

July 98

# CLONTECHniques

Volume XIII, No. 3

Innovative  
Tools to  
Accelerate  
Discovery

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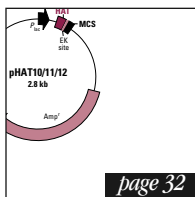
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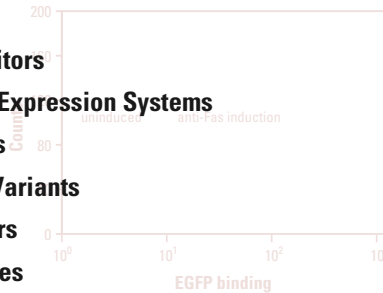
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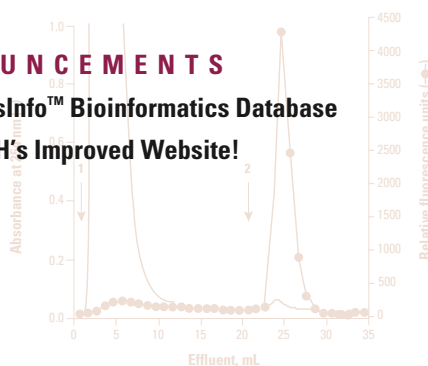


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**CLONTECH**  
NOW YOU CAN.

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UK Japan GmbH Canada Int'l US

# Application-Targeted Atlas™ cDNA Expression Arrays

The intelligent approach to panoramic gene expression profiling

- **Informative gene expression results at an affordable price**
- **Modular product line—mix and match arrays to suit your needs**
- **Comprehensive representation of different functional classes of genes**
- **New AtlasInfo™ Bioinformatics Database**

CLONTECH's Atlas™ cDNA Expression Arrays were designed with a simple idea in mind: nucleic acid array technology should be readily available to all researchers. Our arrays are manufactured on nylon membranes, so they are affordable and do not require any special equipment. Other more expensive arrays generally include as many cDNAs or oligonucleotides as possible, and many of these sequences are poorly characterized. In contrast, the collection of cDNAs featured on each Atlas Array has been carefully selected to produce the most informative gene expression results possible.

With our new application-targeted arrays (Table I), you can focus on specific biological questions. Each of these small arrays includes 100–300 cDNAs that were carefully chosen because of their established relevance to a specific field of research. These application-targeted arrays are even more affordable than our broad-coverage arrays. By mixing and matching these modular arrays, you can create the perfect set of tools for your research needs.

## Apoptosis Array

Apoptosis, or programmed cell death, is central to many fundamental biological processes, including embryonic development, immune response, and tissue repair and renewal. When the mechanisms that regulate apoptosis fail, diseases such as cancer develop. A clearer understanding of the links between apoptosis and cancer will shed new light on the effects and mechanisms of drugs and therapies. The **Atlas Human Apoptosis Array** includes 205 key genes that are known to control apoptosis, including extracellular and cytoplasmic effectors, and provides a complementary technology for our ApoAlert™ customers (see pages 16–18).

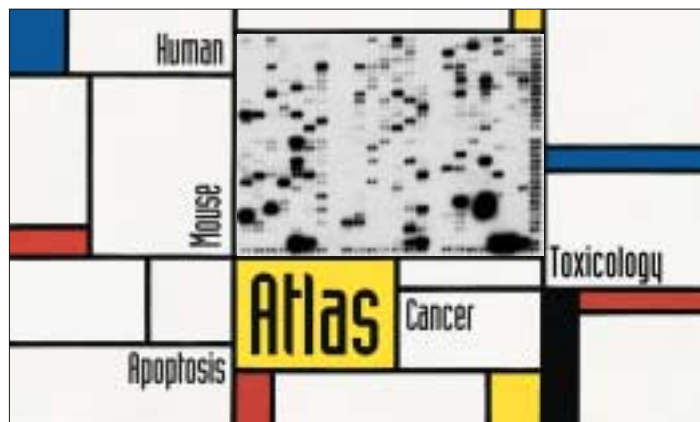


Illustration inspired by the art of Piet Mondrian (1872–1944).

## Stress/Toxicology Array

Apoptosis is often the result of environmental stressors, which activate cellular stress response programs. The **Atlas Human Stress/Toxicology Array** features 236 genes that modulate cellular responses to various stressors, including heat shock, irradiation, infection, and treatment with cytotoxic or genotoxic compounds. In addition, this array includes a number of genes that are responsible for drug/xenobiotic metabolism.

## Cytokine/Receptor Array

Cytokines and their receptors are a diverse group of proteins that control cell growth, differentiation, and survival. The **Atlas Cytokine/Receptor Array** includes 268 representative genes from several cytokine families. By monitoring the expression of these genes in a variety of biological settings, researchers can gain new insights into many areas of biomedical research.

## Cell Cycle Array

The **Atlas Cell Cycle Array** features 111 genes that play integral roles in the complex machinery of the cell cycle. Among these key players are cyclins, cyclin-dependent kinases and their inhibitors, and components of the Rb and p53 pathways. This array also includes mitogen- and stress-activated protein kinases and transcription factors.

## Cell Interaction Array

Cell interactions play a key role in many important cellular events. Interactions between cells are mediated by several families of membrane proteins that perform both structural and signaling functions, helping to regulate development, growth, and differentiation. Interactions of cells with the extracellular matrix are central to myriad biological processes, including cell motility and invasion, tissue homeostasis, and cytokine action. The **Atlas Human Cell Interaction Array** is composed of 265 genes, including membrane receptors, extracellular matrix proteins and proteases, and cytoskeletal proteins.

## Oncogene/Tumor Suppressor Array

The **Atlas Human Oncogene/Tumor Suppressor Array** includes 190 oncogenes and tumor suppressor genes that are involved in the development of various types of cancer. This broad category includes regulators of apoptosis, the cell cycle, gene expression, and DNA damage response and repair. Some of these genes, such as BRCA1 and BRCA2, are known to play important roles in tumorigenesis even though their molecular mechanisms of action are not fully understood.

## Broad-coverage Atlas Arrays

After you have used one of our application-targeted arrays to investigate a specific area of research, you can extend your results using

# Application-Targeted Atlas™ Arrays...continued

one of our larger arrays. Both the Atlas Human (#7740-1) and Atlas Mouse (#7741-1) cDNA Expression Arrays include 588 genes, which are arranged into functional groups representing different areas of research (1, 2). The Atlas Human Cancer cDNA Expression Array (#7742-1) features 588 genes involved in different facets of cancer (3). These arrays provide a broader scope of information. For further exploration of gene expression, you can choose from CLONTECH's selection of innovative tools for gene expression analysis (see pages 12–15).

## Engineered for optimum sensitivity

Atlas Arrays are carefully designed to provide the highest possible ratio of signal to background. We use a proprietary software program to select each cDNA fragment so that it is the optimum size for hybridization, lacks repetitive elements and poly-A sequences, and

has no homology with other sequences on the array. We amplify each cDNA by PCR, then clone and partially sequence inserts for every gene to verify identity. The cloned inserts are then amplified, normalized, and arrayed on the membrane.

The sensitivity of Atlas technology rivals that of fluorescence-based hybridization detection methods. When high-quality poly A<sup>+</sup> RNA is used as starting material and hybridization is performed according to the User Manual, the background level is sufficiently low to permit detection of an mRNA present at only 2–5 copies per cell—an abundance level of about 0.001%. Moreover, Atlas Arrays deliver a linear correlation between hybridization signals and RNA abundance over three orders of magnitude (1), allowing identification of genes that differ in abundance by as little as 3–5 fold.

One of the key features of the Atlas technology is a novel method for the generation of hybridization probes (patent pending). Atlas probes are generated from the RNA samples of interest using gene-specific primers, so they are significantly less complex than probes generated using oligo(dT) or random primers. Reducing probe complexity results in an approximately 10-fold increase in sensitivity, with a concomitant decrease in the level of nonspecific background. Because of this high sensitivity, you can use as little as 2 µg of total cellular RNA as starting material for probe synthesis—a clear advantage when only small amounts of tissues or cells are available. For isolation of total RNA suitable for use with Atlas Arrays, we now offer the Atlas Pure RNA Isolation Kit (see page 5).

## Complete package of reagents ensures accurate results

Atlas Arrays come with everything you need for successful gene expression profiling. Two membranes are included with each purchase, so you can compare the expression patterns of two RNA populations in a single experiment. Each membrane can be used several times. We also provide ExpressHyb™ Hybridization Solution (4; see page 15), reagents for generating and purifying labeled cDNA probes, and a blank membrane for optimizing hybridization conditions.

## Complete list of genes at our website

For a complete list of all the genes included on our Atlas Arrays, please visit our website at [atlas.clontech.com](http://atlas.clontech.com). Like the list included with each Atlas Array, this list includes the gene names and GenBank accession numbers. Genes that are included on several different arrays are indicated. You can easily search this list by the gene name or location on the array.

Table I: CLONTECH's application-targeted Atlas cDNA Expression Arrays

Atlas Human Array*	# of cDNAs	Application or research field
<b>Apoptosis (#7743-1)</b>	205	Cancer Cell cycle Cell death & cytotoxicity Developmental biology DNA damage & repair
<b>Cell Cycle (#7748-1)</b>	111	Cancer Cell cycle Cell death Stress response
<b>Cell Interaction (#7746-1)</b>	265	Cancer Cell motility & invasion Cell signaling & adhesion Cytokine action Developmental biology
<b>Cytokine/Receptor (#7744-1)</b>	268	Cancer Cardiovascular biology Cell & developmental biology Immunology
<b>Oncogene/Tumor Suppressor (#7745-1)</b>	190	Cancer Cell cycle Drug development Environmental toxicology Identifying tumor markers
<b>Stress/Toxicology (#7747-1)</b>	236	Cytotoxicity & toxicology Drug development & response Pathogenicity

\* For a complete list of genes included on each Atlas Array, please visit our website ([atlas.clontech.com](http://atlas.clontech.com)).



# Application-Targeted Atlas™ Arrays...continued

## New AtlasInfo™ Bioinformatics Database

- **Comprehensive information about each gene on our Atlas Arrays**
- **Available free of charge to our Atlas customers**
- **User-friendly format**

With the introduction of CLONTECH's Atlas Human cDNA Expression Array (#7740-1) more than a year ago, nucleic acid array technology has become much more affordable and accessible. Each Atlas Array includes hundreds of cDNAs, and a simple hybridization experiment provides a tremendous amount of information about the expression of these genes. The real challenge lies in sorting through expression data and formulating a biologically meaningful interpretation of the results. To this end, scientists must search through public domain biological databases and journal publications—an arduous task that can take months of concentrated effort.

CLONTECH's new **AtlasInfo Bioinformatics Database** eases this critical informational bottleneck. Designed to support our line of Atlas Arrays, the AtlasInfo database contains up-to-date facts collected from GenBank, Swiss-Prot, and MEDLINE. For each gene, we provide information about the name, other aliases, known functions, chromosomal locations, related cellular disorders and diseases, and homologous sequences in other organisms. This information can be accessed either by the name of the gene or via a logical interface showing its physical location on the array. All information is presented in a user-friendly tabular format and is available free of charge to our Atlas customers.

The AtlasInfo Database currently contains information for all genes on our Atlas Human cDNA Expression Array (#7740-1); we will be adding information for our other arrays soon. We are also planning to expand the information available by adding lists of references and direct links to other sites that have information on specific genes. You can access AtlasInfo directly at [atlasinfo.clontech.com](http://atlasinfo.clontech.com) or via our new Atlas Home Page at [atlas.clontech.com](http://atlas.clontech.com).

Product	Size	Cat. #	
Atlas Human Apoptosis Array	each	7743-1	NEW!
Atlas Human Cell Cycle Array	each	7748-1	NEW!
Atlas Human Cell Interaction Array	each	7746-1	NEW!
Atlas Human Cytokine/ Receptor Array	each	7744-1	NEW!
Atlas Human Oncogene/ Tumor Suppressor Array	each	7745-1	NEW!
Atlas Human Stress/Toxicology Array	each	7747-1	NEW!
Atlas Human cDNA Expression Array	each	7740-1	
Atlas Human Cancer cDNA Expression Array	each	7742-1	
Atlas Mouse cDNA Expression Array	each	7741-1	

### Components

- **Two Atlas™ Array Membranes**
- **10X dNTP Mix** (for [<sup>32</sup>P]-dATP labeling)
- **10X CDS Primer Mix**
- **5X Reaction Buffer**
- **DTT**
- **MMLV Reverse Transcriptase**
- **10X Termination Mix**
- **Control Poly A<sup>+</sup> RNA**
- **ExpressHyb™ Hybridization Solution**
- **C<sub>0</sub>t-1 DNA**
- **CHROMA SPIN™-200 Columns**
- **Deionized H<sub>2</sub>O**
- **Blank Nylon Membrane**
- **Complete User Manual** (PT3140-1)
- **Orientation Grid** (PT3140-2)
- **Atlas™ Array Information List** (PT3140-3)

### Related Products

- **Atlas™ Pure RNA Isolation Kit** (#K1038-1; see facing page)
- **ApoAlert™ Kits and Reagents** (many; see pages 16–18)
- **ExpressHyb™ Hybridization Solution** (#8015-1, -2 & -3; see page 15)
- **RNA Master Blots™** (#7770-1 & #7771-1)
- **Multiple Tissue Northern (MTN™) Blots** (many; see pages 12–15)
- **Premium Poly A<sup>+</sup> & Total RNAs** (many)

### References

1. Atlas Human cDNA Expression Array I (April 1997) *CLONTECHniques* **XII**(2):4–7.
2. Atlas Mouse cDNA Expression Array I (January 1998) *CLONTECHniques* **XIII**(1):2–4.
3. Atlas Human Cancer cDNA Expression Array (April 1998) *CLONTECHniques* **XIII**(2):2–4.
4. Yang, T. T. & Kain, S. R. (1995) *BioTechniques* **18**: 498–503.

### Notice to Purchaser

The Atlas Array products sold by CLONTECH are for research purposes only. Certain isolated DNA sequences included on the 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 Atlas Arrays.

CLONTECH is in the process of patenting certain aspects of the Atlas technology.

# Atlas™ Pure RNA Isolation Kit

For isolation of exceptionally pure total RNA

- The only kit optimized for use with Atlas Arrays
- Virtually eliminates genomic DNA impurities
- Generates high yields of quality RNA

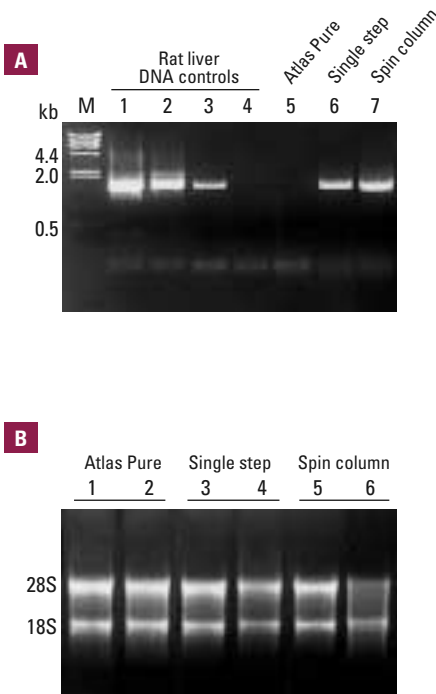
The Atlas™ Pure RNA Isolation Kit yields high-quality total RNA that is virtually free of genomic DNA, nucleases, and other impurities. Specifically designed for use with CLONTECH's Atlas cDNA Expression Arrays (pages 2–4), this kit utilizes guanidinium thiocyanate/phenol extraction methodology. The Atlas Pure Kit can also be used to generate RNA for other applications, such as RT-PCR or SMART™ PCR cDNA synthesis or library construction (see pages 9–10).

## Ideal for use with Atlas Arrays

One of the key features of Atlas Array technology is the use of gene-specific primers for generating highly sensitive hybridization probes (patent pending). This allows the use of total RNA as the starting material for Atlas hybridization experiments without a significant decrease in the ratio of signal to background. However, the quality of RNA is crucial for generating highly sensitive hybridization probes. We strongly recommend that you use only Atlas Pure total RNA for your Atlas experiments.

Total RNA isolated using other commercially available kits often becomes degraded in storage or during cDNA synthesis because of residual RNase contamination. Samples may also contain genomic DNA impurities, which can cause high levels of nonspecific background in Atlas hybridization experiments. Figure 1 shows how Atlas Pure RNA compares to total RNA samples prepared using two of our competitors' kits, a single-step, guanidinium thiocyanate/phenol extraction method and a silica-based spin-column method. Only the Atlas Pure RNA is free of genomic DNA impurities (Panel A).

Atlas Pure RNA is also free of proteins, including RNases. After incubation at 37°C for 8 hours, the Atlas Pure RNA remains intact, exhibiting no visible degradation (Panel B).



**Figure 1. Atlas Pure RNA is free of genomic DNA impurities and nucleases.** Panel A. Each total RNA sample was isolated from 100 mg of rat liver using the indicated kit. Using genomic DNA-specific primers for the MHC class I gene, we performed PCR from 1 µg of each total RNA sample. Lanes 1–4: 1,000, 100, 10, and 0 pg of sheared rat liver genomic DNA, respectively. Lane 5: total RNA isolated using the Atlas Pure RNA Isolation Kit. Lane 6: total RNA isolated using a single-step purification kit. Lane 7: total RNA isolated using a spin-column format kit. Lane M: DNA size markers. Panel B. Each total RNA sample from Panel A was tested for nuclease contamination by incubation at 37°C for 8 hr. Lanes 1, 3, and 5: before incubation. Lanes 2, 4, and 6: after incubation.

Stability of RNA is especially important for RNase-rich tissues such as pancreas, spleen, and liver.

## Optimized reagents provided

The Atlas Pure Kit includes the necessary reagents for isolation of high-quality total RNA and a complete User Manual. We also provide DNase I for complete elimination of DNA contamination so that you can be absolutely confident of your RNA's purity.

Product	Size	Cat. #
Atlas Pure RNA Isolation Kit	each	K1038-1

## Components

- Denaturing Solution
- Saturation Buffer for Phenol
- RNase-free H<sub>2</sub>O
- 2 M NaOAc
- DNase I
- 10X DNase I Buffer
- Complete User Manual (PT3231-1)

Phenol, chloroform, and isopropanol are not included.

