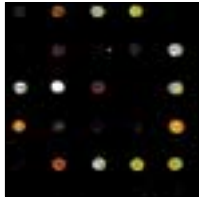


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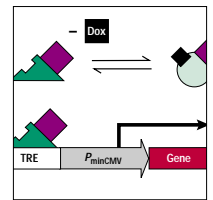
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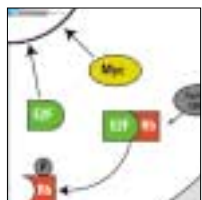
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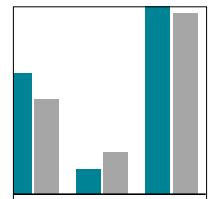
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ABOUT THE COVER

The cover shows an illustration designed for our Creator™ Gene Cloning and Expression System. The illustration was inspired by the art of Wassily Kandinsky (1866–1944).

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Atlas SMART™ Probe Amplification Kit

Accurately amplify cDNA from limited starting material

- Reproducible expression profiling from 1,000 cells or 100 µg of tissue
- Accurately maintains gene representation
- Compatible with any membrane-based array

Now you can generate radioactively labeled cDNA probes for gene arrays from as little as 50 ng of total RNA with the **Atlas SMART™ Probe Amplification Kit**. This kit uses CLONTECH's PCR-based SMART technology (Switch Mechanism At the 5' end of RNA Transcript) to amplify RNA from as little as 1,000 cells or 100 µg of tissue, while maintaining the original representation of the RNA samples. We have optimized the conditions for accurate amplification so that SMART-amplified probes produce results that are comparable to probes made from unamplified total RNA.

In developing this kit, we adapted the protocol used in CLONTECH's **SMART™ PCR cDNA Synthesis Kit** (#K1052-1) specifically for the production of highly sensitive gene array probes. Probes created with this kit can be used with Atlas™ Arrays or any membrane-based array.

From cDNA amplification to probe labeling

A major limitation of gene expression profiling using array technology has been the substantial amount of RNA required for standard probe labeling techniques. SMART amplification bypasses this problem by allowing accurate cDNA amplification from nanogram quantities of total RNA. Figure 1 outlines the process of cDNA amplification and probe labeling using the SMART Kits. The cDNA amplification step uses a modification of the SMART PCR cDNA Synthesis Kit protocol to produce full-length double-stranded cDNA from limited starting material. The Atlas SMART Probe Amplification Kit is then used to label and purify probe DNA.

Some researchers have already successfully used CLONTECH's SMART technology to amplify cDNA for array probes (1–3). We have optimized the conditions for SMART amplification,

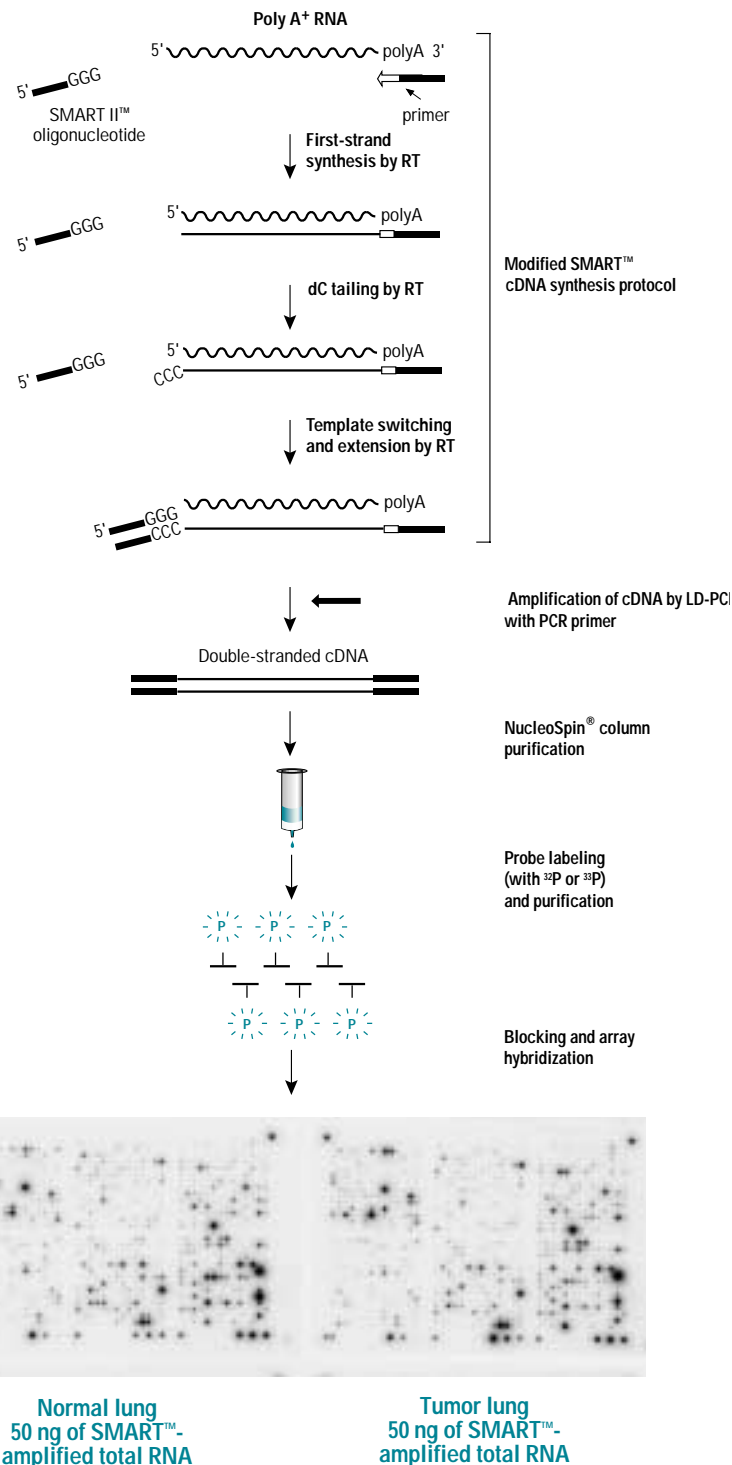


Figure 1. The Atlas SMART™ Probe Amplification Kit modifies the SMART synthesis protocol and includes reagents for probe labeling and blocking before hybridization. Go to smart.clontech.com for more information on the SMART cDNA synthesis process.

Atlas SMART™ Probe Amplification Kit...continued

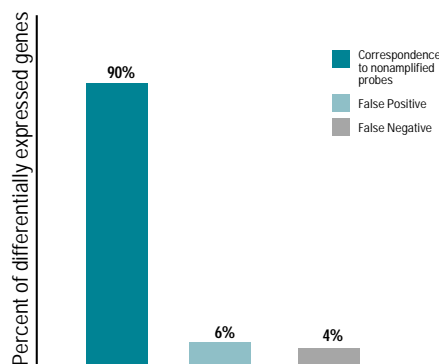


Figure 2. SMART™-amplified probes maintain the complexity and relative abundance when compared to non-amplified probes. Ninety percent of the genes reported as up- or down-regulated using SMART-amplified probes correspond to those identified using nonamplified probes.

cDNA labeling, and hybridization, and now make the procedure available to all researchers. The SMART protocol ensures that the amplified cDNA retains the complexity and relative abundance of the original RNA sample (Figure 2), thus alleviating any worries about using amplified probes for expression analysis.

Accurate representation of genes with SMART™

A common concern in any amplification procedure is that genes expressed at low levels will be lost during the amplification process. With the SMART protocol, we conservatively recommend starting with a minimum of 50 ng of total RNA, which corresponds to about 1,000 cells or 100 µg of tissue. This amount of RNA ensures that SMART amplification yields a pool of cDNA that reflects the sample's original complexity (4, 5). In fact, SMART amplification will retain even single-copy genes.

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 (5).

An additional argument against the use of amplified cDNA for gene expression analyses is that different templates amplify with different efficiencies, based on their sequences. However, because the purpose of an array hybridization experiment is to compare the relative abundance of the same target genes in multiple samples, the amplification efficiency of any given gene is equivalent in 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 arrays hybridized with amplified probes to arrays hybridized with nonamplified probes will not yield accurate results.

The SMART™ way to make array probes

A key component to the Atlas SMART Probe Amplification Kit is the SMART blocking solution, which contains DNA complementary to the SMART oligo used for first-strand cDNA synthesis. This blocking solution ensures that the SMART oligo does not contribute to non-specific hybridization.

The kit also includes a random primer mix, which allows you to generate a probe compatible with any array that uses radioactive detection. However, if you are synthesizing probes for use with our Atlas cDNA Expression Arrays, you should use the gene-specific primer mix (included with each Atlas Array) to produce probes complementary to the genes on that particular Atlas Array. Using the gene-specific primer mix will reduce probe complexity, resulting in an increase in sensitivity and an accompanying reduction in nonspecific background.

The Atlas SMART Probe Amplification Kit contains the SMART blocking solution, Klenow enzyme, dNTP mixes, labeling buffer, random primers, and two Atlas NucleoSpin® Extraction Kits for probe purification. You will also need the SMART PCR cDNA Synthesis Kit and an MMLV RNase H⁻ point mutant reverse transcriptase, such as CLONTECH's PowerScript™ Reverse Transcriptase (#8460-1, -2).

References

1. Livesey, F., et al. (2000) *Curr. Bio.* **2000**(10):301–310.
2. Spirin, K., et al. (1999) *Invest. Ophthalmol. Vis. Sci.* **40**:3108–3115
3. Endege, W., et al. (1999) *BioTechniques* **26**:542–550.
4. Chenchik, A., et al. (1998) In *Gene Cloning and Analysis by RT-PCR*, Eds. Siebert, P. & Larrick, J. (BioTechniques Books, MA), pp. 305–319.
5. Diatchenko, L., et al. (1998) In *Gene Cloning and Analysis by RT-PCR*, Eds. Siebert, P. & Larrick, J. (BioTechniques Books, MA), pp. 213–239.

See page 7 for ordering information.

Atlas™ Glass Mouse and Rat Microarrays

Now screen human, mouse, or rat genes in a glass format

- Minimal cross-hybridization
- Low background—high sensitivity
- Provides consistent results with fluorescently labeled probes
- Works with probes prepared from total or poly A⁺ RNA

CLONTECH now brings the benefits of our glass microarray technology to mouse and rat studies, with the **Atlas™ Glass Mouse and Rat 1.0 Microarrays**. Like the Atlas™ Glass Human 1.0 Microarray, the mouse and rat arrays contain 1,081 single-stranded cDNA fragments. Each sequence is carefully selected for minimal homology with other genes on the array, thus greatly reducing cross-hybridization. In addition, each sequence is individually tested in a hybridization experiment to ensure that it produces a strong signal. The genes chosen for the arrays are all well characterized and span a broad functional range.

The backbone of Atlas Glass Microarrays is our DNA-Ready™ Type II Slides (#7881-1, -2), which have a proprietary surface chemistry to uniformly bind DNA with high efficiency. On this surface, DNA forms discrete spots with negligible spot-to-spot leakage (Figure 1).

Hybridization chamber minimizes non-specific binding

Each glass microarray comes with our user-friendly Atlas™ Glass Hybridization Chamber (Figure 2; #7899-1), designed specifically for glass arrays. Experiments with this convenient screw-top chamber are easier to set up than coverslip hybridizations and also result in a more uniform hybridization with less non-specific binding. Figure 1 shows the much lower level of nonspecific binding using our hybridization chamber compared to hybridizing under a coverslip.

The chamber's 1.8-ml volume also allows you to use total RNA—an option generally not possible with coverslip hybridizations because the small volume under a coverslip makes it difficult to dissolve the amount of cDNA synthesized from total RNA.

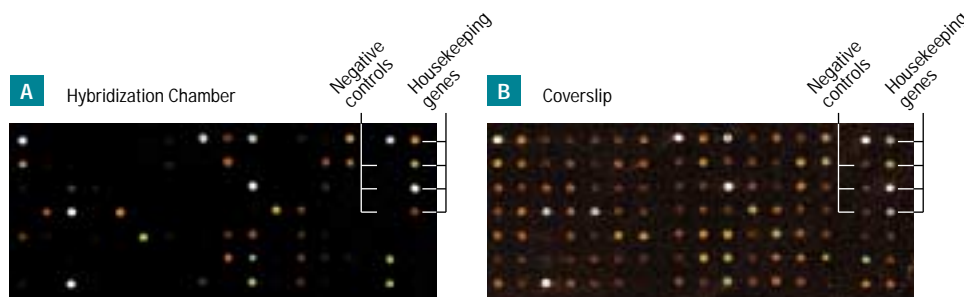


Figure 1. Signal comparison between Atlas™ Glass Hybridization Chamber and coverslip-based hybridization. We generated Cy3-labeled probes from 3 µg of human placenta poly A⁺ RNA using the Atlas Glass Fluorescent Labeling Kit (#K1037-1). The probes were hybridized to the Atlas Glass Human 1.0 Microarray using the Atlas Glass Hybridization Chamber (#7899-1; **Panel A**) or a coverslip (**Panel B**). Results were analyzed using ScanArray 3000 from GSI Lumonics, Inc. Pseudocolors reflect relative hybridization intensity. The brackets show positive and negative control spots. The array hybridized under a coverslip shows high levels of nonspecific cross-hybridization.

High sensitivity & low background

As with our Atlas nylon arrays, the glass arrays are designed to provide the highest possible signal-to-noise ratio. This high-quality signal is achieved by selecting single-stranded cDNA fragments of around 80 bases that lack repetitive elements and have minimal homology with other genes on the array.

