD Adeno-X™ Tet-On™ Expression System (#K1652-1)
- BD Adeno-X™ Rapid Titer Kit (#K1653-1)

BD Adeno-X™ Marker Viruses

Ready-to-use, high-titer adenovirus control stocks

- **Visually determine infection efficiencies**
- **Optimize infection conditions**
- **Compare the infectivity of different cell lines**
- **Use as control virus**

With our new BD Adeno-X™ Marker Viruses, you can quickly and easily determine infection efficiencies, optimize infection conditions, and measure infection rates of any target cell line. These replication-incompetent (ΔE1/ΔE3), first-generation adenoviruses are the perfect infection markers, encoding well-known reporter genes that can be measured by fluorescence microscopy or by direct colorimetric staining. With some of the markers, you can even use flow cytometry to sort for infected cells.

Currently, we offer four different Marker Viruses. Two, **BD Adeno-X™-EGFP** and **BD Adeno-X™-DsRed2**, encode BD Living Colors™ fluorescent proteins—Enhanced Green Fluorescent Protein (EGFP) or *Discosoma sp.* Red Fluorescent Protein (DsRed2). These fluorescent proteins are excellent reporters because their fluorescence can be detected directly in many different species without having to add additional proteins, substrates, or cofactors.

A third virus, **BD Adeno-X™-LacZ**, encodes β-galactosidase, an enzyme whose activity can be measured qualitatively by X-gal staining and quantitatively by luminescence (e.g., using our Luminescent β-gal Reporter System 3 [#K2055-1]). Our fourth Marker, **BD Adeno-X™-Null**, which carries no transgene, can play a critical role in your experiments because it enables you to differentiate between phenotypic changes caused by adenoviral infection and transgene expression. Cells infected with BD Adeno-X-Null represent your true baseline.

All four Marker Viruses were generated using our BD Adeno-X Expression System (#K1650-1), which enables researchers to construct, clone, and propagate recombinant adenovirus using standard restriction and ligation techniques.

Recombinant vectors created with this system contain the immediate early promoter of cytomegalovirus for strong constitutive expression of the transgene—e.g., EGFP, DsRed2, or lacZ. Like other Ad5 adenoviruses, these Marker Viruses can infect a wide variety of dividing and non-dividing cell types, but because they lack the E1 gene, they replicate only in E1 trans-complementing cells such as HEK 293. Each adenovirus was plaque-purified to ensure genetic homogeneity, and then filter-purified using our BD Adeno-X Virus Purification Kit (#K1655-1; see page 10–11). A single tube (100 µl) of BD Adeno-X Marker Virus (~10^{10} ifu) is sufficient for several preliminary experiments and starter amplifications.

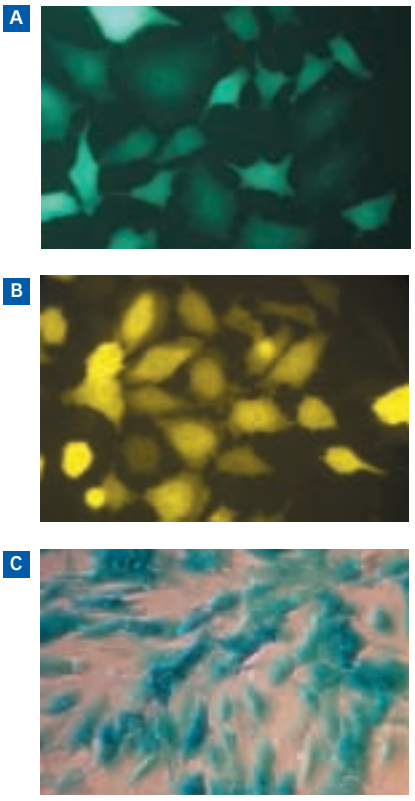


Figure 1. Photomicrographs of cells infected with BD Adeno-X™ Marker Viruses. HeLa cells were infected with either BD Adeno-X-EGFP (**Panel A**) or BD Adeno-X-DsRed2 (**Panel B**) at a multiplicity of 10, incubated for 48 hr, and then observed by fluorescence microscopy using a Zeiss Axioskop equipped with the appropriate light filters. The images were recorded with a color digital camera. BJ human foreskin fibroblasts were infected with BD Adeno-X-LacZ at a multiplicity of 25, incubated for 48 hr, and then stained with X-Gal (**Panel C**).

Product	Size	Cat. #
Adeno-X-EGFP	100 µl	8138-1
Adeno-X-DsRed2	100 µl	8139-1
Adeno-X-LacZ	100 µl	8140-1
Adeno-X-Null	100 µl	8141-1

NEW!

Related Products

- BD Adeno-X™ Viral DNA (PI-*Sce* I & I-*Ceu* I-digested) (#K1650-A)
- BD Adeno-X™ Accessory Kit (#K1650-B)
- BD Adeno-X™ PCR Primer Set 2 (#9133-1)

Notice to Purchaser of BD Living Colors™ DsRed Products

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This product is the subject of pending U.S. and foreign patents.

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Use of BD Biosciences Clontech's BD Living Colors™ products containing DNA sequences coding for mutant *Aequorea victoria* green fluorescent protein (GFP) variants or proteins thereof requires a license from Amersham Biosciences under U.S. Patent Nos. 5,625,048; 5,777,079; 6,054,321 and other pending U.S. and foreign patent applications. In addition, certain BD Biosciences Clontech products are made under U.S. Patent No. 5,804,387 licensed from Stanford University.

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Please contact BD Biosciences Clontech directly for any other assistance, including purchasing and technical support. All companies and institutions purchasing BD Living Colors products will be included in a quarterly report to Aurora Biosciences, as required by the BD Biosciences Clontech/Aurora Biosciences license agreement.

BD Retro-X™ Q Vectors

New self-inactivating vectors engineered to produce higher titers

- **Higher viral titers than with other retroviral vectors**
- **Reliable expression levels due to reduction in promoter interference**
- **Minimize probability of gene interference**
- **Bicistronic expression enables direct selection**

Introducing a new line of self-inactivating retroviral vectors—The **BD Retro-X™ Q Vectors** have uniquely engineered features that provide high viral titers, reliable expression levels, and reduce the possibility of promoter interference (1).