## Related Products

- Atlas™ cDNA Expression Arrays (many; see pages 2–4)
- SMART™ Kits (#K1051-1 & K1052-1; see pages 9–10)
- CLONTECH PCR-Select™ cDNA Subtraction Kit (#K1804-1)
- Delta™ Differential Display Kit (#K1810-1)
- Premium Poly A<sup>+</sup> & Total RNAs (many)
- Multiple Tissue Northern (MTN™) Blots (many)
- RNA Master Blots™ (#7770-1 & #7771-1)
- mRNA Separator (#K1040-2)

# ClonCapture™ cDNA Selection Kit

Novel RecA-based technique for rapid isolation of full-length cDNA clones

- Obtain clones in 2–3 days *without* library screening
- Sequence-specific enrichment for full-length clones
- Works with all plasmid libraries

The ClonCapture™ cDNA Selection Kit is a RecA-based system for rapidly isolating target clones from cDNA libraries without library screening. With the ClonCapture Kit, you can use a partial cDNA fragment as a probe to enrich for specific, full-length cDNA clones, including low- and medium-abundance clones. The ClonCapture procedure can be used with any plasmid library, including those constructed in phagemid, retroviral, and expression vectors. ClonCapture is ideal for isolating full-length clones using partial sequence obtained using the CLONTECH PCR-Select™ cDNA Subtraction Kit (#K1804-1) or using differential display methods such as our Delta™ Differential Display Kit (#K1810-1). ClonCapture can also be used to isolate differentially spliced products. Alternatively, you can use a degenerate probe to isolate clones that are homologous to your gene of interest.

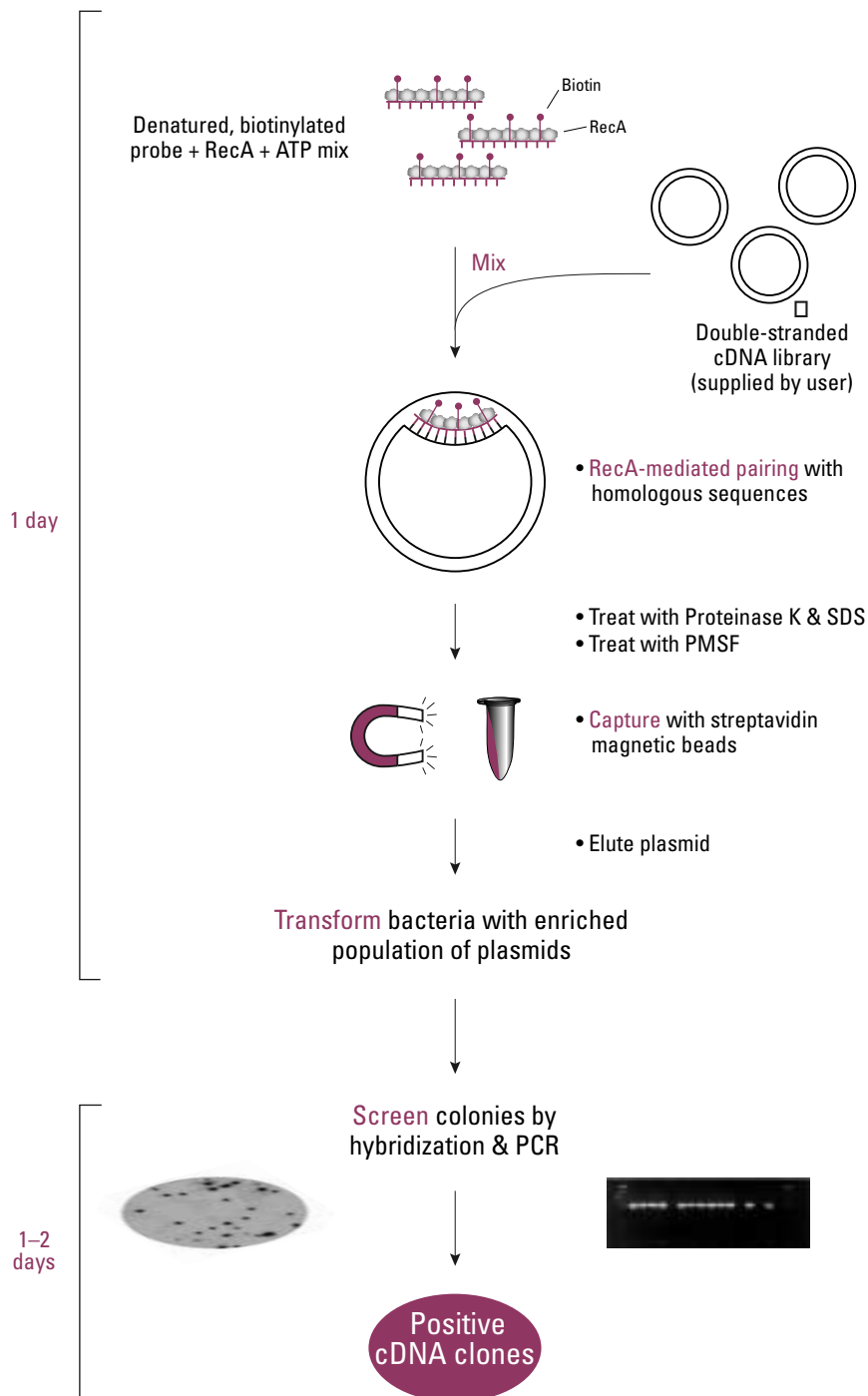
The ClonCapture Kit eliminates the need for conventional, labor-intensive library screening techniques that involve filter preparation and hybridization from large numbers of master plates, a process that can take weeks or months. By efficiently and specifically enriching for clones of interest, ClonCapture makes library screening a straightforward, routine procedure (Figure 1).

## The ClonCapture advantage

ClonCapture is more rapid and efficient than other cDNA enrichment techniques that do not utilize RecA and is more reliable than techniques that require conversion of the library to a single-stranded form. ClonCapture's use of longer probes affords more specificity than methods that use shorter and less specific oligo probes. In addition, although this novel technology is proprietary to CLONTECH, its use does not require a license.

## Full-length cDNA clones in days

ClonCapture facilitates the rapid isolation of cDNA clones by taking advantage of the function of the RecA protein, an essential component of



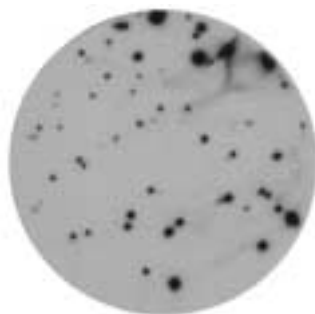
**Figure 1. The ClonCapture cDNA selection system.** ClonCapture allows the rapid isolation of cDNA clones from plasmid libraries by enriching for specific clones as much as  $5 \times 10^4$ -fold. The entire procedure, from synthesis of the biotinylated probe to transformation of the enriched plasmid population, can be performed in one day. Subsequent screening of individual clones takes 1–2 days.

# ClonCapture™ cDNA Selection Kit...continued

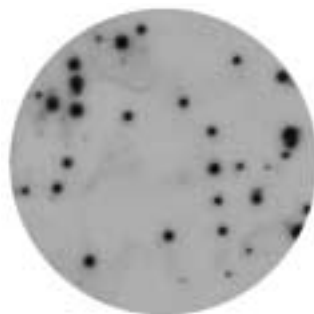
**Table I: ClonCapture enrichment of a synthetic library**

Cm <sup>r</sup> :Cm <sup>s</sup> * plasmid ratio	Cm <sup>r</sup> clones after enrichment	Enrichment factor
1:500	98%	490
	78%	390
	62%	310
1:5,000	48%	2,400
	30%	1,500
	25%	1,250
1:50,000	33%	16,500
	29%	14,500
	17%	8,500

\* Cm<sup>r</sup>/Cm<sup>s</sup> = chloramphenicol resistant/sensitive.  
Data represents three independent experiments at each ratio.



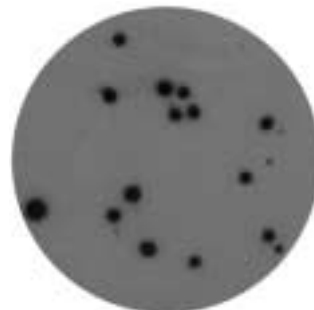
Estrogen receptor



Bcl-x



Dad1



RPA

**Figure 2. Colony hybridization of enriched clones.** ClonCapture was used to enrich for the four indicated genes from a human liver cDNA library. In each case, many positive clones were identified by screening a single plate of bacteria transformed with the enriched ClonCapture products.

the *E. coli* DNA repair system that promotes general genetic recombination. RecA promotes formation of complexes between a single-stranded DNA probe and homologous double-stranded DNA molecules (1–3), thus allowing the direct isolation of double-stranded (ds) plasmids containing a target sequence.

To perform the ClonCapture procedure, all you need is sufficient sequence information from a target gene to design primers for amplification of a 200–300-bp, biotinylated probe. This PCR product is then denatured, complexed with RecA, and used for library enrichment.

The biotinylated probe-RecA complex interacts with homologous sequences of duplex plasmid DNA by displacing one strand of the duplex to form a triple-stranded nucleoprotein complex. In the presence of ATP, RecA would normally catalyze recombination in such a complex. However, the presence of the nonhydrolyzable analog ATPγS stabilizes the triple-stranded intermediate and blocks further strand exchange. After proteolytic removal of RecA, these molecules are selectively captured on streptavidin-coated magnetic beads. The captured ds DNA is recovered by alkaline treatment, leaving the biotinylated probe attached to the magnetic beads. The enriched plasmid population is then precipitated, resuspended, and used to transform bacteria. The resulting colonies are screened by colony hybridization and PCR to identify the desired clones.

### Successful enrichment with ClonCapture

To demonstrate the efficiency of library enrichment using ClonCapture, we performed enrichment on a model library of kanamycin-resistant plasmid spiked with low percentages of a chloramphenicol-resistant plasmid, using a fragment of the chloramphenicol gene as probe. Upon transforming *E. coli* with the enriched clones, the efficiency of the procedure was evaluated by determining the percentage of resulting clones exhibiting chloramphenicol resistance (Table I). After a dilution of  $5 \times 10^4$ , the chloramphenicol-resistant plasmids were enriched more than  $10^4$ -fold, thus comprising more than 25% of the enriched population.

We also used ClonCapture to selectively enrich for seven genes of varying abundance from a human liver cDNA library (see Table II for

# ClonCapture™ cDNA Selection Kit...continued

complete results). After enrichment, colony hybridization was performed on one plate of transformants with each of the seven probes. Filters hybridized with four of these probes are shown in Figure 2. Subsequent PCR (Figure 3) analysis of the positive clones from the Bcl-x and RPA experiments confirmed that 70–80% of the hybridization positives contained the desired inserts, and sequencing confirmed that the majority of these clones were full-length. The final enrichment for these genes ranged from  $5 \times 10^2$ -fold to  $5 \times 10^4$ -fold (Table II).

The ClonCapture Kit provides the necessary reagents to perform five selection and one control experiments. Each kit is provided with a trial-size NucleoSpin® Extraction Kit, sufficient for four probe purifications. NucleoSpin utilizes a unique spin-cup technology that enables the rapid and efficient purification of PCR products away from unincorporated nucleotides and primers. ClonCapture has also been optimized for use with our Advantage® PCR Enzyme Systems, which feature built-in, antibody-mediated hot start for robust, sensitive, reliable performance.

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

NEW!

With every purchase of the ClonCapture Kit, you receive a free trial-size NucleoSpin® Extraction Kit (#K3051-x), sufficient for 4 probe purifications.

### Components

- RecA Protein
- 10X RecA Buffer
- 0.1 M CoCl<sub>2</sub>
- ATP Mix
- Dra I-digested λ DNA
- Proteinase K
- 0.1 M PMSF
- Streptavidin Magnetic Beads
- Binding Buffer
- Washing Buffer
- Elution Buffer
- Precipitation Mix
- Control Biotinylated PCR Fragment
- Control Plasmid Library
- Biotin-21 dUTP
- Complete User Manual (PT3246-1)

### Related Products

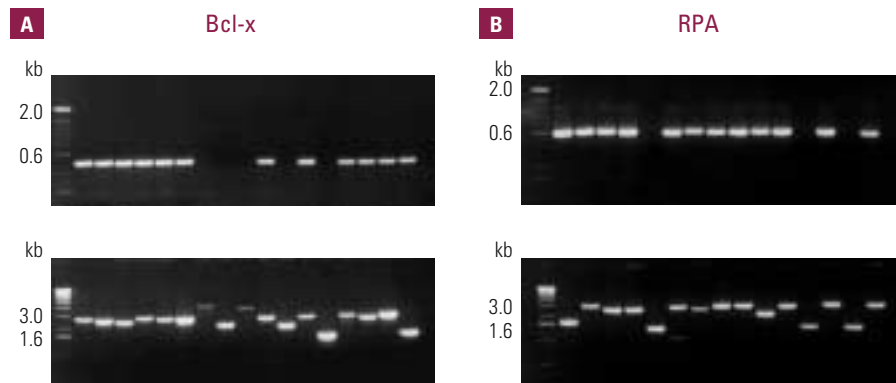
- cDNA Libraries (many)
- SMART™ PCR cDNA Library Construction Kit (#K1051-1; see pages 9–10)
- SMART™ PCR cDNA Synthesis Kit (#K1052-1)
- Advantage® cDNA PCR Kit (#K1905-y, -1)
- Advantage® cDNA Polymerase Mix (#8417-1)
- TaqStart™ Antibody (#5400-1, -2)
- NucleoSpin® Kits (many)

### References

1. Honigberg, S. M., 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. Teintze, M., et al. (1995) *Biochem. Biophys. Res. Comm.* **211**(3):804–811.

### Notice to Purchaser

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



**Figure 3. PCR confirmation of colonies isolated with ClonCapture.** Bcl-x (Panel A) and RPA (Panel B) clones obtained from the colony hybridization shown in Figure 2 were positively identified using gene-specific primers (upper panels). The sizes of the inserts were determined using vector-specific primers (lower panels).

**Table II: ClonCapture cDNA enrichment from a human liver library**

Gene	Representation in original library (%)	Positive clones after hybridization (%)	Confirmation by sequencing (%)	Enrichment
Estrogen receptor	0.005	5	75	$8 \times 10^2$
Bcl-x (apoptosis regulator)	0.005	4.5	88	$8 \times 10^2$
RPA (ribosomal protein)	0.007	3.2	100	$5 \times 10^2$
GBP (GTP-binding protein)	0.0002	10	nd	$5 \times 10^4$
IAP (apoptosis inhibitor)	0.0002	9	nd	$4.5 \times 10^4$
Dad1	0.005	27	nd	$5.4 \times 10^3$
Tob1	0.01	15	nd	$1.5 \times 10^3$

nd = not done

# SMART™ PCR cDNA Library Construction Kit

Improved directional cloning method generates high-quality cDNA libraries

- **High representation of full-length cDNA clones**
- **SMART technology combined with  $\lambda$ TriplEx2 based directional cloning**
- **No adaptor ligation or methylation steps required**
- **Requires as little as 50 ng of total RNA**

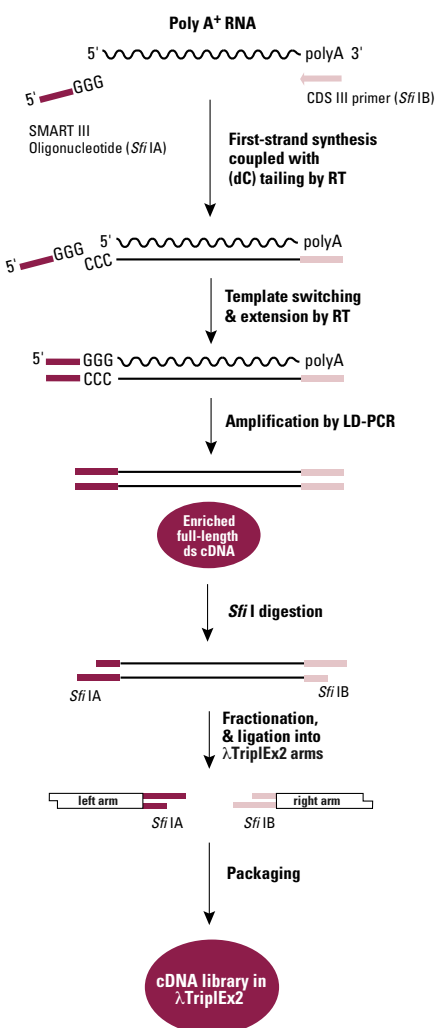
The SMART™ PCR cDNA Library Construction Kit is a PCR-based method for generating high-quality cDNA libraries from as little as 50 ng of total RNA or 25 ng of poly A<sup>+</sup> RNA. This kit utilizes SMART technology and long-distance PCR (LD-PCR; 1, 2) to provide high yields of full-length, double-stranded (ds) cDNA.

The new improved kit incorporates adaptor-free, directional cloning into CLONTECH's  $\lambda$ TriplEx2 Vector. This combination provides a fast and simple method for generating high-quality, directionally cloned cDNA libraries, proven to contain a significantly higher percentage of full-length clones than cDNA libraries constructed by conventional methods, such as Gubler & Hoffman protocols (3, 4).

## Full-length cDNAs with 5' ends

Because reverse transcriptase (RT) cannot always transcribe the entire mRNA sequence, the 5' ends of genes tend to be under-represented in cDNA populations performed by conventional methods. This is particularly true if the first-strand synthesis is primed with oligo(dT) primers or if the mRNA has a persistent secondary structure (5). In addition, T4 DNA polymerase-based conventional adaptor ligation and cloning methods typically result in a loss of cDNA sequence (1–54 bases) at the 5' end. New improved directional cloning method eliminates T4-adaptor procedures, therefore retaining more of the 5' end to obtain full-length cDNA.

In the SMART cDNA synthesis method, CDS III (a modified oligo[dT] primer) is used to begin reverse transcription. When the RT reaches the 5' end of the mRNA, the SMART III™ (patent



**Figure 1. Flow chart of the SMART PCR cDNA Library Construction Kit protocol.** The entire procedure takes only 2 days.

pending) Oligonucleotide serves to extend the template at the 5' end, allowing MMLV-RT to switch templates and continue replicating (Figure 1; 6). The resulting full-length single-stranded (ss) DNA contains the complete 5' end of the mRNA as well as the sequence complementary to the SMART III Oligonucleotide, which serves as a universal PCR priming site (SMART anchor) in the subsequent amplification.

## Selective amplification

Only those ss cDNAs having the SMART anchor sequence at the 5' end can be amplified using the 3' and 5' PCR primers provided. In most cases, incomplete cDNAs and cDNA transcribed from poly A<sup>-</sup> RNA will lack the SMART anchor sequence and will not be amplified. This selective amplification allows you to construct a cDNA library with a higher percentage of full-length clones and eliminates contamination by genomic and poly A<sup>-</sup> RNA (Table I).

