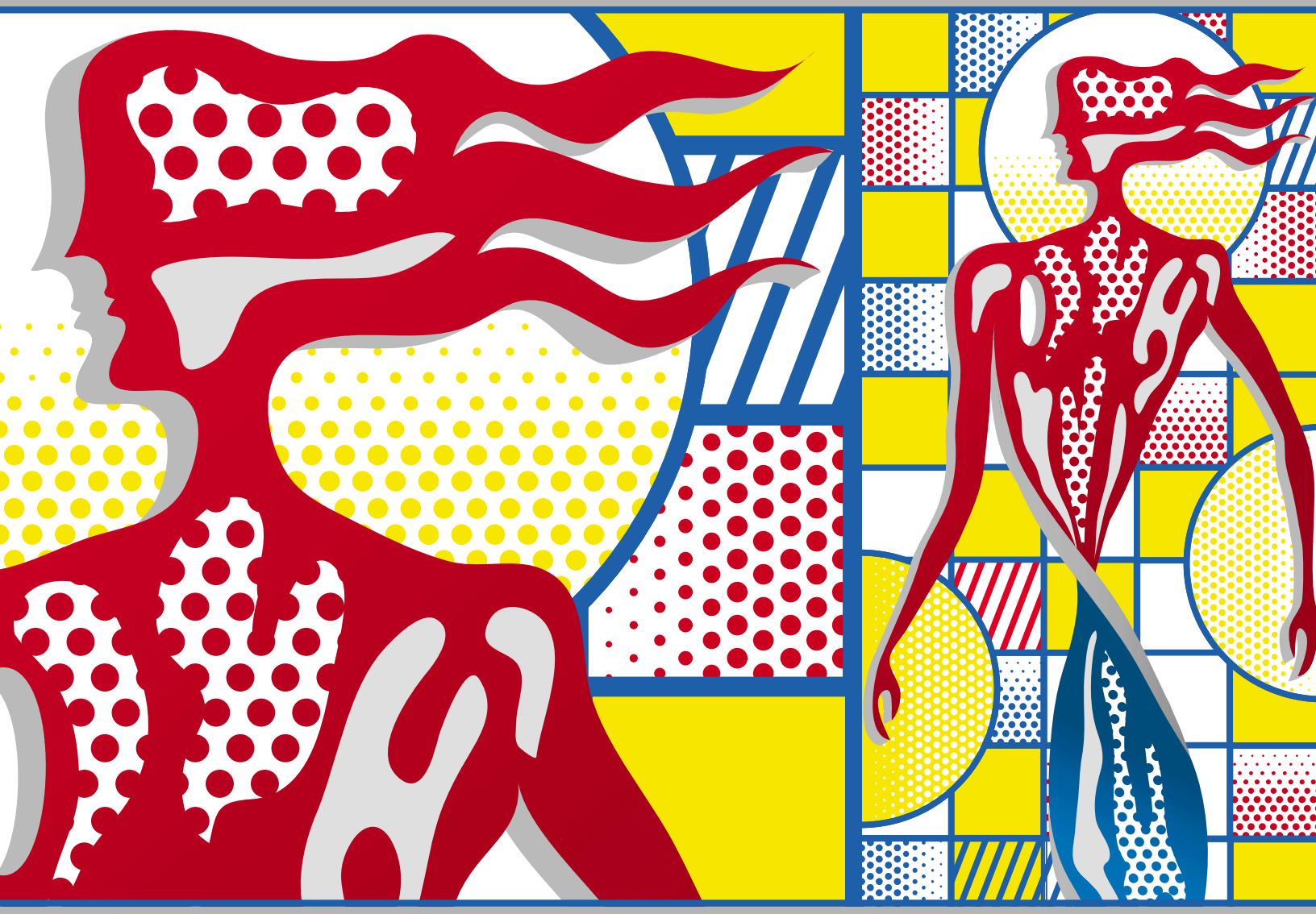
Clontechniques

October 2002



Highlights

Complete Table of Contents on page 1

BD Biosciences
Clontech
Discovery Labware
Immunocytometry Systems
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BD

Illustration inspired by the art of Pablo Picasso (1882–1973).

BD Sprint™ Advantage™ 96 Plate

PCR in a plate—it's that easy.

- 96-well PCR in a fraction of the time—why spend all of your time aliquotting?
- Higher sensitivity, fidelity and yields for all of your high-throughput PCR applications
- 96-well plates that are compatible with all major PCR block and robotics manufacturers

Tired of liquid-handling bottlenecks? Experiencing higher-than-acceptable fail rates? Fed up with optimizing a low performance *Taq* polymerase for high-performance applications? Relax... high-throughput PCR is now easier—and faster!

Introducing the revolutionary new **BD Sprint™ Advantage™ 96 Plate** (#K1950-1), with everything you need to complete 96 PCR reactions—except for water, your primers, and your DNA template! Each well of the BD Sprint Advantage 96 Plate contains a complete, lyophilized master mix comprised of BD TITANIUM™ *Taq*, BD TaqStart™ Antibody, a proofreading enzyme for increased fidelity, dNTPs, and an optimized PCR buffer. To use the plate, simply resuspend the master mix in your diluted primers and template DNA (25 µl total), then go directly to PCR! High-performance, high-throughput PCR in a plate—it's just that easy.

Please see the BD Sprint Kits Notice to Purchaser on page 1.

BD Biosciences Clontech

www.bdbiosciences.com

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October 2002
Volume XVII, No. 4

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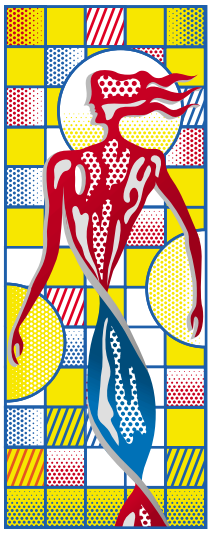


About the Cover

The cover shows an illustration designed for our Disease Profiling Arrays. Illustration inspired by the art of Roy Lichtenstein (1923–1997).

Clontechniques is published quarterly in January, April, July, and October, by BD Biosciences Clontech, 1020 East Meadow Circle, Palo Alto, CA 94303-4230, USA.

BD Atlas™ Plastic Human 12K Microarray

The only calibrated microarray on the market

- **Rely on thoroughly tested long oligos† for optimal specificity and minimal cross-hybridization**
- **Use a microarray that provides greater sensitivity**
- **Our lot-specific Calibration Standards provide more accurate data analysis**

Introducing the most powerful expression profiling tool to date from BD Biosciences Clontech—the **BD Atlas™ Plastic Human 12K Microarray**. This array contains sequences from nearly 12,000 genes printed in duplicate on a plastic support surface. The unique combination of high-throughput gene expression with a plastic format promotes experimental efficiency, lower background, and accurate analysis. In addition, we print thoroughly tested long oligos on each array, which provides superior specificity and sensitivity. All these features make the BD Atlas Plastic Human 12K Microarray the best choice for your expression profiling needs.

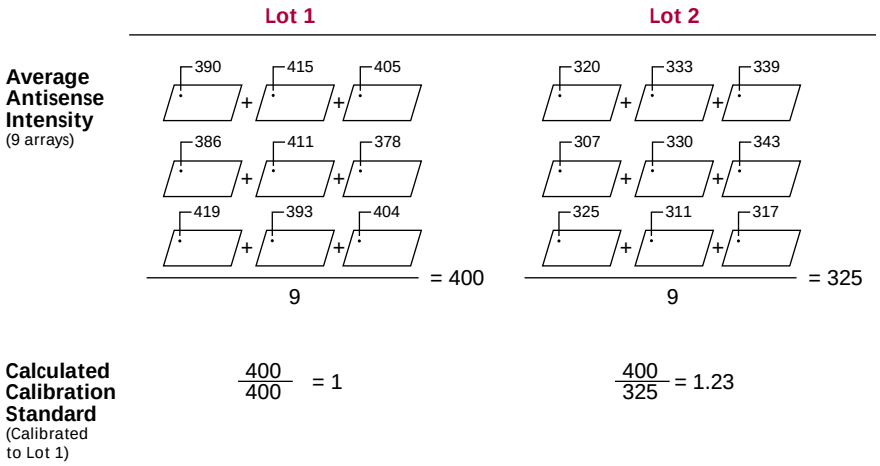
Make accurate and direct comparisons

As a unique added feature of our plastic microarrays, we calculate a Calibration Standard for every gene represented on the array. This means that you can directly compare the results of plastic microarrays from different lots and different experiments with confidence.

With each new lot of microarrays printed, several microarrays (some from the beginning, middle, and end of the printing) are hybridized using an antisense oligo calibration mixture. Following quantitation, the resulting lot-specific calibration values are averaged and listed on our web site, **www.clontech.com/atlas/atlasimage**. These values are easily imported into BD AtlasImage™ Software, which will automatically calculate standardized array signals, yielding the most accurate and meaningful array comparisons. This standardization protocol is ideal for database generation, as it allows statistically significant data to be generated from microarrays printed at different times.

Figure 1 describes the importance of array calibration to generate accurate, meaningful results. Panel A first illustrates the calculation of lot-specific Calibration Standards for a target gene. Then, two different RNA samples are analyzed for target gene expression differences using two arrays—one from each lot (Panel B). Without calibration, the target gene appears upregulated (Raw Signal, Panel B). Our practice of gene standardization demonstrates how the lot-specific value corrects for typical printing variations across lots (Calibrated Signal, Panel B). In this case, array calibration shows an insignificant difference in gene expression.

A **Calibration Procedure for Gene X**



B **Experimental Analysis of Gene X**

Sample A hybridized to array from Lot 1; Sample B hybridized to array from Lot 2

	Sample A (Lot 1 Signal Intensity)	Sample B (Lot 2 Signal Intensity)	Expression Ratio	Interpretation
Raw Signal (No Calibration)	500	350	500/350 = **1.43**	**Misleading**
Calibrated Signal (Intensity x Calibration Standard)	500 x 1 = 500	350 x 1.23 = 430.5	500/430.5 = **1.16**	**Valid**

Figure 1. More accurate expression data using calibrated BD Atlas™ Plastic Microarrays. Panel A. After printing each lot of BD Atlas Plastic Microarrays, sample arrays from the beginning, middle, and end of the printing run are hybridized with a mix of synthetic ^{33}P-labeled antisense oligonucleotides corresponding to all genes on the array. Then, the intensity of each hybridization signal is quantitated by phosphorimaging and averaged. Average antisense intensities are calculated for each gene, as shown above for hypothetical Gene X. Calibration Standards are then calculated for each array lot relative to the initial printing run. All genes in the first printed lot (Lot 1, as shown) are assigned a Calibration Standard of "1.0". **Panel B.** After normalizing arrays based on the overall signal intensities from all genes on the array, experimental intensities for Gene X can then be compared using calculations that correct for array printing variations between lots. Without this correction, gene expression comparisons are less accurate and less reliable.

By eliminating false positives generated by noncalibrated arrays, you save time for further study of real expression differences.