In addition, Atlas Arrays include gene-specific primer mixes for first-strand cDNA synthesis. Probes synthesized using gene-specific primers are significantly less complex than probes generated using oligo(dT) or random primers. Using our gene-specific primers means that you are labeling only those transcripts with a corresponding gene on the array, increasing the sensitivity of your hybridization while minimizing nonspecific hybridization. The combination of our gene-specific primer mix and optimal cDNA fragment design provides the highest sensitivity and lowest background possible.



Figure 2. The Atlas™ Glass Hybridization Chamber.

Choice of detection method

You can label probes for Atlas Glass Microarrays with any fluorescent label. The glass array is compatible with microscope-based fluorescent scanners that accommodates 75 x 25 x 1 mm slides and your chosen fluorescent label.

To synthesize probes for Atlas Glass Microarrays, we recommend using the Atlas™ Glass Fluorescent Labeling Kit (#K1037-1), which generates high-quality fluorescent probes from as little as 20 µg of total RNA. CLONTECH's labeling method uses indirect dye incorporation to ensure efficient and equal labeling with fluorescent dyes, resulting in strong, reproducible signals. The kit is compatible with all fluorescent dyes that are available in N-hydroxy-succinimide reactive form, including fluorescein, rhodamine, Alexa dyes, Cy3, and Cy5.

Simplified troubleshooting

Atlas Glass Microarrays contain housekeeping genes and additional control cDNA spots for troubleshooting problems with cDNA synthesis and labeling. Two of these spots are complementary to the synthesis and labeling controls included in the Atlas Glass Fluorescent Labeling Kit.

Please visit atlas.clontech.com for the complete lists of genes on the glass arrays.

See page 7 for ordering information.

New Atlas™ Mouse Cancer 1.2 Array

Focus on genes implicated in cancer

- Powerful new tool for cancer research
- The highest signal-to-noise ratio
- The most complete murine cancer gene collection on an array

Apoptosis Oncogenes Tumor suppressors	Growth factors Cytokines	DNA damage response, repair, and recombination Cell fate and development receptors
Cell adhesion and motility Angiogenesis	Invasion regulators Cell-cell interactions	Cell-cycle regulators Growth regulators Intermediate filament markers
Controls		

Figure 1. The gene categories on Atlas™ Mouse Cancer 1.2 Array.

The Atlas™ Mouse Cancer 1.2 Array contains 1,176 unique cDNA fragments from genes known to be implicated in cancer development (Figure 1). We assembled genes for this array through literature searches and consultation with cancer researchers. As with all of our Atlas Arrays, this array provides the highest quality expression data of any array on the market. Atlas 1.2 Arrays also offer a lower cost per hybridization than other commercially available arrays, with four reusable arrays included in each purchase.

Engineered for high sensitivity & low background

Atlas Arrays are carefully designed to provide the highest possible ratio of signal to background. We select each cDNA fragment on the arrays so that it is the optimum size for hybridization, lacks repetitive elements and poly A⁺ sequences, and has minimal homology to other sequences on the array.

Atlas Arrays also include gene-specific primer mixes, which incorporate the isotopic label into

only those cDNAs represented on the array. With other methods, such as probe labeling using oligo(dT) or random priming, the probe consists primarily of sequences not represented on the array, resulting in undesirable cross-hybridization and high background.

Label probes using either ³²P or ³³P

Unlike other commercially available arrays, Atlas Arrays can be hybridized with either ³²P or ³³P. ³³P-labeled probes are ideal for generating high-quality images. In contrast, ³²P-labeled probes are more sensitive and are better suited for detection of low-abundance transcripts. With Atlas Arrays, you can choose the detection method that best suits your needs.

See page 7 for ordering information.

Atlas™ Mouse 1.2 II and Rat 1.2 II

Two new members of CLONTECH's extensive Atlas™ nylon membrane array product line give you hundreds more rat or mouse genes for broad expression studies. Atlas™ Mouse 1.2 II and Atlas™ Rat 1.2 II both have 1,176 genes containing no overlap with the earlier 1.2 versions. These arrays contain gene fragments that are carefully selected to have minimal homology, and can be hybridized with either ³²P- or ³³P-labeled probes.

Glass or Nylon?

Should you use glass or nylon arrays? Both have specific advantages (Table I). Nylon arrays hybridized with ³²P probes provide the most sensitive detection and require no special equipment. Glass microarrays, however, offer the convenience of high-resolution fluorescent detection and dual-labeling for hybridizing two probes on a single array*. The slides are also generally easier to handle than nylon membranes, making them better suited for automated, high-throughput applications. Either way, Atlas Arrays give you ready access to a wide range of human, mouse, or rat genes.

Table I: Comparison of nylon & glass arrays

Label	Nylon Membranes		Glass Slides	
	³² P	³³ P	³³ P	Fluorescence
Sensitivity	+++	++	++	+
Resolution	+	++	++	+++
Dual color	no	no	no	yes*
Multiple use	yes		no	
High-throughput	+		++	

+ Indicates relative utility and is not quantitative.

* Using Atlas™ Glass Arrays 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).

Overview of Atlas™ Products

Integrated platform of array products for expression analysis

- Full line of products for gene expression profiling
- Nylon & glass arrays available
- Choose from a wide variety of human, mouse & rat arrays

CLONTECH's Atlas™ Array product line is a platform of high-quality products—including nylon and glass arrays—for expression profiling of well-characterized genes. No other company offers the benefits of this integrated technology at such a competitive price.

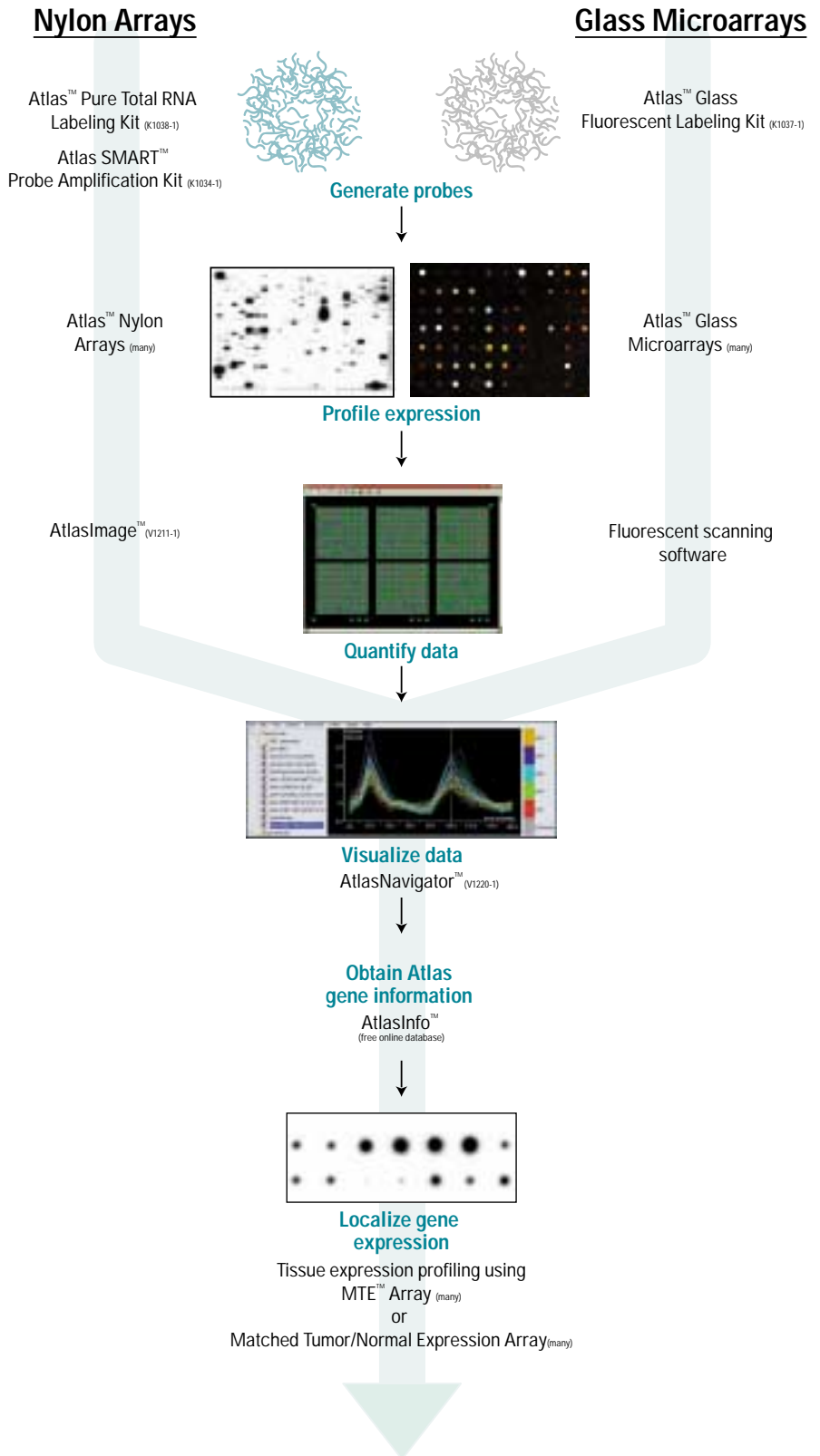
Atlas™ Arrays—affordable technology for cutting-edge research

Although Atlas Arrays provide sophisticated information, they are not expensive or difficult to use. Our nylon arrays are specifically designed to be accessible to all laboratories performing isotopic blot hybridization experiments, and are hybridized and visualized using common techniques. Now, with our new glass arrays, customers with fluorescent scanners can enjoy all the benefits of Atlas in a microarray format.

We offer two types of Atlas Arrays: broad-coverage arrays profile many crucial cellular pathways and functions in a specific species, while application-targeted arrays explore a particular research area using a comprehensive set of genes known to be involved in that process. Each Atlas order comes complete with two or four arrays to generate a differential expression profile, reagents for generating and purifying labeled cDNA probes, hybridization solution, and a User Manual.

Gene expression profiling from start to finish

Atlas Array products fit together to form an integrated, streamlined process for gene expression profiling (Figure 1). At CLONTECH, we are committed to providing a complete line of array products that will suit your needs—whatever the application.



See facing page for ordering information.

Figure 1. Atlas™ Array products take you from RNA isolation to localization of gene expression.

Custom Differential Gene Expression Services

Custom Hybridization/Analysis

Benefiting from the power of Atlas™ cDNA Arrays is even easier with our Atlas Analysis Services for premade and custom Atlas Arrays. We offer both **Atlas™ Hybridization and Analysis Service** (you provide two or more samples and we provide the phosphorimages and AtlasImage™ analysis) and **AtlasImage™ Analysis Service** (you provide a pair of matched phosphorimages and we do the analysis to indicate differential gene expression). We also offer **Custom Atlas SMART™ Probe Amplification**—see page 2 for details about the SMART process.

Custom PCR Subtraction

CLONTECH PCR-Select™ subtraction provides dramatic enrichment of differentially expressed genes in a single round of subtractive hybridization. The resulting subtracted cDNA pool is a normalized, comprehensive set of differentially expressed genes. For each sample pair submitted, we perform two subtractions (forward and reverse), resulting in two

libraries. We verify differential expression by a differential screening process and Virtual Northern analysis. We then sequence the differentially expressed clones. For more information on this service, and a detailed list of services, please contact your local Territory Manager (see back cover). For more information about the PCR-Select process, go to pcr-select.clontech.com.

Atlas™ Product Ordering Information

Atlas Glass Microarrays (# slides)	Genes per array	Cat. #
◆ Mouse 1.0 (2)	1,081	7901-1
◆ Rat 1.0 (2)	1,081	7902-1
Human 1.0 (2)	1,081	7900-1

Atlas Glass Accessory Products	Size	Cat. #
Atlas Glass Fluorescent Labeling Kit	10 rxns	K1037-1
Atlas Glass Hybridization Chamber	each	7899-1
DNA-Ready Type I Slides	5	7880-1
DNA-Ready Type II Slides	25	7880-2
DNA-Ready Type I Slides	5	7881-1
DNA-Ready Type II Slides	25	7881-2
GlassHyb Hybridization Solution	50 ml	8016-1
Atlas Glass Approved DMSO	500 µl	7898-1

Atlas Human Arrays (# membranes)	Genes per array	Cat. #
1.2 Array (4)	1,176	7850-1
1.2 Array II (4)	1,176	7852-1
1.2 Array III (4)	1,176	7855-1
Cancer 1.2 Array (4)	1,176	7851-1
Apoptosis Array (2)	205	7743-1
Cancer cDNA Expression Array (2)	588	7742-1
Cardiovascular Array (2)	588	7734-1
cDNA Expression Array (2)	588	7740-1
Cell Cycle Array (2)	111	7748-1
Cell Interaction Array (2)	265	7746-1
Cytokine/Receptor Array (2)	268	7744-1

Atlas Human Arrays (# membranes)	Genes per array	Cat. #
Hematology/ Immunology Array (2)	406	7737-1
Neurobiology Array (2)	588	7736-1
Oncogene/Tumor Suppressor Array (2)	190	7745-1
Stress Array (2)	234	7747-1
Toxicology Array II (2)	588	7733-1

Atlas Mouse Arrays (# membranes)	Genes per array	Cat. #
◆ Cancer 1.2 Array II (4)	1,176	7858-1
1.2 Array II (4)	1,176	7857-1
1.2 Array (4)	1,176	7853-1
cDNA Expression Array (2)	588	7741-1
Stress Array (2)	140	7749-1

Atlas Rat Arrays (# membranes)	Genes per array	Cat. #
1.2 Array II (4)	1,176	7856-1
1.2 Array (4)	1,176	7854-1
cDNA Expression Array (2)	588	7738-1
Stress Array (2)	207	7735-1
Toxicology Array II (2)	465	7732-1

Atlas Select Arrays (# membranes)	Genes per array	Cat. #
Human Oncogene Array (2)	578	7831-1
Human Tumor Array (4)	437	7830-1

Atlas Array Kits (# membranes)	Size	Cat. #
Atlas Human Array Trial Kit	each	K1840-1
Atlas Human 3.6 Array (6)	each	7870-1
Atlas Human 3.6 Membranes (6)	each	7871-1

Atlas Accessory Products	Size	Cat. #
◆ Atlas SMART cDNA Probe Amplification Kit ^a	each	K1034-1
SMART PCR cDNA Synthesis Kit	7 rxns	K1052-1
AtlasImage 1.5	CD-ROM	V1211-1
AtlasNavigator 1.0	CD-ROM	V1220-1
Atlas Pure Total RNA Labeling System	each	K1038-1

^a used with the SMART PCR cDNA Synthesis Kit.