## Easy directional cloning

The unique SMART III Oligonucleotide and CDS III Primer incorporate asymmetrical *Sfi* I sites (A & B) at the 5' and 3' ends of the cDNA, respectively, during cDNA synthesis (Figure 1). After digestion with *Sfi* I and size fractionation, SMART cDNA is ligated into the *Sfi* I-digested  $\lambda$ TriplEx2 arms which were specially designed to include these sizes (Figure 2). There is no need to blunt-end the cDNA or ligate adaptors. Furthermore, since the *Sfi* I sites are extremely rare in mammalian DNA, there is no need for a methylation step. As a result, all SMART cDNAs remain intact after *Sfi* I digestion while valuable internal restriction sites are preserved.

The  $\lambda$ TriplEx2 MCS is located within an embedded plasmid, which is flanked by *loxP* sites at the  $\lambda$  junctions. Transduction of a  $\lambda$ TriplEx2 lysate into *E. coli* strain BM25.8 promotes Cre recombinase-mediated release and circularization of pTriplEx2 at the *loxP* sites. The two translation start sites and the (dT)<sub>13</sub> slip site are located upstream of the MCS, which allows the insert to be translated in the correct reading frame (Figure 2). This feature makes SMART cDNA libraries well suited for expression-based screening methods in *E. coli*, albeit the 5' untranslated regions of some clones may contain stop codons that would inhibit expression.

The  $\lambda$ TriplEx2 Vector offers the advantage of cloning in a phagemid vector—high-titer libraries, blue/white screening, and regulated expression of cloned inserts. In addition, Cre-*lox*-mediated subcloning provides a simple and easy method of converting clones from phage to plasmid vector.

# SMART™ Library Construction Kit...continued

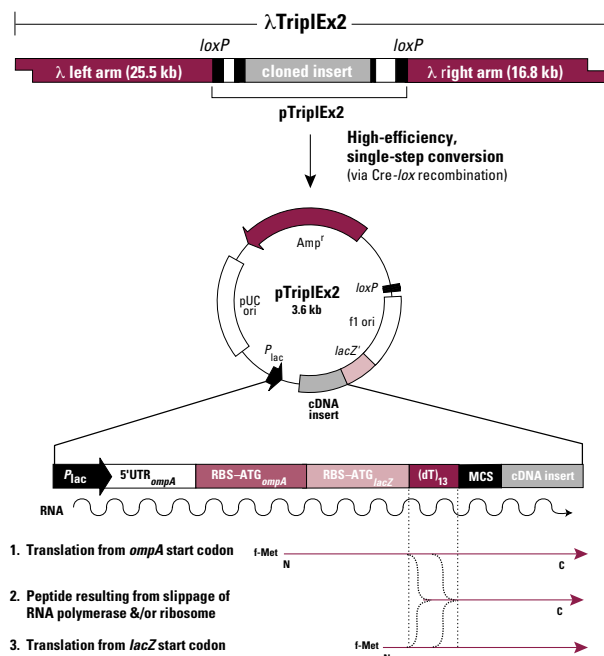
**Table I: 5'-end sequence analysis of SMART cDNA clones**

Clone #	Gene	Size of mRNA (kb)	Additional sequence* (bp)	Additional sequence matches known: genomic sequence	Additional sequence matches known: transcription start site
1	Human transferrin receptor	5.0	+20	Yes	Yes
2	Human cytoskeletal $\gamma$ -actin	1.9	+1	Yes	Yes
3	Human smooth muscle $\alpha$ -actin (aortic)	1.33	+18	Yes	Yes
4	Human smooth muscle $\gamma$ -actin (enteric)	1.28	+11	Yes	Yes
5	Human ribosomal protein L7	0.8	+21	Yes	n/a
6	Human myosin light chain-2	0.77	+11	Yes	n/a
7	Human myosin light chain-2	0.77	+10	Yes	n/a
8	Human LIM domain protein (CLP)	0.6	+19	n/a	n/a
9	Human ATPase factor 6 (F6)	0.5	+54	n/a	n/a
10	Human cytochrome C oxidase (fraction IV, liver)	0.5	+11	n/a	n/a

\* Compared with the longest corresponding cDNA sequence deposited in GenBank; does not include the SMART anchor sequence.

n/a = not available

**Table I.** Two cDNA libraries were constructed in  $\lambda$ gt11 using human skeletal muscle or placenta total RNA and the SMART PCR cDNA Library Construction Kit. 50 recombinant phage plaques from each library were selected at random and their inserts sequenced. Ten of the sequences (clones 1–10 above) were identified in a search of the GenBank database. For clones 1–7, the additional sequence agrees with the known genomic sequence. For clones 1–4, the transcription start sites had been previously determined by primer extension or RNase protection assays. Similar results are expected with  $\lambda$ TriplEx2.



**Figure 2.** The  $\lambda$ TriplEx2 expression system.

Product	Size	Cat. #
SMART PCR cDNA Library Construction Kit	each	K1051-1

With every purchase of the SMART PCR cDNA Library Construction Kit, you receive a free trial-size Advantage® cDNA PCR Kit (#K1905-y), sufficient for 15 SMART PCR reactions. Reverse transcriptase is not included in the kit.

### Components

- CDS III Primer
- SMART III™ Oligonucleotide
- 5X First-Strand Buffer
- DTT
- Control Total RNA
- Glycogen
- 5' PCR Primer
- Proteinase K
- *Sfi* I enzyme & 10X Buffer
- 100X BSA
- $\lambda$ TriplEx2 *Sfi* I (A & B) arms
- T4 DNA Ligase & 10X Buffer
- ATP
- *E. coli* BM25.8 & XL1-Blue
- Control Insert
- 5' & 3' Sequencing Primer
- dNTP mix
- Sodium Acetate
- Deionized H<sub>2</sub>O
- DEPC CHROMA-SPIN™ 400 & 1X Buffer
- Complete User Manual (PT3000-1)
- Protocol-at-a Glance (PT3000-2)
- $\lambda$ TriplEx2 Vector Information Packet (PT3194-5)

### Notice to Purchaser

Use of the *Sfi* 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.

### References

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2. Cheng, S., et al. (1994) *Proc. Natl. Acad. Sci. USA* **91**:5695–5699.
3. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25**: 263–269.
4. Zhu, Y., et al. (July 1996) *CLONTECHniques* **XI**(3): 30–31.
5. CapFinder PCR cDNA Library Construction Kit (January 1996) *CLONTECHniques* **XI**(1):2–4.
6. Furuichi, Y. & Miura, K. (1975) *Nature* **253**:374–375.

# New Retroviral Expression Libraries & Vectors

## New retroviral cDNA libraries & EGFP expression vectors

- **Complete retroviral cDNA cloning systems**
- **New EGFP fusion vectors**
- **Effectively and stably transduce many cell types with high efficiency**

CLONTECH introduces more libraries and vectors as part of our Retro-X™ line of retroviral expression products. Retroviral gene transfer is a well-established technique for stably introducing genes into mammalian cells. The new **Human Mammary Gland** and **Human HeLa Retroviral Libraries** are high-quality, full-length cDNA libraries for retroviral expression cloning (1). CLONTECH's new **pLEGFP-N1** and **pLEGFP-C1 Vectors** allow retroviral expression of fusions to enhanced green fluorescent protein (EGFP), a revolutionary reporter for studying gene expression and protein localization.

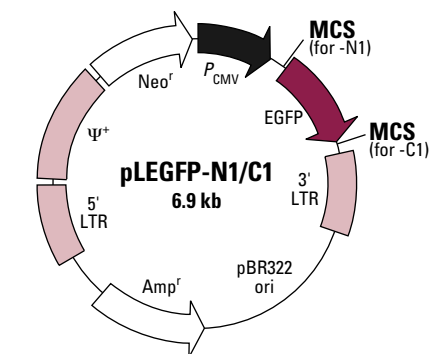
### Retroviral expression cloning

CLONTECH's Retroviral Libraries are constructed in the pLIB vector, which allows efficient transfer of inserts up to 8 kb. cDNA inserts are directionally cloned and are expressed from the 5' LTR of murine leukemia virus. Each Retroviral Library is prescreened by PCR to confirm the presence and full-length nature of several genes of varying abundance.

Transfection of a packaging cell line with a Retroviral Library leads to production of high-titer virus that can be used to infect target cells. You can then select for a desired phenotype and recover positive clones by PCR using the primers provided. Alternatively, these libraries can be used as conventional expression libraries when cotransfected with a vector possessing a selectable marker.

Expression cloning is a powerful approach that should permit the isolation of any gene whose expression is associated with a selectable phenotype. Many oncogenes, cytokines, cytokine receptors, and transcription factors have been identified in this way (2).

Retroviruses allow efficient transfer of high-complexity libraries into virtually all cell types, including primary cells. Transduction efficiencies of up to 100% overcome limitations inherent



### pLEGFP-N1 MCS

```
TCA GAT CTC GAG CTC AAG CTT CGA ATT CTG CAG TCG ACG
      BglII XhoI HindIII SalI/AccI
GTA CCG CGG GCC CCG GAT CCA CCG GTC GCC ACC ATG GTG
      ApaI BamHI EGFP
```

### pLEGFP-C1 MCS

```
EGFP
TAC AAG TCC GGA CTC AGA TCT CGA GCT CAA GCT TCG AAT
      BglII XhoI HindIII
TCT GCA GTC GAC GGT ACC GCG GGC CCG GGA TCC ACC GGA
      SalI/AccI ApaI BamHI
TCT AGA TAA CTG ATC
      STOPs
```

**Figure 1. pLEGFP-N1 & pLEGFP-C1 Vector maps and MCS.** Inserts cloned into pLEGFP-N1 should contain an initiating ATG codon.

in conventional expression libraries. In addition, retroviral vectors integrate at low copy number (~1 copy/cell), facilitating PCR rescue and characterization of positive clones after selection is complete.

### EGFP fusion expression vectors

The pLEGFP-N1 and pLEGFP-C1 Vectors are designed for construction of amino- and carboxy-terminal protein fusions to a gene of interest that are expressed under control of the strong CMV promoter. EGFP, the brightest available variant of green fluorescent protein (3, 4), is an ideal reporter for monitoring gene expression and protein localization in mammalian systems. In addition, the coding sequence of EGFP has been codon optimized for high expression in mammalian cells. Retroviral delivery allows for the single-copy expression of these constructs. Alternatively, these vectors can be used simply to express EGFP as a marker for transfection and infection efficiency.

Product	Size	Cat. #
Human Mammary Gland Retroviral Library	each	HL8001BB
Human HeLa Retroviral Library	each	HL8002BB
pLEGFP-N1 Retroviral Vector	20 µg	6059-1
pLEGFP-C1 Retroviral Vector	20 µg	6058-1

### Library Components

- **Library DNA**
- **Library Culture** (in *E. coli*)
- **pLIB Vector**
- **pLIB-EGFP Control Vector**
- **5' & 3' LIB Primers**
- **Complete User Manual** (PT3230-1)
- **Vector Information Packets** (PT3232-5 & PT3233-5)

### Related Products

- **Retro-X™ System & Vectors** (many)
- **RetroPack™ PT67 Cell Line** (#K1060-D)
- **Human Placenta Retroviral Library** (#HL8000BB)
- **pPUR Vector** (#6056-1)
- **pNeoEGFP Vector** (#6011-1)
- **pHygEGFP Vector** (#6014-1)
- **pEGFP Vectors** (many)
- **CLONfectin™** (#8020-1)
- **CalPhos™ Mammalian Transfection Kit** (#K2051-1)

### References

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2. Whitehead, I., et al. (1995) *Mol. Cell. Biol.* **15**: 704-710.
3. Cormack, B., et al. (1996) *Gene* **173**:33-38.
4. Yang, T. T., et al. (1996) *Nucleic Acids Res.* **24**: 4592-4593.



CLONTECH is proud to announce that we have received an esteemed R&D 100 Award for our Retro-X™ System.

# Tools for Gene Expression Analysis

Made using Premium RNA™—the foundation of scientific success

- **Versatile**—products for a wide range of applications
- **Proven**—more than 3,000 Premium RNA citations
- **Wide selection**—choose from different sources and formats
- **High quality**—saves you time and labor

CLONTECH's Premium RNA™ products provide an integrated platform of tools to accelerate gene expression studies. Because we produce RNA of exceptional quality and established reliability, you save time and labor—and achieve success. Each of our new **Total RNA Panels** includes high-quality total RNA from six human tissues. The new **Human Digestive System 12-Lane Multiple Tissue Northern (MTN™) Blot** and **Multiple Tissue cDNA (MTC™) Panel** are valuable tools for gastrointestinal researchers. Likewise, **QUICK-Clone™ cDNAs** and **Marathon-Ready™ cDNAs** are now available from 14 xenografted human tumors, as well as from adipose tissue.

## Premium RNA sets the standard for quality

CLONTECH has provided scientists with superior RNA products for more than 10 years. Our wealth of experience is evident in the quality and reliability of our tools for gene expression analysis. The foundation of these products is Premium RNA—high-quality total and poly A<sup>+</sup> RNA samples from hard-to-obtain tissues. Each total RNA sample is meticulously prepared using a modified guanidinium thiocyanate method, and each poly A<sup>+</sup> RNA sample is purified via 2–3 rounds of oligo(dT)-cellulose purification. We perform rigorous quality control tests to confirm that each preparation consists of intact, full-length RNA that is virtually free of genomic DNA and nucleases, and that poly A<sup>+</sup> RNA lacks rRNA sequences. Our expertise and attention to detail ensures that you get accurate, dependable results from any of our wide selection of products.

## Versatile tools for many applications

CLONTECH's Premium RNA products can be used for gene cloning and expression analysis



Illustration inspired by the art of René Magritte (1898–1967).

(Figure 1). Table II (page 14) compares these products, each of which is available for a variety of different tissues and cell types. Combining more than one of these approaches is crucial for elucidating the molecular events underlying any complex biological system. In addition, we offer a wide selection of Premium Total and Poly A<sup>+</sup> RNAs prepared from hard-to-obtain tissues. By mixing and matching these products, you can customize your own gene expression toolbox to efficiently and thoroughly investigate your genes of interest.

## New Total RNA Panels—more tissues, better value

Total RNA Panels are the newest Premium RNA products available from CLONTECH. Each panel includes 50-µg samples of high-quality total RNA from six different human tissues (Table I). These panels are economical and

are ideal for applications such as RT-PCR and RNase protection assays, which require only small quantities of each RNA. For customers who need larger samples, each of our Total RNAs is available individually in a 250-µg size.

## Depend on our blots for publication-quality results

MTN Blots are premade Northern blots featuring Premium Poly A<sup>+</sup> RNA from different human, mouse, or rat tissues. Our MTN Blots have been referenced in more than 2,000 journal articles. Premium Poly A<sup>+</sup> RNA, careful preparation, and exacting quality control make them the industry standard. MTN Blots are ready for immediate use with radioactively or nonradioactively labeled probes—simply hybridize, wash, and detect the signals. Each blot can be stripped and reprobed several times. MTN Blots are most suitable for detect-

Table I: Tissue representation in Human Total RNA Panels

Human Panel I (#K4000-1)	Human Panel II (#K4001-1)	Human Panel III (#K4002-1)	Human Panel IV (#K4003-1)	Human Panel V (#K4004-1)
Brain	Bone marrow	Heart	Brain	Adrenal Gland
Heart	Colon	Mammary gland	Cerebellum	Liver
Kidney	Small intestine	Prostate	Fetal brain	Pancreas
Liver	Spleen	Skeletal muscle	Fetal liver	Prostate
Lung	Stomach	Testis	Spinal cord	Salivary gland
Trachea	Thymus	Uterus	Placenta	Thyroid

# Tools for Gene Expression Analysis...continued

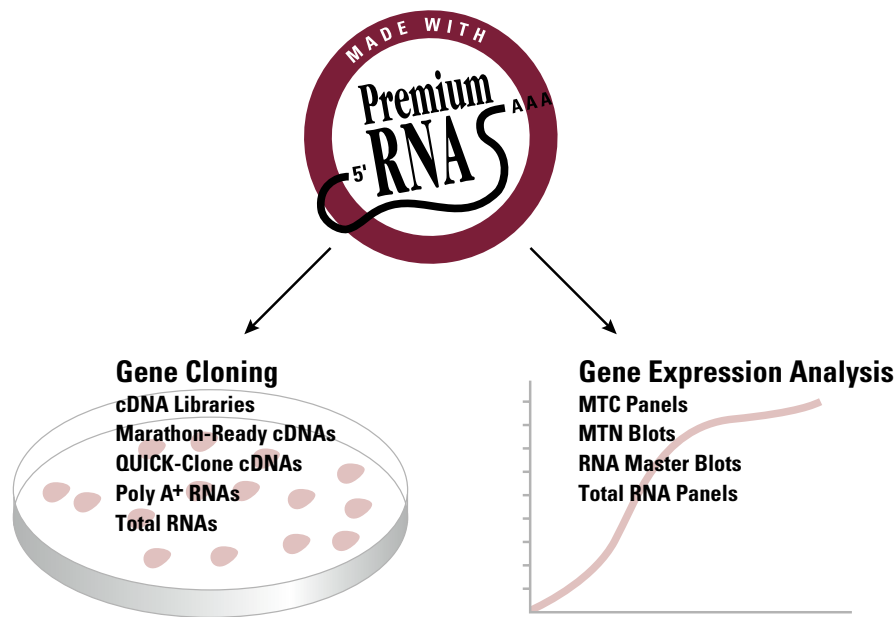


Figure 1. Premium RNA products facilitate gene cloning and expression analysis.

ing transcripts in the range of 0.5–10 kb. A complete User Manual (PT1200-1) and  $\beta$ -actin cDNA probe are included, as well as a free trial size of ExpressHyb™ Hybridization Solution.

CLONTECH offers a wide selection of MTN Blots. The new Human Digestive System 12-Lane MTN Blot features poly A<sup>+</sup> RNA from the following tissues: cecum, colon (ascending, descending, and transverse), duodenum, esophagus, ileocecum, jejunum, ileum, liver, rectum, and stomach.

We also offer RNA Master Blots™ for profiling gene expression in many tissues simultaneously. The Human RNA Master Blot features Premium Poly A<sup>+</sup> RNA from 50 different human tissues, and the Mouse RNA Master Blot includes Premium Poly A<sup>+</sup> RNA from 20 mouse tissues; both include several negative controls. Master Blots are carefully normalized using eight different housekeeping genes, avoiding discrepancies that result when loading is normalized to the expression of any single housekeeping gene (1–3). Master Blots provide semiquantitative results and are more sensitive

than MTN Blots, but they cannot be used to distinguish between mRNAs of different sizes. For obtaining information about transcript size or splice variants, MTN Blots are the better choice.