Depend on superior sensitivity

Of course, even a calibrated array is not an accurate tool if the printed oligos aren't reliable. That's why we rigorously develop and test our oligo sequences to ensure optimal hybridization and sensitivity. To accomplish this, we develop a long oligo for each gene on the array. Each long oligo is an 80-base DNA fragment

BD Atlas™ Plastic Human 12K Microarray...continued

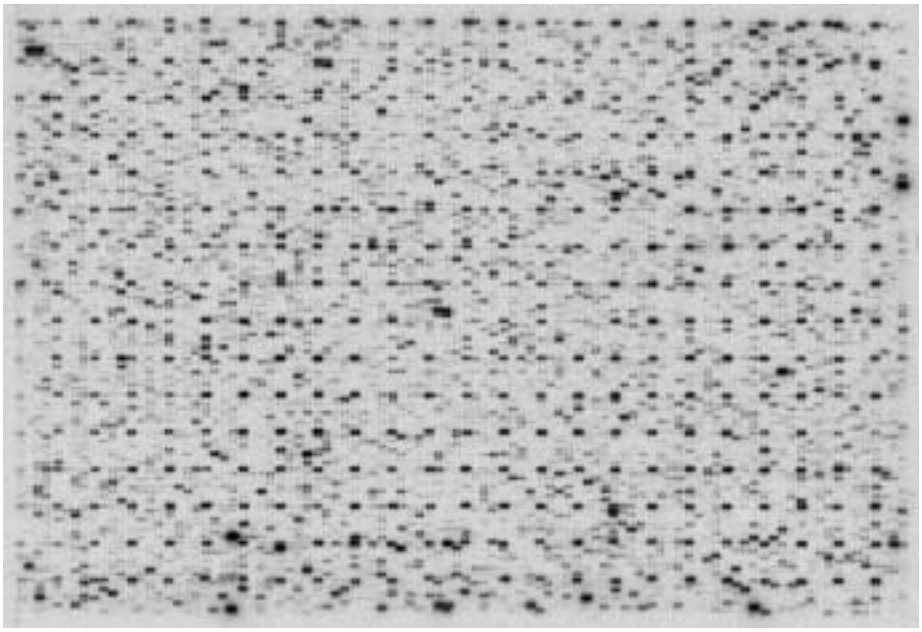


Figure 2. Using the BD Atlas™ Plastic Human 12K Microarray identifies gene expression in human colon total RNA. The array was hybridized using a ^{33}P-labeled probe generated from human colon BD™ Premium Total RNA, according to instructions outlined in the User Manual (PT3591-1).

that combines the high hybridization efficiency of a cDNA fragment with a short oligonucleotide's ability to distinguish between homologous genes. We use antisense hybridization to thoroughly test each oligonucleotide, confirming its identity and ability to produce a strong hybridization signal. Oligos that display weak hybridization signals or exhibit cross-hybridization to other fragments are redesigned. Without these tests, greater than 25% of all oligos would be incapable of producing a unique and usable hybridization signal. BD Biosciences Clontech is the only company performing this type of rigorous antisense testing, giving you a microarray that delivers credible results (Figure 2).

Take advantage of a unique format

BD Atlas Plastic Microarrays offer an unparalleled combination of ease and efficiency. Like nylon arrays, BD Atlas Plastic Microarrays require no special equipment for imaging (just a standard phosphorimager). And like glass arrays, these plastic arrays are nonporous, which greatly decreases nonspecific background and minimizes washing time. The unique quality of the plastic material allows the printing of far more spots than on a nylon membrane, and the spots are more uniform and discrete, facilitating accurate, automated analysis. The plastic support is rigid and resistant to warping at high wash temperatures, so the array does not distort and complicate image analysis. Combine these features with the easier, improved automatic grid alignment featured in our new BD AtlasImage 2.7 Software, and you get a microarray that delivers quality data in little time.

The Plastic Human 12K Microarray also furnishes you with a powerful option in data analysis. Because the gene coordinates represented on the BD Atlas™ Plastic Human 8K Microarray have been maintained on the 12K Microarray, you can easily calibrate your 12K Microarray gene signals to the corresponding values generated on the 8K Microarray. This allows you to further compare your data from 8K Microarray experiments by adding new gene expression data. With this feature, you save time by building upon existing data, instead of starting over.

Product	Size	Cat. #
BD Atlas Plastic Human 12K Microarray	2 arrays	7931-1

NEW!

Components

- 2 Plastic Human 12K Microarrays
- BD PlasticHyb Hybridization Solution
- BD Atlas™ Nucleospin® Extraction Kit
- dNTP Mix
- BD PowerScript™ Reverse Transcriptase
- BD PowerScript™ Reaction Buffer
- Random Primer Mix with Synth. Control
- DTT
- Termination Mix
- Human Placenta Control Poly A$^+$ RNA
- Deionized H_2O
- Gene List CD-ROM (PT3593-CD)
- User Manual (PT3591-1)

Related Products

- BD Atlas™ Plastic Human 8K Microarray (#7905-1)
- BD Atlas™ Plastic Mouse 5K Microarray (#7906-1)
- BD Atlas™ Plastic Rat 4K Microarray (#7909-1)
- BD Atlas™ Plastic Microarray Trial Kit (#K1845-1)
- BD AtlasImage™ 2.7 Software (#V1214-1)
- BD AtlasNavigator™ 2.0 Software (#V1221-1)
- BD Atlas™ Plastic Array Hybridization Box (#7930-1)
- BD Atlas™ Plastic Printing Kit (#K1846-1)
- BD Atlas™ Custom Plastic Arrays (#CS2050-1)
- BD Atlas™ Custom Plastic Hybridization and Analysis (#CS2013-1)
- BD AtlasImage™ Custom Analysis Service (#CS2002)

Notice to Purchaser

† Patent Pending

The BD Atlas™ Array products sold by BD Biosciences Clontech are for research purposes only. These products and the sequences of the polynucleotides thereon are intended to be used for the purchaser's own internal research purposes only and may not be used for diagnostic purposes or for human use.

BD Atlas™ Custom Array Printing Services

Design your own BD Atlas™ Array on-line and let our experts do the rest

- **Print any gene on glass, plastic, or nylon**
- **Choose from our extensive collection of human, mouse, and rat genes—or add your own**
- **Easy-to-use on-line Virtual Array Builder and Gene Search tools**

Having trouble finding a gene array to fit your needs? Maybe you just want to focus on a select set of genes. Then why not design your own expression array using our **BD Atlas™ Custom Array Printing Services**. Simply provide us with the GenBank, LocusLink, or BD Biosciences Clontech ID numbers of the genes you are interested in, and we will print the array for you.

Affordable and flexible, BD Atlas Custom Arrays are meticulously engineered to ensure accurate, reliable, and reproducible results. In fact, genes exhibiting greater than a three-fold change in expression using BD Atlas Arrays are confirmed by RT-PCR with a frequency of over 90%. Thus, custom arrays are ideal for performing new experiments or for confirming results obtained with more extensive arrays that compare thousands of genes.

Design your array on-line

You can design your custom array on-line through our Bioinformatics home page at **bioinfo.clontech.com**. Here, our BD Atlas™ Gene Search & Virtual Array Builder lets you choose from over 13,000 human, 4,000 rat, and 8,000 mouse genes, as it guides you through the design and order process (Figure 1). You can select from our extensive collection of cDNAs and long oligos or submit your own list of genes. If we do not currently have an oligo or cDNA sequence that matches your gene, we will synthesize or clone one for you. After you select the desired genes and enter any of your own, tell us which BD Atlas Array to print: nylon, glass, or plastic—our newest support, designed especially for high-density printing. Like glass, plastic arrays provide a rigid, non-porous surface that resists non-specific binding; but like nylon, they can be analyzed by phosphorimaging.* Finally, tell us how many arrays you would like printed. The price is instantly calculated.

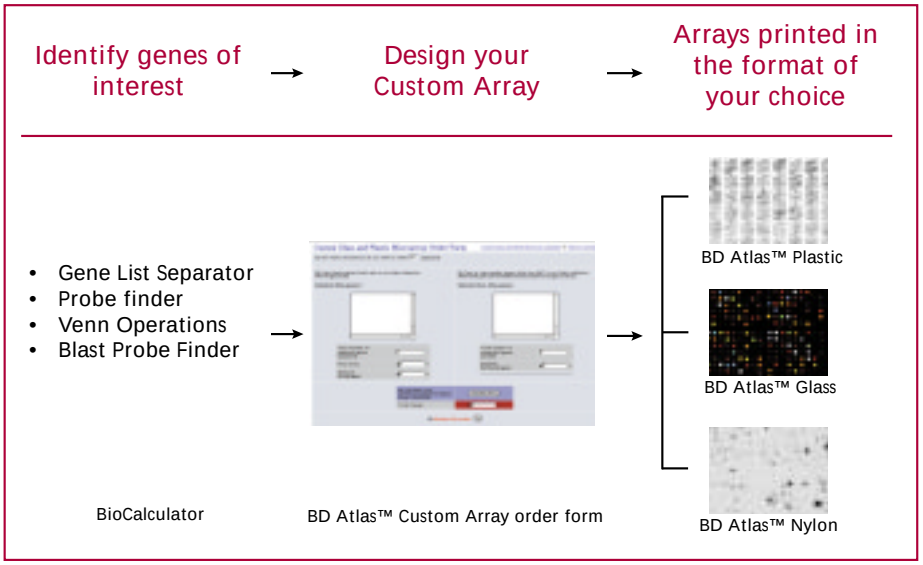


Figure 1. Designing BD Atlas™ Custom Arrays on-line. This simple flow chart illustrates the process for designing custom arrays. If you are starting with your own list of genes—in the form of GenBank, LocusLink, or Unigene Accession numbers, or DNA sequences—you may sort the list using one of our bioinformatics tools. With Venn Operations, you can even compare two lists to produce a single non-redundant list. Next, use our Gene Search and Virtual Array Builder to compile a list of unique sequences for custom printing. Finally, choose the desired surface—nylon, glass, or plastic. To order the array, submit your request on-line. A written confirmation of your order will be sent by e-mail.

Product	Size	Cat. #	Price
BD Atlas Custom Plastic Microarray	each	CS2050-1	inquire
BD Atlas Custom Glass Microarray	each	CS2003	inquire
BD Atlas Custom Nylon Array	each	TP1002	inquire

Notice to Purchaser for BD Atlas™ Products

The BD Atlas™ Array products sold by BD Biosciences Clontech are for research purposes only. Certain isolated DNA sequences included on the BD Atlas Arrays may be covered by U.S. Patents. Presently, it is not clear under U.S. laws whether commercial users must obtain licenses from the owners of the rights to these U.S. patents before using BD Atlas Arrays. These products and the sequences of the polynucleotides thereon are intended to be used for the purchaser's own internal research purposes only and may not be used for drug development or diagnostic purposes, or for human use. Using BD Atlas Glass Microarrays for dual color analysis on a single array in which at least two different samples are labeled with at least two different labels may require a license under one of the following patents: U.S. Patent Nos. 5,770,358 or 5,800,992 (Affymetrix); and U.S. Patent No. 5,830,645 (Regents of The University of California).