The Q Vectors are designed to express a target gene along with an antibiotic selection marker (Figure 1) and to inactivate the promoter in the 5' LTR following integration into the host genome (Figure 3). Self-inactivating vectors provide improved expression due to a reduced chance of promoter interference (2–5). They also have shown more consistent expression in cell types that do not efficiently express transcripts from the MMLV LTR (6), and they are better experimental models because they are less likely to activate cellular sequences upon integration into the genome (7). Also, Q vectors are safer to work with because they are less likely to form replication-competent retrovirus.

Higher viral titers

Previously, self-inactivating retroviral vectors have been reported to produce viral stocks with poor titers (3, 8). To overcome this issue, the Q Vectors include an engineered murine sarcoma virus (MSV) and Cytomegalovirus (CMV) hybrid promoter in the 5' LTR. The CMV enhancer region replaces the less-potent MMSV enhancer region found in the 5' LTR of most retroviral expression vectors. This new configuration takes advantage of E1A expression in HEK 293-derived packaging cells to activate higher viral transcript production. In Figure 2 the percent of infected cells, corresponding to the number of cells expressing green fluorescent protein (Panel A), was measured. Using these methods we find that Q Vectors produce titers of ~3 x 10^7 cfu/ml (Panel B). An SV40 origin of replication included in the Q Vectors may also increase viral titers in packaging cells that express the SV40 T antigen.

Self-inactivating vectors

As described in Figure 3, the self-inactivation feature of Q vectors is provided by a deletion in the U3 region of the 3' LTR. During reverse transcription of the retroviral genome, the partially deleted 3' LTR is copied to the 5' LTR. By duplicating the deletion in the U3, the 5' LTR (MSV/CMV hybrid) promoter is inactivated (9). Thus, once the Q vector expression cassette is integrated in the target cells, transcription can only be driven from the internal CMV promoter immediately upstream of the gene-of-interest (Figure 1). This may reduce the phenomenon known as promoter interference (1, 2) allowing more efficient expression from the internal expression cassette.

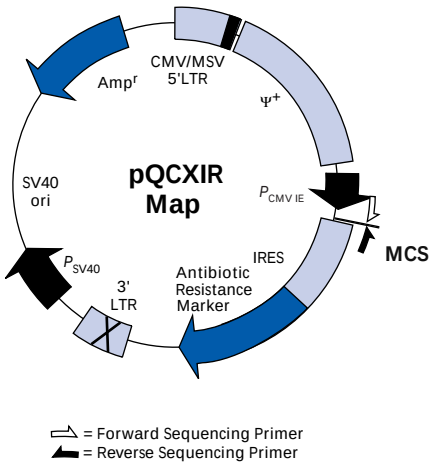
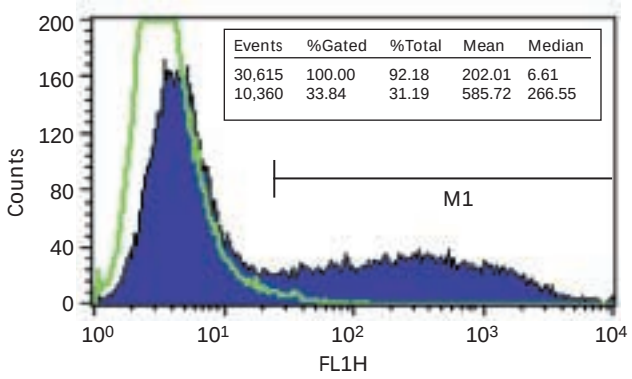


Figure 1. Composite Q Vector Map. In this generalized version of the Q Vector Map, the common elements of all the vectors are represented. Immediately downstream of the CMV immediate early promoter, the multiple cloning site (MCS) is followed by a eukaryotic IRES that ensures a second ORF (an antibiotic resistance marker or another gene in the case of pQCXIX) is co-transcribed with the gene cloned into the MCS. The expression cassette has all of the essential elements for retroviral integration and expression.

A



B

$$\frac{\text{(\% of infected cells) X (cell number at time of harvest)}}{\text{volume of virus used for infection (ml)}}$$

$$\frac{0.3119 \times 1.5\times10^6}{0.0166} = 2.8 \times 10^7 \text{ cfu/ml}$$

cfu = colony forming units

Figure 2. High titers are obtained with the Q Vectors. In this experiment, a pQCXIN construct with Enhanced Green Fluorescent Protein (EGFP) was transfected into GP2-293 cells of the Pantropic Retroviral Expression System (K1063-1) and recombinant virus was harvested 48 hr post-transfection. NIH 3T3 cells were infected with a 10^{-2} dilution of the recombinant virus. 1.5 x 10^6 cells were harvested 72 hr post-infection and evaluated by BD FACS™ analysis for the percent of cells expressing EGFP (**Panel A**). These data indicate a titer of 2.8 x 10^7 cfu/ml (**Panel B**). Negative control indicated in green.

BD Retro-X™ Q Vectors...continued

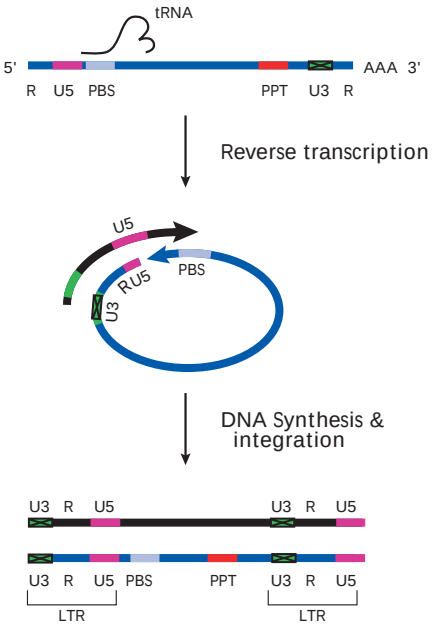


Figure 3. Self-inactivation mechanism of the Q Vectors. Plus strand viral RNA (blue) from the expression cassette is reverse transcribed. During integration, a circular intermediate is formed that results in duplication of the deletion in the U3 region of the 3' LTR. This inactivates the CMV/MSV hybrid promoter in the 5' LTR so that the transcript can only be driven from the internal promoter $P_{CMV\ IE}$ (Figure 1).