### MTC Panels provide a sensitive, semi-quantitative assay

MTC Panels are sets of first-strand cDNAs generated using Premium Poly A<sup>+</sup> RNA from 7–12 different tissues. We carefully normalize each cDNA preparation to the expression of seven housekeeping genes, so gene expression results are extremely accurate. By performing PCR using gene-specific primers, you can analyze gene expression across many tissues—in a fraction of the time required for Northern or dot blot analyses (4). Due to the high sensitivity of RT-PCR, screening of MTC Panels permits detection of mRNAs present at all abundance levels, including very rare transcripts that may not be detectable by Northern blot analysis. Like MTN Blots, MTC Panels may also reveal alternative splicing events.

The Human Digestive System MTC Panel is the newest addition to CLONTECH's selection of MTC Panels. The tissues included are the same

as those represented on the Human Digestive System 12-Lane MTN Blot, so you can quickly verify and extend your hybridization results.

Each MTC Panel cDNA preparation is sufficient for 10 reactions. G3PDH 5' and 3' control PCR primers, control cDNA, complete source information, and a complete User Manual (PT3004-1) are also included. All you need to supply is your gene-specific primers and a polymerase mix for PCR.

### QUICK-Clone cDNAs: Clone without library screening

Traditional library construction and screening procedures may be circumvented by direct amplification of your target cDNA using gene-specific PCR primers—provided that the cDNA template is of the highest quality. QUICK-Clone cDNAs are high-quality, double-stranded cDNA that can be used for cloning by PCR. Each QUICK-Clone cDNA is synthesized from Premium Poly A<sup>+</sup> RNA instead of total RNA, so fewer nonspecific products are generated in PCR. The cDNAs are further purified to eliminate residual RNA and are size-selected to remove fragments smaller than 400 bp.

New QUICK-Clone cDNAs are available from 14 human tumors propagated in athymic nude mice. Xenografted tumors are a superior model of tumorigenesis to cultured cell lines and provide a more consistent source of RNA than do pathological specimens (5). Human, and Rat Fat QUICK-Clone cDNAs are also available, providing new tools for studying obesity and the biology of adipocytes.

Each QUICK-Clone cDNA includes two tubes of double-stranded cDNA sufficient for a total of 20 reactions, and a complete User Manual (PT1150-1). You need only supply your gene-specific primers and a polymerase mix for PCR.

### Marathon-Ready cDNAs are RACE-ready

The same new tissues featured in our QUICK-Clone cDNAs are also available as Marathon-Ready cDNAs—premade, tissue-specific pools of cDNA designed for 5'- and 3'-RACE PCR (6). CLONTECH's Marathon™ cDNA amplification technology has been used successfully by hundreds of researchers to obtain full-length cDNAs, and convenient Marathon-Ready cDNAs

# Tools for Gene Expression Analysis...continued

make it even easier. Each Marathon-Ready cDNA is synthesized from Premium Poly A<sup>+</sup> RNA using a procedure optimized to produce full-length cDNA and eliminate 3' heterogeneity (7). After synthesis, the double-stranded cDNA is ligated to the Marathon Adaptor, specially designed with CLONTECH's patented suppression PCR<sup>†</sup> technology (8). With Marathon-Ready cDNAs, you can use the same template to perform 5'- and 3'-RACE and generate the full-length cDNA.

Each Marathon-Ready cDNA is sufficient for 30 reactions and includes an adaptor primer, nested adaptor primer, 5' and 3' control G3PDH primers, a complete User Manual (PT1156-1), and a Protocol-at-a-Glance (PT1156-2). To perform 5'- and 3'-RACE with Marathon-Ready cDNA, all you need is your

gene-specific primers and a polymerase mix for PCR.

## Rely on CLONTECH's cDNA libraries for results

CLONTECH, the industry leader in libraries, is dedicated to meeting your cloning needs. Our quality cDNA libraries have been cited more than 1,500 times. All libraries are carefully prepared and undergo rigorous quality control testing to ensure that you have a high probability of obtaining full-length cDNA clones.

## Optimized for Advantage<sup>®</sup> PCR Enzyme Systems

For gene expression analysis or cloning with our PCR-based products, we recommend our Advantage PCR Enzyme Systems—optimized for high efficiency and fidelity in conventional and

long-distance PCR (9, 10). Each Advantage cDNA Polymerase Mix contains a primary polymerase, a minor amount of a proofreading polymerase, and TaqStart<sup>™</sup> antibody to provide a built-in hot start (11). This combination increases the efficiency, yield, sensitivity, and specificity of all PCR assays.

For use with MTC Panels, Marathon-Ready cDNAs, and QUICK-Clone cDNAs, we recommend the Advantage cDNA Polymerase Mix, also available as part of the Advantage cDNA PCR Kit. If your gene of interest has a high GC content, use the Advantage-GC Kits, which contain the GC-Melt<sup>™</sup> reagent<sup>††</sup>. For applications that require the highest fidelity, choose the Advantage-HF PCR Kit. See the Related Products on the next page for a list of CLONTECH's Advantage PCR Enzyme Systems.

**Table II: CLONTECH's Premium RNA products**

Product*	Technique	Time	Applications
<b>cDNA Libraries</b>	Nucleic acid, antibody, or two-hybrid screening; expression cloning	3–6 weeks	Obtain full-length cDNA clones for a variety of purposes
<b>Marathon-Ready cDNAs</b>	5' and 3' RACE PCR and full-length cDNA cloning	3 days	Obtain full-length cDNA clones from partial gene sequences generated from cDNA or genomic libraries, expressed sequence tags, differential display, or cDNA subtraction
<b>Multiple Tissue Northern (MTN) Blots</b>	Hybridization of cDNA probes to a Northern blot	1–5 days	Analyze the tissue expression pattern of specific transcripts in 4–12 tissues simultaneously  Obtain information about transcript size, isoforms, and relative abundance levels
<b>Multiple Tissue cDNA (MTC) Panels</b>	PCR amplification of specific sequences	1 day	Obtain semiquantitative data about the expression patterns of specific transcripts in 7–12 tissues simultaneously  Investigate expression of rare transcripts that cannot be detected by Northern blot analysis
<b>QUICK-Clone cDNAs</b>	PCR-based cDNA cloning	1–2 days	Clone cDNAs without library screening  Generate hybridization probes using gene-specific or degenerate primers
<b>RNA Master Blots</b>	Hybridization of probe to mRNA array	1–5 days	Obtain semiquantitative data about the expression pattern of specific transcripts in 20–50 tissues simultaneously
<b>Total RNA Panels</b>	RNase protection assays; RT-PCR	1–3 days	Obtain quantitative data about the expression patterns of specific transcripts in 6 tissues simultaneously  Investigate expression of rare transcripts that cannot be detected by Northern blot analysis

\* See product listing for new Premium RNA products. For a complete list of Premium RNA products, please visit our website ([www.clontech.com](http://www.clontech.com)).

# Tools for Gene Expression Analysis...continued

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## Related Products

- **Advantage® cDNA PCR Kit** (#K1905-1, -y)
- **Advantage® cDNA Polymerase Mix** (#8417-1)
- **Advantage®-GC cDNA PCR Kit** (#K1907-1, -y)
- **Advantage®-GC cDNA Polymerase Mix** (#8419-1)
- **Advantage®-HF PCR Kit** (#K1909-1, -y)
- **Advantage® RT-for-PCR Kit** (#K1402-1, -2)
- **Atlas™ cDNA Expression Arrays** (many; see pages 2-4)
- **CLONTECH PCR-Select™ cDNA Subtraction Kit** (#K1804-1)
- **Delta™ Differential Display Kit** (#K1810-1)
- **Marathon™ cDNA Amplification Kit** (#K1802-1)
- **Premium Poly A+ & Total RNAs** (many)
- **RNA Master Blots™** (#7770-1 & #7771-1)
- **RT-PCR Amplimer Sets** (many)
- **TaqStart™ Antibody** (#5400-1, -2)

## Marathon-Ready cDNA\*

	Rxns	Cat. #
Human Adipose	30	7491-1
Human Subcutaneous Fat	30	7492-1
Mouse White Fat	30	7463-1
Rat Brown Fat	30	7482-1
Human XG Burkitt's Lymphoma (Daudi)	30	7504-1
Human XG Colon Adenocarcinoma (CX-1)	30	7496-1
Human XG Colon Adenocarcinoma (GI-112)	30	7499-1
Human XG Glioblastoma (SF-295)	30	7507-1
Human XG Lung Carcinoma (GI-117)	30	7497-1
Human XG Lung Carcinoma (LX-1)	30	7495-1
Human XG Malignant Melanoma (A375)	30	7508-1
Human XG Malignant Melanoma (GI-105)	30	7505-1
Human XG Mammary Carcinoma (GI-101)	30	7493-1
Human XG Ovarian Carcinoma (GI-102)	30	7503-1
Human XG Pancreatic Adenocarcinoma (GI-103)	30	7494-1
Human XG Prostatic Adenocarcinoma (MRI-H-1579)	30	7509-1
Human XG Prostatic Adenocarcinoma (PC-3)	30	7498-1
Human XG Renal Carcinoma (MRI-H-121)	30	7506-1

Product	Size	Cat. #
Human Digestive System 12-Lane MTN Blot	each	7782-1
Human Digestive System MTC Panel	10 rxns	K1424-1
Human Total RNA Panel I	each	K4000-1
Human Total RNA Panel II	each	K4001-1
Human Total RNA Panel III	each	K4002-1
Human Total RNA Panel IV	each	K4003-1
Human Total RNA Panel V	each	K4004-1

NEW!

## QUICK-Clone cDNA\*

	Rxns	Cat. #
Human Adipose	2 x 10	7216-1
Human Subcutaneous Fat	2 x 10	7217-1
Rat Brown Fat	2 x 10	7161-1
Rat White Fat	2 x 10	7159-1
Human XG Burkitt's Lymphoma (Daudi)	2 x 10	7207-1
Human XG Colon Adenocarcinoma (CX-1)	2 x 10	7202-1
Human XG Colon Adenocarcinoma (GI-112)	2 x 10	7205-1
Human XG Glioblastoma (SF-295)	2 x 10	7213-1
Human XG Lung Carcinoma (GI-117)	2 x 10	7203-1
Human XG Lung Carcinoma (LX-1)	2 x 10	7201-1
Human XG Malignant Melanoma (A375)	2 x 10	7214-1
Human XG Malignant Melanoma (GI-105)	2 x 10	7208-1
Human XG Mammary Carcinoma (GI-101)	2 x 10	7199-1
Human XG Ovarian Carcinoma (GI-102)	2 x 10	7206-1
Human XG Pancreatic Adenocarcinoma (GI-103)	2 x 10	7200-1
Human XG Prostatic Adenocarcinoma (MRI-H-1579)	2 x 10	7215-1
Human XG Prostatic Adenocarcinoma (PC-3)	2 x 10	7204-1
Human XG Renal Carcinoma (MRI-H-121)	2 x 10	7209-1

\* XG indicates a xenografted human tumor propagated in nude mice.

Please visit our website ([www.clontech.com](http://www.clontech.com)) for a complete listing of Premium RNA products.

## Notice to Purchaser

<sup>†</sup> Suppression PCR is covered by U.S. Patents #5,565,340 & 5,579,822; foreign patents pending.

<sup>\*\*</sup> GC-Melt is covered by U.S. Patent #5,545,539; foreign patents pending.

The PCR process is covered by patents owned by Hoffmann-La Roche, Inc., and F. Hoffmann-La Roche, Ltd.

## New 1-Liter ExpressHyb!

CLONTECH's popular ExpressHyb Hybridization Solution is now available in an economical 1-L size. ExpressHyb is an optimized, low-viscosity solution that reduces hybridization time while significantly decreasing background (12). ExpressHyb can be used effectively with either radioactively or nonradioactively labeled DNA or oligonucleotide probes. With ExpressHyb, you can perform Northern, Southern, and colony hybridizations in just one hour. ExpressHyb is also available in 250-ml and 500-ml sizes, and samples are included with MTN Blots, RNA Master Blots, and Atlas™ cDNA Expression Arrays.

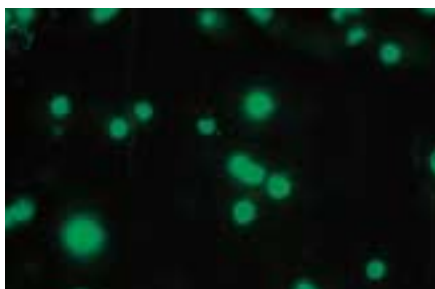
Product	Size	Cat. #
ExpressHyb Hybridization Solution	250 ml	8015-1
	500 ml	8015-2
	1 L	8015-3

NEW!

# ApoAlert™ Annexin V-EGFP

The brightest green fluorescent reagent for early detection of apoptosis

- **Highly sensitive reagent for early detection of apoptosis**
- **Suitable for fluorescence microscopy, flow cytometry, and 96-well assays**
- **Resists photobleaching**



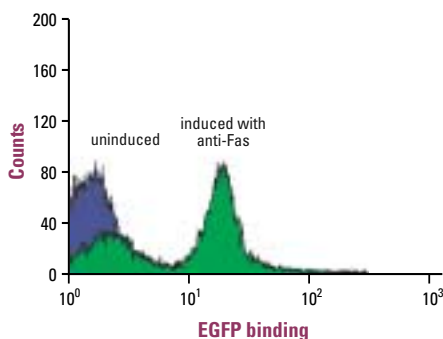
**ApoAlert™ Annexin V-EGFP** (patent pending) is the brightest available green fluorescent reagent for annexin V-based detection of apoptosis. The ApoAlert Annexin V-EGFP Apoptosis Kit provides everything you need to detect the externalization of phosphatidylserine (PS) on the plasma membrane, one of the earliest indicators of the onset of apoptosis (1–3). Annexin V-EGFP is also available separately as a bulk reagent.

## EGFP improves sensitivity and facilitates quantitative analysis

The Annexin V apoptosis assay takes advantage of the fact that PS is translocated from the inner face of the plasma membrane to the cell surface soon after the induction of apoptosis. Once on the cell surface, PS can be bound by annexin V, a protein with a strong, specific affinity for PS (4–6). Using the ApoAlert annexin V assay, you can detect apoptosis in a one-step, 10-minute staining procedure. The assay is nonenzymatic, does not require fixation, and is suitable for both adherent and suspension cells.

ApoAlert Annexin V-EGFP is the best reagent for experiments that require the highest sensitivity because enhanced green fluorescent protein (EGFP; 4, 5) is extraordinarily bright and resistant to photobleaching. Like our annexin V-FITC conjugate, Annexin V-EGFP can be used to detect apoptotic cells either by fluorescence microscopy (Figure 1) or by flow cytometry (Figure 2). Because Annexin V-EGFP is a fusion protein and thus has a 1:1 ratio of EGFP and PS, results obtained using Annexin V-EGFP are quantitative. The Annexin V-EGFP assay can easily be formatted for 96-well assays.

**Figure 1. ApoAlert Annexin V-EGFP generates a bright green fluorescent signal.** Jurkat cells were incubated with 200 ng/ml of Human Fas Monoclonal Antibody (clone CH-11) for 8 hr to induce apoptosis. Cells were centrifuged, resuspended in 1X Binding Buffer, and incubated with 1  $\mu$ l of Annexin V-EGFP for 5 min in the dark. Results were analyzed by fluorescence microscopy. Positive cells exhibit green fluorescence around the plasma membrane.



**Figure 2. Detection of ApoAlert Annexin V-EGFP by flow cytometry.** Cells were treated and analyzed as described in Figure 1.

## Choose the best reagent for your experiments

CLONTECH offers a variety of annexin V reagents for your apoptosis detection experiments. Researchers who want to design their own annexin V reagents can purchase Unlabeled Annexin V or Annexin V-Biotin. We also offer many other kits and reagents for detecting apoptosis; see page 18 for more information.

Product	Size	Cat. #
ApoAlert Annexin V-EGFP	500 assays	8137-1
ApoAlert Annexin V-EGFP Apoptosis Kit	50 assays 200 assays	K2019-1 K2019-2

## Kit Components

- **Annexin V-EGFP**
- **1X Binding Buffer**
- **Propidium Iodide**
- **Complete User Manual (PT3050-1)**

## Related Products

- **ApoAlert™ Annexin V-Biotin (#8131-1)**
- **ApoAlert™ Unlabeled Annexin V (#8130-1)**
- **ApoAlert™ Propidium Iodide (#8135-1)**
- **ApoAlert™ 10X Annexin V Binding Buffer (#8134-1)**
- **Apoptosis-Inducing Reagents (many)**
- **rEGFP Protein (#8365-1)**

## References

1. ApoAlert Annexin V Apoptosis Kit (July 1996) *CLONTECHniques XI*(3):9–11.
2. Zhang, G., et al. (1997) *BioTechniques* **23**:525–530.
3. Kain, S. R. & Ma, J.-T. (1998) *Methods Enzymol.* (in press).
4. Yang, T. T., et al. (1996) *Nucleic Acids Res.* **24**: 4592–4593.
5. Thiagarahan, P. & Tait, J. F. (1990) *J. Biol. Chem.* **265**:17420–17423.
6. Dacharay-Prigent, J., et al. (1993) *Blood* **81**: 2554–2565.
7. Cormack, B. P., et al. (1995) *Gene* **173**:33–38.

See pages 2–4 for information about our new Atlas™ Human Apoptosis Array!

## Notice to Purchaser

The AnnexinV-EGFP reagent is covered by U.S. Patents #5,491,084 and 5,066,787, and is the subject of pending patent applications. All purchasers are granted an automatic license with the purchase of the AnnexinV-EGFP reagent to use it for internal research purposes. However, this license excludes specifically any right to modify the reagent for resale, to use the reagent for the manufacture of commercial products, or to use the reagent in humans or for diagnostic purposes.

# ApoAlert™ Cytotoxicity Assay Kit

Fast, convenient method for assessing cytotoxic effects

- **Convenient 96-well format**
- **Quickly screen candidate compounds for cytotoxicity**
- **Reliable, foolproof colorimetric assay**

The ApoAlert™ Cytotoxicity Assay Kit provides a simple colorimetric method for detecting cell death and is ideal for screening compounds for cytotoxic effects (1). This convenient assay allows you to identify candidate reagents for more in-depth studies of cell death. The 96-well format is ideal for high-throughput testing, and the assay is easy to use and requires no expensive equipment.