Getting organized: Use our bioinformatics web site to manage your gene lists

Along with the Virtual Array Builder, our Bioinformatics home page provides a complete set of tools to help you compare your list of genes to our list of cDNA fragments and long oligos. In the process, you can separate your list into unique and non-unique entries, find corresponding LocusLink and Unigene ID numbers, eliminate redundancies among two or more lists, and BLAST sequences against our database of probes. The results, displayed in text and table formats, can be pasted into the Virtual Array Builder or any other application. These on-line resources—the **Gene List Separator**, **Probe Finder**, **Venn Operations**, and **BLAST Probe Finder**—not only simplify your lists, they also identify the best probes for detecting the gene(s) of interest. The tools are free of charge and require no registration.

*See our catalog for a full comparison of the nylon, glass, and plastic formats.

To find out more about our BD Atlas™ Array products and custom services, log on to **www.clontech.com/atlas**.

Mouse Universal Reference Total RNA

Control RNA for improved microarray standardization

- **Rely on the broadest possible gene representation with minimal lot-to-lot variation**
- **Higher overall gene expression with a control made from various whole tissue sources**
- **Use with any array or labeling method**

Comparisons of your microarray data just got easier with **Mouse Universal Reference Total RNA**. Our Reference Total RNA is made by pooling the total RNA extracts from a collection of different tissues, yielding a mixture with the broadest possible gene representation available. In addition, our Reference Total RNA is produced on an industrial scale, which minimizes variation between lots. Our Reference Total RNA provides you with consistent gene coverage and great flexibility—use it for data normalization with any array and any labeling method.

Easily compare microarray results

Our Reference Total RNA allows you to compare data sets from different microarray experiments. Simply hybridize a probe made with our Reference Total RNA to a microarray each time you perform an experiment, and then normalize your data to the Reference Total RNA. Because we furnish you with enough Reference Total RNA for up to 80 microarray experiments, you can compare results over a series of experiments. Our Reference Total RNA is the best approach to building gene expression databases in which you compare expression profiles from different tissue or cell line models.

To provide you with the best overall gene representation with the least variation in gene expression, we made our Reference Total RNA using a combination of different tissue sources (Figure 1). RNA extracted from a range of different whole tissue sources is purified using our BD™ Premium RNA method. Then the RNA from each tissue is pooled, creating one master stock of high-quality, ultrapure Reference RNA that has a more even gene distribution than any individual tissue tested. We have found that RNA from whole tissues shows higher overall expression with less variation than RNA from cell lines (1). The result is an RNA reference standard that consistently provides homogenous signal intensities across the majority of genes.

Product	Size	Cat. #
Mouse Universal Reference Total RNA	2 x 200 µg	64118-1
Human Universal Reference Total RNA	2 x 200 µg	64115-1

NEW!

Reference

1. Control RNA for Microarray Experiments (April 2002) *Clontechniques* **XVII**(2):6.

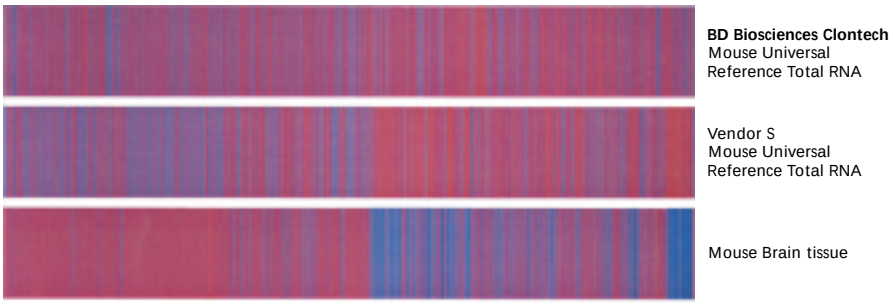


Figure 1. Mouse Universal Reference Total RNA demonstrates more than 90% gene coverage. We generated Cy-3 labeled probes using our Reference Total RNA, another vendor's reference total RNA, and RNA from mouse brain tissue. Probes were hybridized to BD Atlas™ Glass Mouse 3.8 I Microarrays (#7907-1). We analyzed the expression results using GeneSpring® Software (version 3.2.2) to cluster genes according to their expression patterns. A gene is considered expressed when its measured raw intensity is greater than or equal to 100. The red and blue colors indicate high and low expression, respectively. Varying shades of purple indicate the ratio of the intensity of any gene on each array to its median intensity across all arrays. As shown here, nearly all of the expressed genes from the mouse brain tissue were detected using our Reference Total RNA. Furthermore, among the genes detected with the Reference Total RNA, 85% had intensities greater than or equal to the intensity obtained with the hybridization of any single tissue used to prepare our Reference Total RNA (data not shown). Our results indicate that the Reference Total RNA has more than 90% gene coverage with even distribution and outperforms another vendor's RNA mixture.

Cancer Profiling Array II and Tissue-Specific Cancer Profiling Arrays

Obtain reliable gene expression data from a variety of cancer samples

- **Access many hard-to-obtain human tissues at an affordable price**
- **Identify tumor-specific markers, tumor suppressor genes, or potential drug targets**
- **Generate statistically significant data for determining gene relevance in cancer**

BD Biosciences Clontech introduces a new addition to our line of Cancer Profiling Products. The **Cancer Profiling Array II** contains 154 pairs of cDNAs generated from matched normal and tumor tissue samples from individual patients, spotted side by side on a nylon membrane. This new array includes 6 additional tissues not available on our original Cancer Profiling Array, so now you can seek tumor-specific markers in a total of 19 tumor types at once. Most of these tumor types are represented by 10 patients, allowing you to generate statistically significant data for your target gene in a single experiment. Like our other Cancer Profiling Arrays, this array is made using BD SMART™ technology and sample normalization, so you'll obtain reliable, accurate data when identifying cancer-specific expression changes, elucidating tumorigenic pathways, or recognizing potential drug targets.

You can also focus your gene expression study on 30 samples of a specific tumor type. Our **Tissue-Specific Cancer Profiling Arrays** provide the benefits of the Cancer Profiling Array II for specific tissue types, making focused expression profiling of your target gene easy and accurate. These arrays are ideal for researchers studying breast, colon, or lung cancer or for researchers who suspect their target genes are associated with these types of cancer. Since these arrays are manufactured on nylon membranes affixed to glass slides, you can perform high-throughput parallel hybridizations using a minimal amount of a standard radiolabeled probe, and then easily obtain data from multiple slides using normal phosphorimaging techniques.

Acquire tissue diversity at a low price

Eliminate the added time and expense of tissue acquisition, RNA isolation, and membrane manufacture. Our Cancer Profiling Arrays are the ideal choice for high-throughput multiple tumor analysis. With the Cancer Profiling Array II, you can proceed directly to determining your target gene's expression in a variety of tissue types representing various stages of disease. Alternatively, choose a Tissue-Specific Cancer Profiling Array to simultaneously survey the expression pattern of your gene in 30 different tumor samples and their corresponding normal tissues from individual patients. Because each matched pair of cDNAs on these arrays comes from an individual patient, you can be sure that any differential expression you see is due to actual differences between tumor and normal tissue. Pooled samples from multiple patients can mask differences in gene expression patterns between individuals and therefore are not included on our arrays. As an added benefit, we provide clinical information for samples represented on these arrays, so you can investigate possible correlations between expression and patient history.

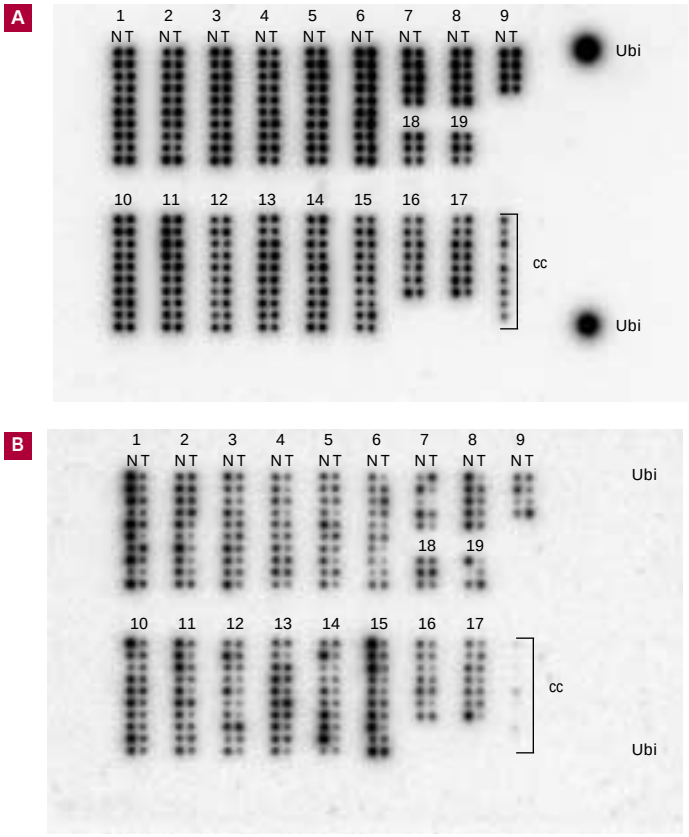


Figure 1. The Cancer Profiling Array II demonstrates tissue-specific expression of gelsolin. The Cancer Profiling Array II was hybridized separately with a radiolabeled probe for the housekeeping gene ubiquitin (**Panel A**) and a radiolabeled probe for gelsolin (**Panel B**). Hybridization signals were detected by phosphorimaging. Numbers indicate tissue types in columns. 1: breast. 2: ovary. 3: colon. 4: stomach. 5: lung. 6: kidney. 7: bladder. 8: vulva. 9: prostate. 10: uterus. 11: cervix. 12: rectum. 13: thyroid gland. 14: testis. 15: skin. 16: small intestine. 17: pancreas. 18: trachea. 19: liver. N = normal. T = tumor. Ubi = ubiquitin cDNA. cc = cancer cell line cDNAs.

