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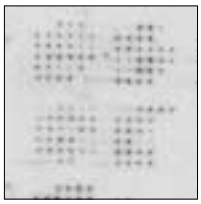

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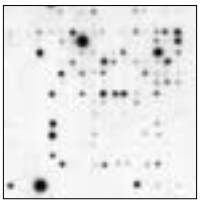

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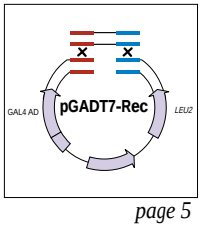

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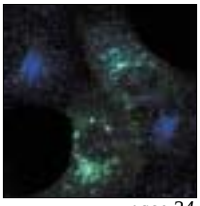

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ABOUT THE COVER

The cover shows an illustration designed for our Adeno-X™ Systems. The illustration was inspired by the art of Hajime Ouchi.

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CLONTECHniques

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Human RNA Chip™

Visualize gene expression across 124 human tissue samples

- **Full human body expression analysis**
- **Two visualization options—fluorescence or radioactivity**
- **Multicolor fluorescence detection**
- **Complements differential gene expression techniques**

CLONTECH now brings RNA to a glass format! The **Human RNA Chip**™ provides quick and accurate quantification of mRNA transcripts from a wide variety of tissues and tumor cell lines. This glass array contains 124 human fetal and adult tissue-specific Premium Poly A$^+$ RNAs, including 20 RNAs derived from different tumor cell lines (Figure 1).

By providing access to a large collection of human tissues and cell lines, the RNA Chip allows you to accurately perform a broad survey of gene expression in one easy hybridization. The glass slide format provides easy high-throughput RNA analysis using standard cDNA glass array scanners and software. This means no new specialized equipment to purchase for a lab already using microarray technology.

With the glass RNA Chip, you can simultaneously hybridize with a gene-specific probe and a housekeeping control gene, labeled with different colors. This technique gives you the accuracy of directly comparing the expression of two genes within the same tissue at the same time.

Multiple probes, one hybridization—complete results

Because of the ability to perform high-throughput analysis, generating an RNA expression profile using RNA Chip hybridization takes only three hours, compared to several days for multiple autoradiographic exposures required for radioactive detection.

With two-color fluorescence detection, in one step, you can determine whether or not your experiment was successful—you can see your control and gene-specific probe on the same chip. There is no need to strip an array and re-probe with a housekeeping gene, as is required with radioactive labeling.

	1 2	3 4	5 6	7 8	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24
A	Cy3	Cerebellum left	Nucleus accumbens	Cy3	Insula	Trachea	Cy3	Atrium left	Liver—left lobe	Ureter	Stomach—fundus	Cy3
B	Whole brain	Cerebellum right	Corpus callosum	Paracentral gyrus	Occipital pole	Lung	Lung—upper right lobe	Atrium right	Liver—right lobe	Thymus	Stomach—pylorus	Colon with mucosa
C	Thalamus	Cerebral cortex	Frontal lobe	Postcentral gyrus	Pituitary gland right	Lung—lower left lobe	Heart	Auricle dextra	Spleen	Esophagus	Pancreas	
D	Hypo-thalamus	Amygdala	Temporal lobe	Medulla oblongata	Tongue	Lung—lower right lobe	Heart—ventricle left	Auricle sinistra	Kidney	Stomach	Colon—ascending	Duodenum
E	Hippo-campus	Putamen	Parietal lobe	Substantia nigra	Tonsil	Lung—upper left lobe	Heart—ventricle right	Heart—pericardium	Bladder	Stomach—cardia	Colon—descending	Jejunum
F	Whole cerebellum	Caudate nucleus	Printing buffer (–)	Pons	Thyroid	Printing buffer (–)	Aorta	Liver	Printing buffer (–)	Stomach—corpus	Colon—transverse	Printing buffer (–)
G	Cy3	Salivary gland	Lymph node mediastinal	Cy3	Adrenal cortex	Bone marrow	Cy3			Whole fetus	Fetal kidney	Cy3
H	Cecum	Small intestine	Prostate	Uterus	Adrenal medulla					Fetal brain	Fetal thymus	Fetal skeletal muscle
I	Ileoccecum	Small intestine w/o mucosal lining	Epididymus	Uterus w/o endometrium	Peripheral leukocytes					Fetal lung	Fetal stomach	
J	Ileum	Epiploon cell	Testis	Placenta	Skeletal muscle					Fetal heart	Fetal intestine	
K	Rectum	Appendix	Uterus Corpus	Mammary gland	Skin					Fetal liver	Fetal adrenal gland	
L	Inter-ventricular septum	Lymph node	Printing buffer (–)	Adrenal gland	Spinal cord	Printing buffer (–)			Printing buffer (–)	Fetal spleen	Fetal spinal cord	Printing buffer (–)
M	Cy3	Lung carcinoma A549	NIH: OVCAR-3	Cy3			Cy3			Yeast total RNA (–)	Human genomic DNA (–)	Cy3
N	Leukemia HL-60	Pancreatic carcinoma	Burkitt's lymphoma Raji	Tumor A-375						Yeast tRNA (–)	HKG (+)	HKG (+)
O	Leukemia K-562	Mammary carcinoma GI-101	Burkitt's lymphoma Daudi	Tumor MR1-H121						*E. coli* rRNA (–)	HKG (+)	HKG (+)
P	Leukemia MOLT-4	Prostate adeno–carcinoma	Tumor ZR75-1	Tumor SF-295						*E. coli* DNA (–)	HKG (+)	HKG (+)
Q	HeLa S3	Colorectal adeno–carcinoma	Tumor G-105	Tumor H-1549						Poly r(A) (–)	HKG (+)	HKG (+)
R	Lung tumor	Colon tumor	Printing buffer (–)			Printing buffer (–)			Printing buffer (–)	Human Cot-1 DNA (–)		Printing buffer (–)

Figure 1. Samples represented on the Human RNA Chip™. The chip will always include 110–125 samples, although the particular samples will be subject to availability. Each sample is printed in duplicate. Cy3 is included as an orientation marker. Samples marked by (–) are negative controls (including spots with printing buffer alone), and samples marked by (+) are positive controls. HKG=housekeeping gene.