Notice to Purchaser

These products were manufactured using the PCR process under license from Roche Molecular Systems and F. Hoffmann-La Roche. No license to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of these products.

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. CLONTECH products and the components thereof may not be resold, modified for resale, or used in any manner in the manufacture of commercial products without prior written approval of CLONTECH. These products were manufactured using the PCR process under license from Roche Molecular Systems and F. Hoffmann-La Roche. No license to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of this product. CLONTECH is in the process of patenting certain aspects of the Atlas technology.

The SMART™ technology is covered by U.S. Patents #5,962,271 & #5,962,272.

For complete lists of the genes on our Atlas™ Arrays, visit atlas.clontech.com.



Premium RNA™ Products



Profile gene expression with CLONTECH's Premium RNA™

- High-quality RNA ensures reliable results
- More than 4,000 Premium RNA™ citations
- Choose from a wide variety of sources and formats

CLONTECH offers an integrated platform of Premium RNA™ Products to accelerate gene cloning and expression studies (Table I). "Premium" refers to the exceptional quality and reliability of our RNAs. With Premium RNA products, you can be confident that your sample has a high percentage of full-length transcripts and that rare transcripts are present. Premium RNA products provide accurate results because our highly intact RNA preparations are representative of expression in their source tissue.

Premium Total RNA sets the standard for quality

The foundation of all Premium RNA products is highly purified total and poly A⁺ RNA. Each total RNA sample is meticulously prepared using a modified guanidium thiocyanate method, and each poly A⁺ RNA sample is enriched for mRNA transcripts with three rounds of oligo(dT)-cellulose purification. We perform rigorous quality control tests to confirm that each preparation consists of intact, full-length RNA, and we guarantee that each contains virtually no genomic DNA. You'll obtain accurate and dependable results with our entire selection of Premium RNA products.

Access hard-to-obtain tissues

CLONTECH offers Premium RNA for many tissues that require special facilities and permits to obtain. In addition, the time and cost required to prepare RNA from these tissues would be prohibitive to most laboratories. A primary benefit of using Premium RNA

products is ready access to multiple tissues at a cost much lower than it would take to isolate the RNA yourself.

Analyze gene expression patterns

We offer RNA from a range of tissues and in a variety of formats for analyzing gene expression. Our RNA Panels, Total RNA, and Poly A⁺ RNA are provided in aqueous solution, and our Multiple Tissue Northern Blots are provided as nylon membranes with lanes of electrophoresed RNA. Some of our Premium RNA products consist of cDNA made from Premium RNA, including Matched cDNA Pairs, Matched Tissue cDNA (MTC™) Panels, and the Matched Tumor/Normal Expression Array.

Visit citations.clontech.com for Premium RNA™ citations.

Table I: Premium RNA™ products and applications

Product	Application
Multiple Tissue Northern (MTN™) Blots	Analyze the transcript size, abundance, and alternative splice forms in 4–12 tissues simultaneously
Human Multiple Tissue Expression (MTE™) Array	Hybridize for high-throughput profiling of gene expression in a broad range of human tissues and cancer cell lines
Matched Tumor/Normal Expression Array	Hybridize for high-throughput profiling of gene expression in a broad range of cancer tissues and corresponding normal tissues
Matched cDNA Pairs	Perform RT-PCR with cDNA derived from individual tumor tissues and the corresponding normal tissues
Multiple Tissue cDNA (MTC™) Panels	Study a wide variety of human, mouse, and rat tissues using PCR
Total RNA Panels	Study gene expression in six different mouse, rat, or human tissues using RT-PCR or RNase protection assays (RPA)
Marathon-Ready™ cDNAs	Directly perform 5' and 3' RACE on cDNA from a variety of tissues
Human Total RNA Master Panel	Analyze gene expression in 24 different human tissues using RT-PCR or RPA
Human Tumor Total RNA	Investigate gene expression in eight human tumor tissues using Atlas™ Array hybridizations, SMART™ cDNA library construction, RT-PCR analysis, RACE, Northern blots, or RPA
Total RNA	Analyze gene expression in a broad range of human, mouse, and rat tissues using Atlas Array hybridization, SMART cDNA synthesis, or RT-PCR analysis
Poly A ⁺ RNA	Analyze gene expression using Northern blotting, RT-PCR, library construction, cDNA amplification, RACE, or RPA



Human Tumor Total RNA

Ready-to-use, high-quality tumor RNA

- Made from Premium RNA™ for accurate results
- Ideal for RT-PCR, RNase protection assays & Northern blots
- Convenient, aqueous format

CLONTECH's **Human Tumor Total RNA** lets you investigate cancer-related gene expression with the application of your choice. The eight new Human Tumor Total RNAs join Matched Tumor/Normal Expression Arrays and Matched cDNA Pairs for studying cancer-related gene expression in human tissues.

Analyze RNA from eight additional tumor tissues

CLONTECH now offers Human Tumor Total RNA from the following eight tumor tissues: **breast, cervix, colon, kidney, lung, ovary, stomach, and uterus**. Each sample is derived from a single tumor and has not been pooled. All samples undergo rigorous quality control

tests to confirm that the RNA preparations are intact and free of contaminants. We ensure that each total RNA shows clear 28S and 18S ribosomal RNA bands when electrophoresed on a denaturing agarose gel—an indication of integrity. Each sample contains 40 µg of high-quality Premium RNA™.

Wide range of applications

These high-quality RNAs can be used for a variety of applications, including SMART™ library construction, RT-PCR, cDNA synthesis, Northern blotting, and RNase protection assays. All samples are provided in aqueous format ready for immediate use.

Related Products

- Multiple Tissue Northern (MTN™) Blots (many)
- Human Total RNA Master Panels (#K4005-1)
- RNA Panels (many)
- Premium Poly A⁺ RNA (many)
- Total RNA Panels (many)
- Matched Tumor/Normal Expression Array (#7840-1)
- Matched cDNA Pairs (many)
- Atlas™ cDNA Expression Arrays (many)
- SMART™ PCR cDNA Synthesis Kit (#K1052-1)

Notice to Purchaser

These products are optimized for use in the Polymerase Chain Reaction (PCR) covered by patents owned by Hoffmann-La Roche and F. Hoffmann-La Roche Ltd. Under these patents no license to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of these products. A license to use the PCR process for certain research and development activities accompanies the purchase of certain reagents from licensed suppliers, such as CLONTECH Laboratories, Inc., when used in conjunction with an authorized thermal cycler, or is available from PE Biosystems. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at PE Biosystems, 850 Lincoln Centre Drive, Foster City, CA-94044 or Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, CA 94501.

Premium RNA™ Product Ordering Information

NEW!

Human Tumor Total RNA	Size	Cat. #
Human Breast Tumor	40 µg	64015-1
Human Colon Tumor	40 µg	64014-1
Human Cervix Tumor	40 µg	64010-1
Human Kidney Tumor	40 µg	64012-1
Human Lung Tumor	40 µg	64013-1
Human Ovary Tumor	40 µg	64011-1
Human Stomach Tumor	40 µg	64009-1
Human Uterus Tumor	40 µg	64008-1
Total RNA	Size	Cat. #
Human Adrenal Gland	250 µg	64016-1
Human Brain, whole	250 µg	64020-1
Human Brain, cerebellum	250 µg	64035-1
Human Fetal Brain	250 µg	64019-1
Human Fetal Liver	250 µg	64018-1

Total RNA	Size	Cat. #
Human Heart	250 µg	64025-1
Human HeLa Cell	250 µg	64021-1
Human Kidney	250 µg	64030-1
Human Liver	250 µg	64022-1
Human Lung	250 µg	64023-1
Human Mammary Gland	250 µg	64037-1
Human Pancreas	250 µg	64031-1
Human Placenta	250 µg	64024-1
Human Prostate	250 µg	64038-1
Human Salivary Gland	250 µg	64026-1
Human Skeletal Muscle	250 µg	64033-1
Human Small Intestine	250 µg	64039-1
Human Spleen	250 µg	64034-1
Human Stomach	250 µg	64090-1
Human Testis	250 µg	64027-1

Total RNA	Size	Cat. #
Human Thymus	250 µg	64028-1
Human Trachea	250 µg	64091-1
Human Uterus	250 µg	64029-1
Mouse Brain	250 µg	64040-1
Mouse Heart	250 µg	64041-1
Mouse Liver	250 µg	64042-1
Mouse Lung	250 µg	64043-1
Mouse Spleen	250 µg	64044-1
Mouse Testis	250 µg	64045-1
Rat Brain	250 µg	64060-1
Rat Heart	250 µg	64061-1
Rat Kidney	250 µg	64062-1
Rat Liver	250 µg	64063-1
Rat Lung	250 µg	64064-1



New Matched cDNA Pairs

Ten new cDNA pairs for convenient gene expression analysis

- New tissue source—breast
- PCR-ready for quick, sensitive analysis of cancer gene expression
- Each pair represents a unique, individual sample

With CLONTECH's **Matched cDNA Pairs** from tumor and normal tissues, you can quickly analyze gene expression in a variety of tumor and corresponding normal tissues from individual patients. Matched Pairs consist of representative first-strand cDNA carefully prepared from a single patient's tumor and normal tissue samples. These cDNA samples let you investigate putative links between specific genes, types of cancer, and tumor development stages. Matched Pairs are ready for immediate use in PCR—simply aliquot the cDNA and add your primers and PCR reagents.

Wide variety of samples for thorough gene analysis

CLONTECH now offers a total of 48 Matched cDNA Pairs from many different tumor tissues. Our ten new Matched Pairs come from breast, ovary, and lung tumors. In each Matched Pair, tumor and normal tissues come from the same individual, so differential expression is due to actual differences between tumor and normal tissue, as opposed to differences between individuals. Pooled samples from multiple patients can mask differences in gene expression patterns between individuals.

We normalize all samples to carefully selected housekeeping genes. The samples come with detailed clinical documentation that includes information regarding tumor size and stage, extent of local invasion, and sites of metastases when available.

Related Products

- Matched Tumor/Normal Expression Array (#7840-1)
- Multiple Tissue cDNA (MTC™) Panels (many)
- Atlas™ cDNA Expression Arrays (many)

Please visit premium-rna.clontech.com for a complete, up-to-date listing of available tissues and clinical sample information.

Notice to Purchaser

These products are optimized for use in the Polymerase Chain Reaction (PCR) covered by patents owned by Hoffmann-La Roche and F. Hoffmann-La Roche Ltd. Under these patents no license to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of these products. A license to use the PCR process for certain research and development activities accompanies the purchase of certain reagents from licensed suppliers, such as CLONTECH Laboratories, Inc., when used in conjunction with an authorized thermal cycler, or is available from PE Biosystems. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at PE Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404 or Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, CA 94501.

Matched cDNA Pair Ordering Information

Matched cDNA Pair	Size	Cat. #	Matched cDNA Pair	Size	Cat. #	Matched cDNA Pair	Size	Cat. #
◆ Human Breast 1	10 rxns	HP101B	Human Kidney 6	10 rxns	HP106K	Human Prostate 1	10 rxns	HP101P
◆ Human Breast 2	10 rxns	HP102B	Human Kidney 7	10 rxns	HP107K	Human Prostate 3	10 rxns	HP103P
◆ Human Breast 3	10 rxns	HP103B	Human Kidney 8	10 rxns	HP108K	Human Rectum 1	10 rxns	HP101R
◆ Human Breast 4	10 rxns	HP104B	Human Kidney 9	10 rxns	HP109K	Human Rectum 2	10 rxns	HP102R
Human Cervix 1	10 rxns	HP101X	Human Kidney 10	10 rxns	HP110K	Human Rectum 3	10 rxns	HP103R
Human Colon 2	10 rxns	HP102C	Human Kidney 11	10 rxns	HP111K	Human Small Intestine 1	10 rxns	HP101I
Human Colon 3	10 rxns	HP103C	Human Lung 1	10 rxns	HP101L	Human Stomach 1	10 rxns	HP101S
Human Colon 4	10 rxns	HP104C	Human Lung 2	10 rxns	HP102L	Human Stomach 3	10 rxns	HP103S
Human Colon 5	10 rxns	HP105C	Human Lung 3	10 rxns	HP103L	Human Stomach 4	10 rxns	HP104S
Human Colon 6	10 rxns	HP106C	◆ Human Lung 4	10 rxns	HP104L	Human Stomach 5	10 rxns	HP105S
Human Colon 7	10 rxns	HP107C	◆ Human Lung 5	10 rxns	HP105L	Human Uterus 1	10 rxns	HP101U
Human Colon 8	10 rxns	HP108C	◆ Human Lung 6	10 rxns	HP106L	Human Uterus 4	10 rxns	HP104U
Human Kidney 1	10 rxns	HP101K	Human Ovary 1	10 rxns	HP101O	Human Uterus 5	10 rxns	HP105U
Human Kidney 2	10 rxns	HP102K	Human Ovary 2	10 rxns	HP102O	Human Uterus 6	10 rxns	HP106U
Human Kidney 3	10 rxns	HP103K	◆ Human Ovary 3	10 rxns	HP103O			
Human Kidney 4	10 rxns	HP104K	◆ Human Ovary 4	10 rxns	HP104O			
Human Kidney 5	10 rxns	HP105K	◆ Human Ovary 5	10 rxns	HP105O			