Rely on accurate sample representation

Each sample cDNA on these arrays was generated from BD™ Premium RNA, which means the original starting material was pure and intact (see pages 8–9). Furthermore, the cDNA was synthesized and amplified using our patented BD SMART™ (**S**witching **M**echanism **A**t the 5' end of the **R**NA **T**ranscript) technology, which ensures that the amplified cDNA retains the original complexity and relative abundance of the tumor and

Cancer Profiling Array II and Tissue-Specific Cancer Profiling Arrays...continued

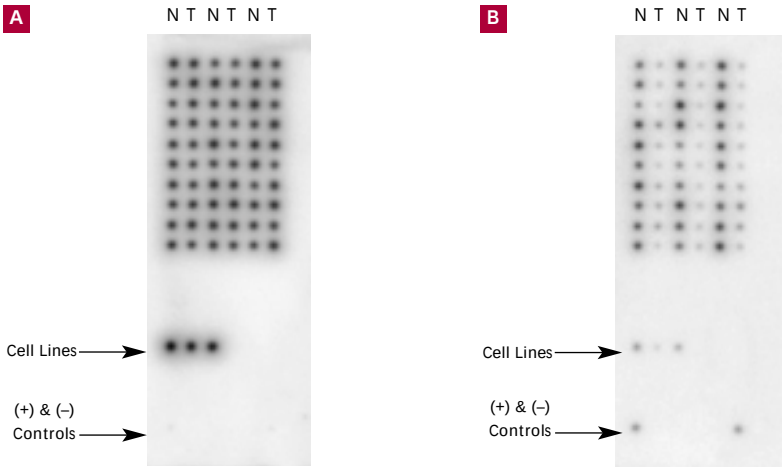


Figure 2. Differential gene expression on the Breast Cancer Profiling Array. The Breast Profiling Arrays were hybridized using a radiolabeled probe for β-actin (**Panel A**) or gelsolin (**Panel B**). Hybridization signals were detected by phosphorimaging. Thirty pairs of BD SMART™ amplified cDNAs generated from breast normal and tumor samples are spotted on the upper portion of the glass slide. The array also includes three breast cancer cell line cDNAs (MCF7, MDA-MB-231, and MDA-MB-435S). Positive controls (ubiquitin cDNA) and negative controls (yeast total RNA, yeast tRNA, *E. coli* DNA, poly A^+, human C_0t-1 DNA, and human genomic DNA) are spotted on the lower portion of the glass slide. N = normal. T = tumor.

normal RNA samples (1, 2). High quality starting materials and accurate sample representation mean you can have complete confidence in your results.

Achieve accurate expression results

All samples on these arrays are normalized to two different housekeeping genes: β-actin and ubiquitin. This normalization process is an integral part of our array quality. Normalization ensures a consistent hybridization signal for all the samples represented on the array while also assuring you that a true differential expression pattern exists for your gene of interest. You can be confident that your results are not due to variances in cDNA content between spots. Figures 1A and 2A demonstrate the uniform quality of these arrays when probed with a constitutively expressed housekeeping gene. Using the candidate tumor suppressor gene gelsolin as a probe, however, distinguishes a differential expression pattern in specific tumor types (Figures 1B and 2B).

Identify disease markers for drug discovery

You can use our Cancer Profiling Arrays to complement your cDNA or oligo microarray studies. These genomic approaches recognize the expression differences of many genes when comparing normal tissues with tumor tissues. When you have identified candidate genes that are either up-regulated or down-regulated in tumors, use our Cancer Profiling Arrays to further define these genes' roles in particular tumor types, and at particular tumor stages (3, 4). Simply generate a radiolabeled probe for your gene of interest and hybridize it to your chosen array.

Using the Cancer Profiling Arrays in this way can serve as a vital step in the identification of potential cancer drug targets. Because development is a costly endeavor, swift validation of candidate genes is essential to focusing on promising therapies. With our Cancer Profiling Arrays, you can generate statistically significant proof of a gene's relevance to cancer and to particular tumor types.

Product	Size	Cat. #
Cancer Profiling Array II	each	7847-1
Breast Cancer Profiling Array	each	7844-1
Lung Cancer Profiling Array	each	7845-1
Colon Cancer Profiling Array	each	7846-1

Components

- Cancer Profiling Array
- Hybridization Chamber (for Tissue-Specific Cancer Profiling Arrays)
- 2 Wash Containers (for Tissue-Specific Cancer Profiling Arrays)
- Human Ubiquitin Control cDNA Probe
- BD ExpressHyb™ Hybridization Solution
- Orientation Grid
- User Manual (PT3578-1)

Related Products

- Matched Tumor/Normal Expression Array (#7840-1)
- Cancer Profiling Array (#7841-1)
- Autoimmune Disease Profiling Array (#7843-1)
- Blood Disease Profiling Array (#7842-1)

References

1. Zhumabayeva, B., *et al.* (2001) *BioTechniques* **30**(1):158–63.
2. Zhumabayeva, B., *et al.* (July 2000) *Clontechniques* **XV**(3):22–23.
3. Sers, C., *et al.* (2002) *Oncogene* **21**:2829–2839.
4. Wiechen, K., *et al.* (2001) *Am. J. Pathol.* **159**:1635–1643.

Notice to Purchaser

Table I: Patient representation for each tissue type included on Cancer Profiling Array II

Tissue type	Number of samples
Breast, ovary, colon, stomach, lung, kidney, uterus, cervix, rectum, thyroid, testis, skin	10
Small intestine, pancreas	7
Bladder, vulva	5
Prostate	4
Trachea, liver	3

BD™ Premium Total RNA Contains Virtually No Genomic DNA, an Important Factor in RNA Quality

Jim Yan, Bakhyt Zhumabayeva, Ph.D., and Michael Herrler, Ph.D.

Gene Cloning and Analysis Group
BD Biosciences Clontech

In this study, we performed RT-PCR and PCR using commercially available total RNA samples and BD™ Premium Total RNA. We found that for expression applications requiring PCR, RNA integrity alone is not sufficient to generate accurate results. The degree of genomic DNA contamination in an RNA sample is an equally important determinant in generating quality data. Our results also show that BD Premium Total RNA contains intact RNA with virtually no genomic DNA.

BD™ Premium Total RNAs are high-quality RNAs useful in a variety of applications, including library construction, BD Atlas™ Array hybridizations, RT-PCR analysis, cDNA synthesis, Northern blotting, and RNase protection assays (RPAs). Each Total RNA sample is prepared using a modified guanidinium thiocyanate method, and rigorous quality control tests confirm that each preparation consists of intact, full-length RNA with virtually no genomic DNA.

Determination of RNA quality is essential to any application that utilizes RNA. RNA quality is usually confirmed by the electrophoresis of a 0.5–1 µg RNA sample on a denaturing formaldehyde/agarose/EtBr gel to check for integrity. Human total RNA samples should produce an even smear between 0.5 and 12 kb, with two bright 28S and 18S rRNA bands at approximately 4.5 and 1.9 kb, respectively. The ratio of the intensities of 28S to 18S rRNA bands should be at least 2:1. A decrease in the intensity ratio to 1:1 or a downward shift in the RNA smear indicates degraded RNA. Poor quality RNA can lead to high background or inaccurate expression results due to the absence of intact, full-length RNA.

However, another important factor in determining RNA quality is the degree of genomic DNA contamination. Genomic DNA is particularly troublesome in gene expression studies using PCR, because its presence in total RNA samples can generate positive expression data that does not truly represent actual expression levels in the RNA sample. To validate expression data in these types of experiments, it is of the utmost importance to ascertain the extent of genomic DNA contamination in the RNA starting material.

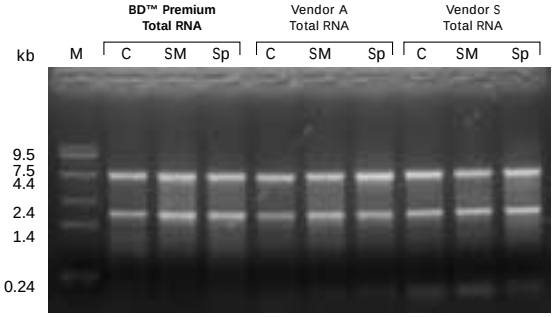


Figure 1. Total RNAs from three commercially available sources appear uniform in RNA integrity. For each sample, 1 µg of human total RNA was heated to 37°C for 2 hr, then subsequently analyzed using a denaturing formaldehyde/agarose/EtBr gel. C: colon. SM: skeletal muscle. Sp: spleen. M: 0.24–9.5 kb RNA ladder (Invitrogen, #15620-016).

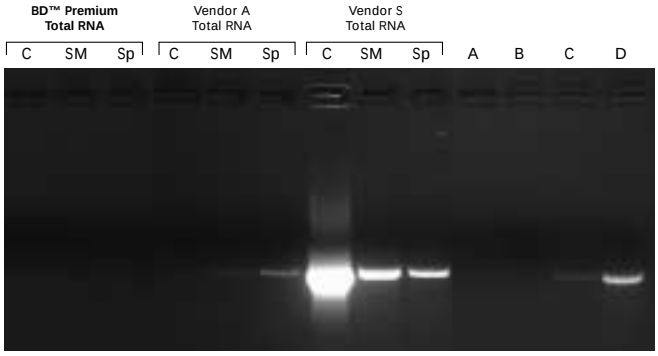


Figure 2. BD™ Premium Total RNA is free of genomic DNA. 1 µg of each human total RNA sample was used directly as a template for PCR using primers that amplify an intronic region of the MHC gene. For human genomic DNA samples, serial dilutions consisting of 1, 10, 100, and 1,000 pg were used as templates for PCR using the same primer set. Products were analyzed using agarose/EtBr gel electrophoresis. C: colon. SM: skeletal muscle. Sp: spleen. Lane A: 1 pg genomic DNA. Lane B: 10 pg genomic DNA. Lane C: 100 pg genomic DNA. Lane D: 1,000 pg genomic DNA.

Assaying for RNA quality

As a first step, we performed a routine RNA integrity test (Figure 1). All samples are fairly uniform in appearance on a denaturing formaldehyde/agarose/EtBr gel, producing bright 28S and 18S rRNA bands and an even smear between 0.5 and 11 kb. Based on this assay alone, all RNA samples contain intact RNA, and thus appear to be equal in quality.

We then investigated differences in these total RNA samples by testing for genomic DNA. A simple method for detecting genomic DNA contamination in RNA samples is to perform a standard PCR using primers designed to amplify an intronic gene region. Parallel comparison of PCR products generated from each RNA sample with those generated from serial dilutions of genomic DNA allows the estimation of genomic DNA present in RNA (Figure 2). Results indicate that total RNA samples from two other commercial sources show considerable amounts of genomic DNA. In contrast, no genomic DNA contamination could be detected in BD Premium Total RNA.