The Q Vector Set includes vectors containing resistance markers for neomycin, hygromycin, or puromycin downstream of the IRES (10). When the bicistronic transcript is expressed in the target cells, the IRES ensures that your gene-of-interest is expressed concomitantly with the antibiotic selection marker. The control vector that is also included in the set, pQCLIN, expresses β-galactosidase (LacZ) from a bicistronic transcript that also encodes neomycin resistance. This control vector provides a means of easily visualizing infectivity (Figure 4). Primers are also included with the BD Retro-X Q Vector Set for easy verification of DNA constructs.

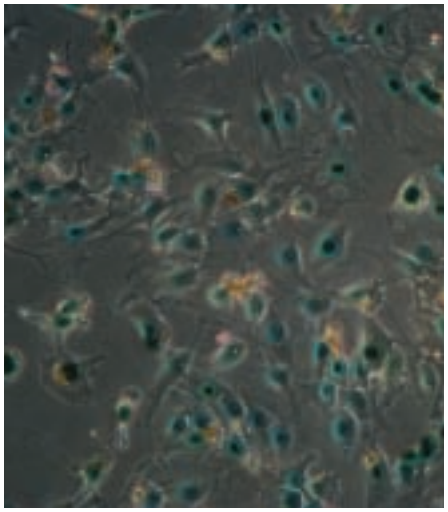


Figure 4. High-efficiency of gene transfer. NIH 3T3 cells infected with retrovirus derived from pQCLIN were stained with X-gal by standard methods and visualized at 48 hr post-infection.

An additional BD Retro-X Q Vector, pQCXIX is available separately. This Q Vector has two multiple cloning sites, one before and another after the IRES. With this vector, two genes can be expressed concomitantly.

Coming Soon!

More BD Retro-X™ Q Vectors

We are expanding our BD Retro-X™ Q Vector line to include versions for use with BD Creator™ Systems for fast, efficient cloning, as well as Q Vectors that express fluorescent marker genes. The BD Creator™ Q Vectors will be available with both a *loxP* site and an MCS for inserting a tissue-specific promoter by your method of choice. A Q Vector already containing a CMV promoter as well as the *loxP* site will also be available for your convenience. BD Retro-X™ Q vectors with fluorescent marker genes will allow you to easily and rapidly identify cell lines that express high levels of your target gene.

Products	Size	Cat. #
BD Retro-X Q Vector Set	Each	9136-1
pQCXIN Retroviral Vector	20 µg	9134-1
pQCXIX Retroviral Vector	20 µg	9135-1

Q Vector Set Components

- pQCXIN Retroviral Vector
- pQCXIH Retroviral Vector
- pQCXIP Retroviral Vector
- pQCLIN Retroviral Vector
- 5' pQC Seq/PCR Primer
- 3' pQC Seq/PCR Primer
- User Manual (PT3132-1)
- Vector Information Packets

Related Products

- BD Retro-X™ System (#K1060-1)
- LRCX Retroviral Vector Set (#K1061-1)
- MSCV Retroviral Expression System (#K1062-1)
- Pantropic Retroviral Expression System (#K1063-1)
- BD RetroPack™ PT67 Cell Line (#K1060-D)
- BD EcoPack2™-293 Cell Line (#C3203-1)
- BD AmphoPack™-293 Cell Line (#C3201-1)
- pLXIN Vector (#6062-1)
- pLEGFP-N1 Vector (#6059-1)
- pLEGFP-C1 Vector (#6058-1)
- RevTet-Off™ System (#K1626-1)
- RevTet-On™ System (#K1627-1)
- pRevTet-On Vector (#6159-1)
- pRevTet-Off Vector (#6140-1)
- pRevTet-Off-IN Vector (#6134-1)
- pRevTRE Vector (#6137-1)

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10. IRES Bicistronic Expression Vectors (January 2002) *Clontechniques* **XVII**:21.

Notice to Purchaser

Use of the IRES sequence is covered by U.S. Patent #4,937,190 and is limited to use solely for research purposes. Any other use of the IRES sequence requires a license from Wisconsin Alumni Research Foundation.

BD Mercury™ TransFactor Profiling Kit—Oncogenesis 2

Simultaneously profile 6 cancer-related transcription factors

- **Faster and more sensitive than gel-shift assays**
- **DNA binding studies in high-throughput format**
- **New cancer-related transcription factors**

Introducing the newest **BD Mercury™ TransFactor Profiling Kit†—Oncogenesis 2**. Changes in gene expression patterns result from changes in DNA-binding properties of nuclear transcription factors. Using this TransFactor Profiling Kit, you can quickly assess changes in transcription factor-DNA binding in response to the physiological and pathological events that lead to tumor formation (Figures 1 & 2). With the addition of this TransFactor Kit, the DNA-binding activities of more than 20 different transcription factors can be profiled (Table I).

The TransFactor Kits provide a quick and sensitive immunosorbent assay for studying DNA binding by proteins that is superior to gel-shift assays (1, 2). After extracts are incubated in the wells coated with DNA binding sequences, the bound proteins are detected with specific antibodies. The entire procedure can be performed in about 3–4 hours and is up to ten times more sensitive than gel-shift assays (3, 4). These kits have a flexible 96-well format for examining multiple samples in a single experiment. Sixteen wells are coated with wild-type oligo for each transcription factor. You can use the whole plate, or remove and analyze just some of the wells without losing track of which wells correspond to which transcription factor.

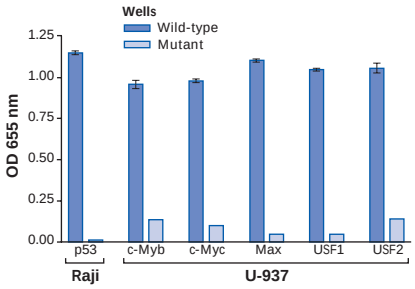

Figure 1. Profiling of the transcription factors in the BD Mercury™ TransFactor Profiling Kit—Oncogenesis 2. Six transcription factors involved in oncogenesis were assayed simultaneously. 30 µg of nuclear extract was used in each binding assay. Binding assays to mutant sequences are also included as controls in this experiment. The experiments were performed in triplicate as described in our User Manual. Transcription factor and nuclear extracts are indicated below the graph.