## Complete kit for detecting cell death

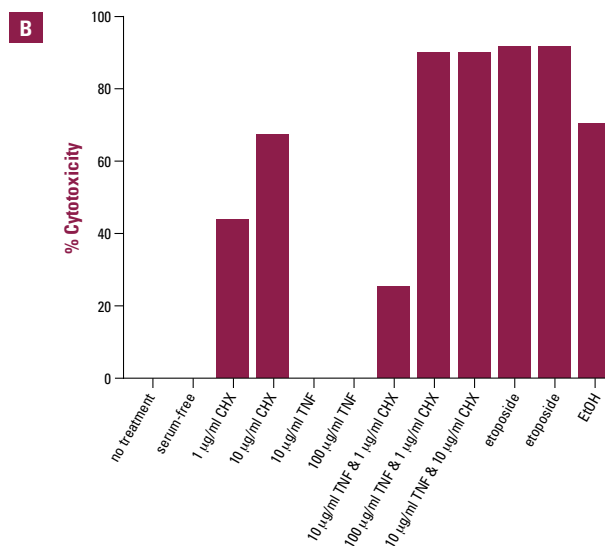
The ApoAlert Cytotoxicity Assay Kit provides all the necessary reagents for cytotoxicity testing, including two 96-well plates and a handy Protocol-at-a-Glance. The first step is to culture your choice of adherent cells in a 96-well plate. To test a compound of interest, simply add it to the cell culture medium and incubate for 24–48 hours. Then rinse with Cell Buffer, incubate with Cell Fixative followed by Cell Stain, and terminate the assay by rinsing with water. During this procedure, dead cells detach from the plate, so only viable, adherent cells are stained with dye. Results can be visualized directly (Figure 1A) or quantified using a standard plate reader (Figure 1B). See next page for ordering information.

## Choose ApoAlert for your in-depth studies

After you have identified candidate cytotoxic compounds, you can embark on a more in-depth investigation of cell death. CLONTECH offers many ApoAlert Kits and Reagents (Table I) for studying very early to late stages of apoptosis. For information about Annexin V-EGFP, the newest addition to the ApoAlert product line, see previous page.

## Reference

1. Matthews, N. & Neale, M. L. (1987) In *Lymphokines and Interferons: A Practical Approach*, Eds. Clemens, M. J., et al. (IRL Press, Oxford), pp. 221–225.



**Figure 1. The ApoAlert Cytotoxicity Assay Kit provides visual and quantitative results.** NIH 3T3 cells were seeded in a 96-well plate and cultured for 24 hr. Duplicate wells were treated for 24 hr with the various agents indicated in Panel B. The ApoAlert cytotoxicity assay was performed according to the User Manual. **Panel A.** Living cells are stained purple. Cytotoxicity is indicated by clear areas in the well. **Panel B.** The plate shown in Panel A was read at 570 nm using a standard plate reader. CHX = cycloheximide. TNF = tumor necrosis factor.

## New high-concentration apoptosis inhibitors

CLONTECH's apoptosis inhibitors are cell-permeable, noncleavable peptide analogs of caspase (ICE-family protease) substrates. We now offer our inhibitors in an economical 10-mM concentration to facilitate their use in tissue culture. Because smaller volumes are required, you minimize the cytotoxic effects of the DMSO in which the inhibitors are dissolved. We still offer our inhibitors at the original, 1-mM concentration for use in experiments with cell-free extracts. Please see our catalog or our website ([www.clontech.com](http://www.clontech.com)) for a complete product list.

# ApoAlert™ Cytotoxicity Assay Kit...continued

NEW!

**Table I: CLONTECH's ApoAlert products**

Product	Cat. #	Detects	Method	Approximate time after induction*
<b>ApoAlert Caspase-3 Assay Kits</b>	K2026-1, -2 K2027-1, -2	ICE-family protease activity	fluorometric or colorimetric	2 hr
<b>ApoAlert Caspase-8 Assay Kits</b>	K2028-1, -2	Caspase-8 activity	fluorometric	2 hr
<b>PARP Monoclonal Antibody</b>	8192-1	PARP cleavage by caspases	Western blot	2-3 hr
<b>ApoAlert Annexin-V Apoptosis Kits &amp; Reagents</b>	many	PS externalization	fluorescence microscopy or flow cytometry	3-4 hr
<b>ApoAlert DNA Fragmentation Assay Kit</b>	K2024-1, -2	nuclear DNA fragmentation	TUNEL; fluorescence microscopy or flow cytometry	4-6 hr
<b>ApoAlert LM-PCR Ladder Assay Kit</b>	K2021-1	nucleosomal ladder formation	LM-PCR	4-6 hr
<b>ApoAlert Cytotoxicity Assay Kit</b>	K2023-1	cell death	colorimetric assay in 96-well plate	>24 hr

Product	Size	Cat. #
ApoAlert Cytotoxicity Assay Kit	200 assays	K2023-1
ApoAlert Caspase Inhibitor, VAD-fmk (10 mM)	40 µl	8183-1
ApoAlert Caspase-1 Inhibitor, YVAD-cmk (10 mM)	40 µl	8181-1
ApoAlert Caspase-3 Inhibitor, DEVD-CHO (10 mM)	40 µl	8180-1
ApoAlert Caspase-3 Inhibitor, DEVD-fmk (10 mM)	40 µl	8182-1
ApoAlert Caspase-8 Inhibitor, IETD-fmk (10 mM)	40 µl	8184-1

#### Related Products

- **Apoptosis Antibodies & Inhibitors** (many)
- **Apoptosis-Inducing Reagents** (many)

\* These experiments were conducted using Jurkat cells treated with anti-Fas mAb.

## MDCK Tet-Off™ Cell Line

For inducible, high-level expression with the Tet Systems

- **The fastest way to establish a Tet System expressing your gene of interest**
- **Well-characterized cell line offers proven Tet responsiveness**

CLONTECH introduces the **MDCK Tet-Off™ Cell Line**, developed for use with our Tet Gene Expression Systems or RevTet Retroviral Expression Systems. This line is derived from the Madin Darby Canine Kidney (MDCK) Type II epithelial parental line and stably expresses the Tet-Off transactivator, tTA (1, 2). MDCK cells have been used extensively in the pharmaceutical industry as they support the growth of vesicular exanthema virus, vesicular stomatitis, infectious canine hepatitis, vaccinia, Coxsackie B-5, adenovirus, and reoviruses. This particular MDCK cell line was established to examine the regulation of

beta-catenin distribution between cadherin and adenomatous polyposis coli (APC) protein complexes and the functions of those complexes (3, 4). This line expresses pIgR and is thus resistant to both G418 and puromycin.

All Tet Cell Lines are carefully selected for their ability to exhibit the tightly regulated, high-level gene expression originally described by Gossen & Bujard (1, 2). These time-saving cell lines display low background expression and as much as 20,000-fold induction of protein expression—hallmarks of the Tet Systems.

Our expanded collection of Tet Cell Lines provides the most convenient means to achieve regulated expression of your gene of interest. All Tet Cell Lines are extensively tested for high inducibility and low basal activity. See our catalog or website for a complete listing.

Product	Size	Cat. #
MDCK Tet-Off Cell Line	2 x 1 ml	C3017-1

NEW!

#### 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. Barth, A. I. M., et al. (1997) *J. Cell Biol.* **136**: 693-706.
4. MDCK Tet-Off Cell Line provided by Y. Altschuler & K. Mostov, UCSF.

Use tetracycline-free Tet System Approved FBS (#8630-1) to ensure optimal results with the Tet Systems.

#### Notice to Purchaser

Use of the Tetracycline-controllable expression systems (the "Tet System") is covered under U.S. patent #5,464,758, which has been assigned to BASF Aktiengesellschaft. Academic research institutions are granted an automatic license with the purchase of this product to use the Tet System only for internal, academic research purposes, which license specifically excludes the right to sell, or otherwise transfer, the Tet System or its component parts to third parties. In accepting this license, all users acknowledge that the Tet System is experimental in nature. BASF makes no warranties, express or implied, of any kind, and hereby disclaims any warranties, representations, or guarantees of any kind as to the Tet System, patents, or products. All others are required to obtain a license from BASF prior to purchasing these reagents or using them for any purpose. CLONTECH is required by its licensing agreement to submit a report of all purchasers of the Tet-controllable expression systems to BASF Bioresearch Corporation. For license information, contact: Director of Business Development, BASF Bioresearch Corporation, 100 Research Drive, Worcester, MA 01605-4314. Fax: 508-755-8506.

# Protein L Antibody Purification Tools

Powerful new products for all your antibody purification and immunodetection needs

- **Protein L binds Ig subclasses IgM, IgA, IgD, IgG, IgE & IgY**
- **Ideal for humanized antibody purification**
- **Agarose conjugate simplifies purification and immunoprecipitation**

**Protein L** exhibits a unique combination of species-specific, immunoglobulin-binding characteristics and high affinity for many classes of antibodies. These characteristics make Protein L a more flexible and effective tool than either Protein A or Protein G in immunoglobulin purification procedures. In addition to better performance in many conventional applications, the specific binding characteristics of Protein L facilitate difficult purification strategies, such as humanized antibody purification.

## An ideal mix of binding properties

Protein L is a recombinant form of a *Peptostreptococcus magnus* cell-wall protein that binds immunoglobulins (Ig) through  $\kappa$  light-chain interactions that do not interfere with the Ig antigen-binding site. A majority of Ig subclasses, including IgG, IgM, IgA, IgD, IgE, and IgY, from human, mouse, rat, rabbit, and chicken possess  $\kappa$  light chains and can thus be bound with high affinity by Protein L (1–3). Protein L also binds Ig fragments, including scFv and Fab.

Table I shows a comparison of the broad range of Ig classes and subclasses that can be purified using Protein L, compared to Proteins A or G. Protein L displays an impressive ability to bind Ig from a variety of species including human, mouse, and chicken. However, the utility of Protein L also derives from what it does *not* bind. Unlike Protein A and Protein G, Protein L does not bind bovine, goat, or sheep Ig. Protein L is thus ideal for the specific purification of humanized antibodies from these transgenic animals, a primary source for therapeutic antibody production. Additionally, Protein L is uniquely suited for direct purification of monoclonal antibodies from media supplemented with FBS or BSA.

## Two convenient forms

Protein L is available as an agarose conjugate in buffered solution and as whole protein in

lyophilized form. **Protein L Agarose Beads** are cross-linked beaded agarose complexes that have been optimized for rapid and convenient single-step purification and immunoprecipitation of immunoglobulins. They are suitable for purification from plasma or serum from a variety of sources (Table I). Lyophilized Protein L is a versatile tool that can be conjugated to marker molecules for immunodetection, coupled to gel matrices for single-column affinity purification, and adsorbed to plates for antibody capture.

## References

1. Bjorck, L. (1988) *J. Immunol.* **140**:1194–1197.
2. Nilson, B. H. K., *et al.* (1993) *J. Immunol. Meth.* **164**: 33–40.
3. Akerstrom, B., *et al.* (1989) *J. Biol. Chem.* **264**: 19740–19746.

Products	Size	Cat. #
Protein L Agarose Beads	2 ml	3901-1
	5 ml	3901-2
Protein L	1 mg	3900-1

## Related Products

- **Molecular & Cell Biology Support Antibodies** (many; see page 10)
- **Oncogene & Tumor Suppressor Antibodies** (many)
- **Cell Cycle Antibodies** (many)
- **Phosphokeratin Antibodies** (many)

See our website ([www.clontech.com](http://www.clontech.com)) for a complete listing.

## Notice to Purchaser

Protein L and Protein L Agarose are licensed from ACTigen Ltd.

**Table I: Binding of immunoglobulins to Protein L, Protein A & Protein G**

	Protein L <sup>a</sup>	Protein A <sup>b</sup>	Protein G <sup>c</sup>
Human Ig			
IgG	++	+++	+++
IgM	++	+	–
IgA	++	+	–
IgE	++	+	–
IgD	++	+	–
Fab	++	+	+
F(ab') <sub>2</sub>	++	+	+
$\kappa$ light chains	++	–	–
scFv	++	+	–
Mouse Ig			
IgG1	++	+	++
IgG2a	++	++	++
IgG2b	++	++	++
IgG3	++	+	++
IgM	++	+	–
IgA	++	++	+
Polyclonals			
Mouse	++	++	++
Rat	++	+	++
Rabbit	+	++	+++
Sheep	–	++	++
Goat	–	+	++
Bovine	–	+	++
Porcine	++	++	++
Chicken IgY	++	–	–

<sup>a</sup> Protein L binds primarily to  $\kappa$  light chains and  $\lambda$ V1 chains.

<sup>b</sup> Protein A binds to  $\gamma$ Fc and VHIII variable domains.

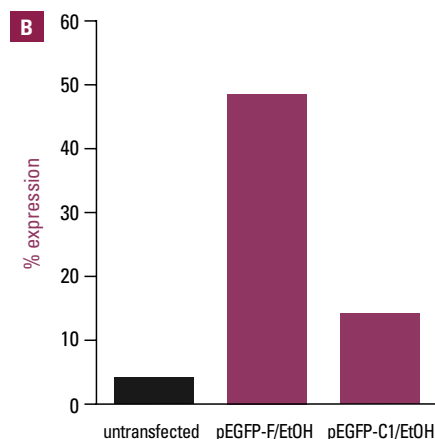
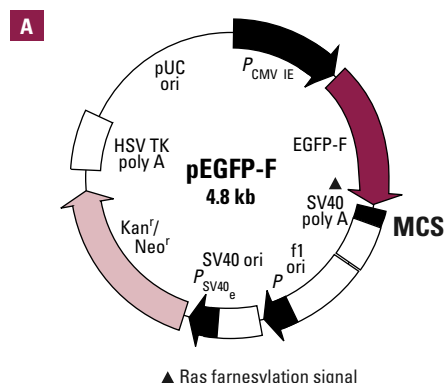
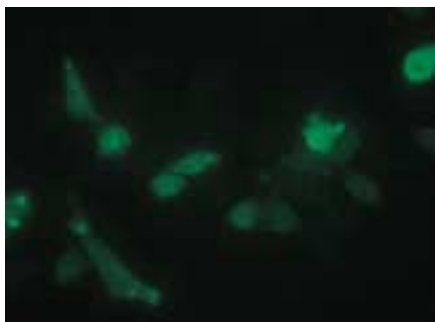
<sup>c</sup> Protein G binds to  $\gamma$ Fc and cy1 chains.

+++ = very strong binding, ++ = strong binding, + = moderate binding, – = no binding

# New Living Colors™ Vectors

## The evolution of a revolution continues

The color revolution is upon us. CLONTECH—the leading innovator in GFP technology—introduces new Living Colors™ Vectors that greatly increase the opportunities for using fluorescent proteins in a wide variety of experiments. The following four pages feature exciting new vectors that employ modified green fluorescent protein—either as color variants or as proteins with unique signaling and destabilization tags. These new vectors will give you the tools to monitor gene expression more easily in your system of choice and by your detection method of choice.



**Figure 2. Panel A.** Map of pEGFP-F. MCS: *Xho* I, *Sac* I, *Eco* 136 II, *Hind* III, *Eco* R I, *Pst* I, *Sal* I, *Acc* I, *Kpn* I, *Asp* 718 I, *Sac* II, *Apa* I, *Bsp* 120 I, *Xma* I, *Sma* I, *Xba* I. The *Xba* I site is methylated in the DNA provided. **Panel B.** EGFP-F fluorescence remains intact after trypsinization and ethanol fixation. CHO cells were transfected with pEGFP-F or pEGFP-C1 (containing unmodified EGFP) using CLONfectin. 24 hr post-transfection, cells were trypsinized, collected by centrifugation, and incubated with 80% EtOH on ice. Cells were sorted by flow cytometry. Cells transfected with pEGFP-F retain high intracellular levels of the fluorescent protein while those transfected with pEGFP-C1 exhibit a partial reduction in fluorescence.

### EGFP and EGFP-F have identical spectral properties

Like EGFP, the EGFP-F chromophore exhibits a single, strong excitation peak at 488 nm (blue light) and a strong emission peak at 507 nm. It is human codon-optimized for efficient translation and bright fluorescence in mammalian cells under a variety of conditions. The protein can be co-expressed with other enhanced fluorescent

### pEGFP-F—farnesylated EGFP

- **Localized to plasma membrane; withstands ethanol fixation**
- **Cotransfection marker for flow cytometry or microscopy in living or fixed samples**
- **Positive control for EGFP fusion protein membrane localization experiments**

The pEGFP-F Vector represents a significant breakthrough in GFP technology. Now you can easily fix and ethanol-treat GFP-expressing cells without protein leakage and loss of fluorescence intensity. EGFP-F is a modified form of the enhanced green fluorescent protein that remains bound to the plasma membrane in both living and fixed cells. EGFP-F contains the 20-amino-acid farnesylation signal from the c-Ha-Ras protein (1, 2). Normally, this farnesylation sequence targets Ha-Ras to the plasma membrane. When this C-terminal signal is fused to EGFP, the resulting farnesyl modification allows EGFP-F to attach to the plasma membrane in a similar manner. This specific localization allows EGFP-F to be viewed easily by fluorescence microscopy (Figure 1; 3).

### No GFP leakage during ethanol fixation

Unmodified EGFP is a versatile and convenient reporter and cotransfection marker in living cells. However, because it is expressed as a soluble, cytoplasmic protein, it can leach out of permeabilized cells during ethanol fixation, resulting in decreased fluorescence intensities. In contrast, EGFP-F remains attached to the inner

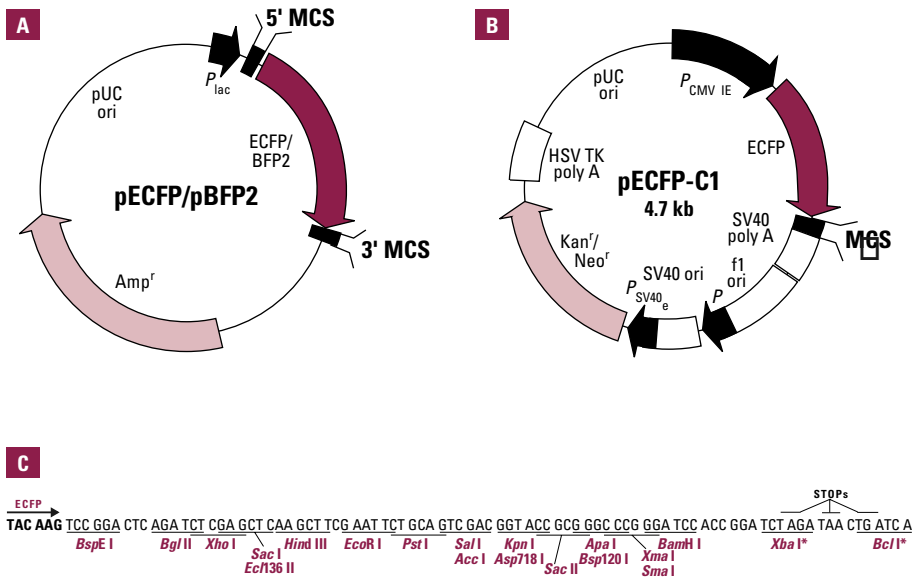
**Figure 1. EGFP-F expression in formalin-fixed CHO cells.** pEGFP-F Vector was transfected using CLONfectin™ (#8020-1). 24 hr post-transfection, cells were rinsed in PBS, fixed in neutral buffered formalin for 20 min, and observed by fluorescence microscopy. Note that the fluorescent signal is strongly localized to the plasma membrane.

face of the plasma membrane without leakage, even in ethanol-permeabilized cells. Figure 2B illustrates the striking difference in fluorescence intensities of cells that were transfected with EGFP-F or EGFP, followed by trypsinization and fixation in ethanol. The fluorescence intensity of EGFP-F-transfected, permeabilized cells is significantly higher than that of EGFP-expressing cells, as determined by flow cytometry.