Human RNA Chip™ ...continued

Your choice of detection options

While fluorescent labeling has many advantages, the RNA Chip also allows you to obtain the highly sensitive measurement of low-abundance genes that can only be achieved with radioactivity (Figure 2; 1). Imaging for the RNA Chip is straightforward—standard phosphorimaging for ^{33}P, or a fluorescence scanner for fluorescently labeled probes.

Accurate expression profiling

RNA Chip samples are normalized to not one, but six different housekeeping genes. The strict normalization and quantitation of RNAs on the RNA Chip ensures accurate conclusions about the presence and relative abundance of a given mRNA (2, 3).

As demonstrated in Figure 2, the RNA Chip can be used to establish the expression profile for a gene of interest. You can see if a gene is broadly expressed in many tissues (such as G3PDH, a housekeeping gene), or if it is tissue-specific (such as TAT, a liver-specific gene). Even low-abundance genes (such as lactoferrin, less than 0.001% of an mRNA population)can be observed.

Knowing where a gene is expressed gives you insight into the potential function of that gene's protein. The RNA Chip can show you which tissues to use for obtaining full-length cDNA for further analysis, or where to focus on more detailed spatial expression using *in situ* hybridization.

From differential expression to tissue localization

The RNA Chip is an integral part of CLONTECH's comprehensive approach to gene discovery and functional analysis—it links upstream techniques of examining differential gene expression to downstream methods of determining protein function.

CLONTECH offers a number of options for detecting differential expression, such as Atlas™ cDNA Expression Arrays, CLONTECH PCR-Select™ cDNA subtraction (#K1804-1), or Delta™ Differential Display (#K1810-1). Once you identify a differentially expressed gene, you can create a probe from the cDNA (Figure 3) and hybridize it to the RNA Chip to see which tissues express your gene. You can then perform further analysis using Northern blots, full-length cDNA cloning, and gene expression.

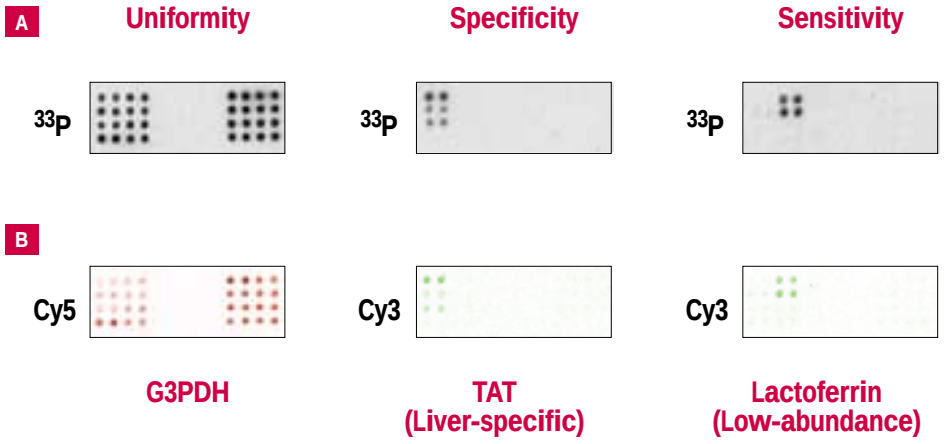

Figure 2. RNA Chip™ performance. Panel A. Three different probes were labeled using ^{33}P and hybridized to the RNA Chip. The images were captured using a phosphorimager**. Panel B.** The three gene-specific probes were labeled using Cy3 or Cy5 and hybridized to the RNA Chip. The slides were scanned using a fluorescence scanner. The images show a subset of samples on the Human RNA Chip.

Figure 4 demonstrates how differential expression and tissue localization can be combined to identify a gene and begin to dissect that protein's function. CLONTECH offers a full line of products to take you through the entire process (see Related Products).

Access difficult-to-obtain poly A$^+$ RNAs

The RNA Chip provides easy access to poly A$^+$ RNAs from the widest range of tissues and cancer cell lines available. Because of the time and expense of preparing all the poly A$^+$ RNAs included on the RNA Chip, it is a practical and convenient option for quickly screening gene expression.

The 124 tissues on this glass array represent an expansion of CLONTECH's nylon membrane MTE™ Array (Multiple Tissue Expression Array, #7775-1). The type of RNA may change from lot to lot and is subject to availability, but will always span 110–125 samples.

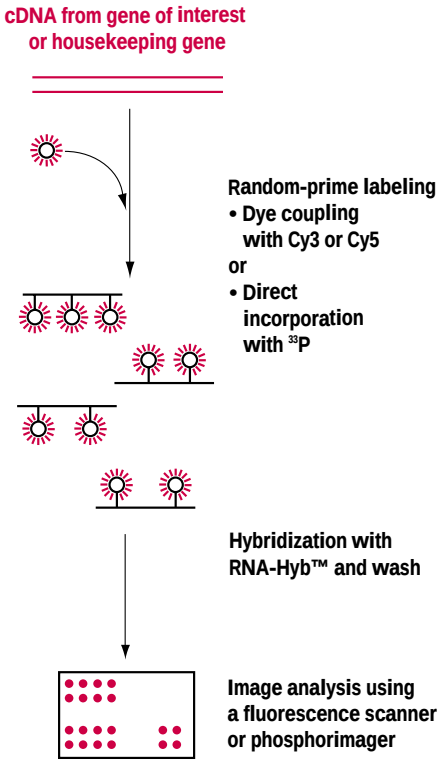

Figure 3. The RNA Chip™ probe-labeling process.