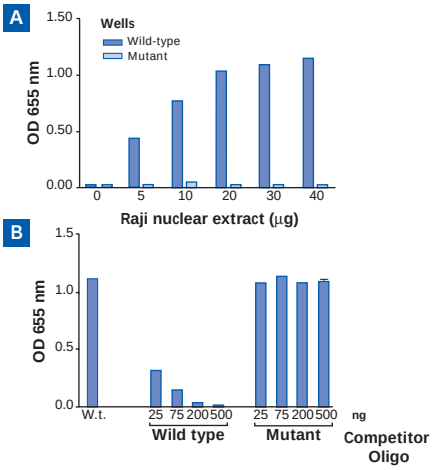

Figure 2. Dose response and competition assays of p53. Panel A: Dose Response. Increasing concentrations of Raji nuclear extract generated an increase in signal for binding to wild type, but not mutant oligos. **Panel B: Competition assay.** The signal was decreased with the increasing amount of wild type competitor oligo, but not with mutant oligo.

The transcription factors featured in our Oncogenesis 2 profiling plate have important roles in the control of cell growth and division. The tumor suppressor p53 and the oncoproteins c-Myc and c-Myb are modified in a large variety of cancers. The kit also includes wells for studying three Myc-related proteins: Max, that forms heterodimers with c-Myc; as well as USF1 and USF2, that bind DNA as dimers. Detecting changes in the DNA binding of these transcription factors is important for understanding their role in tumor formation, as well as for developing treatments to the related cancers.

Table I: Transcription factors studied with the TransFactor profiling kits

Inflammation 1
ATF2, CREB-1, c-Fos, c-Rel, NFκB p50, NFκB p65

Inflammation 2
c-Fos, FosB, c-Jun, JunD, Sp-1, STAT1

Oncogenesis 1
DP-1, E2F-1, E2F-2, Rb, p107, Sp-1

Oncogenesis 2
c-Myb, c-Myc, Max, USF1, USF2, p53

BD Mercury™ TransFactor Products

	Size	Cat. #
Profiling Kit—Oncogenesis 1	96 rxns	K2073-1
Profiling Kit—Oncogenesis 2	96 rxns	K2075-1
Profiling Kit—Inflammation 1	96 rxns	K2062-1
Profiling Kit—Inflammation 2	96 rxns	K2072-1

NEW!

Components

- Color-coded TransFactor Plate
- TransFactor Rack
- Primary Antibodies
- Secondary Antibody
- TransFactor Buffer
- Blocking Reagent
- TMB Substrate
- Stop Solution
- Positive Control Nuclear Extract
- Wild-Type Competitor Oligos
- Mutant Competitor Oligos
- User Manual (PT3549-1)
- Protocol-at-a-Glance (PT3549-2)

Related Products

- BD Mercury™ TransFactor CREB-1 Kit (#K2066-1)
- BD Mercury™ TransFactor DP-1 Kit (#K2069-1)
- BD Mercury™ TransFactor c-Fos Kit (#K2065-1)
- BD Mercury™ TransFactor c-Jun Kit (#K2061-1)
- BD Mercury™ TransFactor NFκB p50 Kit (#K2058-1)
- BD Mercury™ TransFactor NFκB p65 Kit (#K2067-1)
- BD Mercury™ TransFactor Rb Kit (#K2068-1)
- BD Mercury™ TransFactor STAT1 Kit (#K2059-1)
- TransFactor Extraction Kit (#K2064-1)

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†Patent Pending

BD Living Colors™ DsRed-Express

Engineered for rapid fluorescence development

- **Develops fluorescence as fast as Enhanced Green Fluorescent Protein (EGFP)**
- **Ideal for detecting the onset of promoter activity**
- **Reduced green emission**

BD Biosciences Clontech introduces **DsRed-Express**—a variant of *Discosoma sp.* red fluorescent protein (DsRed; 1). DsRed-Express contains nine amino acid substitutions, which drastically reduce the time from transfection to detection of red fluorescence and diminish the level of green emission (2).

Because it attains fluorescence so quickly, DsRed-Express can be used as a marker to rapidly assess transfection efficiencies or as a reporter to detect the onset of promoter activity. It can also be used in combination with EGFP, variants of EGFP, or HcRed—a far red-shifted fluorescent protein (3, 4)—to monitor gene expression from different promoters. Studies are underway to determine the suitability of DsRed-Express as a protein tag.

More rapid development of red fluorescence

When DsRed-Express is expressed in mammalian cell cultures, red-emitting cells can be detected by either fluorescence microscopy or flow cytometry within 8–12 hours of transfection—a maturation rate comparable to that of Enhanced Green Fluorescent Protein (EGFP; Figures 1 & 2). Maximum fluorescence is usually achieved within 24–30 hours. The rapid acquisition of red fluorescence may be partly due to an increase in the protein's folding efficiency (2). Though DsRed-Express appears to have a lower quantum yield and extinction coefficient than either DsRed2 or DsRed1 (2), its improved folding efficiency more than makes up for the loss.

Reduced level of green emission

DsRed-Express is especially well adapted for flow cytometry; the recombinant protein emits a very low level of green fluorescence. This is critical for cell sorting applications, since flow cytometers interpret low level green emissions as if the cell were expressing a true green fluorescent protein such as EGFP. When this residual green emission is absent, however, the instrument is able to fully resolve EGFP and DsRed-expressing populations, making dual-color experiments easy to analyze (Figure 3).

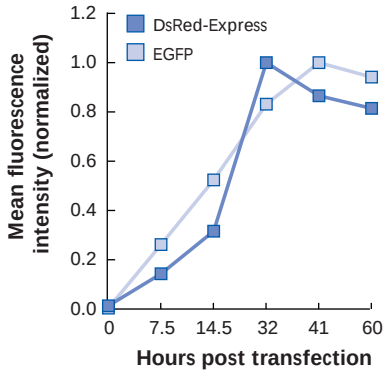

Figure 1. The maturation of DsRed-Express is comparable to that of EGFP. HeLa cells were transiently transfected with 3 µg of pDsRed-Express-N1 or pEGFP-N1 (#6085-1) and analyzed over time by flow cytometry on a BD FACSCaliber™ instrument using the 488-nm laser line.