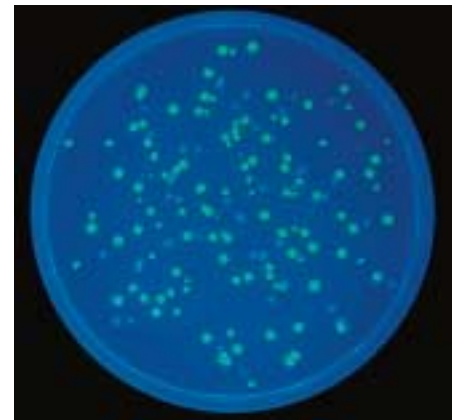
### pEGFP-F makes cotransfection easier to monitor

The pEGFP-F Vector was created by fusing the C-terminal Ras farnesylation signal in frame with the C-terminus of EGFP (Figure 2A). The vector is designed to be used primarily as a cotransfection marker for determining efficiency of transfection with a vector containing your gene of interest. After transfection, sorting cells for EGFP-F fluorescence by flow cytometry can decrease the need for multiple rounds of selection using antibiotic. The vector contains a multiple cloning site (MCS) downstream of the EGFP-F coding sequences to allow generation of protein fusions to the C-terminus. EGFP-F is expressed under control of the CMV immediately promoter, and the vector contains a kanamycin/neomycin resistance cassette for propagation and selection in *E. coli* and mammalian cells, respectively.

# New Living Colors™ Vectors...continued



**Figure 3. pECFP, pBFP2, and pECFP-C1 Vectors.** Panel A. Map of pECFP and pBFP2. The fluorescent-protein coding region is flanked by two MCSs for easy excision (restriction sites not shown). Panel B. Map of pECFP-C1. Genes inserted into the MCS of pECFP-C1 are expressed as fusions to the C-terminus of ECFP. Panel C. MCS of pECFP-C1. \*The *Xba* I and *Bcl* I sites are blocked by methylation in the DNA provided. All restriction sites shown are unique.



**Figure 4. BFP2 and GFPuv fluorescence.** Long-wave UV irradiation of a mixture of JM109 cells transformed separately with pBFP2 and pGFPuv Vectors reveals both blue and green fluorescent colonies. Cultures were plated on LB/amp.

proteins (like EYFP or EBFP) for use in dual-labeling studies by flow cytometry. In addition, CLONTECH's Monoclonal and Polyclonal GFP Antibodies (#8362-1 & #8363-1, -2, respectively) can be used to detect EGFP-F and EGFP-F-fusion proteins.

## ECFP—enhanced cyan fluorescent protein

- **Ideal variant for double and triple labeling experiments**

The **pECFP** and **pECFP-C1 Vectors** encode a cyan variant of enhanced green fluorescent protein. The ECFP gene contains six amino-acid substitutions, one of which (Tyr66→Trp) shifts the emission of the fluorophore from green to cyan. ECFP's emission spectrum consists of a large peak at 475 nm and a smaller peak at 501 nm (4). Like EGFP, ECFP is human codon-optimized for high expression in mammalian cells (6).

With the introduction of ECFP, we now offer *four* different enhanced color variants of GFP: green, blue, yellow, and cyan. ECFP fluorescence is stronger than EBFP fluorescence (4) and is expected to be more resistant to photobleaching than EBFP because it is excited at a longer, less destructive wavelength. (See Table I on page 24 for excitation and emission spectra of all the enhanced variants.) Because of ECFP's unique characteristics, ECFP and EYFP make the ideal fluorescent protein pair for standard dual-color fluorescence microscopy. By using digital imaging microscopy, it is also possible to triple-label cells with ECFP in conjunction with EBFP and EYFP, although researchers should be aware that EBFP photobleaches more rapidly than any of the other variants. Finally, ECFP makes a superior donor fluorophore for fluorescence resonance energy transfer (FRET) systems when EYFP is used as the acceptor molecule (5).

Like our pEGFP Vector, the pECFP Vector (Figure 3A) is designed so that the ECFP coding regions can easily be excised using the flanking restriction sites, or amplified via PCR. You can

use the pECFP-C1 Vector (Figure 3B) to generate ECFP fusion proteins for expression and localization studies in mammalian cells. Fusions are expressed at high levels from the CMV promoter.

## BFP2—blue fluorescent protein

- **More color choices for use in bacteria**

Our new **pBFP2 Vector** (Figure 3A) expands your expression options for bacteria. You can now easily perform double-labeling experiments using GFPuv and BFP2, or GFP and BFP2, in bacterial cultures to simultaneously study the effects of two different promoters or to screen for positive colonies expressing the color variant of choice (Figure 4). BFP2 is expressed more efficiently than EBFP in bacteria; it is not toxic to cells and is expressed at high levels. Partly because of its higher quantum yield, BFP2 also resists photobleaching better than EBFP, and therefore can be detected using a hand-held (long-wave) UV lamp. The BFP2 gene can easily be excised from the vector and inserted into another bacterial expression vector of choice. We recommend using JM109 cells for double-labeling experiments.

# New Living Colors™ Vectors...continued

## New destabilized vectors give you more choices for transient expression analysis

In April, we introduced two pd2EGFP Vectors (7; patent pending), marking an exciting beginning to a brand new line of destabilized green fluorescent protein vectors. Now CLONTECH introduces additional destabilized variants that will greatly enhance the utility of green fluorescent protein for studying gene expression in either mammalian or bacterial cells.

### pd1EGFP & pd4EGFP

- **Rapid-turnover EGFP now with three different half-lives—refine your expression studies**
- **Optimized for mammalian expression**

The **pd1EGFP-N1 Vector** and **pd4EGFP-N1 Vector** encode EGFP with fluorescent half-lives of one hour and four hours, respectively, allowing you to fine-tune your studies of transient gene expression. Like d2EGFP, d1EGFP and d4EGFP were constructed by fusing amino acids 422–461 of the mouse ornithine decarboxylase protein (MODC) to the C-terminus of EGFP. This region of MODC contains a PEST degradation domain that targets the protein for rapid turnover. d2EGFP, our first destabilized variant, has a half-life of two hours *in vivo* (7). By making a few amino-acid substitutions in the MODC region of d2EGFP (for d1EGFP: E428A, E430A, E431A; for d4EGFP: T436A) we effectively altered d2EGFP's half-life to one hour (d1EGFP) or four hours (d4EGFP). Comparative fluorescence half-lives were determined by flow cytometry (Figure 5A).

All three destabilized variants retain the intrinsic fluorescent properties of the EGFP chromophore. dEGFP can be excited at 488 nm with an argon-ion laser to produce a single, strong emission peak at 507 nm. Both the destabilized and the stable variants are suitable for fluorescence microscopy and flow cytometry. The pdEGFP-N1 vectors are designed for generating fusions of a gene of interest with the N-terminus of dEGFP; they can also be used to constitutively express dEGFP. We also offer the **pd2EGFP Vector**,

which can be used as a source of the d2EGFP gene for insertion into other vectors.

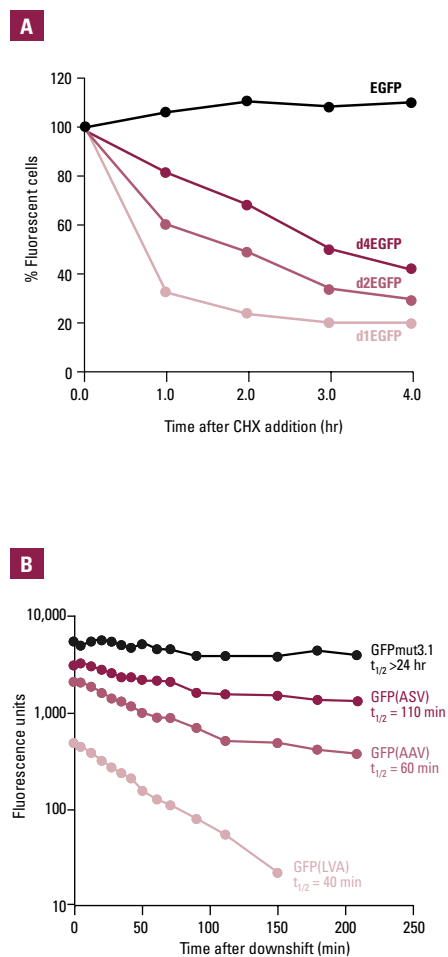
### Destabilized GFP for use in bacteria

- **Study transient gene expression**
- **Convenient vector set**

Now you can also study transient gene expression in bacteria using destabilized GFP as a reporter. Our new **Bacterial Destabilized GFP Vector Set** contains three destabilized GFP variants, each with a different half-life (Figure 5B). It also contains the **pGFPmut3.1 Vector**, the parent vector that encodes a stable GFP gene. By providing a set of three variants with different half-lives, we enable you to choose the variant that is appropriate for your particular bacterial host, experimental design, and detection method. The vectors contain the *lac* promoter for convenient inducibility of GFP or destabilized GFP using IPTG.

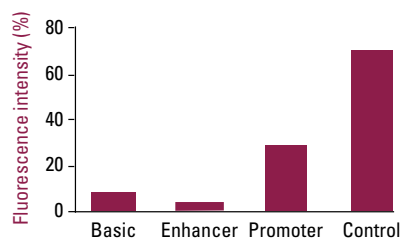
Each destabilized GFP variant contains a different peptide tag at the C-terminus (ASV, AAV, or LVA) that targets the fluorescent protein for degradation by endogenous tail-specific proteases (8, 9). The tail-specific degradation systems are apparently conserved in both Gram-negative and Gram-positive bacteria (8). Note that because the tail-specific proteases function differently across species lines, actual half-lives of the destabilized GFP proteins may vary somewhat in your particular host. Figure 5B demonstrates the half-lives of the three destabilized variants as measured in an *E. coli* host strain.

The GFPmut3.1 variant of GFP is designed to provide stronger fluorescence in bacteria and yeast than any other variant we sell (8, 10). Unlike human codon-optimized EGFP, the GFPmut3.1 gene is based on jellyfish codons and is therefore better suited than EGFP for expression in both *E. coli* and yeast. GFPmut3.1 is extremely stable (half-life of >24 hr) and the vector can be used either as a control or as a source of the GFPmut3.1 gene for insertion into other vectors. The pGFPmut3.1 Vector is also sold separately.



**Figure 5. Fluorescence stabilities of CLONTECH's destabilized GFP variants.** **Panel A.** EGFP mammalian variants. CHO-K1 Tet-Off™ cells were transiently transfected with pTRE-EGFP, pTRE-d1EGFP, pTRE-d2EGFP, or pTRE-d4EGFP. 24 hr post-transfection, cells were treated with 100 µg/ml cycloheximide (CHX) and collected for flow cytometry analysis. Percent fluorescence after treatment is plotted on the y-axis. Other mammalian cell lines exhibit similar fluorescence half-lives (data not shown). **Panel B.** GFP bacterial variants. Each variant has its own unique peptide tag (ASV, AAV, or LVA); the tag imparts a specific half-life to each destabilized variant. *E. coli* cells were induced to express destabilized GFP with 200 µM IPTG. Cultures were then transferred to minimal media without IPTG and cells were harvested at various time points. Fluorescence intensities were measured using a fluorometer set to detect emission at 515 nm. GFPmut3.1, the unmodified variant, has a half-life of >24 hr. Data provided by Dr. Claus Sternberg, Technical University of Denmark.

# New Living Colors™ Vectors...continued



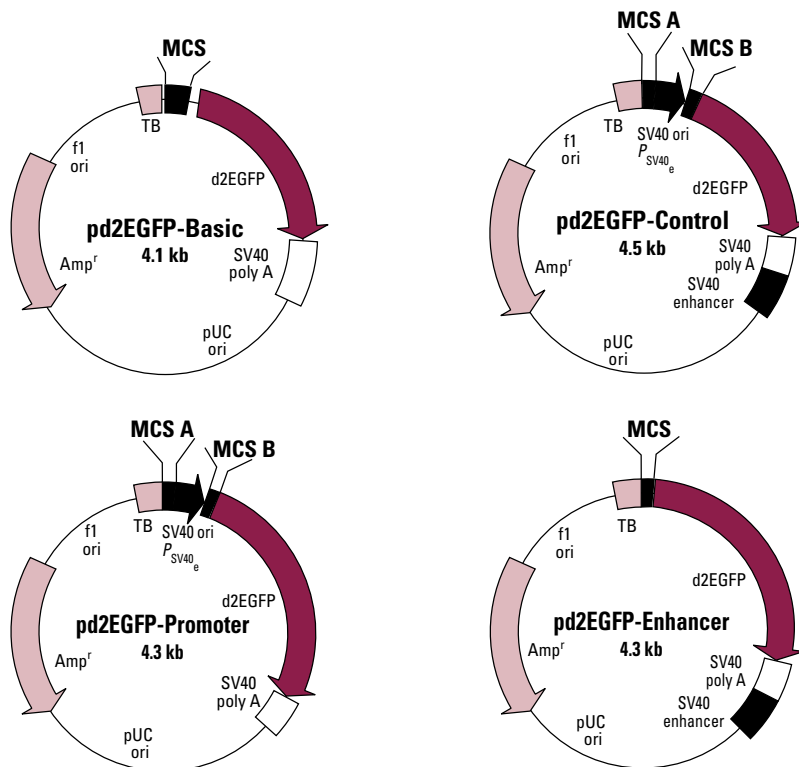
**Figure 6. d2EGFP as a reporter of transcription.** HEK293 cells were transfected with pd2EGFP-Basic, pd2EGFP-Enhancer, pd2EGFP-Promoter, or pd2EGFP-Control. 24 hr post-transfection, cells were harvested and fluorescence was measured by flow cytometry. Percent fluorescence intensity indicates the fraction of fluorescent cells in each sample.

## d2EGFP Reporter Vectors

- Versatile new mammalian vectors for promoter & enhancer studies

Because d2EGFP is so well suited as a reporter of transient gene expression, we have incorporated this protein into the versatile pSEAP2 Vector backbone to create our new **d2EGFP Reporter Vectors** (Figure 7). These vectors are useful for cloning promoter or enhancer elements of interest upstream of the d2EGFP coding sequences. Like our pSEAP2 Reporter Vectors, which use secreted alkaline phosphatase for chemiluminescent or fluorometric detection, the pd2EGFP Reporter Vectors provide an accurate assessment of *cis*-element activity. However, the d2EGFP reporter offers several advantages over the SEAP reporter. First, d2EGFP is nonenzymatic and requires nothing but blue light (488 nm) for fluorescence. Second, it can be detected in living or fixed cells either by microscopy or by flow cytometry. Third, because d2EGFP is so rapidly turned over, it provides a real-time measure of transient promoter activity. Fourth, d2EGFP can be used to monitor changes in gene expression in living animals (e.g., in transgenic mice).

The **pd2EGFP-Basic Vector** lacks any eukaryotic promoter or enhancer sequences; it can be used either as a negative control or as a cloning



**Figure 7. pd2EGFP Reporter Vectors.** TB = transcription blocker.

vehicle for strong mammalian promoters. The **pd2EGFP-Control Vector** contains the SV40 early promoter upstream, and the SV40 enhancer downstream, of the d2EGFP coding regions. It is designed for use as a positive control. The **pd2EGFP-Promoter Vector** contains the SV40 early promoter (with no enhancer) located upstream of the d2EGFP gene, and the **pd2EGFP-Enhancer Vector** contains the SV40 enhancer (but no promoter) located downstream of d2EGFP. Each vector is sold separately. Figure 6 shows relative expression levels of d2EGFP from each of the different reporter vectors.

## References

1. Aronheim, A., *et al.* (1994) *Cell* **78**:949–961.
2. Hancock, J. F., *et al.* (1991) *EMBO J.* **10**: 4033–4039.
3. Jiang, W. & Hunter, T. (1998) *BioTechniques* **24**: 348–354.
4. Heim, R. & Tsien, R. Y. (1996) *Curr. Biol.* **6**:178–182.
5. Miyawaki, A., *et al.* (1997) *Nature* **388**:882–887.
6. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
7. Living Colors Destabilized EGFP Vectors (April 1998) *CLONTECHniques XIII*(2):16–17.
8. Andersen, J. B., *et al.*, in press.
9. Keiler, K. C., *et al.* (1996) *Science* **271**:990–993.
10. Cormack, B. P., *et al.* (1996) *Gene* **173**:33–38.

Checking your Living Colors vectors? The following sequence can be used as a convenient sequencing primer to confirm the presence of the correct fluorescent chromophore (EYFP, ECFP, EBFP, or EGFP):

**5' GGC GCG GGT CTT GTA GTT GCC G 3'**

This primer anneals to the coding strand about 100 bp downstream of the chromophore mutations.

Please see page 24 for ordering information and related products.

# New Living Colors™ Vectors...continued

NEW!

**Table I: Living Colors fluorescent proteins**

Protein	Host Cells	Excitation/Emission Maxima (nm)	Stability	Recommended Filter Sets	Features/Applications
<b>Living Colors Variants</b>					
EBFP	mammalian <sup>a</sup>	380/440	>24 hr (some photo-bleaching)	Omega XF10 Omega XF06	Use for double labeling
ECFP	mammalian <sup>a</sup>	433 & 453/ 475 & 501	>24 hr	Chroma 31044 Omega XF78 <sup>b</sup> Omega XF114	Use for double & triple labeling
EGFP	mammalian <sup>a</sup>	488 /507	> 24 hr	Omega VIVID Set XF100	Most widely used color variant
EGFP-F	mammalian	488 /507	>24 hr	Omega VIVID Set XF100	Cotransfection marker; localized to PM
EYFP	mammalian <sup>a</sup>	513/527	>24 hr	Omega VIVID Set XF104	Receptor molecule for FRET with ECFP
BFP2	bacterial	380/440	>24 hr	n/a	High quantum yield; use for double labeling
GFPmut3.1	bacterial	395 & 470/509	>24 hr	n/a	
GFPuv	bacterial	395/509	>24 hr	n/a	
GFP	bacterial	395 & 470/509	>24 hr	n/a	
<b>Destabilized GFP Variants</b>					
d1EGFP	mammalian	488 /507	t <sub>1/2</sub> = 1 hr	Omega XF100	Use destabilized variants to measure transient gene expression
d2EGFP	mammalian	488 /507	t <sub>1/2</sub> = 2 hr	Omega XF100	
d4EGFP	mammalian	488 /507	t <sub>1/2</sub> = 4 hr	Omega XF100	
GFP(ASV)	bacterial	395 & 470/509	t <sub>1/2</sub> = 110 min <sup>c</sup>	n/a	Sold as vector set with GFPmut3.1
GFP(AAV)	bacterial	395 & 470/509	t <sub>1/2</sub> = 60 min <sup>c</sup>	n/a	
GFP(LVA)	bacterial	395 & 470/509	t <sub>1/2</sub> = 40 min <sup>c</sup>	n/a	

<sup>a</sup> Enhanced fluorescent proteins are human codon-optimized. However, four pEFP Vectors (pEBFP, pEGFP, pECFP, & pEYFP) have a bacterial promoter.