Tet Products—An Overview

The most widely used inducible mammalian expression system

- Tight regulation of gene expression
- Highest fold induction
- Proven system for studying toxic proteins
- Ideal for transgenics

CLONTECH's **Tet-On™** and **Tet-Off™ Gene Expression Systems** provide tightly regulated control of gene expression that is both reversible and quantitative (1, 2). Both systems offer:

- Precise control of gene expression using doxycycline (Dox)
- Maximal expression that is far higher than that provided by the full-length constitutive CMV promoter (3, 4)
- Tight off-state for expressing toxic proteins (5, 6)
- No pleiotropy from the inducer, so only your target protein is regulated by Dox (5)

How the Tet systems work

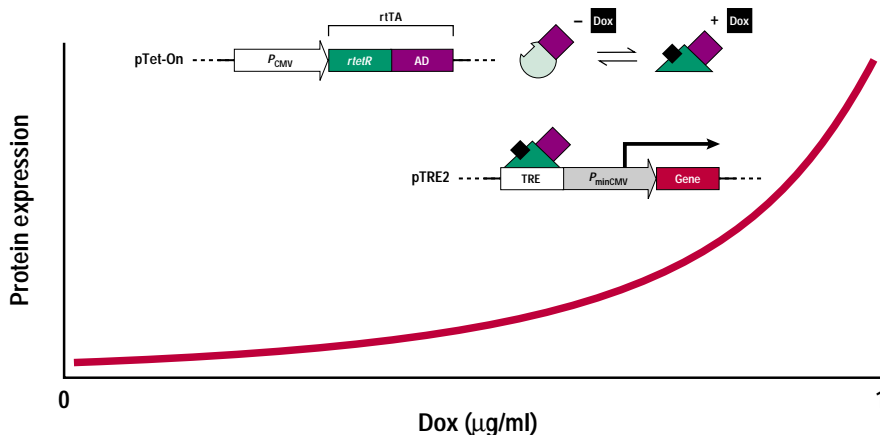
Developing Tet-On and Tet-Off Systems requires the integration of both a regulatory plasmid (pTet-On™/pTet-Off™) and a response plasmid (pTRE2; Figure 1). The pTet-Off regulatory plasmid expresses the tetracycline-controlled transactivator (tTA)—a chimeric protein composed of the Tet repressor protein (TetR) fused to the VP16 activation domain (AD) of the herpes simplex virus. The response plasmid contains a tet response element (TRE) upstream of a silent promoter driving the gene of interest. The TetR portion of the tTA binds to TRE in the absence of Tc or Dox, allowing the VP16 domain to activate transcription.

In the Tet-On System, the regulatory plasmid includes four point mutations in TetR. This change yields the reverse transactivator (rtTA), which binds TRE only in the presence of Dox—the target gene stays off until you add Dox.

Tet-On™ vs. Tet-Off™

Tet-On is ideal for studying toxic or apoptosis-inducing proteins that you want turned on rapidly at a critical point in the experiment. This system is also good for experiments in which you do not want to maintain your culture or transgenic organism with doxycycline.

Tet-On™



Tet-Off™

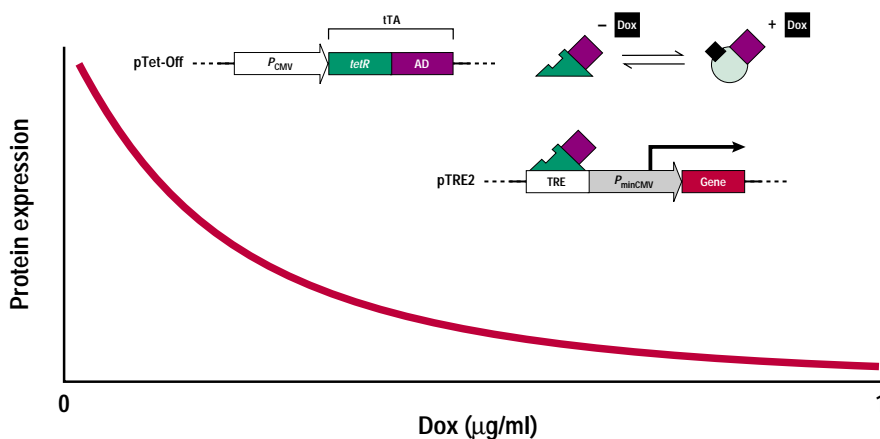


Figure 1. Tet-On™ and Tet-Off™ provide tightly-regulated, reversible gene expression.

Viral Tet Systems

The **RevTet-On™** and **RevTet-Off™ Systems** provide high-efficiency retroviral gene transfer to a range of cell types, including hard-to-transfect cell lines such as Jurkat, PC12, and primary cultures. We also offer the **Adeno-X™ Tet-Off™ Expression System** for the highest-level transient protein expression. Adeno-X Tet-Off uses adenoviral gene transfer to infect dividing or nondividing mammalian cells with nearly 100% efficiency.

See pages 12–15 for new Tet Cell Lines and Vectors.

References

1. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**:5547–5551.
2. Gossen, M., *et al.* (1995) *Science* **268**:1766–1769.
3. Yin, D. X., *et al.* (1996) *Anal. Biochem.* **235**:195–201.
4. Kanamori, H. & Shapiro, J. (1999) *BioTechniques* **26**:1018–1020.
5. Harkin, D. P., *et al.* (1999) *Cell* **97**:575–586.
6. Lee, *et al.* (1998) *Proc. Natl. Acad. Sci. USA* **95**:11371–11376.

New Tet-On™ Cell Lines

Quickly establish stable, inducible expression of your protein

- Saves time by eliminating the first clonal selection
- Screened for high inducibility & minimal background
- Ideal for use in cancer, cell cycle, & apoptosis studies
- Guaranteed *Mycoplasma*-free

CLONTECH introduces two new cell lines for use with our Tet-regulated gene expression system—U-2 OS Tet-On™ and MCF7 Tet-On™. These cell lines stably express the reverse tetracycline-controlled transactivator (rtTA) for high-level gene expression in the presence of the tetracycline-derivative doxycycline. Like all of our Tet Cell Lines, these show low background gene expression and high inducibility (Figure 1).

Save time & energy

Our Tet-On and Tet-Off™ Cell Lines save time by eliminating the need to establish stable expression of the rtTA or rTA regulatory constructs (Figure 2). This saves you as much as two months of work. To complete the tet-regulated system, you transfect these cell lines with a vector containing your gene of interest under the control of the tet inducible promoter. In these double-stable cell lines, your gene of interest is placed under tight tet regulation.

Study cancer, cell cycle, growth arrest, & apoptosis

U-2 OS Tet-On and MCF7 Tet-On are derived from cancer cell lines that have been the basis for a wide variety of studies. Our related Tet-Off versions of these cell lines expanded the range of research options by allowing researchers to tightly regulate their gene of interest. This tight regulation allows researchers to study proteins that disrupt cell cycle or induce apoptosis—options that are not possible without the ability to fully suppress the gene's transcription. Tet Cell Lines also provide an internal control by allowing you to compare a single cell line before and after induction.

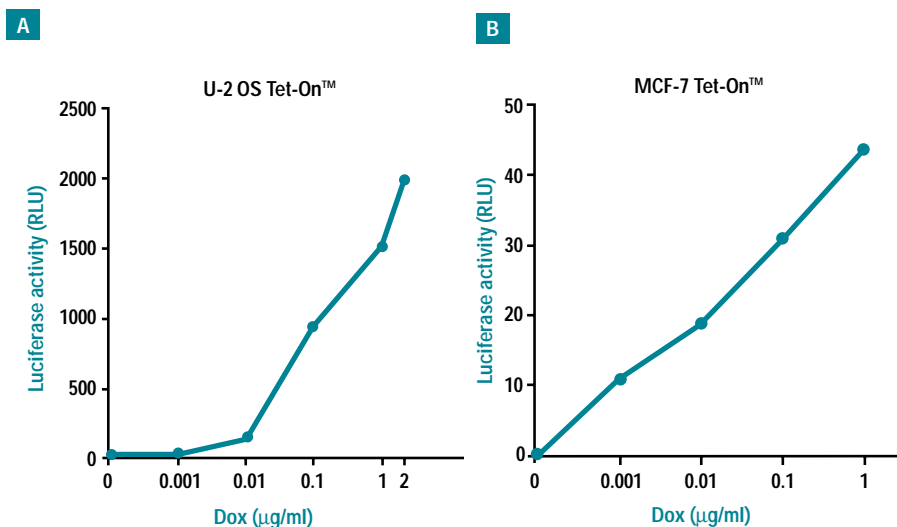


Figure 1. Tet-On™ Cell Lines show dose-dependent induction. U-2 OS and MCF7 Tet-On Cell Lines were transfected with pTRE-Luc. Cells were incubated for 48 hr in various concentrations of doxycycline. They were then harvested and assayed for luciferase activity. RLU = relative light units.

U-2 OS Tet-On is a human osteosarcoma cell line that is ideal for studying cancer and the cell cycle. It lacks the tumor suppressor gene Rb, making it possible to specifically study this pathway. Ookawa *et al.* (1) used our related osteosarcoma Tet-Off cell line Saos-2 Tet-Off*—which lacks both p53 and Rb—to study the role of Rb. They put Rb under tetracycline control and found that its expression led to an increased percentage of cells in the G₀/G₁ phase of the cell cycle and enlargement of the cells. They also found some differentiation as a result of Rb expression. This study would not be possible without regulated gene expression because an osteosarcoma cell line constitutively expressing Rb would not survive.

MCF7 Tet-On is derived from a human breast adenocarcinoma cell line. Like our Tet-Off version of this cell line, MCF7 Tet-On is ideal for studying the effects of gene expression in cancer cells. In one experiment using MCF7 Tet-Off, Fan & Bertino put *K-ras* under tetracycline control (2). When *K-ras* was expressed, they found up and down regulation of several cell cycle proteins, and an accelerated entry into S phase. Other groups have used MCF7 Tet-Off to examine the effects of overexpressing genes

that are commonly up- or down-regulated in cancer cells. In one study, Wagener *et al.* (3) found that overexpression of the apoptosis-regulator gene bax-alpha increased the cell's sensitivity to a chemotherapeutic drug, suggesting a reason why cancer cells that down regulate this protein respond poorly to chemotherapy. Another group used MCF7 Tet-Off to study the radiosensitivity of cancer cells overexpressing cyclin D1 (4).

Functionally tested

We functionally test every Tet Cell Line for high inducibility and extremely low background. Tet Cell Lines are also certified to be free of bacteria, fungus, and *Mycoplasma*, and demonstrate excellent viability. Each cell line comes with the CHO-AA8-Luc Tet-Off Control Cell Line, a User Manual (PT3001-1), and a Protocol-at-a-Glance (PT3001-2).

For other products used in studying the cell cycle, see the Mercury™ Pathway Profiling Luciferase System 4 on pages 16–17.

New Tet-On™ Cell Lines...continued

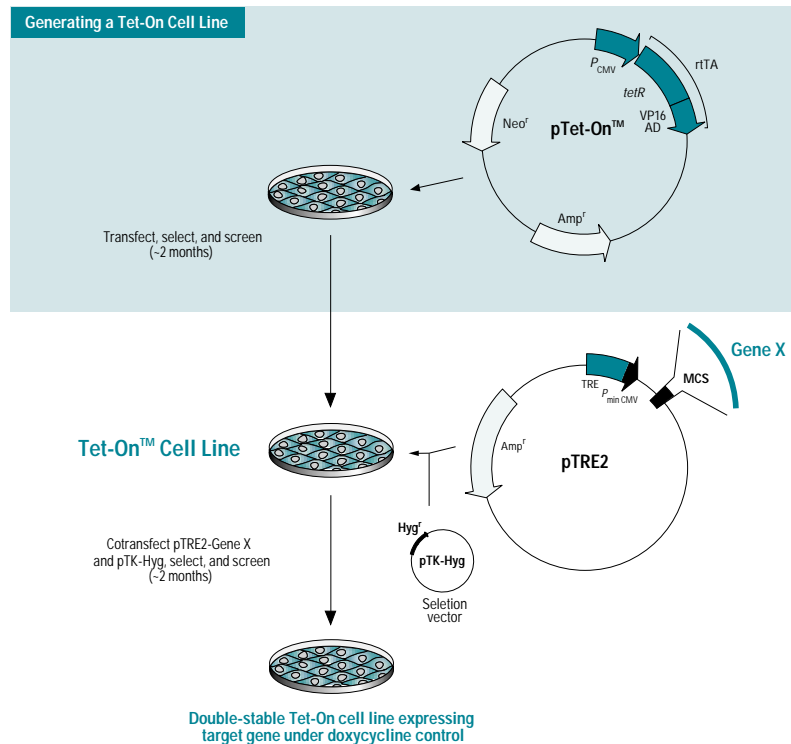


Figure 2. Premade Tet-On™ Cell Lines save you a considerable amount of work.