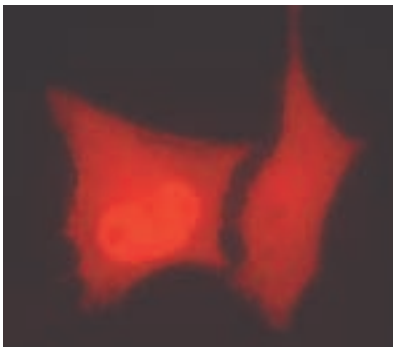

Figure 2. Photomicrograph showing HeLa cells 14 hr after transfection with pCMV-DsRed-Express. HeLa cells were transiently transfected with 0.7 µg pCMV-DsRed-Express (#6995-1) and grown for 14 hours. Results were visualized using Chroma Technology Corp. filter sets d540/40x, 570dcpl, and d600/50m; a cooled CCD camera (MicroMax Interline Transfer Camera, Roper Scientific) and Metamorph Software (Universal Imaging Corp.). This micrograph was taken on a Zeiss Axioskop using the 40X objective and a 300-ms exposure.

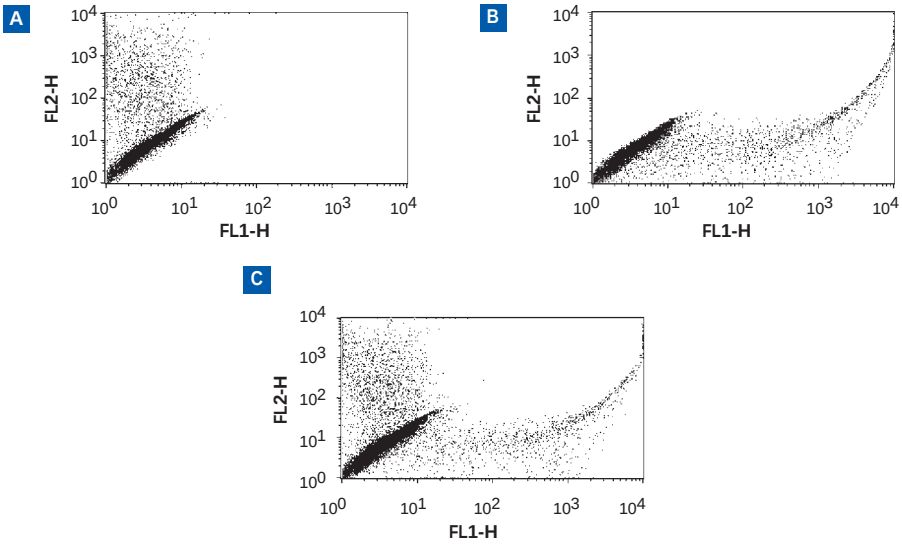

Figure 3. Cells transfected with DsRed-Express have a lower level of residual green emission, making multi-color analyses more accurate. HEK 293 cells transiently transfected with either pCMV-DsRed-Express or pEGFP-N1 were analyzed by flow cytometry as described in Figure 1. Cells were transfected as follows: **Panel A.** pCMV-DsRed-Express. **Panel B.** pEGFP-N1. **Panel C.** A mixture of cells transfected with either pCMV-DsRed-Express or pEGFP-N1. Note that cells expressing DsRed-Express distribute almost exclusively along the FL-2 (red emission channel) axis and show no "bleed-through" into the FL-1 (green emission) channel.

BD Living Colors™ DsRed-Express...continued

Same excitation and emission maxima as DsRed2

The mutations made to create DsRed-Express do not significantly alter the excitation and emission maxima (Table I), so it can be detected with the same filter combinations used for DsRed2. Furthermore, DsRed-Express is recognized by our BD Living Colors™ DsRed Monoclonal Antibody (#8374-1, -2), the same antibody used for detecting DsRed2 and DsRed1-E5, the Fluorescent Timer.

We currently offer three DsRed-Express vectors: **pCMV-DsRed-Express**, designed for use as a cotransfection marker in mammalian cells; **pDsRed-Express**, which serves primarily as a source of DsRed-Express cDNA; and **pDsRed-Express-1**, a promoterless vector that can be used to monitor the activity of different promoter/enhancer elements cloned into the multiple cloning site. For more information about these and other BD Living Colors™ Proteins, please visit **www.bdbiosciences.com** and navigate to our vector information page.

Product	Size	Cat. #
pCMV-DsRed-Express Vector	20 µg	6995-1
pDsRed-Express Vector	20 µg	6993-1
pDsRed-Express-1 Vector	20 µg	6994-1

Which BD Living Colors™ red fluorescent protein is right for you?

Table I: Comparison of DsRed2, DsRed-Express, and HcRed1

	DsRed2	DsRed-Express	HcRed1
Excitation maximum (nm)	563	557	588
Emission maximum (nm)	582	579	618
Brightness[a]	nd	17,100	600
Time to detection[b]	24 hr	8–12 hr	16 hr
Quaternary structure	tetramer[c]	tetramer[c]	dimer[d]
Relative utility for:			
General reporter	–	++	+
Fusion proteins	+[e]	n/d	+[e]
Multicolor applications			
Fluorescence microscopy	+	++	++
Flow cytometry	+ (Can be excited with 488-nm laser line)	++ (Can be excited with a 488-nm laser line)	++ (Excitation requires a 568-nm laser line)

[a] brightness = quantum yield x extinction coefficient.
[b] Measured by fluorescence microscopy.
[c] DsRed2 and DsRed-Express are believed to form the same tetrameric structure as wild-type DsRed.
[d] See reference 4.
[e] known to work with some fusion proteins (our unpublished data).
n/d = not determined

Related Products

- BD Living Colors™ EGFP Vectors (many)
- BD Living Colors™ ECFP Vectors (many)
- BD Living Colors™ EYFP Vectors (many)
- BD Living Colors™ DsRed2 Vectors (many)
- BD Living Colors™ HcRed Vectors (many)
- BD Living Colors™ Subcellular Localization Vectors (many)

References

1. Matz, M. V., *et al.* (1999) *Nat. Biotechnol.* **17**:969–973.
2. Bevis, B. J. & Glick B. S. (2002) *Nat. Biotechnol.* **20**:83–87.
3. BD Living Colors™ HcRed (April 2002) *Clontechniques* **XVII**(2):12-13.
4. Gurskaya, N. G., *et al.* (2001) *FEBS Letters* **507**:16–20.

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This product is the subject of pending U.S. and foreign patents.