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Human RNA Chip™ ...continued

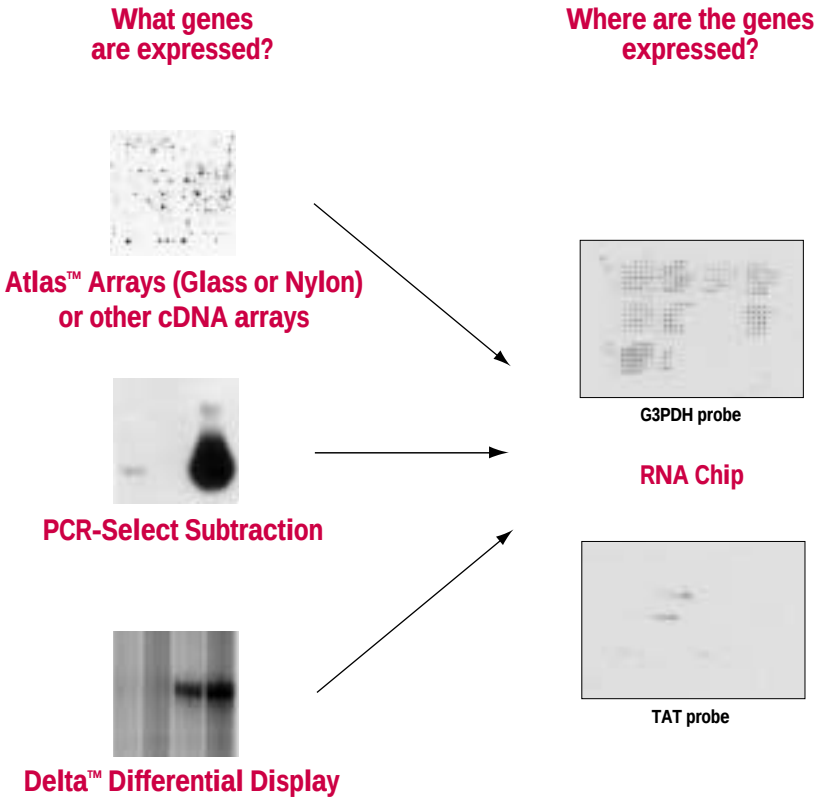

Figure 4. The RNA Chip™ links differential screening strategies with functional analysis. Using a cDNA array, PCR subtraction, or differential screening, a gene can be identified to be up- or down-regulated in a sample. A probe made from that cDNA can be used to identify where the gene is expressed. In the example above, the gene was terminal acetyltransferase (TAT), which is shown to be active in the liver samples of the RNA Chip. G3PDH was used as a housekeeping control gene. The next step in the analysis could be to figure out the function of the protein.

High-quality RNA

The "Premium" of CLONTECH's Premium RNA™ line refers to the exceptional quality and reliability of the highly purified total and poly A^+ RNAs used in all Premium RNA products. Each total RNA sample is meticulously prepared using a modified guanidinium thiocyanate method, and each poly A^+ RNA sample is enriched for mRNA transcripts with three rounds of oligo(dT)-cellulose purification. Using CLONTECH's Premium Poly A^+ RNA guarantees the presence of even rare transcripts. We perform rigorous quality control assays to confirm that each preparation consists of intact, full-length RNA, and we ensure that each RNA sample contains virtually no genomic DNA.

Complete hybridization kit

The Human RNA Chip comes as a complete hybridization system to ensure consistency and accuracy. RNA Chips are shipped hybridization-ready, with RNA-Hyb™ Buffer (optimized for RNA Chip use). The human G3PDH cDNA probe is provided as a positive control. Detailed source information for each sample is also supplied.

Product	Size	Cat. #
Human RNA Chip	each	7950-1

Components

- **Two Human RNA Chips**
- **Human G3PDH Control cDNA Probe**
- **RNA-Hyb™ Hybridization Solution**
- **RNA Chip™ Hybridization Chamber**
- **Wash Containers**
- **Klenow Mix**
- **Radioactive Labeling dNTP Mix**
- **Fluorescent Labeling dNTP Mix**
- **Fluorescent Labeling Buffer**
- **DMSO**
- **RNase-Free Deionized Water**
- **Lithium Chloride**
- **Sodium Acetate**
- **EDTA**
- **Complete User Manual** (PT3420-1)

Related Products

- **Atlas™ Arrays** (many; see page 12)
- **Human Multiple Tissue Expression (MTE™) Array** (#7775-1)
- **Mouse RNA Master Blot™** (#7771-1)
- **Multiple Tissue cDNA (MTC™) Panels** (many)
- **Multiple Tissue Northern (MTN™) Blots** (many)
- **Human β-Actin cDNA Control Probe** (#9800-1)
- **NucleoSpin® Extraction Kit**

References

1. Duggan, D. J., *et al*. (1999) *Nature Genet.* **21**:10–14.
2. Spanakis, E. & Brouty-Boye, D. (1994) *Nucleic Acids Res.* **22**:799–806.
3. Spanakis, E. (1993) *Nucleic Acids Res.* **21**:3809–3819.

MATCHMAKER Library Construction & Screening Kit

A complete two-hybrid library construction and screening kit

- **Efficient—construct & screen a two-hybrid library in less than a week**
- **Practical—requires just 25 ng of poly A⁺ RNA**
- **Universal—generate two-hybrid libraries from any tissue source**
- **Simple & reliable—perfect for the first-time two-hybrid user**

In response to numerous requests, CLONTECH has developed the **MATCHMAKER Library Construction & Screening Kit**, a PCR-based system for building high-quality cDNA libraries in a GAL4-activation domain (AD) vector. This novel library construction protocol integrates the efficiency of our SMART™ technology with the sensitivity of our MATCHMAKER Two-Hybrid System 3. Using this powerful combination, library construction and screening can be completed in less than one week.

The MATCHMAKER Library Construction Kit relies on established techniques for the generation of complex two-hybrid cDNA libraries. Even researchers approaching this task for the first time will appreciate the dependable design of this system. Our protocol (Figure 1) features a novel, one-step *in vivo* cloning procedure that is both quick and efficient. This technique exploits the homologous recombination gap repair pathway in yeast to join DNA segments. Because the cloning step takes place *in vivo* in a MATCHMAKER yeast reporter strain, library construction and screening can be completed in rapid succession without the need for any bacterial transformation or amplification steps.