Tet-On Cell Line	Cell Type	Size	Cat. #
MCF7 Tet-On	Breast adenocarcinoma, human	1 ml	C3022-1
U-2 OS Tet-On	Osteosarcoma, human	1 ml	C3023-1
CHO-K1 Tet-On	Ovary, Chinese hamster, proline deficient	1 ml	C3021-1
HeLa Tet-On	Cervical epithelioid carcinoma, human	1 ml	C3000-1
Jurkat Tet-On	Acute T-cell leukemia, human	1 ml	C3019-1
PC12 Tet-On	Adrenal pheochromocytoma, rat	1 ml	C3015-1
293 Tet-On	Transformed primary embryonal kidney, human	1 ml	C3003-1
Tet-Off Cell Line	Cell Type	Size	Cat. #
CHO-AA8 Tet-Off	Ovary, Chinese hamster	1 ml	C3004-1
COS-7 Tet-Off	Transformed kidney, African green monkey	1 ml	C3011-1
HeLa Tet-Off	Cervical epithelioid carcinoma, human	1 ml	C3005-1
HeLa S3 Tet-Off	Cervical epithelioid carcinoma, human	1 ml	C3001-1
HT-1080 Tet-Off	Fibrosarcoma, human	1 ml	C3020-1
Jurkat Tet-Off	Acute T-cell leukemia, human	1 ml	C3009-1
MCF7 Tet-Off	Breast adenocarcinoma, human	1 ml	C3007-1
MDCK Tet-Off	Kidney epithelioid, canine	1 ml	C3017-1
MEF/3T3 Tet-Off	Embryonic fibroblast, mouse	1 ml	C3018-1
PC12 Tet-Off	Adrenal pheochromocytoma, rat	1 ml	C3006-1
Saos-2 Tet-Off*	Osteosarcoma, human	1 ml	C3013-1
293 Tet-Off	Transformed primary embryonal kidney, human	1 ml	C3008-1

* Saos-2 Tet-Off is hygromycin-resistant. Use pPUR (#6156-1) for selection rather than pTK-Hyg.

Related Products

- Tet-On™ Gene Expression System (#K1621-1)
- Tet-Off™ Gene Expression System (#K1620-1)
- Adeno-X™ Tet-Off™ Expression System (#K1651-1)
- pTRE2 Vector (#6241-1)
- pTRE2-d2EGFP Vector (#6242-1)
- pTRE2-HA Vector (#6249-1; see pages 14–15)
- pTRE2-6xHN Vector (#6246-1; see pages 14–15)
- pBI Tet Vector (#6152-1)
- pBI-EGFP Tet Vector (#6154-1)
- pBI-G Tet Vector (#6150-1)
- pBI-L Tet Vector (#6151-1)
- pTK-Hyg Vector (#6153-1)
- pTRE2 Sequencing/PCR Primers (#9130-1)
- TetR Monoclonal Antibody (#8632-1)
- VP16 Polyclonal Antibody (#3844-1)
- Doxycycline (#8634-1)

References

1. Ookawa, K., *et al.* (1997) *Oncogene* 14(12):1389–1396.
2. Fan, J. & Bertino, J. R. (1997) *Oncogene* 14(21):2595–2607.
3. Wagener, C., *et al.* (1996) *Intl. J. Cancer* 67(1):138–141.
4. Martin, C., *et al.* (1999) *Cancer Res.* 59(5):1134–1140.

Notice to Purchaser

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EU office: Knoll AG, Knollstrasse 50, 67061 Ludwigshafen, Germany
Fax: +49 621-589-1901

US office: BASF Bioresearch Corporation, 100 Research Drive,
Worcester, MA 01605-4314, U.S.A., Fax: +1 508-755-8508

or use our electronic licensing request form via
<http://www.knoll.de/tet/licensing/index.html>.

This product is sold under a patent sublicense for research purposes only. Licenses for commercial manufacture or use may be obtained directly from Harvard University.

New Tagged Vectors for Tet-Off™ & Tet-On™

Combine high-level regulated expression with easy detection and purification

- Affinity tags allow easy detection of positive clones
- Tight regulation, high protein expression
- Combine with the TALON™ system for purification

Two new vectors expand CLONTECH's Tet-Off™ and Tet-On™ Expression Systems. The Tet response vectors **pTRE-6xHN** and **pTRE-HA** join pTRE2 and pTRE-Myc, all of which can be used in Tet-Off or Tet-On Cell Lines for high-level tetracycline-regulated expression. The new vectors contain either an HA or 6xHN epitope tag upstream from the multiple cloning site (Figure 1). When using one of the vectors to express a protein, you can screen for your protein with antibodies against the tag—saving you the time and labor of creating a separate unique antibody for each protein.

The pTRE-6xHN vector expresses proteins tagged with six (His-Asn) repeats. If you want to purify a protein from mammalian cells in large quantity, pTRE-6xHN is optimized for use with CLONTECH's TALON™ Resin or other immobilized metal affinity column (IMAC) resins. Using TALON under mild elution conditions, you get only your pure, functional protein.

With the ability to suppress transcription, Tet expression vectors provide a built-in negative control, and can be induced to high levels of expression. When fully induced, stably transfected Tet cell lines can express proteins at levels more than 30-fold higher than when driven by the standard CMV promoter (1, 2). Like the original Tet Vectors, pTRE-6xHN and pTRE-HA provide tightly controlled gene expression when combined with a Tet-Off or Tet-On regulatory plasmid or one of the premade Tet-Off or Tet-On Cell Lines (see pages 12–13).

Tet Vector tags make protein detection easy

Using the pTRE2 sequencing primers, you can confirm that your protein is in frame with the tag. The 6xHN or HA tags fused to your protein let you easily identify inducible clones expressing your protein, investigate protein interactions by coimmunoprecipitation, and detect subcellular localization of your protein. These tags also provide a way to screen colonies directly for protein expression by Western analysis using our polyclonal antibody generated specifically against the 6xHN and HA tags. Figure 2 shows that HA fusion proteins can be detected directly from cell lysates using CLONTECH's polyclonal antibody against HA.

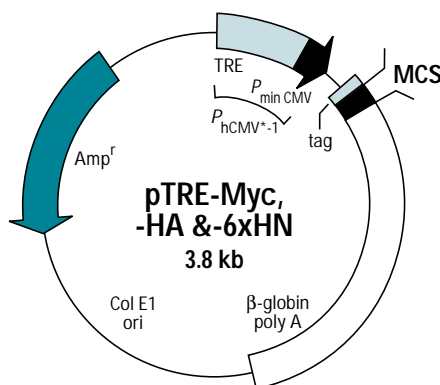


Figure 1. Map of pTRE tagged vectors. MCS = *Sfi* I, *Hind* III, *Sal* I, *Acc* I, *Not* I, *Eco* R V & *Xba* I.

Control expression of your protein

Tet Systems provide precisely regulated control of transgene expression that is reversible and quantitative. Using doxycycline, you control how much and when proteins are expressed. Figure 3 shows the tight regulation of the pTRE Vectors in a Tet-Off Cell Line—with the addition of doxycycline, luciferase expression of pTRE-6xHN-Luc and pTRE-HA-Luc drops to baseline at a concentration of less than 1 ng/ml. Figure 2 is a Western blot of the same samples as Figure 3B, again demonstrating the dose-response of pTRE-HA-Luc.

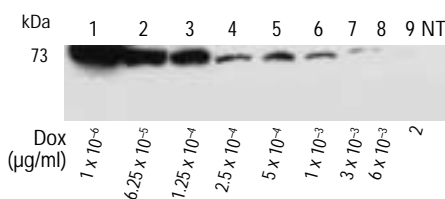


Figure 2. Detection of Tet-regulated HA-luciferase. Cell lysates from the samples used in Figure 3B were screened for protein expression by Western blot. Equal amounts of total protein for each sample were separated by SDS-PAGE, blotted onto a nylon membrane, and probed with the HA Polyclonal Antibody (#3808-1). The blot was developed using a chemiluminescent detection system. NT = nontransfected.

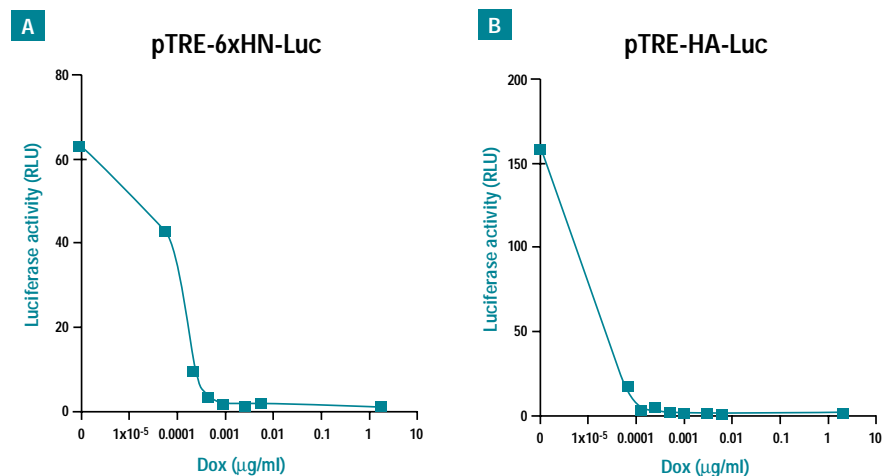


Figure 3. The pTRE-HA and pTRE-6xHN Vectors provide highly inducible gene expression. HeLa Tet-Off cells were transiently transfected with pTRE-6xHN-Luc (Panel A) or pTRE-HA-Luc (Panel B) and grown for 48 hr in the presence of doxycycline in a range of concentrations. Cells were lysed and assayed for luciferase activity. RLU = relative light units.

New Tet Vectors...continued

TALON™ for Purifying 6xHN-Tagged Proteins

TALON™ is a cobalt IMAC resin designed for purification of polyhistidine-tagged proteins like 6xHN (3–5). It is compatible with many commonly used buffers, and allows protein purification under native or denaturing conditions—with virtually no loss of metal, allowing TALON Resin to be reused.

With its minimal metal leaching, high resolution, and low background, TALON overcomes many of the problems associated with other IMAC resins. TALON Resin also requires fewer washes and uses milder elution conditions. Unlike other IMAC resins, TALON has a remarkably high affinity for His-tagged proteins, and a very low affinity for other proteins (Figure 4). In most cases, the binding properties of the cobalt in TALON allow protein purification without the use of a separate wash solution, under pH conditions that protect protein integrity.

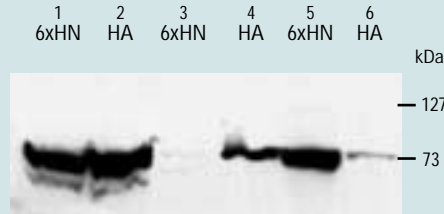


Figure 4. Purification of 6xHN-luciferase using TALON™ Metal Affinity Resin. Total cell lysate from cells expressing either HA-tagged luciferase (pTRE-HA-Luc) or 6xHN-tagged luciferase (pTRE-6xHN-Luc) were bound for 1 hr to 100 µl of TALON Resin (#8901-1) equilibrated with 20 mM MOPS, pH 7.0, 0.2M NaCl. The beads were then washed extensively with the same buffer containing 20 mM imidazole. The beads were suspended in loading buffer containing SDS, centrifuged, and the supernatant was loaded on an SDS-PAGE gel, and analyzed by Western blot using an anti-luciferase polyclonal antibody. Lanes 1 & 2: cell extract. Lanes 3 & 4: supernatant after binding and washing. Lanes 5 & 6: material bound to TALON beads.

Tet Vectors	Size	Cat. #
pTRE-HA Vector	20 µg	6249-1
pTRE-6xHN Vector	20 µg	6246-1
pTRE-Myc Vector	20 µg	6247-1
pTRE2 Vector	20 µg	6241-1
pTRE2 Sequencing/PCR Primers	100 rxns	9130-1

TALON Products	Size	Cat. #
TALON Metal Affinity Resin	10 ml	8901-1
	25 ml	8901-2
	100 ml	8901-3
	250 ml	8901-4
TALON Superflow Metal Affinity Resin	25 ml	8908-1
	100 ml	8908-2
TALONspin Columns	10 col.	8902-1
	25 col.	8902-2
	50 col.	8902-3
	100 col.	8902-4
TALON CellThru	10 ml	8910-1
	100 ml	8910-2
TALON 2-ml Disposable Gravity Columns *	50 col.	8903-1
TALON Buffer Kit	each	K1252-1
TALON Purification Kit	each	K1253-1
CellThru 2-ml Disposable Columns *	50 col.	8914-1
CellThru 10-ml Disposable Columns *	20 col.	8915-1

Fetal Bovine Serum	Size	Cat. #
Tet System Approved Fetal Bovine Serum	500 ml	8630-1

* These columns contain no resin.

Tet System Approved FBS

Tetracycline-free fetal bovine serum

- Each lot pretested for optimal results with Tet Systems
- Eliminates interference from residual tetracycline or its derivatives

Tet System Approved Fetal Bovine Serum helps ensure the success of your Tet experiments by enhancing the tight regulation of the Tet System. Due to the sensitivity of the Tet System, residual tetracyclines in serum can severely inhibit the full range of induction—even picogram amounts of tetracyclines can decrease maximum expression levels in the Tet-Off System and increase background expression levels in the Tet-On System.

Many lots of commercially available FBS contain trace amounts of tetracyclines because of the widespread use of this antibiotic in cattle. Quality controls on serum include assaying for antibiotic activity of tetracyclines; however, a tetracycline that shows no antibiotic activity can still bind to the (r)TetA and regulate gene expression. Thus, serum considered tetracycline-free can still affect the Tet Systems.

Tet System Approved FBS is high-quality fetal bovine serum that has been functionally tested to ensure that it permits the full range of tetracycline-regulated induction with well-characterized Tet cell lines. The use of Tet System Approved FBS allows the highest possible level of induction.

Related Products

- HA-Tag Polyclonal Antibody (#3808-1)
- 6xHN Polyclonal Antibody (#8940-1)
- c-Myc Monoclonal Antibody (#3800-1)
- c-Myc Monoclonal Antibody-Agarose Beads (#3843-1)
- Tet-On™ & Tet-Off™ Gene Expression Systems (#K1621-1 & #K1620-1)
- Tet-On™ & Tet-Off™ Cell Lines (many; see page 13)
- pTet-Off™ Vector (#K1620-A)
- pTet-On™ Vector (#K1621-A)

References

1. Yin, D., *et al.* (1996) *Anal. Biochem.* 235: 95–201.
2. Kanamori, H. & Shapiro, J. (1999) *BioTechniques* 26:1018–1020.
3. Chaga, G. S., *et al.* (1999) *Biotechnol. Appl. Biochem.* 29(1):19–24.
4. Porath, J. (1992) *Protein Express. Purif.* 3:263–281.
5. Yang, T., *et al.* (1997) *Amer. Biotechnol. Lab.*, pp. 12–14.