BD ApoAlert™ Caspase Assay Plates

Caspase activity profiling in a convenient 96-well format

- **Simultaneously profile multiple caspases**
- **Monitor caspase-3 activity in up to 96 samples at once**
- **Fast and easy fluorescent assay**
- **No handling of caspase substrates required**
- **Convenient 96-well format**

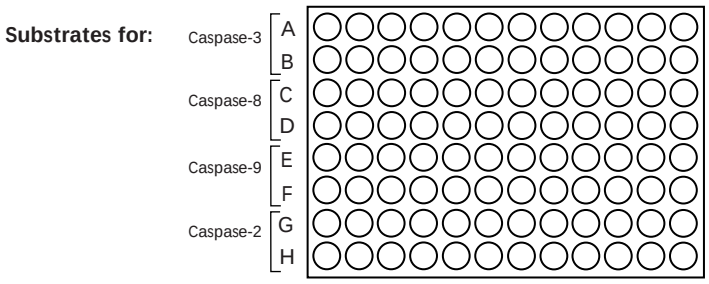

Figure 1. BD ApoAlert™ Caspase Profiling Assay Plate layout. Caspase Profiling Assay Plates contain immobilized substrate for each of caspases-3, -8, -9 & -2 as shown. Caspase-3 Assay Plates contain immobilized caspase-3 specific substrate in Rows A-H.

Apoptosis studies have just ramped up with our **BD ApoAlert™ Caspase Assay Plates**. Interested in finding out much more about the apoptotic caspase response? Use this convenient and fast format to simultaneously monitor and profile the activity of up to four different caspases with the BD ApoAlert Caspase Profiling Assay Plate or study caspase-3 activation in depth with the BD ApoAlert Caspase-3 Assay Plate.

Caspases are members of a large family of cysteine proteases that mediate programmed cell death (apoptosis). Upon activation, caspases disable cellular housekeeping and repair programs and cleave important structural components (1). With the Caspase Profiling Assay Plate (Figure 1), you can measure the activation of four different caspases —caspase-2, -3, -8, and -9—using a fluorometric method. Now you can easily and conveniently profile caspase activities in different cell lines after treatment with different apoptotic stimuli.

Simultaneously analyze many apoptotic events in just one experiment!

The 96-well assay format is designed to increase the amount of information obtained at the same time. Caspase Assay Plates allow for convenient fluorometric analysis of various samples using a single 96-well plate. Because the fluorogenic substrates specific for particular caspases are already immobilized in the wells of our 96-well plates, testing a variety of samples is easy, eliminating the need for additional handling of caspase substrates. Upon addition of a cell lysate containing activated caspase to the well, the immobilized fluorogenic substrate is cleaved by the corresponding activated caspase, releasing fluorescent product that can be easily detected by a standard fluorescence plate reader (Figure 2).

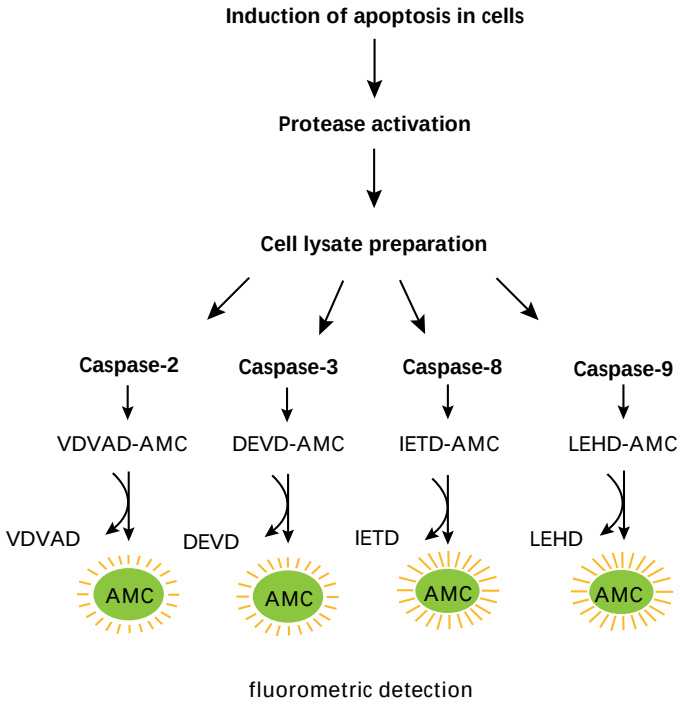

Figure 2. Fluorometric detection of caspase activity during apoptosis. After protease activation, the activated caspases recognize their respective substrates which are covalently linked to the fluorogenic dye, 7-amino-4-methyl coumarin (AMC). Upon cleavage by the respective caspase, the free dye can be detected using a plate reader with a 380 nm Excitation and 460 nm Emission filter.

Rapid fluorescence-based analysis

With Caspase Assay Plates, you can detect caspase activation 90 minutes after cell collection. In Figure 3, apoptosis was induced in Jurkat cells either by addition of staurosporine (Panel A) or exposure to UV light (Panel B). Get definitive results quickly with BD ApoAlert™ Caspase Assay Plates! Cells were collected at regular time points following apoptosis induction and cell lysates were analyzed using the Caspase Profiling Assay Plate. For cells treated with the same apoptotic stimulus, you can now easily detect cell type differences during caspase activation.

Versatile profiling tool—2 different formats provided

Whether you're analyzing several different caspases simultaneously or you have a targeted area of research, there is a