MATCHMAKER gets SMARTer

In designing the MATCHMAKER Library Construction Kit, we have paid particular attention to the method used for preparing cDNA. The quality of the cDNA used to build a two-hybrid library is vital to the success of your experiments. Should the cDNA synthesis procedure yield an unusually high number of incomplete cDNAs or if the procedure fails to copy certain transcripts, the result is a population of clones that lacks the complexity

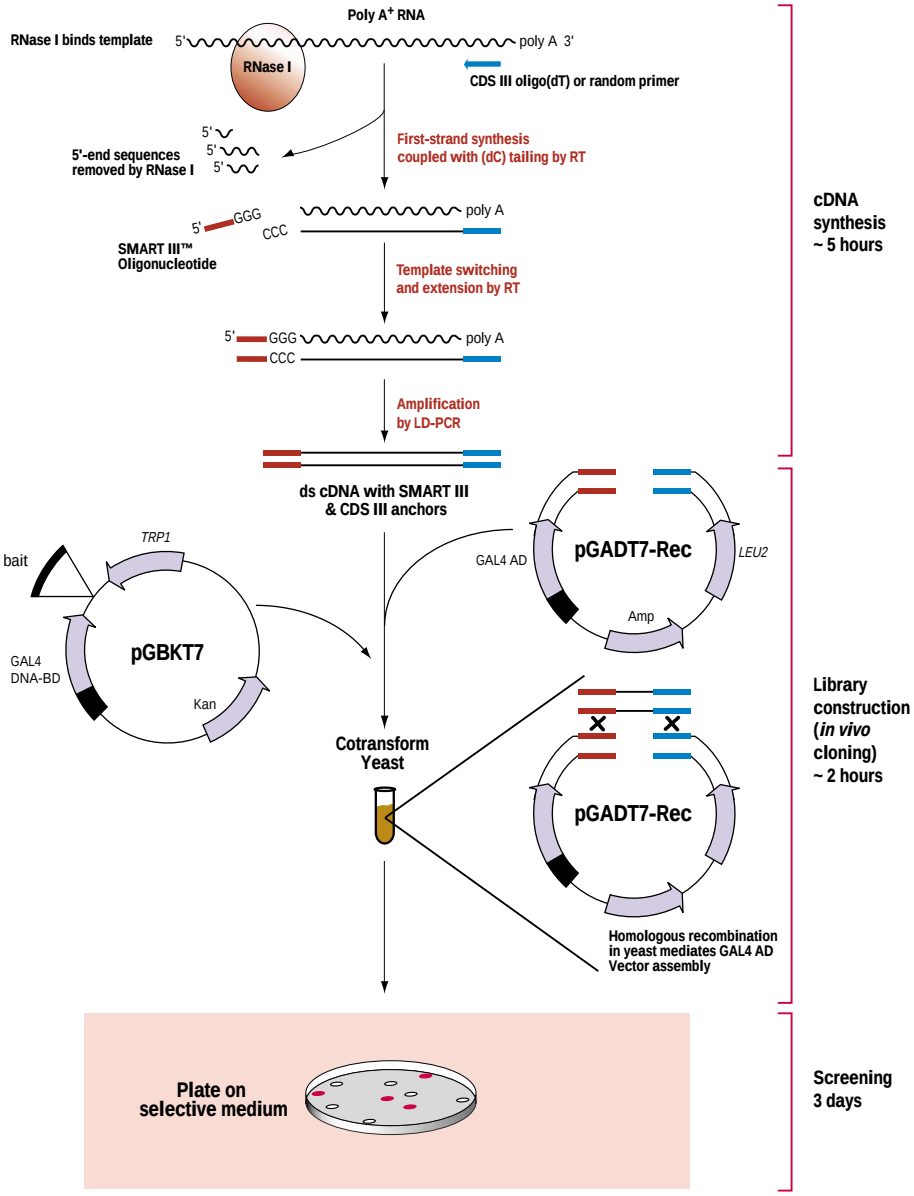

Figure 1. The MATCHMAKER Two-Hybrid Library Construction & Screening Kit procedure. Library construction begins with just nanograms of either total or poly A⁺ RNA. An oligo(dT) or random primer (CDS III) is used to prime the first-strand cDNA synthesis reaction, and RNase I is added to remove untranslated regions from the 5'-end of the transcript. When reverse transcriptase (RT) reaches the 5' end of the mRNA, the enzyme's terminal transferase activity adds a few deoxycytidines (dC). The 3' end of the SMART III Oligonucleotide anneals with the (dC) stretch, forming an extended template for RT. The resulting single-stranded cDNA is complementary to the mRNA template as well as the CDS III and SMART III oligos, which serve as universal priming sites for LD-PCR. Next, double-stranded cDNA, a linearized GAL4 AD vector (pGADT7-Rec), and a GAL4 DNA-BD/bait vector (pGBKT7) are cotransformed into a yeast reporter strain, where the cDNA recombines with homologous sequences in pGADT7-Rec. Transformants are then screened for two-hybrid interactions by plating on the appropriate selective medium. Your cDNA/AD fusion library can also be screened by mating with a pretransformed bait strain.

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MATCHMAKER Library Construction & Screening Kit...*continued*

of the original tissue. In screening such a population for interacting proteins, you are likely to miss important and potentially novel interactions. Fortunately, there is a method for generating high-quality cDNA while maintaining sequence representation: CLONTECH's SMART cDNA synthesis technology.

SMART, or **S**witching **M**echanism **a**t the 5' end of the **R**NA **T**ranscript, is a cDNA synthesis and amplification technology that lets you produce a complete cDNA library from just nanograms of RNA. SMART is an essential component of a number of our cDNA synthesis products including the SMART™ cDNA Library Construction Kit. Now it has been adapted for use with our MATCHMAKER Two-Hybrid System 3 to construct two-hybrid libraries.