Notice to Purchaser

See page 13 for the Tet Systems licensing statement.

Mercury™ Cell Cycle Profiling System

Study the activation of cell cycle enhancer elements *in vivo*

- Analyze four distinct cell cycle regulation pathways
- Quantify change in pathway activity
- Save time with a quick & easy *in vivo* system

The **Mercury™ Pathway Profiling Luciferase System 4** provides a simple and effective way to study cell cycle regulation *in vivo*. The system includes a set of four vectors that each contain a distinct *cis*-acting enhancer element—responsive to the Rb, E2F, Myc, or p53 proteins—upstream from the luciferase reporter gene. When a pathway leading to one of these four proteins is active, the transactivator binds the *cis*-acting enhancer element in the vector and activates luciferase transcription. In this way, you can study the effects of different drugs, stimuli, or cotransfected genes on a given pathway by assaying for luciferase expression.

Luciferase System 4 is the latest in our line of Mercury Pathway Profiling Kits. Our SEAP system and four luciferase systems provide *cis*-acting reporter vectors for studying 13 different signaling pathways, including vectors for the AP1, CRE, GRE, SRE, and NFκB pathways (Table I). Together, they give you a sensitive system for detecting activation of pathways involved in key research areas, such as cancer, apoptosis, stress, and cell growth (Table II).

The cell cycle

Figure 1 shows the four stages of the cell cycle for normal cells undergoing division: G₁ (gap 1), S (synthesis), G₂ (gap 2), and M (mitosis). Some cells also enter a nondividing G₀ phase. Molecular checkpoints regulate when the cell progresses from G₁ to S phase or from G₂ to M phase. These checkpoints are tightly regulated by cell cycle proteins such as cyclins, cyclin-dependent kinases (CDKs), and other proteins, including Rb, E2F, Myc, and p53.

Key pathways for cell cycle, cancer, or apoptosis research

The vectors included in the Mercury Pathway Profiling Luciferase System 4 allow you to quantitatively assay for the activity of Rb, E2F, Myc, or p53 in your system of interest. They provide

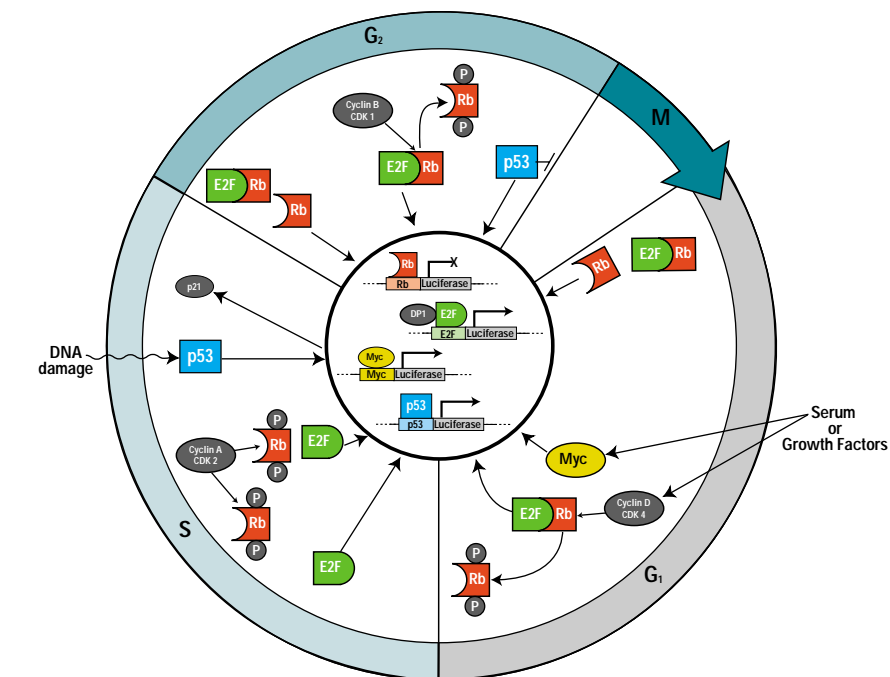


Figure 1. Rb, E2F, Myc, and p53 regulate a cell's normal progression through the cell cycle.

an accurate indication of whether a given pathway is affected by drugs or cotransfected genes.

- Rb (retinoblastoma protein) is a tumor suppressor that inhibits gene transcription when it is unphosphorylated. It also binds E2F and other transcription factors and prevents them from activating transcription of their target genes. CDKs phosphorylate Rb before S phase and M phase, causing Rb to dissociate from bound transcription factors.
- E2F is a family of transcription factors that form heterodimers with DP1. Together, they bind Rb during much of G₁ and G₂. When Rb is phosphorylated, E2F dissociates and is free to bind its response element and regulate gene expression.
- c-Myc is a transcription factor that regulates transcription of many genes and is particularly important in the transition into S phase. It also positively regulates some CDK complexes, increasing their ability to phosphorylate proteins such as Rb and allow a cell to move through the cycle.
- p53 is a tumor suppressor that inhibits cell proliferation when DNA damage occurs. It binds to other transcription factors, but also acts as a transcription factor itself—most notably increasing expression of the cell cycle inhibitor p21.

Table I: *Cis*-acting enhancer elements*

System	Response elements
Mercury Pathway Profiling Luciferase System	AP1, CRE, GRE, HSE, NFκB, SRE
Mercury Pathway Profiling Luciferase System 2	AP1(PMA), NFAT, NFκB
Mercury Pathway Profiling Luciferase System 3	AP1, CRE, E2F, ISRE, SRE
Mercury Pathway Profiling Luciferase System 4	p53, Myc, Rb, E2F
Mercury Pathway Profiling SEAP System	AP1, CRE, GRE, HSE, Myc, NFAT, NFκB, SRE

*Each system also includes the appropriate negative control vector. These vectors can be used to clone a putative enhancer element.

Low basal activity, high induction

Each vector in this system has low background and high inducibility. In Figure 2, we cotransfected NIH 3T3 cells with a *cis*-acting reporter vector and a vector encoding the appropriate signaling protein. In each case, the cotransfected cells showed extremely high fold induction. Because Rb expression inhibits transcription, cells cotransfected with the Rb vector in Panel D of Figure 2 show decreased luciferase activity.

Mercury™ Cell Cycle Profiling System...continued

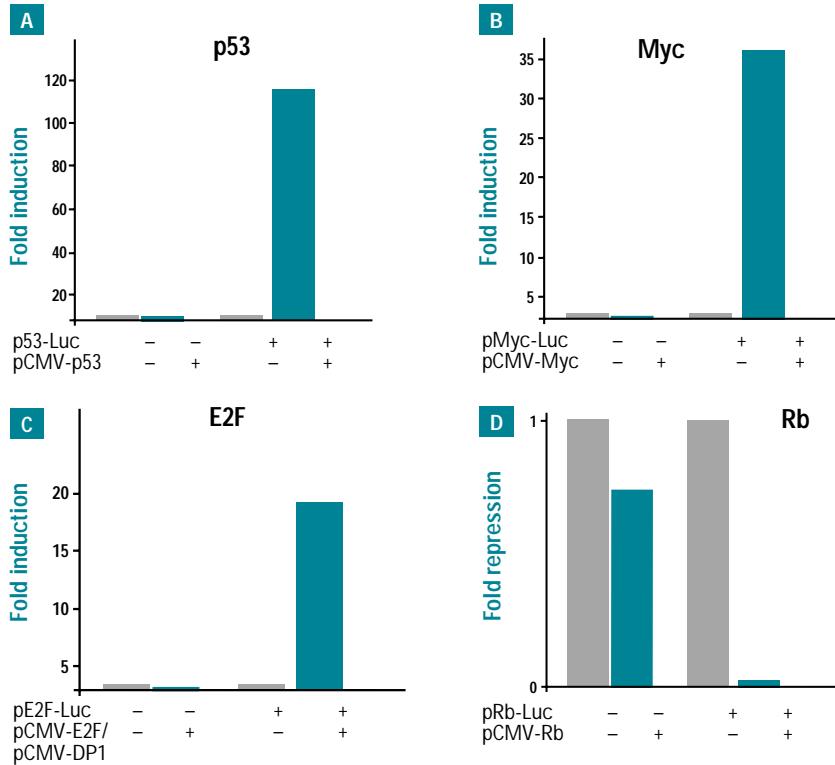


Figure 2. Mercury™ Pathway Profiling Luciferase System 4 vectors produce high fold induction. NIH 3T3 cells (Panels B, C, and D) or fibroblast 10(1) cells (Panel A) were transiently cotransfected with the indicated vectors. Luciferase activity was assayed 24 hr after transfection using the Luciferase Reporter Assay Kit (#K2039-1). RLU = relative light units.

Choose from three highly sensitive reporters

Several vectors from Mercury Pathway Profiling Kits are available separately with either luciferase, SEAP, or destabilized enhanced green fluorescent protein (d2EGFP) as a reporter. d2EGFP has a short half life for high

fold induction and accurate real-time analysis and allows you to use flow cytometry to profile signaling pathways. SEAP makes an excellent reporter for time course studies because it is secreted—it can be measured by collecting samples of the medium without lysing the cells or needing duplicate cultures.

Products	Size	Cat. #
Mercury Pathway Profiling Luciferase System 4	each	K2056-1
Mercury Pathway Profiling Luciferase System	each	K2049-1
Mercury Pathway Profiling Luciferase System 2	each	K2052-1
Mercury Pathway Profiling Luciferase System 3	each	K2053-1
Mercury Pathway Profiling SEAP System	each	K2044-1

Components

- p53-TA-Luc Vector
- pMyc-TA-Luc Vector
- pE2F-TA-Luc Vector
- pRb-TA-Luc Vector
- pTA-Luc Vector
- 2 M Calcium Solution
- 2X HEPES-Buffered Saline (HBS)
- Sterile H₂O
- Complete User Manual (PT3286-1)

Related Products

- Mercury™ *In Vivo* Kinase Assay Kits (many)
- Mercury™ Signaling Probes (many)
- Mercury™ Dominant-Negative Vector Sets (many)
- Luciferase Reporter Assay Kit (#K2039-1)

Table II: Mercury™ Pathway Profiling Vectors for different areas of research

Research area	Cis-acting response element
Apoptosis	p53
Cancer	AP1, p53, Rb
Cell cycle	E2F, Myc, p53, Rb
Cell growth	AP1, E2F, ISRE, Myc, SRE, p53, Rb
Cytokine response	GRE, ISRE
Hormone response	GRE
Inflammation	ISRE, NFAT, NFκB
Neuroscience	CRE
Stress	AP1, CRE, HSE, Myc, NFAT, NFκB, SRE

Large-Insert cDNA Libraries

SMART™ library construction technology enriches for larger full-length cDNAs

- Average insert size twice that of other libraries
- Ideal for cloning larger full-length cDNAs
- Ready to screen

Now you can obtain full-length cDNA clones from even more human and mouse tissues with CLONTECH's new **Large-Insert cDNA Libraries**. We now offer a mouse brain library as well as human placenta, skeletal muscle, and heart libraries with average insert sizes that are longer than 3 kb. Our library construction process combines SMART™ technology with size fractionation to ensure an average insert size that is twice that of other libraries. These libraries are constructed in λ TriplEx2™, a novel phagemid cDNA cloning vector that results in higher library complexity. Together, these features greatly increase the chance of finding your full-length clones.

Advanced library construction technology

Figure 1 illustrates how we generate the Large-Insert cDNA Libraries. First, we use the SMART III™ Oligonucleotide and long-distance PCR (1, 2) to generate full-length, double-stranded cDNA. SMART (Switching Mechanism at the 5' end of RNA Transcript) ensures high representation of full-length cDNAs (3). After synthesizing and digesting the cDNAs with *Sfi*I, we enrich for large, full-length clones by size fractionation on a low-melting-point agarose gel. Finally, we excise cDNAs greater than 3 kb and directionally clone them into our λ TriplEx2 phagemid, which is optimized for efficient cloning of large inserts.

Larger inserts than conventional libraries

As Table I and Figure 2 demonstrate, our Large-Insert Libraries have an average insert size of >3 kb, with an insert size range between 0.7–8.0 kb. In contrast, even the best conventional libraries have an average insert size of <2 kb. Because of the large average insert size, you are more likely to obtain a clone for your full-length cDNA. Although our Large-Insert

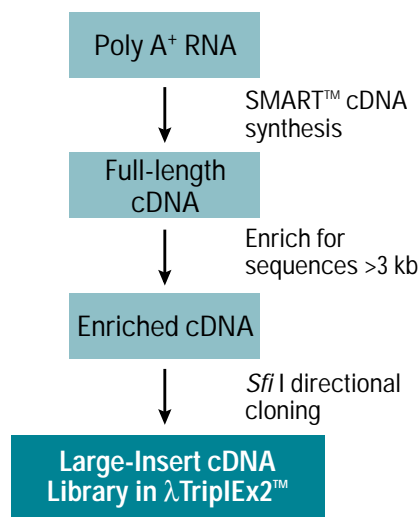


Figure 1. Large-Insert cDNA Library construction process.

cDNA Libraries are enriched for cDNAs >3 kb, the size-fractionation procedure does not completely exclude shorter fragments (Table I), so these libraries can be a good resource for cloning full-length cDNAs in the 2–3-kb range. These libraries are not recommended for cloning cDNAs smaller than 1 kb.