BD ApoAlert™ Caspase Assay Plates...continued

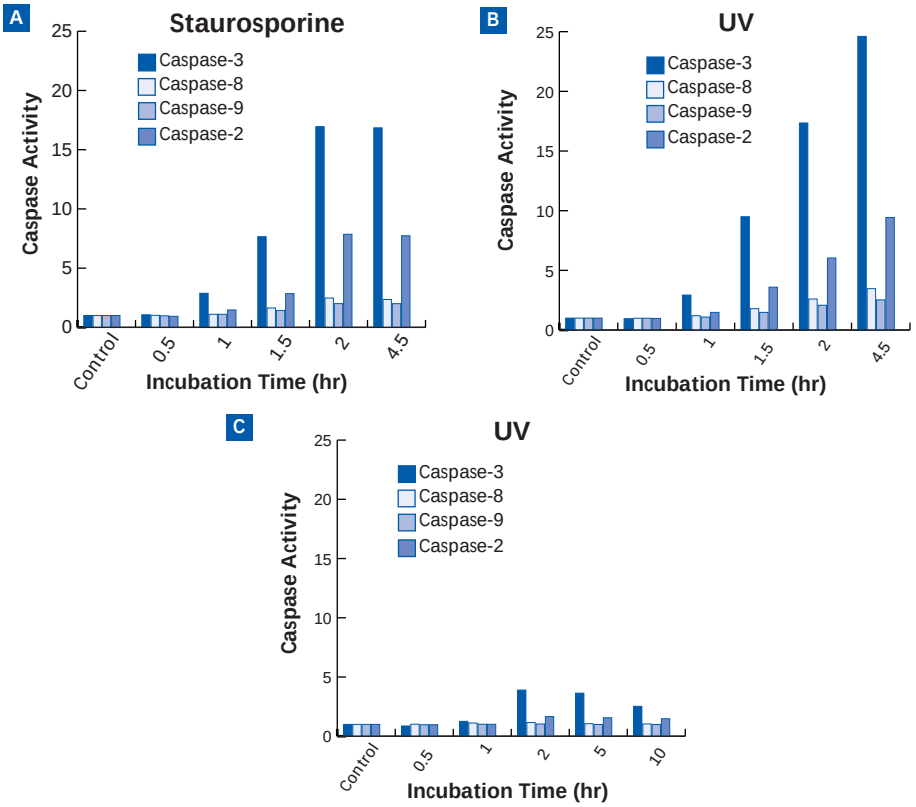

Figure 3. Time course study of caspase activation in induced cells. Jurkat cells were induced with Staurosporine (700 nM; **Panel A**) or UV light (5 min exposure prior to chase; **Panel B**) and harvested at defined time points following induction. At each time point, cells were collected, lysed, centrifuged (5 min, 14,000 rpm) and the supernatant collected. After all time points were collected, 50 µl of the lysates (equivalent to $2x10^5$ cells/well) were transferred into each well of a Caspase Profiling Assay Plate and then incubated at 37°C for 2 hr. A CytoFluor II Fluorescence Plate Reader set to 380 nm (excitation), 460 nm (emission) was used for fluorescence measurements. The increase in fluorescence by the various activated caspases is shown as a fold increase relative to uninduced cells. Induced cells show an increase in caspase activity over time. The increase in caspase activity in Staurosporine-induced cells plateaus between 2 and 4.5 hr post-induction; however, the caspase activity of cells induced with UV light continues to increase during that time frame. This implies the presence of different mechanisms at work for the regulation of caspase activity in cells induced to undergo apoptosis via UV or Staurosporine. The induction of apoptosis in MCF-7 cells (caspase-3 deficient) by UV light (same method described above) results in a very low rate of caspase activation (**Panel C**). MCF-7, a Caspase-3 deficient breast cancer cell line, has a very low rate of caspase activation (2), a finding consistent with our results. This low but detectable rate of transient caspase-3 substrate cleavage may be caused by an activation of caspase-7. Caspase-7 has a low but measurable affinity to the DEVD sequence contained in the caspase-3 substrate and has been shown to be activated in apoptotic MCF-7 cells (2).

Caspase Assay Plate to meet your needs. Why profile multiple caspases at once? Different caspases become activated via different apoptotic pathways. Depending upon the specific cell type as well as apoptotic stimuli used, one or more caspases will be activated. Use our Caspase Profiling Assay Plate to analyze several different caspases in your samples at once, with up to twenty-four wells available for each caspase. We also offer the Caspase-3 Assay Plate containing the same substrate in each well, allowing you to detect caspase-3 activity simultaneously in a variety of samples. Either way you get reliable, easy-to-read fluorometric detection results. Caspase-specific inhibitors are included for your control needs.

Product	Size	Cat. #
Caspase Profiling Assay Plate	1 plate	K2033-1
	5 plates	K2033-2
Caspase-3 Assay Plate	1 plate	K2032-1
	5 plates	K2032-2

Caspase Profiling Assay Plate Components

- Caspase Assay Plate(s)
- Reaction Buffer
- DTT
- Cell Lysis Buffer
- Caspase-3 Inhibitor
- Caspase-8 Inhibitor
- Caspase-9 Inhibitor
- Caspase-2 Inhibitor

Caspase-3 Assay Plate Components

- Caspase-3 Assay Plate(s)
- Reaction Buffer
- DTT
- Cell Lysis Buffer
- Caspase-3 Inhibitor

References

1. Earnshaw, W. C., *et al.* (1999) *Annu. Rev. Biochem.* **68**:384–424.
2. Kottke *et al.* (2002) *Journal of Biological Chemistry* **27**:804–815.

NucleoFast® 96 PCR Kits

Superior technology for PCR product purification & recovery

- **Purification of up to 96 PCR products in under 20 minutes**
- **Up to 90% recovery**
- **Ultrafiltration technology—requires no wash step**

Do you need quick, high-recovery purification for your PCR products? The **NucleoFast® 96 PCR Plates** are the new solution for high recovery of nucleic acids in just 20 minutes. For added convenience, our **NucleoFast® 96 PCR Clean-Up Kit** also includes recovery solutions and Collection Plates for storing purified samples. NucleoFast technology is based on ultrafiltration: The nucleic acids from the PCR reaction are collected on the filter membrane while the contaminants are filtered directly to the waste. By eliminating the intermediate wash steps required with silica-based purification systems, NucleoFast streamlines the purification process. The purified PCR products are suitable for most downstream applications including capillary sequencing, microarray spotting, restriction analysis, labeling, or cloning.

Optimized filter for high recovery

The NucleoFast filter has several features that make it uniquely suited for its purpose. The NucleoFast filter's most notable feature is a high recovery rate—50–90% for PCR products of 150–10,000 bp (Figure 1). With the specially-treated hydrophilic filter no detergents wash out of the membrane during purification. With other commercial filters, detergents wash out of the membrane and cause problems in downstream applications such as microarray spotting. In addition, the recovery process is easier because the filter membrane is durable enough to tolerate contact with disposable tips.