<sup>b</sup> This filter not suitable for double labeling.

<sup>c</sup> Half-lives measured in *E. coli*.

Product	Size	Cat. #
pEGFP-F Vector	20 µg	6074-1
pECFP Vector	20 µg	6075-1
pECFP-C1 Vector	20 µg	6076-1
pBFP2 Vector	20 µg	6038-1
pd1EGFP-N1 Vector	20 µg	6073-1
pd4EGFP-N1 Vector	20 µg	6072-1
pd2EGFP Vector	20 µg	6010-1
pd2EGFP-Control Vector	20 µg	6012-1
pd2EGFP-Basic Vector	20 µg	6013-1
pd2EGFP-Enhancer Vector	20 µg	6015-1
pd2EGFP-Promoter Vector	20 µg	6016-1
Bacterial Destabilized GFP Vector Set	each	K6002-1
pGFPmut3.1 Vector	20 µg	6039-1

## Bacterial Destabilized GFP Vector Set Components

- pGFP(AAV) Vector
- pGFP(LVA) Vector
- pGFP(ASV) Vector
- pGFPmut3.1 Vector
- Vector Information Packets
- Living Colors™ User Manual (PT2040-1)

## Related Products

- CLONfectin™ Transfection Reagent (#8020-1)
- pd2EGFP-N1 Vector (#6009-1)
- pd2EGFP-1 Vector (#6008-1)
- pEGFP, EYFP & EBFP Vectors (many)
- pGFP Vector (#6097-1)
- pGFPuv Vector (#6079-1)
- GFP Monoclonal Antibody (#8362-1)
- GFP Polyclonal Antibody (#8363-1, -2)

Coming soon: Look for more modified EGFP variants with localization signals to intracellular organelles and the cytoskeleton!

## New cell biology books available!

### Bioluminescence Methods and Protocols

Robert A. LaRossa, Ed.  
Humana Press, 1998

An indispensable resource for anyone who wants to learn more about using luciferase and GFP as bioluminescent reporters in applied fields. Each protocol contains step-by-step instructions and detailed troubleshooting notes. Applications are relevant to such diverse fields as molecular toxicology, cell physiology, clinical chemistry, and environmental monitoring.

320 pp. hardcover Cat. #V2372-1

### Fluorescence Microscopy

B. Herman  
BIOS Scientific Publishers, 1998

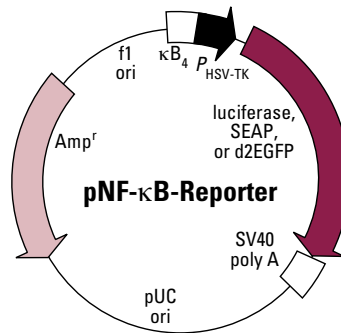
Want to improve your microscopy technique? Like to learn more about immunofluorescence, *in situ* hybridization, and photomicrography? Then this book is for you. Techniques included in this work focus on the applications of using fluorescent markers to label cells or other molecules. Troubleshooting guides and helpful hints are also included.

144 pp paperback Cat. #V2373-1

# NF- $\kappa$ B Transcription Reporter Vectors

Analyze induction of a well-studied signal transduction pathway

- Quickly identify regulators of NF- $\kappa$ B signal transduction
- Choose from three popular reporters—luciferase, SEAP, or d2EGFP
- Rapid, high-level induction *in vivo*
- Compatible with many mammalian cell types



CLONTECH introduces **NF- $\kappa$ B Transcription Reporter Vectors**—three new vectors designed especially for monitoring activation of the NF- $\kappa$ B signal transduction pathway. This pathway, evolutionarily conserved in various forms across a wide range of species, is an essential component of the basic immune response in mammals. Identifying agonists and antagonists of the NF- $\kappa$ B pathway may lead to new therapies for certain cancers or immunological disorders, and our new vectors are convenient screening tools for finding these potential NF- $\kappa$ B regulators. The vectors are available in three forms: **pNF- $\kappa$ B-Luc** contains the firefly luciferase gene; **pNF- $\kappa$ B-SEAP** contains the secreted alkaline phosphatase (SEAP) gene; and **pNF- $\kappa$ B-d2EGFP** (patent pending) contains the gene encoding destabilized enhanced green fluorescent protein.

After transfection of the reporter vector into an appropriate cell line, you can activate the NF- $\kappa$ B pathway using various stimuli. Induction of the pathway enables endogenous NF- $\kappa$ B to bind to the four tandem copies of the kappa enhancer element ( $\kappa$ B<sub>4</sub>) located upstream of the reporter gene on the vector. Binding of NF- $\kappa$ B enhances the association of the cells' general transcription machinery with the herpes simplex virus thymidine kinase (HSV-TK) promoter fused downstream of  $\kappa$ B<sub>4</sub>, resulting in high induction levels of reporter gene transcription.

## Widely studied signal transduction pathway

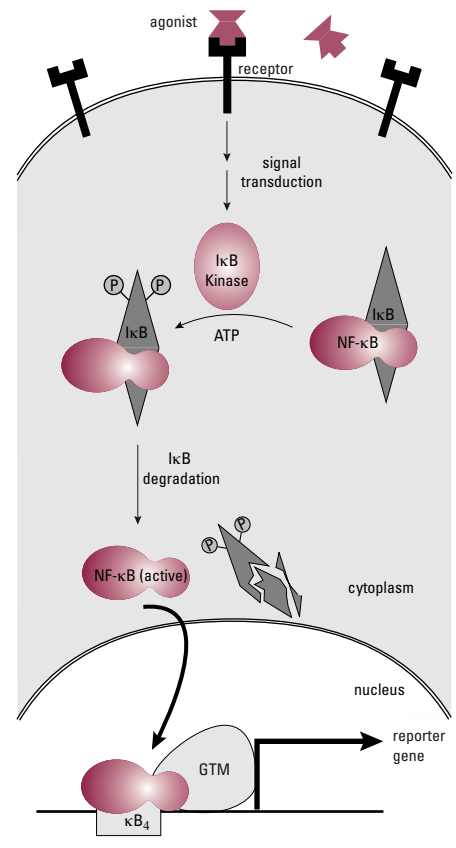
In mammalian systems, the NF- $\kappa$ B signal transduction pathway is induced by stimulation of the TNF or IL-1 (or other) lymphokine receptors, either by their respective ligands, by

**Figure 1. Map of pNF- $\kappa$ B Reporter Vectors.** The reporter gene is either luciferase, SEAP, or d2EGFP. The NF- $\kappa$ B Vectors contain four tandem copies of the  $\kappa$  enhancer element. When the plasmid is transfected into an appropriate cell line, expression of the reporter gene can be induced by adding TNF, IL-1, or another stimulus that activates the NF- $\kappa$ B signal transduction pathway.

lipopolysaccharide (LPS), or by phorbol esters. Through a series of intracellular steps, the activation of the receptor promotes the phosphorylation and subsequent dissociation of the I $\kappa$ B inhibitor protein from the inactive NF- $\kappa$ B complex, allowing liberated NF- $\kappa$ B to translocate to the nucleus (Figure 2). Once active and inside the nucleus, NF- $\kappa$ B binds to the  $\kappa$  enhancer element on the DNA and activates transcription of several apoptosis-related, cell growth-dependent, and B-cell-proliferative genes (1–3). Our vectors provide researchers with a direct assay of induction of this pathway.

## Convenient assay of NF- $\kappa$ B activity

Figure 3 shows induction levels of the luciferase, SEAP, and d2EGFP reporter genes after adding TNF $\alpha$  to cells transfected with the appropriate reporter vector. For each reporter gene, expression levels generally increase more than four-fold within six hours, and expression can be detected as little as two hours post-induction. Note that luciferase and SEAP permit detection of particularly high levels of induction in comparison to d2EGFP. These two reporters should be used instead of d2EGFP if sensitivity is a critical issue in designing your assay. Also note that the expression levels of SEAP and d2EGFP peak about



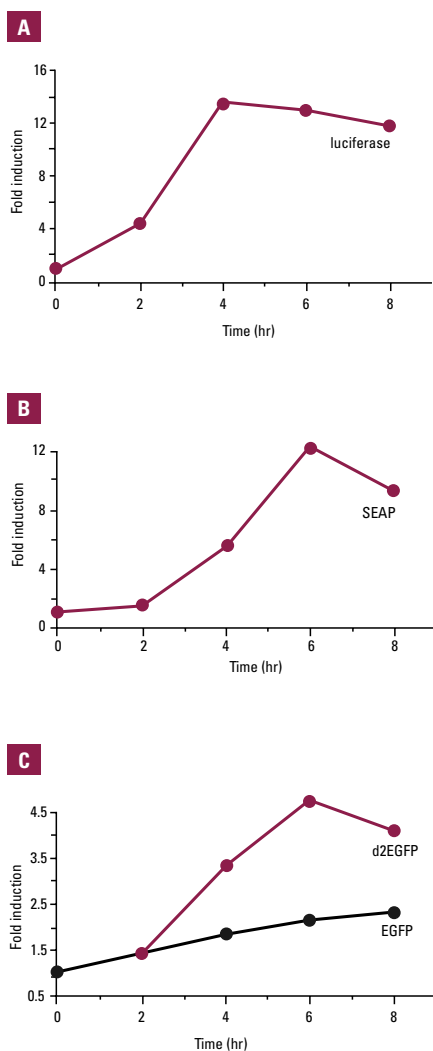
**Figure 2. The NF- $\kappa$ B signal transduction pathway.** In mammals, activated NF- $\kappa$ B protein binds to the  $\kappa$  enhancer element, which controls expression of several genes involved in humoral immune response. After transfection of a pNF- $\kappa$ B Reporter Vector into an appropriate cell line, NF- $\kappa$ B binds to the  $\kappa$ B<sub>4</sub> element on the vector and initiates transcription of luciferase, SEAP, or d2EGFP. GTM = general transcription machinery.

two hours after the peak in luciferase expression. This lag time may be due to additional posttranslational modifications needed for proper folding of d2EGFP or the secretion of SEAP into the culture medium.

## Three reporters, many advantages

A choice of three vectors allows you to select the reporter gene that is best suited for your preferred method of detection. Both SEAP and luciferase are highly sensitive enzymatic reporters that can provide quantitative data on absolute induction levels, but both require addition of substrate for measuring activity.

# NF- $\kappa$ B Transcription Reporter Vectors...continued



**Figure 3. Induction of reporter genes.** 293 cells were transiently transfected with pNF- $\kappa$ B-Luc, pNF- $\kappa$ B-SEAP, or pNF- $\kappa$ B-d2EGFP. 24 hr post-transfection, cells were treated with 100 ng/ml TNF $\alpha$ . Cells were harvested at various time points after induction. **Panels A and B.** Luciferase and SEAP reporter genes exhibit approximately 12-fold induction when activated by TNF $\alpha$  as measured by enzymatic assays. **Panel C.** d2EGFP exhibits approximately 4-fold induction. d2EGFP and EGFP fluorescence levels were measured by flow cytometry.

Luciferase permits detection of the highest induction levels of all three reporters. SEAP is useful for time-course studies in intact cell cultures; because the enzyme is secreted into the culture medium, you can take samples of the

culture supernatant at various time points without harvesting the cells. d2EGFP detection is nonenzymatic, can be detected in living or formaldehyde-fixed cells, and provides real-time analysis of relative induction levels by fluorescence microscopy or flow cytometry with minimal sample manipulation.

d2EGFP is a modified form of EGFP designed for rapid turnover in mammalian cells (4; see page 22). In contrast with EGFP, which is extremely stable, d2EGFP's half-life is two hours. Therefore, it provides a more accurate assessment of transient activation by NF- $\kappa$ B *in vivo* and is ideal for kinetic studies of gene activation. Because unmodified EGFP accumulates in the cell, even under levels of basal expression (i.e., without NF- $\kappa$ B activation), it is relatively insensitive to induction (Figure 3C). In contrast, d2EGFP, which is rapidly turned over, is almost undetectable when expressed at basal, uninduced levels; it is highly sensitive to induction and exhibits a much higher range of fold-increase in fluorescence intensity. Note that Figure 3C reports fold-induction, not absolute induction levels; actual fluorescence intensity of EGFP is higher at uninduced levels than that of d2EGFP.

Each vector comes with a Vector Information Packet that describes the features and optimum detection methods for assaying expression from each vector. The pNF- $\kappa$ B-d2EGFP Vector is also supplied with the Living Colors™ User Manual (PT2040-1), which provides detailed information on detection of EGFP fluorescence. Vector maps and sequences are also available at our website ([www.clontech.com](http://www.clontech.com)).

The NF- $\kappa$ B Vectors are the first in a series of reporter vectors we'll be offering for measuring activation of various signal transduction pathways. You can help us design future signal transduction reporter vectors. Got a hot signal transduction pathway you'd like to assay with luciferase, SEAP, or d2EGFP? Let us know and we may incorporate it into future vectors. Contact us at our website ([www.clontech.com/clontech/TAP.html](http://www.clontech.com/clontech/TAP.html)) or simply e-mail us at [tap@clontech.com](mailto:tap@clontech.com).

Product	Size	Cat. #
pNF- $\kappa$ B-Luc Vector	20 $\mu$ g	6053-1
pNF- $\kappa$ B-d2EGFP Vector	20 $\mu$ g	6054-1
pNF- $\kappa$ B-SEAP Vector	20 $\mu$ g	6055-1

#### Related Products

- pSEAP2 Vectors (many)
- p $\beta$ gal Vectors (many)

#### References

1. Baeuerle, P. A. & Baltimore, D. (1996) *Cell* **87**: 13–20.
2. Baeuerle, P.A. (1998) *Curr. Biol.* **8**:R19–R22.
3. Peltz, G. (1997) *Curr. Opin. Biotechnol.* **8**:467–473.
4. Living Colors Destabilized EGFP Vectors (April 1998) *CLONTECHniques* **XIII**(2):16–17.

#### Notice to Purchaser

NF- $\kappa$ B-d2EGFP is covered by U.S. Patent #5,491,084, and is the subject of pending patent applications. All purchasers are granted an automatic license with the purchase of NF- $\kappa$ B-d2EGFP to use it for internal research purposes. However, this license excludes specifically any right to modify the reagent for resale, to use the reagent for the manufacture of commercial products, to use in drug discovery processes, or to use the reagent in humans or for diagnostic purposes.

NEW!

# HAT™ Protein Expression & Purification System

A versatile new system compatible with TALON™ Resin

- **Novel polyhistidine epitope tag allows purification at neutral pH**
- **Versatile purification—use with native or denaturing conditions**
- **High-expression prokaryotic vectors in all three reading frames**
- **Delivers optimum purification using TALON™ IMAC resin**

Our new **HAT™ (Histidine Affinity Tag) Protein Expression & Purification System** (patent pending) provides a more convenient and efficient way to express and purify proteins. The HAT Vectors encode a novel polyhistidine epitope tag (see Figure 1 legend) that enables purification of proteins expressed in bacteria under the mild conditions of neutral or physiological pH. In concert with our cobalt-based TALON IMAC (immobilized metal affinity chromatography) resin, the HAT Vectors facilitate simplified protein purification under either native or denaturing conditions.

## The HAT sequence

The HAT epitope is a naturally occurring, 19-amino-acid sequence from the chicken lactate dehydrogenase protein. This sequence of non-adjacent histidine residues possesses less overall charge than tags with consecutive His residues, such as the 6xHis tag. As a result, HAT-protein fusions exhibit solubility that more closely resembles that of wild-type proteins while still possessing strong affinity for immobilized metal ions. The unique binding characteristics of the HAT sequence allow both imidazole- and pH-gradient purification of proteins under native conditions at neutral pH (7), as well as under denaturing conditions.

The HAT System offers advantages for native protein purification compared to 6xHis-tag purification protocols that require the use of alkaline buffers (pH 8). Purification at neutral pH is more efficient due to the reduction in binding and elution of impurities such as non-His-tagged proteins. In addition, purification at neutral pH decreases the activity of basic proteases and generally results in

higher protein stability. For proteins that exhibit lower solubility, the HAT System is also suitable for purification under denaturing conditions.

## The pHAT Vectors—optimized for TALON purification

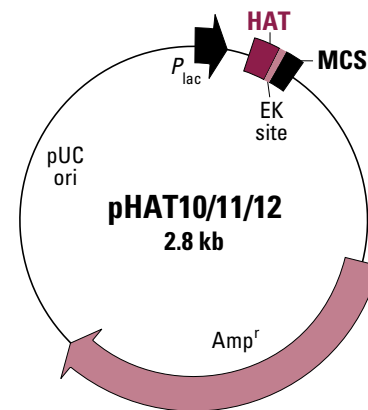
Proteins expressed from the pHAT Vectors can be purified with TALON Resin (1), using batch or gravity-flow protocols, or with our TALON Superflow™ Resin, using medium-pressure FPLC (2). TALON has a remarkably high affinity for His-tagged proteins and very low affinity for other proteins. The unique binding properties of the cobalt metal in TALON and of the HAT sequence combine to allow purification without the need for an additional wash solution and under pH conditions that preserve protein integrity. TALON incorporates a tetradentate metal chelator that firmly binds the cobalt metal ion and virtually eliminates metal leakage under a variety of conditions, including high salt and pH extremes.

The HAT sequence and an enterokinase (EK) cleavage site have been incorporated into the pUC19 backbone to create the pHAT Vectors (Figure 1). The EK site allows optional removal of the HAT sequence from the purified protein by treatment with enterokinase. Convenient restriction sites allow excision of the HAT sequence, with or without the EK site, for cloning in other vectors.