The λ TriplEx2™ advantage

The λ TriplEx2 vector offers the advantages of cloning in a phagemid vector—higher titer libraries, blue/white screening, and high complexity. λ TriplEx2 also expresses library inserts in all three reading frames, to provide you with the best chance of finding positive clones when screening with antibodies.

Quickly screen for clones

Large-Insert cDNA Libraries are ready to screen on arrival, saving you the time required to prepare a cDNA library. You simply transduce individual λ TriplEx2 phagemid clones into one of the supplied *E. coli* strains and screen with either a DNA probe or antibody.

High-quality libraries

CLONTECH rigorously tests all Large-Insert cDNA Libraries to provide you with the highest quality product available. To verify that inserts were accurately amplified, we sequenced 10 kb of library inserts from one library and found only a single mutation. Furthermore, CLONTECH guarantees that each library has greater than 10^6 independent clones and that the average insert size is >3 kb. All libraries are provided at a titer greater than 10^9 pfu/ml.

References

1. Barnes, W. M. (1994) *Proc. Natl. Acad. Sci. USA* 91:2216–2220.
2. Chang, S., et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:5695–5699.
3. SMART cDNA Library Construction Kit (October 1998) *CLONTECHniques XIII*(4):12–13.

Table I: Features of Large-Insert cDNA Libraries

Library	Ave. insert size	Insert size range (kb)	Insert rate	Independent clones	%White/Blue
Human					
Placenta	3.6	0.9–5.3	14/15	4.7 x 10 ⁶	90%
Skeletal Muscle	3.4	1.1–6.0	20/20	2.0 x 10 ⁶	87%
Heart	3.1	1.0–8.0	20/20	5.0 x 10 ⁶	89%
Testis	3.4	2.1–5.0	15/15	4.8 x 10 ⁶	99%
Brain	3.8	1.8–5.5	11/15	3.9 x 10 ⁶	99%
Fetal Brain	5.0	3.0–7.0	14/15	4.8 x 10 ⁶	99%
Mouse					
Brain	3.0	0.7–5.0	19/20	5.5 x 10 ⁶	92%

Large-Insert cDNA Libraries...continued

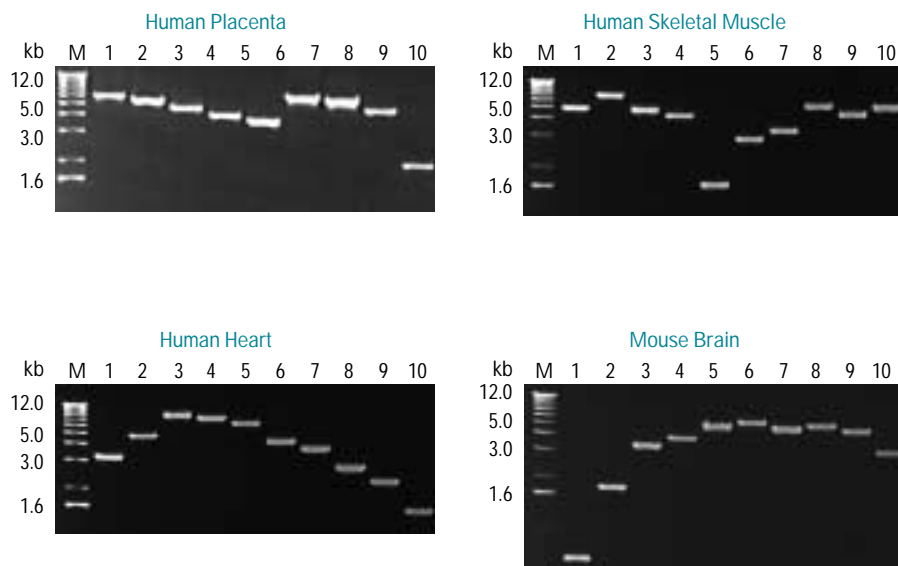


Figure 2. Insert screening by LD-PCR of random Large-Insert cDNA Library clones. Inserts from 10 random clones were amplified by LD-PCR using the λ TriplEx insert screening primers. Insert size was determined by electrophoresis on a 1.1% agarose/EtBr gel, and the average size was found to be >3 kb for all libraries. Lane M = Markers.

cDNA Libraries	Vector	Cat. #
Mouse Brain	λ TriplEx2	ML5500u
Human Placenta	λ TriplEx2	HL5502u
Human Skeletal Muscle	λ TriplEx2	HL5505u
Human Heart	λ TriplEx2	HL5506u
Human Brain	λ TriplEx2	HL5500u
Human Testis	λ TriplEx2	HL5503u
Human Fetal Brain	λ TriplEx2	HL5504u

NEW!

NEW!

NEW!

NEW!

Components

- Large-Insert cDNA Library Lysate
- *E. coli* BM25.8 & XL1-Blue
- 5' & 3' Sequencing Primers
- Complete User Manual (PT3003-1)

Notice to Purchaser

The SMART™ technology is covered by U.S. Patents #5,962,271 & #5,962,272. Use of the *Sfi*I cloning strategy is licensed under U.S. Patent #5,595,895. The PCR process is covered by patents owned by Hoffmann-La Roche, Inc., and F. Hoffmann-La Roche, Ltd.



Employment Opportunities

At CLONTECH Laboratories, Inc., we develop and manufacture tools for life science research. Our mission is to be a global leader in high-quality innovative products for the life science market. Our expertise in cDNA array technology, PCR, two-hybrid analysis, gene expression, GFP and other reporter systems, nucleic acid chemistry, and other molecular biology applications enables us to be a leader in cutting-edge technologies. We firmly believe that our employees and our customers are our most valuable assets.

Our continued success has created the following opportunities:

- Market Development Manager
- Technical Support Specialist
- Associate Scientist
- Research Scientist I/II
- Technical Writer

If you would like to join an elite group of talented individuals, send your resume indicating the position of interest to:

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Palo Alto, CA 94303-4230
Attn: Human Resources

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EOE/AA/M/F

Living Colors™ D.s. Monoclonal Antibody

Highly specific monoclonal antibody for detecting DsRed

- Recognizes DsRed & fusions to DsRed
- Excellent for immunoprecipitation & Western blot experiments
- Does not cross-react with GFP or GFP variants

CLONTECH's new **Living Colors™ D.s. Monoclonal Antibody** specifically detects wild-type or human codon-optimized red fluorescent protein (DsRed) and fusions to these proteins. The antibody is ideal for Western blot or ELISA experiments, and is particularly useful for immunoprecipitating DsRed.

We purified the new monoclonal antibody from the culture media of a mouse hybridoma cell line. It recognizes only a single epitope of DsRed, so the antibody produces a strong, specific signal with low background and does not crossreact with any *Aequorea victoria* GFP variant.

Highly specific antibody

To evaluate the antibody's specificity, we performed Western and coimmunoprecipitation experiments. Figure 1 shows a Western analysis of cell lysate from HEK 293 cells that were transiently transfected with pDsRed1-C1 (#6924-1). The antibody detected DsRed with high specificity and very low background.

Both Figures 2 and 3 show the D.s. Monoclonal Antibody's specificity in coimmunoprecipitation experiments. In Figure 2, the antibody specifically immunoprecipitated recombinant DsRed added to HEK 293 whole cell lysate. In Figure 3, we transiently transfected HEK 293 cells with pDsRed1-C1 or pEGFP (#6077-1) and immunoprecipitated DsRed using the D.s. Monoclonal Antibody. To visualize the bands, we used the D.s. Peptide Antibody in a Western analysis. Both antibodies show a strong specificity for DsRed and no crossreaction with EGFP.

Two antibodies for all your needs

With our new monoclonal antibody, we now offer antibodies to DsRed isolated from both rabbit and mouse. Although both antibodies are excellent for Western analysis, we recommend the monoclonal antibody for immunoprecipitation experiments.

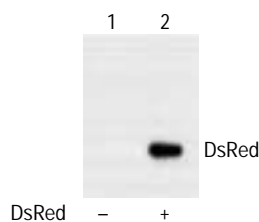


Figure 1. The Living Colors™ D.s. Monoclonal Antibody provides a strong, specific signal in Western analysis. HEK 293 cells were transiently transfected with pDsRed1-C1 (#6924-1) as indicated. Whole cell lysates (10 µg/well) were then subjected to Western analysis using the Living Colors D.s. Monoclonal Antibody.

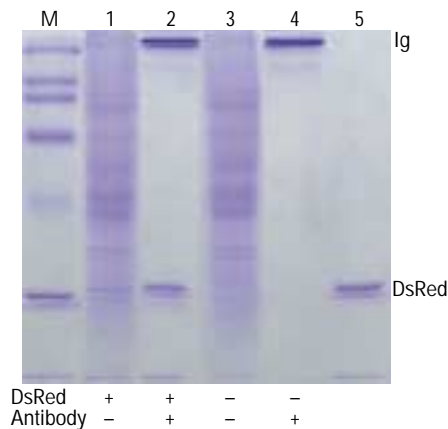


Figure 2. The Living Colors™ D.s. Monoclonal Antibody specifically precipitates DsRed added to whole cell lysates. Coomassie blue-stained 10% SDS-PAGE run under nonreducing conditions. HEK 293 cell lysate (10 µg/well) with 10 µg of recombinant DsRed added to Lanes 1 and 2, and 20 µg antibody added as indicated. Lane 5: recombinant DsRed. Lane M = Marker.

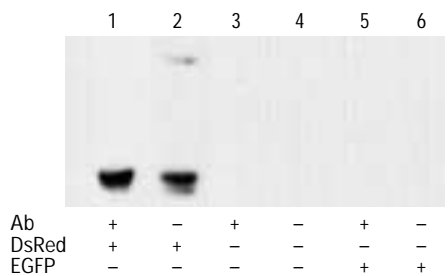


Figure 3. The Living Colors™ D.s. Monoclonal Antibody specifically precipitates DsRed from transiently transfected cells. Whole cell lysate from HEK 293 cells was transiently transfected with pDsRed1-C1 (#6924-1; Lanes 1 & 2), pEGFP-1 (#6086-1; Lanes 5 & 6), or untransfected (Lanes 3 & 4). DsRed was precipitated with 3 µl of D.s. Monoclonal Antibody as indicated. Western analysis using the D.s. Peptide Antibody (#8370-1, -2) specifically detected DsRed in Lanes 1 and 2, but did not detect EGFP in Lanes 5 and 6.

Products	Size	Cat. #
Living Colors D.s. Monoclonal Antibody	20 µg 100 µg	8373-1 8373-2
Living Colors D.s. Peptide Antibody	20 µg 100 µg	8370-1 8370-2



Related Products

- Living Colors™ A.v. Monoclonal Antibody (JL-8; #8371-1, -2)
- Living Colors™ Full-Length A.v. Polyclonal Antibody (#8372-1, -2)
- Living Colors™ A.v. Peptide Antibody (#8367-1, -2)
- Living Colors™ A.v. Peptide Antibody-AP Conjugate (#8368-1)
- Living Colors™ A.v. Peptide Antibody-HRP Conjugate (#8369-1)

ClonCapture™ cDNA Selection

Screen, isolate, and express in less than one week

- Rapid, reliable method for identifying full-length clones
- Cloning & mammalian expression from a single vector
- Extensive library collection

ClonCapture™ is a better way to screen libraries. ClonCapture allows you to quickly isolate clones based on a partial cDNA sequence and then directly introduce them into mammalian cells for expression studies—bypassing both library screening and subcloning into another vector. You can screen many clones at a time, making the process especially useful for extracting rare and low-abundance clones.

There are several options for using the ClonCapture strategy. We offer **ClonCapture™ Libraries** from one of seven tissues that are ready to be purified and screened with the **ClonCapture™ cDNA Selection Kit** (#K1056-1). The kit also works with any other plasmid-based library. **ClonCapture-Ready™ Super DNAs** provide an even faster route to expression studies—they are supercoiled DNA preparations already isolated from ClonCapture Libraries, ready for immediate use with the ClonCapture cDNA Selection Kit.

The ClonCapture™ Advantage

ClonCapture technology uses the RecA protein, which forms complexes between single-stranded DNA and homologous double-stranded DNA (1–4). With ClonCapture, RecA mediates pairing between the double-stranded cDNA clone and a biotinylated probe. Then, you capture those clones with streptavidin magnetic beads and recover the clones with alkaline treatment (Figure 1).

ClonCapture is extremely efficient, even for low-abundance transcripts. It is more reliable than techniques that require converting the library to single-stranded form, and uses longer probes for higher specificity. A puromycin selection step ensures that nearly all surviving colonies will stably express the cDNA inserts—as a result, you screen fewer colonies to find functional, high-expressing clones.

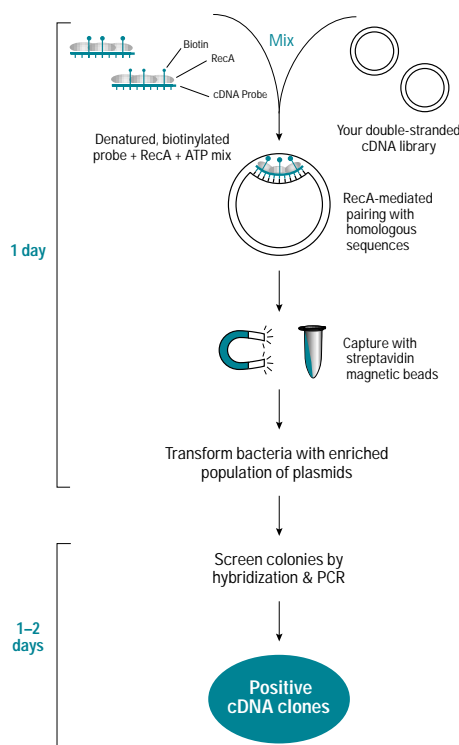


Figure 1: The ClonCapture™ process. The ClonCapture procedure starts with a 200–600-bp biotinylated probe. The probe is complexed with RecA and mixed with your library where it interacts with plasmid DNA to form a triple-stranded nucleoprotein complex. After proteolytic removal of RecA, the complexes are captured on streptavidin-coated magnetic beads. The captured DNA with your clone of interest is then recovered by alkaline treatment.