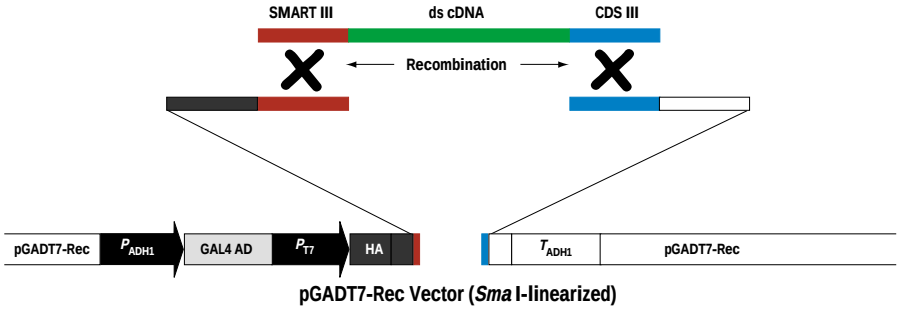

Figure 3. Large-scale transfer of cDNA to a GAL4 AD yeast expression vector, pGADT7-Rec, by homologous recombination. LD-PCR-amplified double-stranded cDNA is mixed with linearized pGADT7-Rec DNA (provided) and cotransformed into yeast cells. Homologous sequences at the ends of the cDNA are used by yeast repair enzymes to fill the gap in pGADT7-Rec, restoring the plasmid to its circular form. The outcome is a fully functional GAL4 AD/cDNA expression vector.

cDNA synthesis with SMART™

To meet the specific requirements of two-hybrid analysis, we modified the SMART procedure so that it is possible to remove noncoding 5'-end sequences from the mRNA template before they can be copied by reverse transcriptase. This step prevents 5'-end regions from being translated as GAL4 AD fusions.

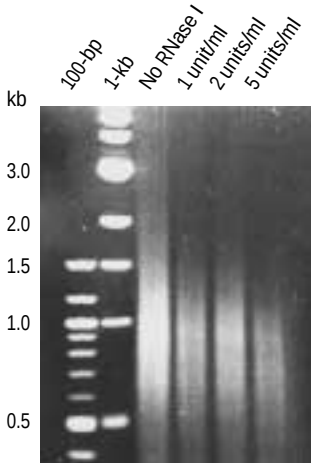

Figure 2. Noncoding 5'-end sequences can be removed from mRNA before two-hybrid library construction. RNase I degrades single-stranded RNA. When it is included in the first-strand cDNA synthesis reaction buffer, the enzyme removes 5'-end nucleotides before they can be copied to cDNA. As shown here, you can control the average cDNA length by changing the RNase I concentration.

These intervening sequences, not normally translated as part of the native protein, may block crucial binding domains, hiding them from interacting partners during the two-hybrid screen. With this modified procedure, you can remove 5'-end sequences by adding RNase I to the first-strand cDNA synthesis reaction (Figure 2).

We've built the MATCHMAKER Library Construction Kit around SMART because of its versatile design and economical protocol. SMART is versatile because it allows you to produce two-hybrid libraries from any tissue using total or poly A^+ RNA; it's economical because it lets you synthesize a library from just 25 ng of poly A^+ RNA. SMART has one other important advantage: it eliminates the need for adaptor ligation.

You don't need adaptors (or unique restriction sites) because cDNA synthesis and amplification are primed by the SMART III™ and CDS III Oligonucleotides. The CDS III oligo primes reverse transcription of poly A^+ RNA; the SMART III oligo attaches to the dC-rich cDNA tail—formed when our PowerScript™ Reverse Transcriptase (RT) reaches the end of the RNA molecule—and serves as an extended template for cDNA synthesis (Figure 1). After RT switches templates from the mRNA to the SMART oligo, a complete cDNA is synthesized with the additional SMART III sequence at the end. Double-stranded cDNA is produced and then amplified by long distance PCR (LD-PCR), the final step in creating a library of double-stranded cDNAs that contain SMART III and CDS III sequence anchors. With these anchors in place, cDNA can be transferred *directly* into the GAL4 AD yeast expression vector, pGADT7-Rec.

Let the yeast clone for you

The SMART double-stranded cDNA can be homologously recombined with pGADT7-Rec *in vivo* in a yeast reporter strain (Figure 1). Simply cotransform yeast with your SMART cDNA and the pGADT7-Rec vector (*Sma* I-linearized). Fast, convenient, and efficient, this one-step cloning procedure is possible because the SMART III and CDS III anchor sequences have also been engineered into the pGADT7-Rec plasmid. In its linear form, pGADT7-Rec is repaired in yeast by recombination with overlapping sequences at the ends of the SMART cDNA (Figure 3). Plasmid repair is manifest as a positive—i.e., as a $Leu2^+$ transformant. Cloning by this method is extremely efficient, yielding a complex collection of distinct clones (Figure 4). In two easy steps, you create a complete two-hybrid library, pretransformed and ready for screening, and all you need to provide is the RNA.

Library construction & screening in less than one week

With the MATCHMAKER Library Construction & Screening Kit, library construction and two-hybrid screening are seamlessly coordinated,

MATCHMAKER Library Construction & Screening Kit...*continued*

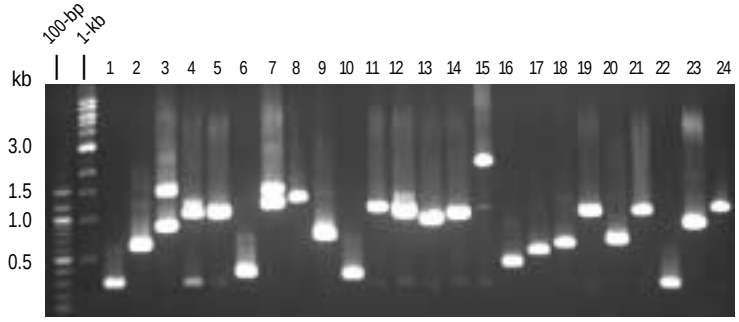

Figure 4. Rapid cloning by homologous recombination is extremely efficient and reliable. After cotransforming yeast with pGADT7-Rec (*Sma* I-linearized) and cDNA (prepared with the SMART method outlined in the text), transformants were selected on SD/–Leu plates. Randomly picked clones were screened for inserts by PCR. In this analysis, 24 out of 24 transformants carried plasmids with cDNA inserts. Note the wide range of insert sizes.

saving you time and effort. In fact, cloning and screening can be carried out in the same host strain on the same day! If you prepare a GAL4 DNA-binding domain (BD) fusion—e.g., pGBKT7/bait—in advance, you can include it in the cotransformation reaction together with your cDNA library and the pGADT7-Rec DNA. With a single transformation step, all three DNA components can be introduced into the yeast reporter strain (Figure 1). As soon as the pGADT7-Rec AD vector is assembled by the host's recombination processes, screening begins—automatically. Positive two-hybrid interactions can be identified immediately after transformation by plating transformants on medium that selects for the GAL4-responsive nutritional reporter gene.