Determining genomic DNA effect

Our next step was to determine the contribution of genomic DNA contamina-

BD™ Premium Total RNA Contains Virtually No Genomic DNA, an Important Factor in RNA Quality...continued

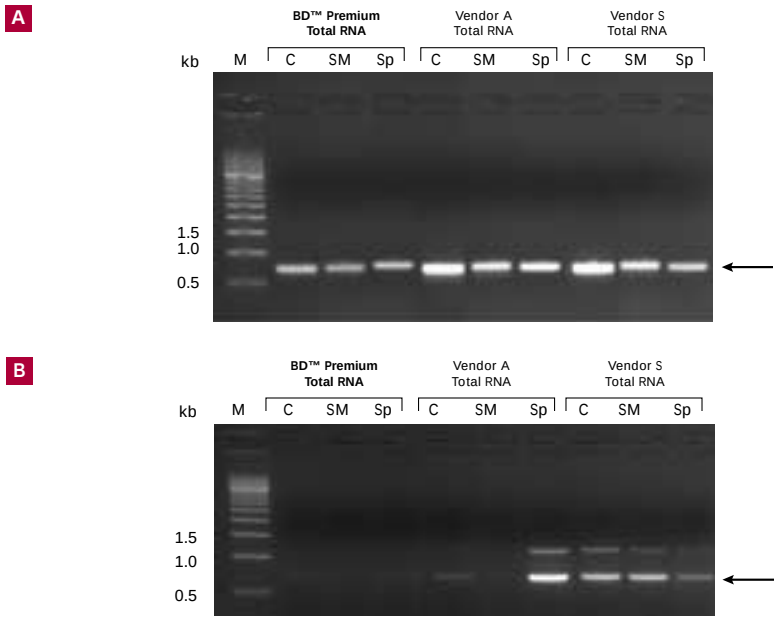


Figure 3. Positive RT-PCR results from total RNA samples containing genomic DNA. Panel A. First strand cDNA was synthesized using BD PowerScript™ Reverse Transcriptase (#8460-1) and 1 µg of total RNA. PCR was subsequently performed using an aliquot of the RT reaction with primers for a cDNA fragment of the phospholipase A2 gene. After 28 cycles, RT-PCR products were analyzed using agarose/EtBr gel electrophoresis. C: colon. SM: skeletal muscle. Sp: spleen. M: 500 bp Molecular Ruler (Bio-Rad Laboratories, #170-8203). Arrow denotes the 700-bp product. **Panel B.** Total RNA samples were used directly as a template for PCR (omitting prior RT step) using the same primer set. After 35 cycles, PCR products were analyzed using agarose/EtBr gel electrophoresis. Sample lanes are assigned as in Panel A.

tion to a standard RT-PCR experiment. We first performed a parallel RT-PCR analysis of all total RNA samples using primers that amplify a cDNA fragment of the housekeeping gene phospholipase A2 (Figure 3, Panel A). All samples generate the expected 700–bp fragment. However, a separate experiment in which all RNA samples were used directly as a template for a standard PCR (omitting the prior RT step and using the same primers as before) also produces the same fragment for some samples, thus showing evidence of genomic DNA contamination (Figure 3, Panel B). These results indicate that a portion of the product generated in the RT-PCR is in fact due to the presence of genomic DNA in the RNA starting material for some samples. Furthermore, these results suggest that genomic DNA contributes to an inaccurate representation of this gene's expression. Notably, however, BD Premium Total RNA demonstrates the least genomic DNA contamination among commercially available RNA tested in this study.

We conclude that RNA integrity testing is not sufficient to guarantee RNA quality. Genomic DNA contamination is also a critically important parameter. Although all RNA samples tested in this study are intact, without RNA degradation, these samples vary widely in their genomic DNA content. Genomic DNA contributes to a false representation of gene expression within a given RNA sample, and as shown here this type of contamination has a strong bearing on expression studies involving PCR. By extension, genomic DNA contamination would affect quantitative applications such as cDNA probe synthesis for microarrays and real-time PCR, yielding higher background and inaccurate results. Our data here show that of the three commercially available sources of total RNA, BD Premium Total RNA consists of intact RNA that is virtually free of genomic DNA, resulting in a more accurate representation of gene expression using RT-PCR.

Table I: Products that use BD™ Premium RNA

Gene Expression Analysis
BD MTN™ Blots, BD MTE™ Arrays, BD MTC™ Panels, Total RNA Panels, Cancer and Disease Profiling Arrays, Tumor/Normal Matched cDNA Pairs and Panels

Gene Cloning
BD™ Premium Total RNAs and Poly A$^+$ RNAs, BD QUICK-Clone™ cDNAs, BD™ Marathon-Ready cDNAs

Product	Size	Cat. #	Price
BD Premium Human Total RNA			
	50 µg	many	
	250 µg	many	
BD Premium Mouse Total RNA			
	250 µg	many	
BD Premium Rat Total RNA			
	50 µg	many	
	250 µg	many	

Also Available: BD™ Premium Reserve RNA

Our Premium Reserve RNA samples are isolated from extremely rare or difficult-to-obtain tissues and are available for custom packaging. More than 100 human RNAs are available, and we offer both Premium Total and Poly A$^+$ RNAs. In addition, our collection features a number of matched tumor and normal RNAs from individual patients. Because quantities are so limited, we are unable to offer these Premium Reserve RNAs through our regular catalog. For Premium Reserve RNA selection and ordering details, contact your BD Biosciences Clontech sales representative or visit **www.clontech.com/premium-rna**. Supplies are limited, so please inquire about availability.

BD In-Fusion™ PCR Cloning Kit

Precise, directional cloning of PCR products—without restriction enzymes

- **No restriction enzyme or ligase required**
- **Compatible with the BD Creator™ System for immediate expression analysis**
- **No A-overhang requirement—Use any thermostable polymerase for amplification**
- **Robust performance—easily clone up to 8 kb**

The **BD In-Fusion™ PCR Cloning Kit** is designed for fast, high-throughput cloning of PCR products without the need for restriction enzymes, ligase, or blunt-end polishing. This kit includes our proprietary BD In-Fusion Enzyme and pDNR-Dual Donor Vector for generating precise, directional constructs that are immediately ready for expression analysis with our BD Creator™ Gene Cloning & Expression System.

The BD In-Fusion™ PCR cloning method

The BD In-Fusion method consists of a simple 30 min benchtop incubation of the PCR product with the linearized pDNR-Dual Vector, followed by transformation of *E. coli* (Figure 1). Optional blue/white selection on X-Gal plates can be used to screen out rare non-linearized vector background. Although linearized pDNR-Dual is provided, the BD In-Fusion enzyme action is universal and allows cloning of PCR products into any vector.

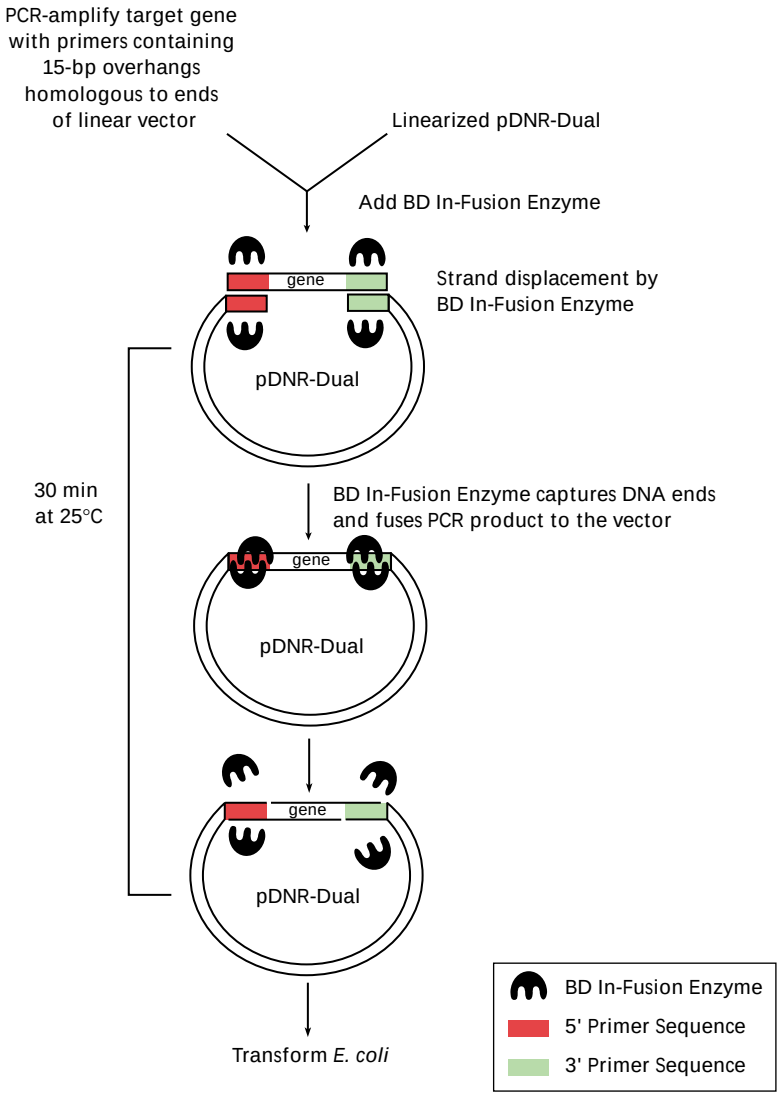


Figure 1. The BD In-Fusion™ cloning method.

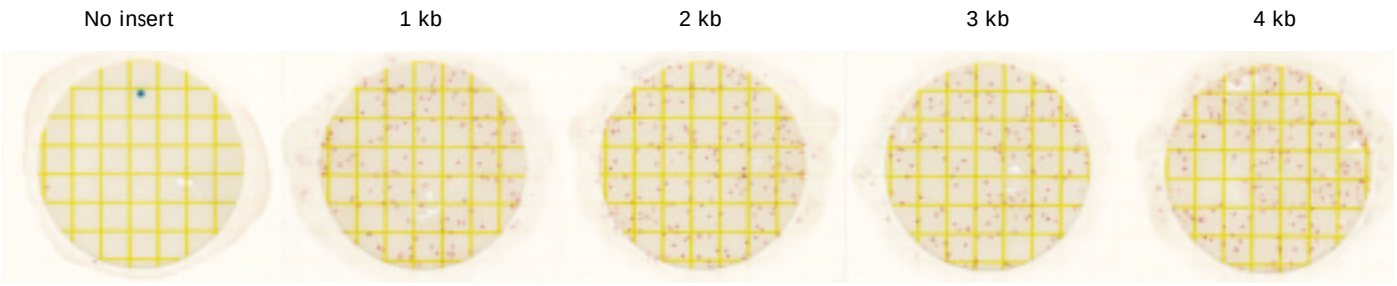


Figure 2. BD In-Fusion™ Method efficiently clones a range of insert sizes. BD In-Fusion Cloning was performed using 100 ng linear vector and 50 ng of each of the PCR products indicated. 1 µl of each 20 µl reaction was then transformed into BD Fusion-Blue™ competent cells. After 1 hr of outgrowth, 1/10 of the volume of each transformation was plated on BD CLONdisc™ plates.

BD In-Fusion™ PCR Cloning Kit...continued

Table I: BD In-Fusion™ is the most flexible and comprehensive cloning system available

	BD In–Fusion™ PCR Cloning Kit	TOPO TA cloning kits	Directional TOPO	T-A cloning
Directional	✓		✓	
Primer extension required	✓		✓	
Reaction time	**10–30 min**	5–30 min	5–30 min	Overnight
Blue-white screening	✓	Some kits		✓
Proofreading polymerase	✓	Some kits	✓	
Ligase not required	✓	✓	✓	
Gene transfer capabilities	**BD Creator™ Systems**		Gateway	None
High efficiency cloning of long fragments	✓			

In addition, virtually any PCR fragment can be cloned with this kit. The BD In-Fusion PCR cloning method does not require the presence of A-overhangs, so you can use any thermostable polymerase for amplification, including proofreading enzymes such as *Vent* and *Pfu*.

For PCR amplification we recommend our BD Advantage™ 2 Polymerase Mix (#8430-1), a robust enzyme mix that is ideally suited for long-distance (LD) PCR and has been thoroughly tested with the BD In-Fusion protocol.