References

1. Honigberg, S., et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:9586–9590.
2. Rigas, B., et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:9591–9595.
3. Treintze, M., et al. (1995) *Biochem. Biophys. Res. Comm.* 211:804–811.
4. Zhumabayeva, B., et al. (1999) *BioTechniques* 27:834–845.

For more information and order forms for the custom service, go to www.clontech.com and select ClonCapture's product page.

Product	Size	Cat. #
ClonCapture cDNA Selection Kit	6 rxns	K1056-1

ClonCapture cDNA Libraries	Size	Cat. #
Human Brain	each	HL9002CC
Human Heart	each	HL9000CC
Human Kidney	each	HL9003CC
Human Liver	each	HL9001CC
Human Placenta	each	HL9005CC
Human Skeletal Muscle	each	HL9006CC
Human Testis	each	HL9004CC

ClonCapture-Ready Super DNA	Size	Cat. #
Human Brain	each	HC9002
Human Heart	each	HC9000
Human Kidney	each	HC9003
Human Liver	each	HC9001
Human Placenta	each	HC9005
Human Skeletal Muscle	each	HC9006
Human Testis	each	HC9004

Custom ClonCapture Services	Cat. #
Custom ClonCapture Library Screening	CS1100
Custom ClonCapture Library Screening & Sequencing	CS1101
Custom ClonCapture Library Prescreening	CS1102

Notice to Purchaser

Use of the RecA technology is licensed under U.S. Patent #4,888,274.

Let Us Find Your Clones!

CLONTECH offers **Custom ClonCapture Library Screening Services** for isolating the longest clones of your gene of interest. You provide a nucleotide sequence or DNA fragment (>300 bp) and we will prepare the biotinylated probes and perform the ClonCapture process using our extensive collection of plasmid cDNA libraries.

Our first step is to prescreen a pooled library to verify the presence of your clone, unless you specify a particular library for screening.* Then, using ClonCapture technology and the libraries indicated in the prescreening, we identify the longest clones to which your sequence hybridized. For an additional fee, we will provide partial sequences for your clones.

*The prescreening service charge applies even if your submitted sequence fails to hybridize to our libraries.

Gene Expression Profiling of Cancer Tissues Using Matched Tumor/Normal Expression Arrays

Bakhyt Zhumabayeva, Luda Diatchenko, Alex Chenchik, & Paul D. Siebert

Gene Cloning & Analysis Group
CLONTECH Laboratories, Inc.

We used SMART™ PCR-amplified cDNAs arrayed on a nylon membrane for high-throughput expression profiling with limited starting material. We show that SMART (Switch Mechanism At the 5' end of the RNA Template) cDNAs maintain the complexity of the original mRNA population. We used CLONTECH's Matched Tumor/Normal Expression Array to demonstrate that SMART-amplified cDNAs are suitable for high-throughput studies—to compare the relative abundance of target genes and to detect differentially expressed genes in a wide variety of tissues simultaneously.

Identifying differentially expressed genes is a common starting point for understanding the molecular mechanisms underlying biological processes such as differentiation, development, and tumorigenesis. Some techniques that researchers employ to determine differential expression include subtractive hybridization, differential display, and cDNA arrays. In order to yield accurate results, each of these techniques requires a sufficient amount of RNA from the tissue or cell under investigation.

SMART™ PCR cDNA synthesis provides an elegant method for amplifying cDNA samples (see page 2 for a diagram of the process). Using PCR amplification, however, brings into question whether the amplified cDNA is accurately represented in the same proportion as in the original sample. CLONTECH scientists and other researchers have successfully used SMART PCR-generated cDNA for library construction, subtractive hybridization, and virtual Northern blotting. After the initial identification of a differentially expressed gene, the next step is to determine if that expression is statistically different in multiple samples, or to localize the expression to specific tissues.

We now report that SMART PCR-generated cDNA provides suitable material for high-throughput expression profiling using such products as CLONTECH's Matched Tumor/Normal Expression Array. We confirm that SMART retains the original mRNA message profile (1, 2).

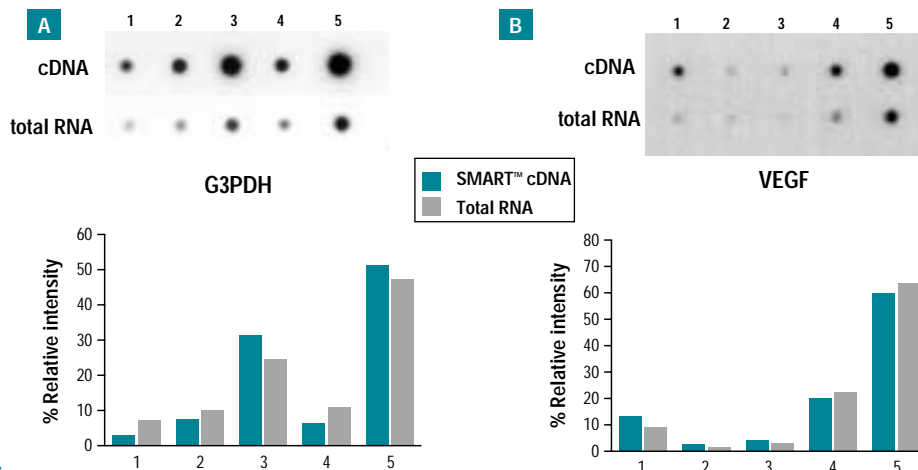


Figure 1. Array of SMART™ cDNAs and original total RNA samples probed with G3PDH and VEGF. 50-ng samples of SMART-amplified cDNAs and 1 µg of corresponding total RNAs from five different tissues were spotted on nylon membranes. The membranes were hybridized with radiolabeled probes for G3PDH (Panel A) and VEGF (Panel B). Hybridization signals were detected by phosphorimaging. Due to the different hybridization characteristics of cDNA and RNA, hybridization signal values for both G3PDH and VEGF were quantified and represented as a percentage of the total signal from all five spots for either cDNA or RNA (bottom graph). 1: liver. 2: placenta. 3: brain. 4: kidney. 5: HeLa cells.

SMART™ cDNAs accurately reflect gene expression

We amplified cDNAs using SMART technology from five human total RNA samples purified from liver, placenta, brain, kidney, and HeLa cells. We then spotted 50 ng of the amplified cDNA on nylon membranes alongside 1 µg of each corresponding total RNA. We hybridized the membranes with radioactively labeled cDNA probes for glyceraldehyde-3-phosphate dehydrogenase (G3PDH; Figure 1A), and vascular endothelial growth factor (VEGF; Figure 1B). The results show that, in all five tissues, 50 ng of SMART cDNA produces the same relative signal intensities and gene expression profile across the samples as 1 µg of the original total RNA samples. Furthermore, a comparison of hybridization signal intensities of 50 ng/dot of cDNA and 1 µg/dot of total RNA showed that SMART cDNA yields a 5–10-fold higher sensitivity of detection than the corresponding total RNA.

SMART™ cDNA yields signals that are both specific & quantitative

To evaluate the specificity of SMART PCR amplification, we synthesized SMART cDNA from spleen, placenta, kidney, lung, adult liver, testis, whole brain, fetal liver, thymus, pancreas, prostate, and HeLa cell total RNA. We arrayed 50, 30, and 10 ng of each purified cDNA on a

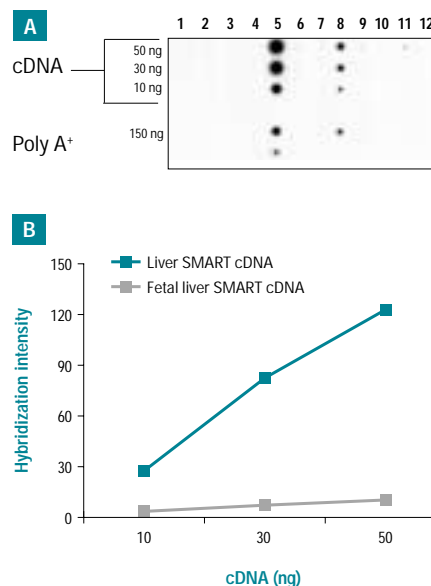


Figure 2. Array of different amounts of SMART™ cDNA hybridized with a liver-specific TAT probe. cDNAs were prepared from 12 different human total RNA samples. Different amounts of each purified cDNA were arrayed on a nylon membrane along with 150 ng of corresponding poly A⁺ RNA. The membrane was hybridized with a radiolabeled probe for TAT and visualized by phosphorimaging (Panel A). Panel B shows the linear correlation between the intensity of each hybridization signal and the amount of spotted cDNAs for adult liver and fetal liver. 1: spleen. 2: placenta. 3: kidney. 4: lung. 5: adult liver. 6: testis. 7: whole brain. 8: fetal liver. 9: HeLa cells. 10: thymus. 11: pancreas. 12: prostate.

Gene Expression Profiling of Cancer Tissues Using Matched Tumor/Normal Expression Arrays...continued

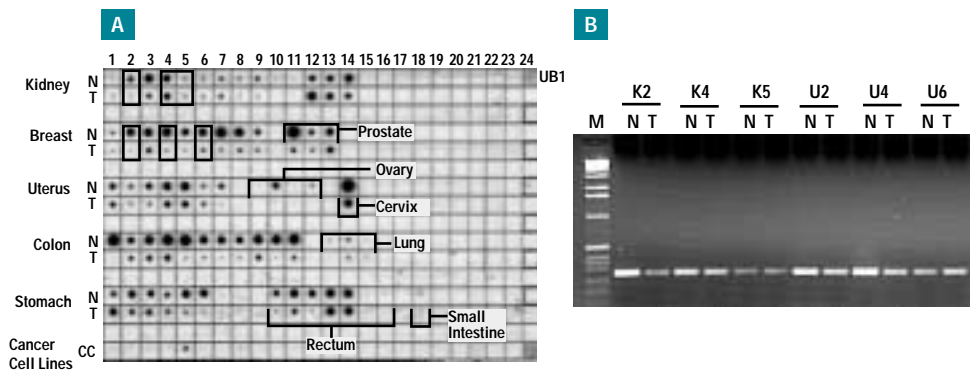


Figure 3. Array hybridization and RT-PCR confirmation of differential expression using SMART[™]-amplified cDNAs from matched normal and tumor total RNAs. Normalized membranes were hybridized with a radiolabeled probe for gelsolin (Panel A). Three cDNA pairs from kidney (K2, K4 & K5) and three cDNA pairs from uterus (U2, U4 & U6) were used in RT-PCR to confirm the differential expression of gelsolin (Panel B). Lane M = Marker. N = cDNA from normal tissue. T = cDNA from tumor tissue. UBI = Positive control cDNA for ubiquitin (10 ng per spot) on the right top and bottom corners of the membrane. Six negative controls are included on the right of the membrane.

nylon membrane, and hybridized it with a cDNA probe for the liver-specific gene, tyrosine aminotransferase (TAT). Figure 2 shows that TAT specifically hybridizes with only cDNAs synthesized from adult liver and fetal liver (Panel A). This figure also demonstrates a near linear correlation between each hybridization signal intensity and the spotted amount of cDNA (Panel B). Again, these experiments show that SMART cDNAs generate much stronger hybridization signals than corresponding total RNAs, providing higher sensitivity for detecting the expression of low abundance RNA transcripts.

SMART[™] cDNAs for high-throughput gene expression profiling

We used Matched Tumor/Normal Expression Arrays to detect differentially expressed genes. These are nylon arrays of SMART-generated cDNAs from 68 matched tumor and normal total RNA samples. The RNA samples come from breast, kidney, lung, uterus, cervix, ovary, prostate, stomach, colon, rectum, and small intestine.

We hybridized three arrays with cDNA probes for three genes that have been shown to be involved in cancer: glutathione peroxidase (GP), gelsolin (GSN), and Kunitz-type protease inhibitor (KTPI). Figure 3 shows the results for GSN.

Down regulation of GP was previously detected in lung tumors by subtractive hybridization (3).

Our cDNA array revealed the down regulation of GP in lung tumors of three independent patients. In addition to that, our hybridization data revealed the down regulation of GP in all of the kidney, breast, and colon tumors (4).

GSN expression has been shown to be down regulated in human breast ductal carcinoma by immunohistochemistry using a monoclonal antibody (5). Our Matched Tumor/Normal Expression Array confirmed the down regulation of GSN in breast tumors, and revealed its down regulation in prostate and colon tumors (Figure 3). RT-PCR results for some of the kidney and uterus cDNA samples confirmed the hybridization results for all three genes.

KTPI has been shown to be overexpressed in pancreatic cancer (6). We detected the up-regulated expression of KTPI in breast tumor cDNAs and some uterus tumor tissues. Conversely, KTPI expression was down regulated in some of the kidney, rectum, and colon tumor cDNAs (4).

We conclude that the Matched Tumor/Normal Array gives accurate results for quick detection of differentially expressed genes between tumor and normal tissues. The array could also be used to confirm differential expression of genes identified by other techniques, or to screen for differentially expressed genes in a large number of independent samples.

Product	Size	Cat. #
Matched Tumor/Normal Expression Array	each	7840-1
Atlas SMART Probe Amplification Kit [†]	each	K1034-1
SMART PCR cDNA Synthesis Kit	7 rxns	K1052-1

[†] used with the SMART PCR DNA Synthesis Kit.

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The SMART[™] technology is covered by U.S. Patents #5,962,271 & #5,962,272.