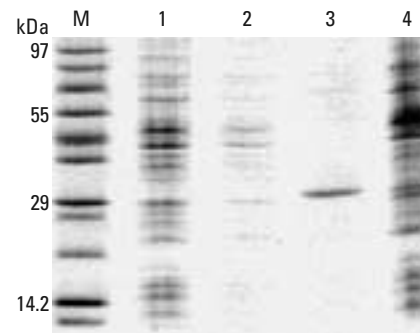
## Efficient purification of HAT-GFP

Figure 2 demonstrates the highly specific purification of a HAT-GFP (green fluorescent protein) fusion by pH-gradient elution from TALON Resin under gravity-flow conditions. Whole lysate from cells expressing the HAT-GFP construct was loaded and washed in the same step at pH 7.0. The HAT-GFP fusion was then eluted at high purity at pH 6.0 (Lane 3). More than 80% of the fusion was eluted under these mild conditions (Table I), in contrast to other tags that require lower pH for elution.

TALON Superflow Resin combines all the benefits of TALON with improved flow characteristics that are ideal for production-scale applications. HAT-GFP was rapidly purified using TALON Superflow and FPLC (Figure 3A). Elution was performed at pH 7.0 in the presence of imidazole. Again, these mild conditions



**Figure 1. The pHAT Vectors.** These vectors allow efficient protein purification due to an improved polyhistidine affinity tag (HAT) sequence (Lys, Asp, His, Leu, Ile, His, Asn, Val, His, Lys, Glu, Glu, His, Ala, His, Ala, His, Asn, Lys). An enterokinase (EK) site between the HAT sequence and the MCS allows isolation of the native protein. MCS: *Sal*I, *Bam*H I, *Sma*I, *Kpn*I, *Sac*I, *Eco*R I.



**Figure 2. Batch purification of HAT-GFP by pH gradient elution from TALON Resin.** SDS/PAGE analysis of HAT-GFP fusion protein purified on TALON Resin. Lane 1: flow-through after sample loading in 50 mM sodium phosphate, 0.3 M NaCl, pH 7.0. Lane 2: wash with same buffer. Lane 3: elution with 50 mM sodium phosphate, 0.3 M NaCl, pH 6.0. Lane 4: whole cell extract. Lane M: size markers.

discriminate against nonspecific binding and allow impressive adsorption and recovery (Figure 3B & Table 1).

The HAT System contains three pHAT Vectors (same construct in all three reading frames),

# HAT™ Protein Expression & Purification System...continued

NEW!

**Table I: TALON purification of HAT-GFP fusion protein**

Batch/gravity-flow purification		FPLC purification	
Nonadsorbed	14%	Nonadsorbed	16%
Eluted at pH 6.0	82%	Adsorbed	84%
Eluted at pH 5.0	4%		
Eluted at pH 4.0	0%		
<b>Purification factor</b>	<b>&gt;18</b>	<b>Purification factor</b>	<b>&gt;20</b>

Product	Size	Cat. #
HAT Protein Expression & Purification System	each	K6050-1
HAT Polyclonal Antibody	100 µl	8909-1

### Kit Components

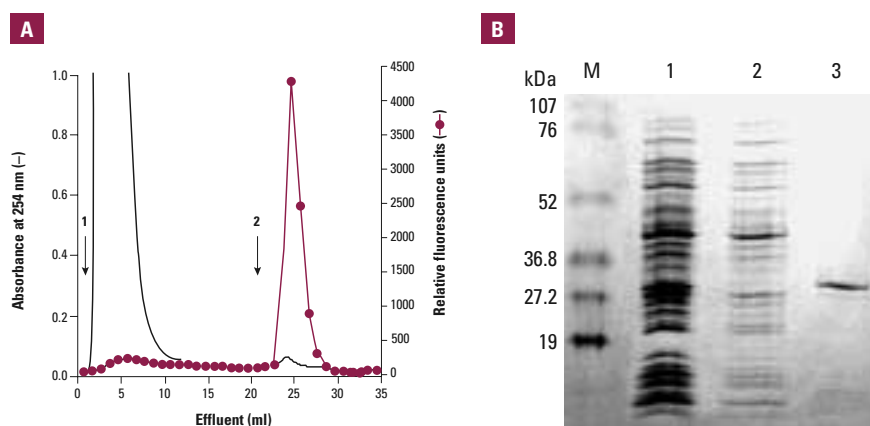
- pHAT10 Vector
- pHAT11 Vector
- pHAT12 Vector
- pHAT-DHFR Control Vector
- TALON Resin
- Buffer A
- Buffer B
- Buffer C
- Guanidine HCl
- Disposable Plastic Columns
- Complete User Manual (PT3250-1)
- Vector Information Packet (PT3251-5)

### Related Products

- TALON™ Resin & Columns (many)
- TALON™ Superflow™ Metal Affinity Resin (#8908-1, -2)

### References

1. TALON Metal Affinity Resin (April 1995) *CLONTECHniques X*(2):8-9.
2. TALON Superflow Metal Affinity Resin (January 1998) *CLONTECHniques XII*(1):14.



**Figure 3. FPLC purification of HAT-GFP using TALON Superflow Resin.** Panel A. Crude extract from 150 mg of cells expressing the HAT-GFP fusion was loaded onto a 3 x 1-cm column containing TALON Superflow Resin in 50 mM sodium phosphate, 0.3 M NaCl, 5 mM imidazole, pH 7.0 (Arrow 1) and eluted with 50 mM sodium phosphate, 0.3 M NaCl, 150 mM imidazole, pH 7.0 (Arrow 2). Panel B. SDS/PAGE analysis. Lane 1: whole cell extract. Lane 2: flow-through. Lane 3: elution. Lane M: size markers.

the pHAT-DHFR Control Vector, TALON Resin, a complete set of loading and elution buffers, disposable gravity-flow columns, and a complete User Manual.

### HAT Polyclonal Antibody

CLONTECH also offers the **HAT Polyclonal**

**Antibody**—a valuable tool for researchers purifying HAT-proteins on TALON Resin. This antibody can be used to detect and quantify HAT-proteins with minimal background, before and after purification, in Western blotting, immunoprecipitation, and ELISA applications.

# NucleoSpin® High-Throughput Plasmid Purification Kits

Eight-well strips for convenient preparation of plasmid minipreps



**Figure 1. The NucleoVac™ vacuum manifold.** This high-quality acrylic manifold is easy to operate and maintain. It holds 12 Multi-8 strips at a time and can also accommodate individual NucleoSpin columns. NucleoSpin Multi-8 Plus Filter Strips (pink; on top of manifold) filter the bacterial lysate and deliver the cleared suspension containing plasmid DNA into NucleoSpin Multi-8 Strips (white; inside manifold) for further processing. The NucleoVac manifold is sold separately.

- **State-of-the-art purification system**
- **Process up to 96 minipreps in less than 90 minutes**
- **Yields plasmid pure enough for fluorescent sequencing**

The new **NucleoSpin® Multi-8 Plus and NucleoSpin Multi-8 Plasmid Kits** provide uncompromised performance and convenience for purifying high- and low-copy plasmids. Both kits contain 8-well strips of NucleoSpin miniprep columns that can be easily processed by vacuum filtration. The molded acrylic, 96-well format **NucleoVac™ Vacuum Manifold** (sold separately) is designed exclusively for use with the Multi-8 and Multi-8 Plus Kits. It allows simultaneous processing of up to 96 samples in less than 90 minutes or batches of eight strips in less than 30 minutes. The NucleoSpin Multi-8 Plus Kits also contain special 8-well Filter Strips that reduce prep time even further by enabling you to clear bacterial lysates directly in the NucleoVac instead of clearing by centrifugation. The NucleoSpin Multi-8 Kits (but not the Multi-8 Plus Kits) can also be used to purify large constructs such as BACs and PACs.

## High-quality DNA

Like the columns in our original NucleoSpin Plasmid Purification Kits, the new Multi-8 Columns contain a unique silica membrane that binds DNA in the presence of chaotropic salt. After a modified alkaline lysis procedure, bacterial lysates are loaded into individual columns arranged on the NucleoVac manifold. Filtrates are drawn out of the columns by vacuum, eliminating the need for centrifugation during the washing and elution steps and significantly reducing prep time. The manifold is easy to use and assemble. Together, the kits and the manifold can be used to purify high-quality DNA suitable for radioactive and fluorescent sequencing, library screening, and cloning.

## Flexible system

NucleoSpin columns are designed for purifying up to 20 µg of plasmid DNA from 1–5-ml *E. coli* overnight cultures. As many as 96 preparations can be performed at once in a 96-well plate format. The strips can also be used in multiples of eight, or each strip can be separated into individual columns.

The NucleoSpin Multi-8 Kits and the NucleoVac are supplied with a complete User Manual that provides illustrated instructions for performing successful minipreps.

Product	Size	Cat. #
NucleoSpin Multi-8 Plasmid Kit	96 preps 5 x 96 preps	K3057-1 K3057-2
NucleoSpin Multi-8 Plus Plasmid Kit	96 preps 5 x 96 preps	K3058-1 K3058-2
NucleoVac	each	4071-1

NEW!

## Kit Components

- **NucleoSpin® Multi-8 Plasmid Strips**
- **NucleoSpin® Multi-8 Filter Strips** (Multi-8 Plus Kit only)
- **Collection Tubes**
- **Caps** (in strips of eight)
- **Buffers A1, A2, A3, A4, AW & AE**
- **RNase A** (lyophilized)
- **Foam, wash plate, filter paper & foil** (for vacuum manifold)
- **Complete User Manual** (PT3225-1)

## Related Products

- **NucleoBond® Plasmid Purification Products** (many)
- **NucleoSpin® Miniprep Kits** (#K3050-1 & #K3050-2)
- **NucleoSpin® Genomic DNA Purification Kits** (many)

Our NucleoSpin and NucleoBond Kits are manufactured with strict adherence to exacting quality control standards to ensure maximum reliability and performance. These kits provide you with the most advanced purification technologies at competitive prices.

To request a brochure on our Nucleic Acid Purification Products, please see the back cover for Literature Request information.



# Pretransformed Human MATCHMAKER cDNA Libraries

Faster, simpler approach to two-hybrid library screening

- Save several weeks in two-hybrid library screening
- Eliminates library-scale transformations for screening
- New host strain simplifies analysis of putative clones
- Fewer false positives

CLONTECH's **Pretransformed MATCHMAKER Libraries** are high-complexity GAL4 cDNA libraries that have already been amplified and introduced into yeast strain Y187. Thus, these libraries significantly reduce the labor and time required to screen a MATCHMAKER Library because you do not have to perform a library transformation.

Figure 1 illustrates the convenient, easy Pretransformed Library screening protocol. Simply mix the library with a suitable MAT $\alpha$  strain, such as PJ69-2A, that has been transformed with your bait construct. The next day, plate the mating mixture on medium that selects for two-hybrid interactions, and then analyze positive clones.

The advanced yeast mating partner strain PJ69-2A (1), included with each Pretransformed Library, contains two nutritional reporter genes (*ADE2* & *HIS3*) under the control of different promoters that significantly reduce the incidence of false positives (1, 2). The library host strain Y187 provides a third reporter gene (*lacZ*) for optional quantification of signal strength.

All libraries are provided with control strains to test for a positive two-hybrid interaction. A complete User Manual (PT3183-1), the Yeast Protocols Handbook (PT3024-1), and a Vector Information Packet are also included. CLONTECH also offers a complete line of yeast media and dropout supplements—see our website or catalog.

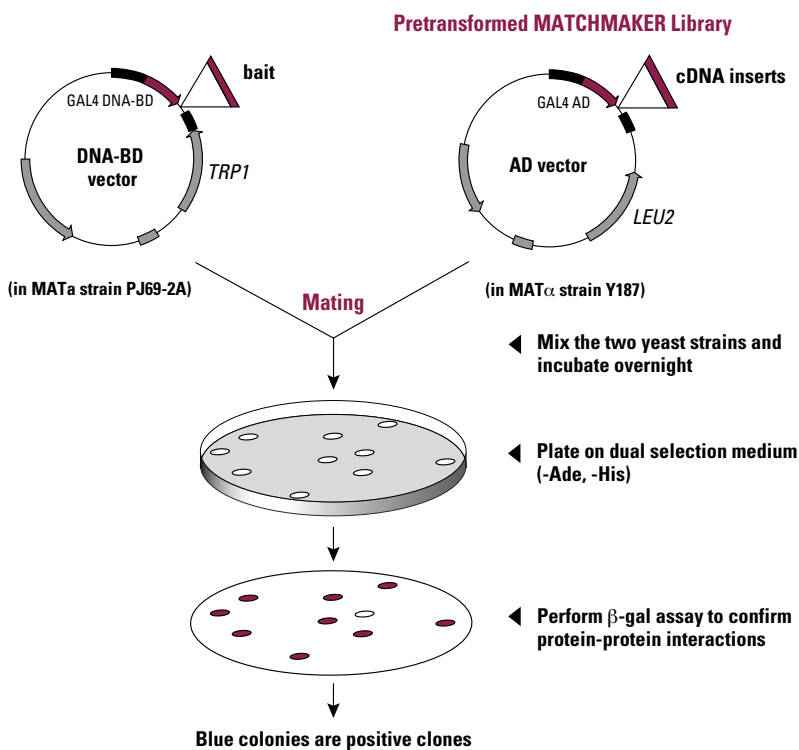
## References

1. James, P., et al. (1996) *Genetics* **144**:1425–1436.
2. Pretransformed MATCHMAKER cDNA Libraries (October 1997) *CLONTECHniques* **XII**(4):12–13.

<sup>a</sup> All libraries were prepared using plasmid DNA amplified from the corresponding original MATCHMAKER Library in *E. coli*. For information on the original libraries, see our Catalog or visit our website.

<sup>b</sup> Tissue source may vary from lot to lot.

<sup>c</sup> Each 1-ml aliquot is sufficient for a complete library screening of  $>1 \times 10^6$  independent clones.



**Figure 1. Two-hybrid screening using Pretransformed MATCHMAKER cDNA Libraries.** Your bait protein is expressed as a fusion with the GAL4 DNA-BD in PJ69-2A. pGBT9 (#K1605-A) and pAS2-1 (#K1604-B) and pBridge (#6184-1) are appropriate DNA-BD vectors. The high-complexity cDNA library, which expresses fusions with the GAL4AD, is provided in yeast strain Y187. When the two transformant cultures are mated, diploid cells are formed which contain three reporter genes: *HIS3*, *ADE2*, and *lacZ*. The GAL4 DNA-BD binds to the GAL UAS and, if the fusion proteins interact, the AD is brought into proximity of the reporter genes' promoters, thereby activating transcription and permitting growth on selection medium and expression of  $\beta$ -gal.

## Pretransformed MATCHMAKER Libraries<sup>a</sup>

Poly A <sup>+</sup> RNA Source <sup>b</sup>	Vector	Size <sup>c</sup>	Cat. #
<b>Human Brain</b> whole; 37-yr-old Caucasian male	pACT2	5 x 1 ml	HY4004AH
<b>Human Fetal Brain</b> whole; pooled from 9 Caucasian fetuses	pACT2	5 x 1 ml	HY4028AH
<b>Human HeLa</b> HeLa S3; ATCC #CCL 2.2	pGAD GH	5 x 1 ml	HY4000AA
<b>Human Liver</b> 40-yr-old Caucasian male	pACT2	5 x 1 ml	HY4024AH
<b>Human Ovary</b> pooled from 5 Caucasians, ages 30–60	pACT2	5 x 1 ml	HY4051AH
<b>Human Placenta</b> pooled from 4 Caucasians, ages 25–41	pACT2	5 x 1 ml	HY4025AH
<b>Human Testis</b> pooled from 11 Caucasians, ages 10–61	pACT2	5 x 1 ml	HY4035AH

## Notice to Purchaser

Practice of the two-hybrid system is covered by US Patents #5,283,173 and #5,468,614 assigned to the Research Foundation of the State University of New York. Purchase of any CLONTECH two-hybrid reagent does not imply or convey a license to practice the two-hybrid system covered by these patents. Commercial entities purchasing these reagents must obtain a license from the Research Foundation of the State University of New York before using them. CLONTECH is required by its licensing agreement to submit a report of all purchasers of two-hybrid reagents to SUNY Stony Brook. Please contact Carol Dempster, Ph.D., at the Long Island Research Institute for license information (Tel: 516-361-6800; Fax: 516-361-6840).

# Molecular Biology Support Antibodies

New specificities for use with Tet Systems, phage display, luciferase & common epitope tags

The **VP16 Polyclonal Antibody (Affinity Purified)** can be used to detect the tetracycline-responsive transactivator (tTA) and the reverse tTA (rtTA) that are encoded by the various regulator plasmids in CLONTECH's Tet-On™, Tet-Off™, RevTet-On™, and RevTet-Off™ Systems. Researchers using phage display will

want to try our new antibodies raised against intact **Bacteriophage M13** and against **Protein VIII** of Bacteriophage M13. Both antibodies can be coated onto plates for use as the "capture antibody" in phage display experiments, or they can be used as the primary detection antibody for captured phage after screening.

Our new offerings also include a **Luciferase Polyclonal Antibody (pAb)-HRP Conjugate**, as well as antibodies to the widely used **HA** and **HSV epitope tags**. All our antibodies are high quality and pretested. For a complete, up-to-date listing, please visit [www.clontech.com](http://www.clontech.com).

Antibody	Source/Type	Applications*	Size	Cat. #
<b>VP16 Polyclonal Antibody (Affinity Purified)</b>	Rabbit Ig	WB, IP, IF, ELISA	100 µg	3844-1
<b>fd Bacteriophage M13 Polyclonal Antibody (IgG)</b>	Rabbit IgG	WB, IP, ELISA	1 mg	3837-1
<b>Protein VIII Polyclonal Antibody (Affinity Purified)</b>	Rabbit Ig	WB, IP, ELISA	100 µg	3838-1
<b>HA-Tag Polyclonal Antibody (IgG)</b>	Rabbit IgG	WB, IP, ELISA	500 µg	3832-1
<b>HSV-Tag Polyclonal Antibody (Affinity Purified)</b>	Rabbit Ig	WB, IP, IF, ELISA	100 µg	3828-1
<b>Luciferase pAb-HRP Conjugate (IgG)</b>	Rabbit IgG	WB, ELISA	500 µg	3836-1

\* WB = Western blot; IP = immunoprecipitation; IF = immunofluorescence; ELISA = enzyme-linked immunosorbent assay.

NEW!

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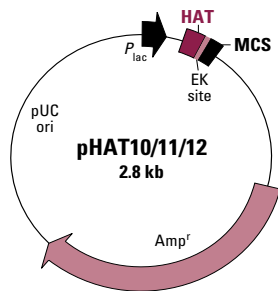
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At CLONTECH, we're constantly improving our website to provide you with the most complete, up-to-date information available about our products and services.

Our newly redesigned Home Page makes browsing for product information even easier. New Product Family Home Pages (coming soon) now provide quick links to our major product lines, like our Atlas cDNA Arrays.

Got technical questions? Our website now includes Technical Tips for answers to your most frequently asked questions about select product lines.

Visit us at [www.clontech.com](http://www.clontech.com) today!

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