BD Creator™ System

The BD In-Fusion PCR Cloning Kit makes it easy to clone and characterize products (Figure 2). After you obtain a cDNA of interest, the BD Creator System enables directional, single-step, fast, and precise transfer of genes from pDNR-Dual to any one of our Acceptor Vectors. Then our wide variety of expression systems allow you to express the gene to study protein-protein interactions, protein localization, gene expression patterns, gene function, and more.

BD Fusion-Blue™ Competent Cells

High-efficiency, competent cells optimized for use with cutting-edge cloning and expression technologies.

- One-shot transformation aliquots
- PCR cloning
- BD Creator™ recombination
- Low background
- High efficiency

Product	Size	Cat. #
BD In-Fusion PCR Cloning Kit	50 rxns	K1916-1
	100 rxns	K1916-2
BD Fusion-Blue Competent Cells	22 tubes	C5004-1

BD In-Fusion™ Kit Components

- BD In-Fusion Enzyme concentrate
- BD In-Fusion Enzyme Dilution Buffer
- 10X BD In-Fusion Reaction Buffer
- 10X BSA
- pDNR-Dual, linearized
- 1.1-kb Control Insert

Related Products

- BD Advantage™ PCR Kits (many)
- BD Sprint™ Advantage™ 96 Plate (#K1950-1)
- pLP-CMV Acceptor Vector (#8901-1)
- pLP-EYFP-C1 Acceptor Vector (#6341-1)
- pLP-EGFP-C1 Acceptor Vector (#6342-1)
- pLP-ECFP-C1 Acceptor Vector (#6343-1)
- pLP-LNCX Acceptor Vector (#6344-1)
- pLP-IRES2-EGFP Acceptor Vector (#6345-1)
- pLP-IRESneo Acceptor Vector (#6346-1)
- pLP-RevTRE Acceptor Vector (#6347-1)
- pLP-TRE2 Acceptor Vector (#6348-1)
- pLP-GADT7 AD Acceptor Vector (#6349-1)
- pLP-GBKT7 DNA-BD Acceptor Vector (#6350-1)
- pLP-CMV-Myc Acceptor Vector (#6351-1)
- pLP-PROTet-6xHN Acceptor Vector (#6352-1)
- BD Creator™ Acceptor Vector Construction Kit (#K1690-1)
- pLPS-3' EGFP Acceptor Vector (#6360-1)
- pLP-CMVneo Acceptor Vector (#6361-1)
- pLP-CMV-HA Acceptor Vector (#6362-1)
- pLP-BacPAK9 Acceptor Vector (#6211-1)
- pLP-BacPAK9-6xHN Acceptor Vector (#6212-1)

BD Creator™ BacPAK9 Shuttle Vectors

Easy preparation of baculoviral shuttle constructs via Cre-*loxP* recombination

- **BD Creator™ cloning is fast and efficient**
- **Vectors for native expression of proteins under optimal folding conditions**
- **Tagged expression vector provides easy purification with BD TALON™ Resins**

Do you need to express your protein in a baculoviral system, use vectors that eliminate complicated subcloning procedures and let you proceed directly to expression in the shortest time possible? Our new **pLP-BacPAK9** and **pLP-BacPAK9-6xHN** Vectors do just that. These BacPAK9 Shuttle Vectors are BD Creator™ Acceptor Vectors that provide efficient subcloning and compatibility with Baculoviral expression systems like our BD BacPAK™ Baculovirus Expression System (#K1601-1) and BD Biosciences Pharmingen's Baculo-Gold™ Expression System.

BD Creator™ technology ensures high-efficiency cloning

These vectors act as BD Creator Acceptor Vectors because they contain the *loxP* sequence from the P1 bacteriophage (1), instead of a multiple cloning site. In BD Creator cloning, Cre Recombinase transfers a gene of interest from any BD Creator Donor Vector into any BD Creator Acceptor Vector in just 15 minutes without restriction digestion or ligation (1). This method of subcloning is extremely efficient (Figure 1).

Quickly focus on protein expression

After transferring your gene of interest to the expression cassette of the shuttle vector, you can express the protein as part of the Baculoviral genome (Figure 2). The

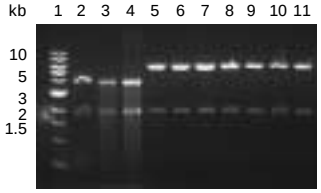


Figure 1. Efficient BD Creator™ transfer of EGFP gene from Donor Vector to pLP-BacPAK9-6xHN Acceptor Vector. Lane 1: 1-kb molecular weight marker. Lane 2: pLP-BacPAK9-6xHN Acceptor Vector digested with *Aat* II. Lane 3: Donor Vector digested with *Aat* II. Lanes 4–11: recombinants from Cre reaction digested with *Aat* II. Seven out of eight recombinants contain the correct insert.

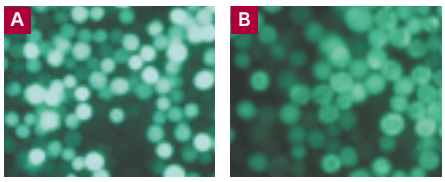


Figure 2. Expression of Enhanced Green Fluorescent Protein (EGFP) and 6xHN-tagged EGFP from BacPAK9 Shuttle Vector constructs in *Spodoptera frugiperda* (Sf21) cells. pLP-BacPAK9 and pLP-BacPAK9-6xHN were used to generate pLP-BacPAK9-EGFP and pLP-BacPAK9-6xHN-EGFP respectively by rapid transfer of the EGFP gene from a Donor Vector. These recombinant vectors were then used to make virus using our BD BacPAK™ Baculoviral Expression System (K1601-1). **Panel A.** Shown above are Sf21 cells infected with recombinant virus. pLP-BacPAK9-EGFP. **Panel B.** pLP-BacPAK9-6xHN-EGFP.

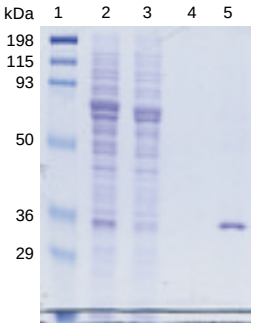


Figure 3. Purification of 6xHN-tagged EGFP from baculovirus using BD TALON™ Resin. Lane 1: markers. Lane 2: soluble lysate from Sf21 cells. Lane 3: flowthrough. Lane 4: wash with 5 mM imidazole. Lane 5: elution with 150 mM imidazole. Lysis, wash, and elution buffers all contain 20 mM Tris, pH 8.0 and 100 mM NaCl. The theoretical MW of 6xHN-EGFP is 31.2 kDa.

AcMNPV sequences flanking the *loxP* site promote recombination with baculoviral DNA to transfer the expression cassette to the polyhedrin locus of the baculoviral genome. The BD BacPAK Baculoviral Expression System has special features that promote high recombination efficiency as well as high yields of protein for a eukaryotic system (see inset).

Easy purification of 6xHN-tagged proteins with BD TALON™ Resins

You can express a protein bearing a 6xHN tag with pLP-BacPAK9-6xHN. Once this protein is expressed, it can be easily purified using BD TALON™ Resin, our patented cobalt-based immobilized metal affinity resin. (Figure 3).

Product	Size	Cat. #
pLP-BacPAK9 Acceptor Vector	20 µg	6211-1
pLP-BacPAK9-6xHN Acceptor Vector	20 µg	6212-1

NEW!

Related Products

- BD BacPAK™ Baculovirus Expression System (#K1601-1)
- BD BacPAK™ Baculovirus Rapid Titer Kit (#K1599-1)
- BD Creator™ pDNR Cloning Kit (#K1670-1)
- Cre Recombinase (#8480-1)
- BD TALON™ Metal Affinity Resin (#8901)
- BD TALON™ Superflow Resin (#8908)
- BD TALONspin™ Columns (#8902)
- BD TALON™ CellThru Resin (#8910)
- BD TALON™ CellThru Disposable Columns (#8914)
- BD TALON™ Purification Kit (#K1253-1)
- BD TALON™ 2-ml Disposable Gravity Columns (#K8903-1)
- BD TALON™ Buffer Kit (#K1252-1)

Reference

1. Sauer, B. (1994) *Curr. Opin. Biotechnol.* **5**:521–527.

BD BacPAK™ Expression System Features

- High yield compared to mammalian expression systems
- Greater similarity to naturally occurring protein due to the eukaryotic folding conditions
- High recombination efficiency due to the design of the BacPAK6 Viral DNA

New NucleoSpin® Nucleic Acid Purification Kits

Medium- and high-throughput DNA purification from Blood and Virus

- **Fast, easy protocol completed in under 30 minutes**
- **Medium- and high-throughput formats**
- **No phenol-chloroform extraction**

Introducing three new kits for medium- or high-throughput nucleic acid purification from blood and virus. The **NucleoSpin® Multi-8 Blood Kits** allow you to purify genomic DNA from 200-µl samples of whole blood, plasma, serum, or other biological fluids. The **NucleoSpin® Multi-8 Virus Kits** and **NucleoSpin® Multi-96 Virus Kits** allow you to purify viral RNA or DNA from 100-µl samples of plasma, serum, or cell-free biological fluids.

These kits are designed for fast processing of a flexible number of samples without the inconvenience of phenol-chloroform extraction. The Multi-8 format comes with strips of 8 purification columns that allow you to process n x 8 samples at once (Figure 1). The Multi-96 format includes 96-column plates that allow you to process n x 96 samples at once.

NucleoSpin® Starter Kits

Both the Multi-8 kits are offered in a starter kit format which includes additional components required when using these kits for the first time. A specially designed Tube Rack is included for holding the 8-column strips in the centrifuge. Also, Dummy Strips are provided for balancing and stabilizing the strips when fewer than 96 samples are being purified. These components can be re-used with non-starter kits.

Quick and easy protocols

The NucleoSpin protocols are designed to streamline the DNA purification process. Once the biological fluids are loaded, the purification usually takes less than 30 minutes, depending on your centrifuge.

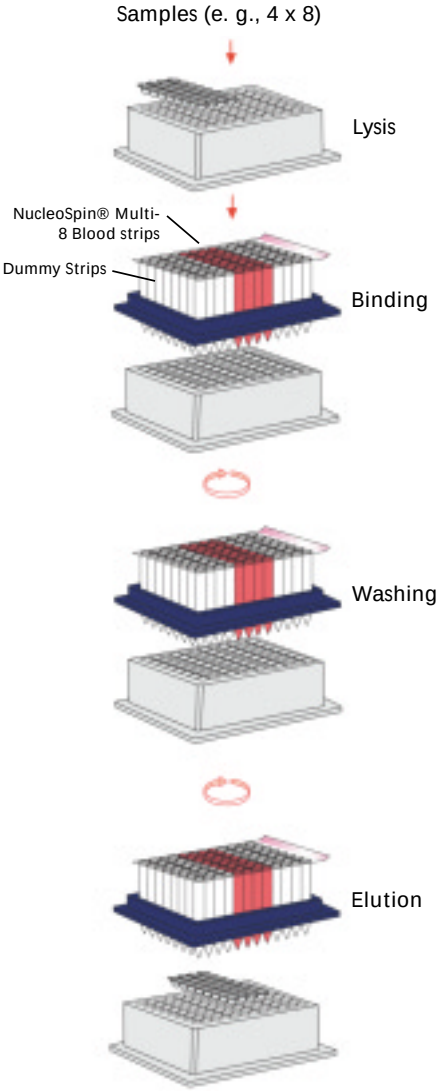


Figure 1. NucleoSpin® Multi-8 Blood purification procedure.