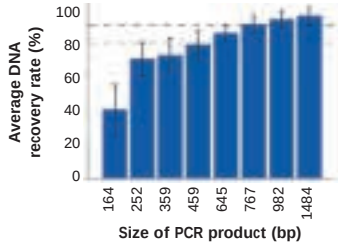

Figure 1. High recovery. The NucleoFast ultrafiltration membrane has a high rate of recovery of even small PCR products. Recovery rate depends on the PCR product length.

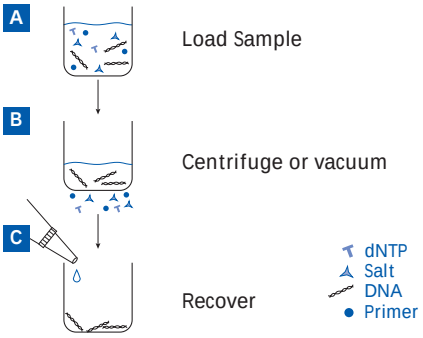

Figure 2. Ultrafiltration purification. Panel A. Add the reaction mixture directly to the well. **Panel B.** The DNA binds, while the contaminants (dNTPs, primers, salts, and other additives) pass through the filter membrane. **Panel C.** The purified DNA is then recovered by resuspending with buffer or water on the membrane.

New technology saves time and money

The NucleoFast procedure takes just two steps. The PCR reactions are simply loaded on the filter membrane, and while nucleic acids bind to the membrane, the contaminants pass through. Buffer is then added to the surface of the membrane to release the nucleic acids (Figure 2). The pure DNA is recovered from the surface of the membrane with water or low-salt buffer.

Wash steps to remove impurities are unnecessary with NucleoFast Kits, because buffers containing chaotropic salts are not required. With the ultrafiltration membrane there is no longer a need for prolonged washing with ethanol-containing buffers or for the subsequent drying step that is necessary with silica membranes. After loading the PCR reactions, proceed to direct elution of the nucleic acids from the filter membrane. The two-step recovery of the purified product saves you time and the reduction in buffers saves you money.

NucleoFast 96 PCR Plates are designed to recover PCR products as small as 150–300 bp, with either manual (vacuum or centrifuge) or automated purification methods, in less than 20 minutes. These plates have a simple and sturdy, one-piece plate design that is compatible with many different vacuum manifolds and can easily be adapted for automated processing.

Product	Size	Cat. #
NucleoFast 96 PCR Clean-Up Kit		
	4 x 96 preps	K3099-1
	24 x 96 preps	K3099-2
NucleoFast 96 PCR Plates*		
	10 plates	K3100-1
	50 plates	K3100-2

NEW!

* Trial size available

NucleoFast 96® PCR Clean-Up Kit Components

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- Collection Plates
- Buffer RB
- Nuclease-free water
- Self-adhesive Plate Seal

Related Products

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- NucleoTrap® Gel Extraction Kit (#K3070-1)*
- NucleoSpin® 8 Extract Kit (#K3059-1, -2)
- NucleoSpin® 96 Extract Kit (#K3065-1, -2)*
- NucleoTrap® PCR Purification Kit (#K3071-1)*
- NucleoTrap® PCR Suspension (#4081-1)
- NucleoVac™ (#4071-1)
- BD Sprint™ Advantage™ 96 Plate (#K1950-1)

*Trial sizes available

New BD Matchmaker™ Custom Pretransformed Two-Hybrid Libraries

Now available in a BD Creator™ Donor Vector: pDNR-GADT7-Rec

- **High-complexity two-hybrid libraries**
- **Quickly move any library insert into multiple expression systems**

Our **BD Matchmaker™ Custom Pretransformed Library Service** now offers library construction in a Creator Donor Vector. Starting with RNA, tissue, or cells that you provide, we construct a high-complexity cDNA library in pDNR-GADT7-Rec, a newly developed GAL4 activation domain (GAL4 AD) cloning vector uniquely engineered for two-hybrid screening and Cre-*loxP* recombination (1, 2). Your library is pretransformed into yeast strain Y187 (*MAT*α) or AH109 (*MAT***a**). pDNR-GADT7-Rec contains all the elements necessary for identifying and confirming protein-protein interactions, plus two *loxP* sites, which flank the library insert. The *loxP* sites enable you to transfer the insert to any BD Creator™ Acceptor Vector using Cre recombinase (Figure 1). The Cre-catalyzed reaction, which takes just 15 min, maintains the reading frame and orientation of the insert, so the Acceptor Vector expresses the same gene product. And because you can transfer the insert to multiple Acceptor Vectors simultaneously, you can can quickly switch from two-hybrid analysis to studies of protein localization (BD Living Colors™), Co-IP with tagged mammalian expression vectors, Tet-regulated expression, retroviral gene delivery, and more. Visit **www.bdbiosciences.com** or ask your Territory Manager for the custom library ordering form.

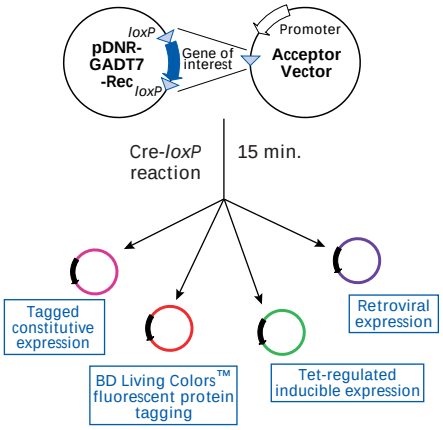

Figure 1. Blaze a direct path from two-hybrid screening to expression studies. After identifying and confirming novel protein-protein interactions, you can easily transfer the library inserts from positive clones to any other BD Creator™ Acceptor Vector. The constructs cover a wide range of functional studies.

NEW!

BD Matchmaker™ Custom Pretransformed cDNA Library Products

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pDNR-GADT7-Rec/Y187		
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For-Profit	each	CS1034-2
pDNR-GADT7-Rec/AH109		
Not-for-Profit	each	CS1035-1
For-Profit	each	CS1035-2

References

1. Creator System Overview (October 2001) *Clontechniques* XVI(4):5–6.
2. Creator™ Gene Cloning & Expression System (April 2002) *Clontechniques* XV(2):7–11.

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To learn more about our BD Creator™ Gene Cloning & Expression System, please visit our Creator home page, accessible from **www.bdbiosciences.com**.

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