MATCHMAKER vectors facilitate downstream characterization

The System 3 vectors pGADT7-Rec (a derivative of pGADT7) and pGBKT7, supplied with the MATCHMAKER Library Construction & Screening Kit, contain c-Myc and HA epitope tags, bacterial selection markers, and T7 promoters to help expedite the discovery and verification of new interactions. Inclusion of the epitope tags eliminates the need to generate antibodies to new proteins—CLONTECH'S c-Myc and HA antibodies (#3800-1 & #3808-1, respectively) will recognize fusion proteins *in vivo* and *in vitro*. The T7 promoter allows you to transcribe and translate epitope-tagged protein *in vitro* and also serves as a priming site for DNA sequencing.

Construct & screen your library one week, verify interactions the next

After identifying positive two-hybrid interactions using selective medium, researchers frequently evaluate their clones further using a variety of *in vitro* and *in vivo* biochemical assays. Our MATCHMAKER product line supports you in this logical next step. To test putative interactions outside of the yeast environment, try the pCMV-Myc and pCMV-HA Vector Set (#K6003-1), which allows you to confirm protein interactions by coimmunoprecipitation from mammalian cells.

It's easy to see why so many choose MATCHMAKER for their two-hybrid studies: library construction, screening, and analysis—MATCHMAKER covers it all.

For additional information on MATCHMAKER products, visit **matchmaker.clontech.com**

Product	Size	Cat#
MATCHMAKER Library Construction & Screening Kit	each	K1615-1

With every purchase of the MATCHMAKER Library Construction & Screening Kit, you receive a free trial-size Advantage™ 2 PCR Kit (#K1910-y), sufficient for 30 SMART PCR reactions.

Components

- **pGADT7-Rec Cloning Vector** (*Sma* I-linearized)
- **pGBKT7 (DNA-BD) Cloning Vector**
- **SMART III™ Oligonucleotide**
- **CDS III oligo(dT) and random primers**
- **PowerScript™ Reverse Transcriptase**
- **RNase I & RNase H**
- **Control Poly A⁺ RNA Template**
- **5' & 3' cDNA Amplification Primers**
- **First-Strand Buffer**
- **dNTP Mix**
- **CHROMA SPIN™+TE-400 Columns**
- **AH109 & Y187 Competent Yeast Cells**
- **PEG/ LiAc Solution**
- **Yeast Minimal Media Dropout Supplements**
- **pGBKT7-53 Control Vector**
- **pGBKT7-Lam Control Vector**
- **SV40 Large T PCR Fragment**
- **Complete User Manual** (PT3529-1)
- **Vector Information Packets** (PT3530-5 & PT3248-5)
- **Yeast Protocols Handbook** (PT3024-1)

Related Products

- **Advantage™ 2 PCR Kit** (#K1910-1, -y)
- **Advantage™ 2 Polymerase Mix** (#8430-1, -2)
- **SMART™ cDNA Library Construction Kit** (#K1051-1)
- **cDNA Libraries & Poly A⁺ RNA** (many)
- **HA-Tag Polyclonal Antibody** (#3808-1)
- **c-Myc Monoclonal Antibody** (#3800-1)
- **pCMV-Myc & pCMV-HA Vector Set** (#K6003-1)

SpotLight™ Chemiluminescent Kits

Nonradioactive alternative for array and Northern blot hybridizations

- **The only kits optimized for use with Atlas™ cDNA Arrays**
- **Fast—expose for just 10 minutes**
- **Produces signal comparable to radioactive probes in a fraction of the time**
- **Low background results in a stronger, cleaner signal**

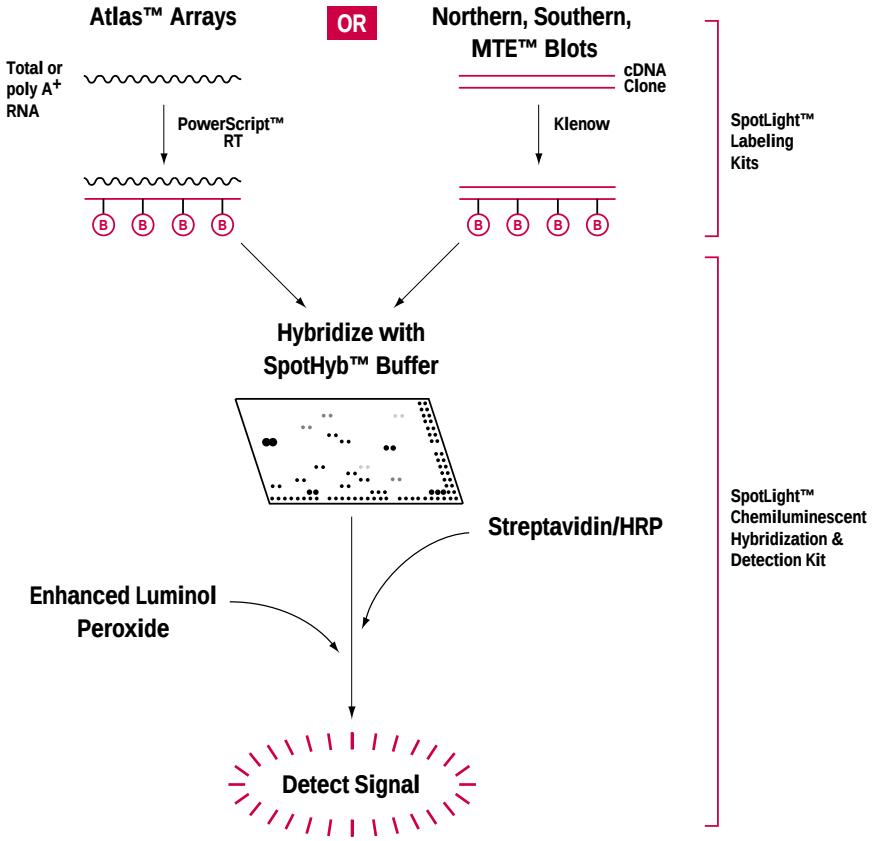

Figure 1. CLONTECH's SpotLight™ Labeling Kits use biotinylated dCTP for direct incorporation of label. After labeling your probe with either our SpotLight Atlas Labeling Kit or SpotLight Random Primer Labeling Kit, perform both hybridization and detection with our SpotLight Chemiluminescent Hybridization & Detection Kit.