NEW!

NucleoSpin® Products	Size	Cat. #
Multi-96 Virus Kit	1 x 96	K3096-y
	4 x 96	K3096-1
Multi-8 Virus Starter Kit	12 x 8	K3097-1
Multi-8 Virus Kit	60 x 8	K3097-2
Multi-8 Blood/Tissue Starter Kit	12 x 8	K3098-1
Multi-8 Blood/Tissue Kit	60 x 8	K3098-2

Related NucleoSpin® Products

- Blood Mini (#K3052-1, -2)
- Blood Midi (#K3054-1)
- Blood XL (#K3095-1)
- Blood QuickPure (#K3082-1)
- Multi-96 Blood (#K3062-1)
- Virus (#K3055-1)
- Virus Midi (#K3061-1)

Notice to Purchaser

NucleoSpin® products are offered by BD Biosciences Clontech through a partnership with MACHEREY-NAGEL GmbH, Inc. a major manufacturer of products for analytical research. This partnership allows us to provide the high-quality products that you've come to expect from BD Biosciences Clontech to meet your nucleic acid purification needs. MACHEREY-NAGEL's strict adherence to quality control standards maintains the reliability and performance of these purification products.

Destabilized DsRed-Express and HcRed Vectors

Red and far-red fluorescent proteins engineered for rapid turnover

- **Detect transient changes in gene expression**
- **Develop stable cell lines**
- **Monitor multiple events simultaneously—choose from destabilized cyan, green, yellow, and red fluorescent proteins**

Our newest BD Living Colors™ vectors—**pDsRed-Express-DR** and **pHcRed1-DR**—encode destabilized variants of our red and far-red fluorescent proteins DsRed-Express and HcRed1. In contrast to the original proteins, these destabilized variants—**DsRed-Express-DR** and **HcRed1-DR**—have short half-lives, making them well suited for studies that require rapid reporter turnover. These new promoterless vectors can be used to accurately analyze *cis*-acting regulatory elements in studies of gene regulation, and may facilitate routine generation of stable transfectants.

DsRed-Express-DR and HcRed1-DR were constructed by fusing the fluorescent proteins to amino acid residues 422–461 of mouse ornithine decarboxylase (MODC), one of the most short-lived proteins in mammalian cells (1). This C-terminal region of MODC contains a PEST sequence that targets the protein for degradation, resulting in rapid protein turnover (1, 2).

Many potential applications

Because of their rapid turnover, DsRed-Express-DR and HcRed1-DR are useful as transcription reporters for measuring both the up- and down-regulation of promoter activity. For example, by placing DsRed-Express-DR or HcRed-DR under the transcriptional control of a *cis*-acting regulatory element, you can develop assays to study the induction and repression of gene expression during signal transduction (Figure 1). Similar constructs could also be designed to explore the programmed changes that occur during embryogenesis and cell differentiation. Such studies have been carried out with destabilized green fluorescent protein (3, 4). And because the fluorescence can be detected without the addition of substrates or cofactors, you can measure the events non-invasively in real time—a real advantage over other transcriptional reporters such as luciferase or β-galactosidase.

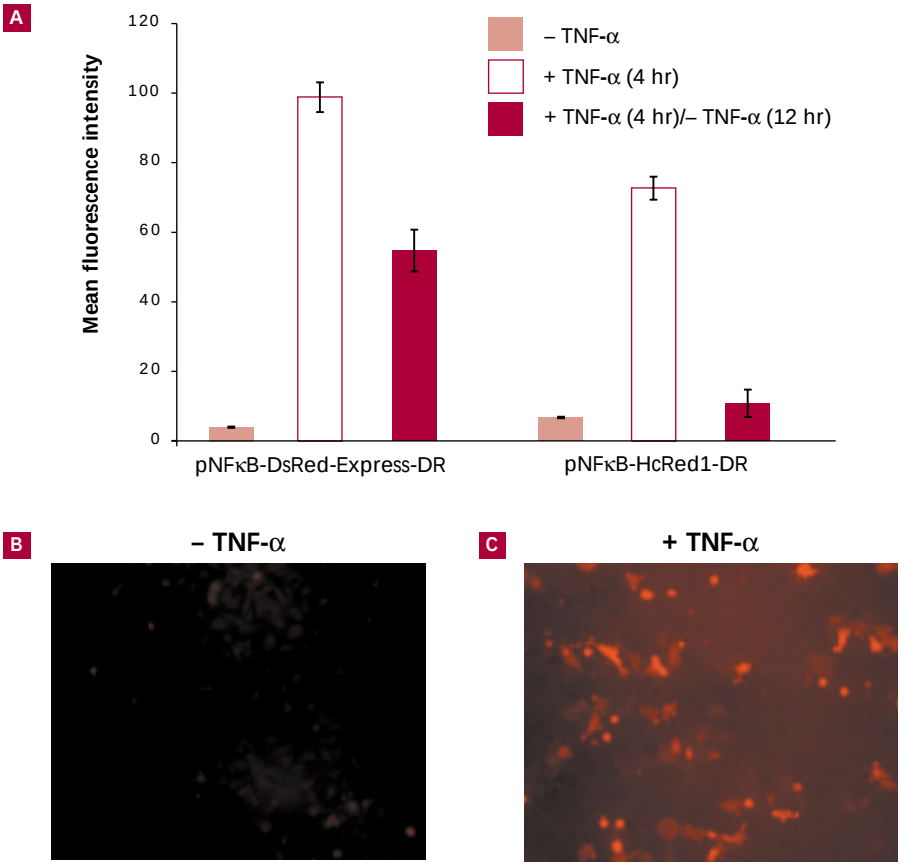


Figure 1. Destabilized red fluorescent proteins measure both the up- and down-regulation of promoter activity. Panel A: To measure the activation of NFκB—a transcription factor known to regulate several genes involved in inflammation, immune response, and apoptosis (7, 8)—the NFκB DNA response element was cloned into the MCS upstream of the fluorescent reporter gene in pDsRed-Express-DR and pHcRed1-DR. The constructs were then transiently transfected into HeLa cells. After overnight incubation, cells were analyzed by flow cytometry using a BD FACSVantage™ SE at three separate times: first to establish the baseline fluorescence; second to measure the fold induction after 4 hours of treatment with 100 ng/ml TNF-α; and third to measure down-regulation 12 hours after withdrawing TNF-α from the culture. In this example, HcRed1-DR was excited with a 568-nm laser line; DsRed-Express with a 488-nm line. **Panels B & C:** Photomicrographs of cells transiently transfected with pNFκB-DsRed-Express-DR before (**Panel B**) and after (**Panel C**) induction. The image was recorded with a Zeiss Axioskop using Chroma Technology Corp filters hq545/50X, 580dcxr, and hq630/60M.

Red fluorescence and rapid turnover heighten the sensitivity of your assays

Destabilized fluorescent proteins have clear advantages over their long-lived counterparts. First, when placed under the control of an inducible promoter, destabilized variants exhibit a higher fold-induction upon activation (Figure 1). That's because the small amount of protein expressed in the uninduced state is rapidly degraded, so the baseline fluorescence in the uninduced state is low—and the lower the baseline, the greater the sensitivity of your assay. Second, with their long-wavelength excitation maxima, destabilized *red* fluorescent proteins such as DsRed-Express-DR and HcRed1-DR eliminate the need for intense, high-energy radiation that may damage cells and tissues, and their long-wavelength emissions stand out sharply against the green autofluorescent background from media, culture ware, and cellular components. (For detailed information about the spectral properties of HcRed1 and DsRed-Express, please see References 5 and 6.)

In some kinetic assays, rapid activation is as important as rapid inactivation. Our data (Figure 1) show that these new

Destabilized DsRed-Express and HcRed Vectors...continued

transcription reporters develop fluorescence soon after induction, as expected from past studies of DsRed-Express and HcRed1, the parent proteins, whose maturation rates compare favorably to that of enhanced green fluorescent protein (EGFP; 5, 6). Similarly, when the inducer is withdrawn, the fluorescence quickly declines due to the rapid turnover of the reporter (Figure 1).

Many destabilized vectors to choose from, cyan, green, yellow—and now red

pDsRed-Express-DR and pHcRed1-DR (Figure 2) join a growing line of BD Living Colors™ cyan, green, and yellow fluorescent vectors. Like our other promoterless vectors, pDsRed-Express-DR and pHcRed1-DR contain an upstream multiple cloning site so that you can join any promoter/enhancer element to the red reporter of your choice: DsRed-Express-DR or HcRed1-DR. The coding sequence for each reporter has been human codon-optimized for efficient translation in mammalian cells. Whether viewed by fluorescence microscopy (Figure 1) or measured by flow cytometry, these reporters emit at distinctive wavelengths that are easily resolved from our cyan, green, and yellow fluorescent variants. So why not take your experiments one step further and combine reporters to monitor two or even three different events simultaneously? The tools are now at hand.

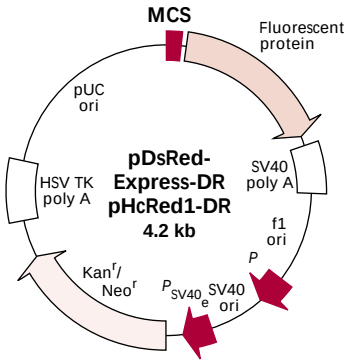


Figure 2. Plasmid map for pHcRed1-DR and pDsRed-Express-DR.

Product	Size	Cat. #
pDsRed-Express-DR Vector	20 µg	6996-1
pHcRed1-DR Vector	20 µg	8114-1

References

1. Li, X., *et al.* (1998) *J. Biol. Chem.* **273**:34970–34975.
2. Rechsteiner, M. (1990) *Sem. Cell. Biol.* **1**:433–440.
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7. Jesenberger, V. & Jentsch, S. (2002) *Nature Reviews* **3**:112–121.
8. Baldwin, A. S. (1996) *Annu. Rev. Immunol.* **14**:649–681.

Notice to Purchaser of DsRed and HcRed Products

Not-For-Profit-Entities: Orders may be placed in the normal manner by contacting your local representative or BD Biosciences Clontech Customer Service at either 800-662-2566 or 650-424-8222, extension 1. BD Biosciences Clontech grants not-for-profit research entities a worldwide, non-exclusive, royalty-free, limited license to use this product for non-commercial life science research use only. Such license specifically excludes the right to sell or otherwise transfer this product or its components to third parties. Any other use of this product will require a license from BD Biosciences Clontech. Please contact our licensing hotline by phone at either 800-662-2566 or 650-424-8222, extension 7816; or by e-mail at licensing@clontech.com.