CLONTECH's new SpotLight™ Chemiluminescent Kits allow you to perform fast, sensitive screening of nylon membrane-based blots and arrays without the hassles of working with radioactivity. To meet a variety of research needs, we offer two SpotLight probe labeling kits. Combine the appropriate labeling kit with our universal hybridization and detection kit, and you have a complete system for nonradioactive analysis.

The **Atlas™ SpotLight™ Labeling Kit** is ideal for use with our extensive selection of Atlas™ cDNA Arrays. Designed specifically for synthesizing probes from RNA samples, the Atlas SpotLight Labeling Kit is compatible with any nylon membrane-based cDNA array. For Northerns, Southerns, and other nylon membrane hybridization applications, use our **SpotLight™ Random Primer Labeling Kit** to generate probes with the same safe, fast technology. The **SpotLight™ Chemiluminescent Hybridization & Detection Kit** is optimized for use with all of our nylon membrane-based blots and arrays and contains our novel SpotHyb™ Hybridization Buffer, ensuring the highest possible signal-to-noise ratio.

Advanced chemiluminescent technology

CLONTECH's SpotLight Kits combine our extensive experience in gene expression analysis with advanced chemiluminescent technology. SpotLight couples our unique SpotHyb Hybridization Buffer with robust luminescent detection using biotin/streptavidin-HRP (Figure 1). This formulation eliminates problems with low sensitivity and high background that plague other nonradioactive methods. Our SpotHyb Hybridization Buffer is specially formulated to minimize nonspecific

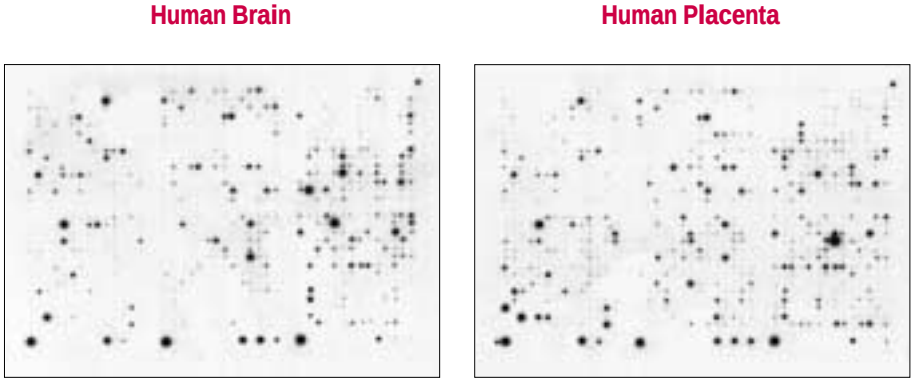

Figure 2. The Atlas™ Human 1.2 Array II (#7852-1) visualized using chemiluminescent detection. Array hybridization was performed with biotinylated cDNA probes prepared from 2 µg of human brain poly A+ RNA or 2 µg of human placenta poly A+ RNA. Hybridization and detection were performed using the SpotLight Hybridization & Detection Kit. Results were visualized by exposing blots to x-ray film for 10 min.

SpotLight™ Chemiluminescent Kits...continued

hybridization. The strong and specific affinity of the HRP-conjugated streptavidin for the biotinylated probe further reduces background problems during detection.

For signal visualization, our HRP/luminol-based chemiluminescent method offers both high sensitivity and flexibility. In practice, other methods that use colorimetric detection allow only one exposure per blot. If the colorimetric reaction is allowed to proceed too far, background becomes saturated and the experiment has to be repeated with a fresh blot. With HRP-mediated chemiluminescent detection, the light signal is fast, strong and remains constant for up to six hours, permitting multiple exposures.

Screen Atlas™ Arrays safely & quickly

The combination of low background and high signal intensity make CLONTECH's SpotLight Kits ideal for use with cDNA arrays (Figure 2). Our Atlas SpotLight Labeling Kit allows you to quickly generate biotin-labeled array probes from either 2 µg of poly A^+ or 50 µg of total RNA using our PowerScript™ Reverse Transcriptase and the gene-specific CDS primer mix included with each Atlas Array. If required, additional CDS primer mix is available separately. Researchers who wish to generate probes for use with other types of cDNA membrane arrays can simply substitute a standard random primer mix.

Development of results is faster than radioactivity—a typical exposure time is only 10 minutes. To quantify results, just scan the developed film and analyze with our AtlasImage™ software. Since biotinylated probes are stable for up to six months, the same probe can be used with multiple arrays to confirm results.

Use SpotLight™ for Northerns, Southerns & dot blots

With CLONTECH's SpotLight Random Primer Labeling Kit, biotin labeling of your clone is as straightforward as conventional ^{32}P labeling, but with safer, simpler handling and cleanup. Starting with a double-stranded cDNA clone representing your gene of interest, the SpotLight Random Primer Labeling Kit uses Klenow enzyme to incorporate biotinylated dCTP, generating highly sensitive, biotin-labeled probes for use with all Northern and Southern blots.

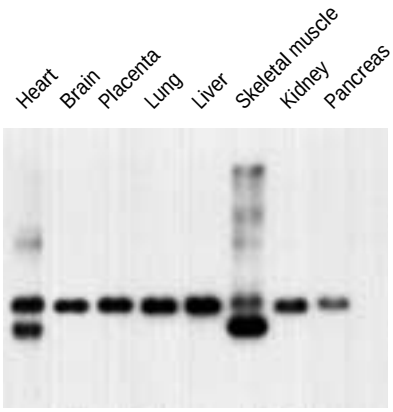

Figure 3. Hybridization of a Human MTN™ Blot (#7760-1) probed with biotinylated human β-actin probe. Human β-actin control probe was labeled with the SpotLight Random Primer Labeling Kit. Signal was visualized with the SpotLight™ Hybridization & Detection Kit and the blot was exposed to x-ray film for 60 sec.