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

Attention Drug Discovery Customers!

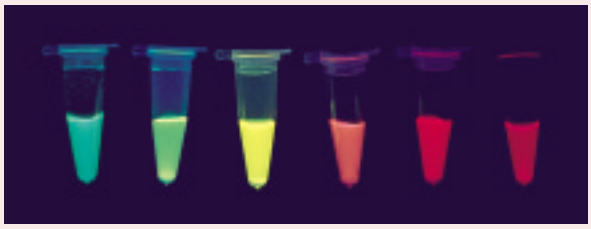

Figure 3. BD Living Colors™ Reef Coral Fluorescent Proteins under UV light. From left to right: AmCyan, ZsGreen, ZsYellow, Dsred, AsRed, and HcRed.

Are you interested in using BD Living Colors Fluorescent Proteins for your internal research? BD Biosciences Clontech offers flexible research and drug discovery licenses that provide access to a complete set of novel Reef Coral Fluorescent Proteins (RCFPs): AmCyan, ZsGreen, ZsYellow, DsRed, AsRed, and HcRed (Figure 3). Six distinct proteins, four brilliant colors—cyan, green, yellow, and three spectrally distinct reds—available exclusively to pharmaceutical and biotech companies through the **BD Living Colors™ Licensing Program**.

To learn more about how these reporters can illuminate your research, log on to the RCFP family home page at **www.clontech.com/products/families/RCFP**. While there, be sure to download a free copy of the BD Living Colors™ Licensing Program brochure, which describes all six RCFPs in vivid detail. To reach us directly, call our Licensing Hotline at 800-662-2566, extension 7816 (outside the U.S., contact your local BD Biosciences representative); or e-mail us at licensing@clontech.com.

BD Mercury™ TransFactor Profiling Kit—Oncogenesis 3

A high-throughput assay for detecting DNA-protein interactions

- **Analyze the DNA-binding activity of multiple transcription factors simultaneously with one assay**
- **Faster and more sensitive than gel-shift assays**
- **Flexible 96-well format**

A key step in the regulation of gene expression is the binding of a transcription factor to its *cis*-acting DNA response element. In the past, researchers routinely measured such activities using an electrophoretic mobility gel-shift assay (EMSA). Today, however, many are discovering that DNA-protein interactions can be measured with greater sensitivity and in shorter time using non-radioactive, 96-well plate assays developed by BD Biosciences Clontech (1, 2).

BD Mercury™ TransFactor Profiling Kits† are the new high-throughput alternative to EMSA and supershift assays. These kits provide a highly specific immunoassay for detecting and quantifying the DNA-binding of several transcription factors involved in inflammation and oncogenesis (3, 4). Our newest kit, **Oncogenesis 3**, lets you measure HIF-1α, HIF-1β, Egr-1, c/EBP, Oct I, and Oct II, adding six more entries to the long list of factors you can now profile with our ready-to-use kits (Table I).

TransFactor Profiling Kits are ideal for studying transcriptional regulation in different cell lines and tissues, and for investigating potential drug targets. Each kit contains a 96-well plate (Figure 1) for measuring the DNA-binding behavior of six different transcription factors. Individual wells have been precoated with the DNA consensus binding sequence for a specific factor. To perform an assay, add nuclear extract from mammalian cells to the wells and incubate to allow the transcription factor to bind its sequence. Wash away the unbound proteins, and add primary antibody. Then, add HRP-conjugated secondary antibody, incubate with HRP substrate, and measure the color intensity.

Figure 1. TransFactor Kits are available in individual and profiling formats. Individual Kits let you investigate a single transcription factor in depth. Profiling Kits, on the other hand, enable you to screen the DNA-binding activities of multiple factors involved in specific biological processes such as inflammation and oncogenesis. Profiling plates (shown above) are divided into six sets of color-coded wells; each set contains the cis-acting DNA element for a specific transcription factor. All TransFactor Plates consist of unique snap-off wells, so you can reconfigure the plates to fit your experimental design. You may perform all 96 reactions at once, or remove wells (individually or in strips) for use at a later time.

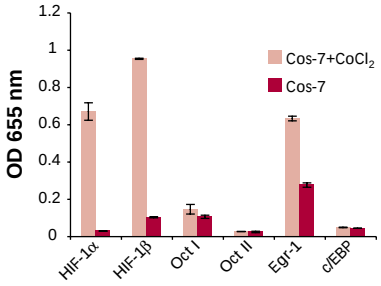

Figure 2. Transcription Factor Profiling with Oncogenesis 3. Cos-7 cells were treated with 0.15 mM $CoCl_2$ for 23 hr. Nuclear extracts were then prepared using the BD TransFactor Extraction Kit (#K2064-1), and assayed according to the protocol in the BD Mercury TransFactor Kits User Manual (PT3594-1).

TransFactor assays typically take 3–4 hours, and are 10 times more sensitive than EMSA (1, 2). The flexible 96-well format gives you the ability to compare multiple samples simultaneously (Figure 2). You can even perform competition assays to assess binding specificity and to determine the key bases in the protein-binding DNA consensus sequence.

BD Mercury™ TransFactor Profiling Kits	Size	Cat. #
Profiling Kit—Oncogenesis 1	96 rxns	K2073-1
Profiling Kit—Oncogenesis 2	96 rxns	K2075-1
Profiling Kit—Oncogenesis 3	96 rxns	K2076-1
Profiling Kit—Inflammation 1	96 rxns	K2062-1
Profiling Kit—Inflammation 2	96 rxns	K2072-1

BD Mercury™ Individual TransFactor Kits	Size	Cat. #
NFκB p50 Kit	96 rxns	K2058-1
STAT1 Kit	96 rxns	K2059-1
c-Jun Kit	96 rxns	K2061-1
c-Fos Kit	96 rxns	K2065-1
CREB-1 Kit	96 rxns	K2066-1
NFκB p65 Kit	96 rxns	K2067-1
Rb Kit	96 rxns	K2068-1
DP-1 Kit	96 rxns	K2069-1

Related Product

- TransFactor Extraction Kit (#K2064-1)

References

1. BD Mercury TransFactor Kits (January 2002) *Clontechniques* **XVII**(1):8–9.
2. Shen, Z., *et al.* (2002) *Biotechniques* **32**:1168–1177.
3. Two New BD Mercury TransFactor Profiling Kits (April 2002) *Clontechniques* **XVII**(2):20.
4. BD Mercury TransFactor Profiling Kit–Oncogenesis 2 (July 2002) *Clontechniques* **XVII**(3):15.

† Patent Pending

Table I: Transcription factors profiled by BD Mercury™ TransFactor Kits

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

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

Oncogenesis 3
HIF-1α, HIF-1β, Egr-1, c/EBP, Oct I, Oct II

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

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

Tet System Approved FBS

More options to achieve the best results

- **Functionally tested for optimal Tet induction**
- **The only choice for Tet-induced expression of toxic proteins**
- **Two options:**
  - **US-Sourced, our premium FBS**
  - **USDA-Approved, our economical alternative**

The problem: Trace tetracycline contaminants in standard Fetal Bovine Serum (FBS) can alter experimental results by enabling background expression in BD Tet-On™ Systems and suppressing maximum expression levels in BD Tet-Off™ systems.

The solution: Tet System Approved FBS from BD Biosciences Clontech, the only functionally-tested FBS approved for use with our BD Tet-On and BD Tet-Off Systems. The functional testing makes our FBS superior because it ensures that you will be able to achieve the full range of inducibility and the lowest background possible with our Tet Systems (Figure 1). Just testing for antibiotics in FBS may not detect trace levels of tetracycline and its derivatives. Trace amounts can significantly alter the inducibility of the Tet Expression Systems. Some Tet cell lines can be affected by as little as picograms/ml concentrations of tetracyclines.

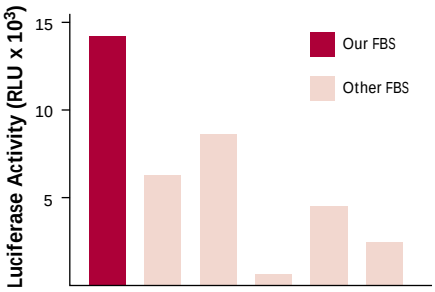

Figure 1. Serum source affects luciferase expression levels in BD Tet-Off™ Cells. BD Tet-Off Cells grown in the presence of 10% Tet System Approved FBS were compared to identical cultures grown in the presence of 10% of other lots of "antibiotic-free" FBS. In BD Tet-Off cell lines, gene expression is normally maximal in the absence of Tetracyclines (Tc). Tc levels are high enough in some lots of commercial serum to completely shut off TRE-regulated genes in a BD Tet-Off Cell Line, and to fully induce TRE-regulated genes in a BD Tet-On cell line (data not shown). RLU = relative light units.

We now offer two different versions of Tet System Approved FBS. The US-Sourced FBS is the same high-quality product that we have been supplying for years. The new USDA-Approved FBS undergoes all the same testing as US-Sourced FBS, is collected in USDA-approved facilities, and meets USDA standards for quality. Both of our Tet System approved FBS types are now available in a trial size. See for yourself the difference functional testing can make!

Product	Size	Cat. #
Tet Approved FBS, US-Sourced		
	50 ml	8630-y
	500 ml	8630-1
Tet Approved FBS, USDA-Approved		
	50 ml	8637-y
	500 ml	8637-1

NEW!

Related Products

- BD Tet-On™ System (#K1621-1)
- BD Tet-Off™ System (#K1620-1)
- BD RevTet-On™ System (#K1627-1)
- BD RevTet-Off™ System (#K1626-1)
- BD Adeno-X™ Tet-On™ System (#K1652-1)
- BD Adeno-X™ Tet-Off™ System (#K1651-1)

Coming Soon from BD Biosciences Clontech!

For more information on these products visit **www.clontech.com**.

Profile the effects of cancer treatments on gene expression

With our **Cancer Cell Line Profiling Array**, you will soon be able to quickly determine the effects of a wide variety of cancer treatments on your genes of interest. This nylon array includes cDNA samples prepared from 26 different cancer cell lines that were each treated with 26 agents, including chemotherapies, stress inducers, and radiation. Simply hybridize a radiolabeled probe to assess a gene's expression in response to treatment, to investigate its role in disease, or to predict novel gene function based on the expression profile of known genes. Eleven different tissue types are represented on the array to provide a broad sampling of different cancer types.

Ultra high-throughput PCR in a fraction of the time

Last Spring we launched our revolutionary BD Sprint™ Advantage™ 96 Plate for high-throughput PCR. Soon we will be going to the next level with our **BD Sprint™ TITANIUM *Taq* 384 Plate**. This 384-well plate provides everything you need for PCR in lyophilized form and is ideal for genotyping and SNP studies, as well as any other ultra high-throughput PCR application in which sensitivity and yield are critical. The TITANIUM *Taq* 384 Plate is compatible with all ultra high-throughput PCR machines and robotic systems. Simply resuspend the lyophilized mix with 10-µl of water containing primers and template and go directly to PCR—and results!

Please see the BD Sprint Kits Notice to Purchaser on page 1.

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