You can use your probes to screen our Human Multiple Tissue Expression (MTE™) Array for the presence and relative abundance of a target mRNA in a broad range of fetal and adult tissues. You can also use your probes with our Multiple Tissue Northern (MTN™) Blots to analyze transcript size and relative abundance (Figure 3). SpotLight biotinylated probes detect even low-abundance transcripts—the lack of background means that you can clearly detect even faint signals. Results are fast—most signals are clearly visible within five minutes and often sooner.

Convenient, complete kit for hybridization and detection

Our SpotLight Chemiluminescent Hybridization & Detection Kit contains all the necessary components for performing hybridization and detection with biotinylated probes. Optimized for use in even the most sensitive applications, our SpotHyb buffer is compatible with all our membrane-based gene expression analysis products including our Atlas cDNA Arrays, and our MTN blots and MTE array.

NEW!

Product	Size	Cat. #
Atlas SpotLight Labeling Kit	16 rxns	K1033-1
SpotLight Random Primer Labeling Kit	10 rxns	K1027-1
SpotLight Chemiluminescent Hybridization & Detection Kit	each	K1032-1

Atlas™ SpotLight™ Labeling Kit Components

- **PowerScript™ Reverse Transcriptase**
- **RT Labeling Mix**
- **RT Labeling Buffer**
- **DTT**
- **Biotin dT_{100} Control**
- **Control RNA**
- **Complete User Manual** (PT3517-1)

SpotLight™ Random Primer Labeling Kit Components

- **Random Primer Mix**
- **Klenow Labeling Mix**
- **Klenow Labeling Buffer**
- **Klenow Enzyme**
- **β-actin Control cDNA**
- **Nuclease-Free Water**
- **Biotin dT_{100} Control**
- **Complete User Manual** (PT3516-1)

SpotLight™ Chemiluminescent Hybridization & Detection Kit Components

- **Stabilized Streptavidin-Horseradish Peroxidase Conjugate**
- **Luminol/Enhancer Solution**
- **SpotHyb™ Buffer**
- **Stable Peroxide Solution**
- **Blocking Buffer**
- **Wash Buffer**
- **Substrate Equilibration Buffer**
- **Complete User Manual** (PT3518-1)

Related Products

- **Atlas™ cDNA Expression Arrays** (many)
- **Multiple Tissue Northern (MTN™) Blots** (many)
- **Human Multiple Tissue Expression (MTE™) Array** (#7775-1)
- **AtlasImage™ 1.5** (#V1211-1)

See page 12 for a complete list of Atlas™ Arrays and accessory products.

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Custom Atlas™ Hybridization & Analysis

Send us your samples—we'll do the rest!

- **Take advantage of our extensive array experience and expertise in RNA isolation**
- **Analyze samples with any of our 29 unique nylon membrane arrays**

With CLONTECH's Custom Atlas™ Analysis Services, you receive the highest quality gene expression results possible, with minimal effort. These unique services not only save you time, but also let you take advantage of our scientists' extensive experience in Atlas Array experimentation and troubleshooting. The following services are now available using any of our 29 unique nylon membrane arrays: **Custom Atlas™ Hybridization & Analysis**, **Custom Atlas SMART™ Probe Amplification** (for analysis when RNA is limited), and **Custom AtlasImage™ Analysis** (Table I).

Table I: Details of Custom Atlas™ Analysis Services

Service	You Provide	We Deliver
Custom Atlas™ Hybridization & Analysis	2 samples: • $>10^7$ cells or • >50 mg tissue	• Results of RNA quality analysis • Phosphorimages of hybridized membranes • AtlasImage software analysis output, indicating differential gene expression
Custom Atlas SMART™ Probe Amplification (must be combined with Custom Hybridization & Analysis Service)	2 samples: • 10^3–10^7 cells or • 100 µg–50 mg tissue	• Same data as above service
Custom AtlasImage™ Analysis	2 matched phosphorimages or scanned autorads (TIFF or GEL format)	• AtlasImage software analysis output indicating differential gene expression.

Custom Atlas™ Hybridization & Analysis

For standard gene expression profiling needs, we recommend Custom Atlas Hybridization & Analysis. Simply provide two samples—either frozen cells ($>10^7$) or tissue (>50 mg) and indicate which Atlas Array to probe. We isolate RNA from your samples, generate ^{33}P-labeled cDNA probes (which produce the highest quality images), perform the hybridization, generate phosphorimages, and perform a thorough analysis using our AtlasImage software (Figure 1). Although we also accept RNA samples for this service, we prefer to purify RNA ourselves due to the sensitive nature of array experiments. After our analysis is complete, we provide you with the results of our RNA quality assessment, phosphorimages of hybridized membranes, and annotated AtlasImage results, indicating differential gene expression. See Figure 1 and the Custom AtlasImage Analysis section below for a further description of the AtlasImage report.

Custom Atlas SMART™ Probe Amplification

If you have less than 10^7 cells or 50 mg of tissue, add Custom Atlas SMART Probe Amplification to the Custom Atlas Hybridization & Analysis Service. The combination of these services enables a reliable analysis of samples from as few as 1,000 cells or 100 µg of tissue (1).

The Atlas SMART amplification procedure employs CLONTECH's widely used PCR-based SMART technology to amplify cDNA samples. This system is the only one available that reliably produces representative amplified probes(1–6). SMART amplification even retains single-copy genes.

Custom AtlasImage™ Analysis

Our AtlasImage analysis software provides a convenient solution for comparing gene expression profiles generated with Atlas Arrays. With our Custom AtlasImage Analysis Service (included with Custom Atlas Hybridization and Analysis, described above), you need only provide matched phosphorimages or scanned autoradiograms in TIFF or GEL format, and we perform the array comparison for you. When we complete our analysis, we provide you with a spreadsheet containing the AtlasImage results. To make the data as accessible as possible, the report is divided into two sets: a list of all genes showing differential expression and a comprehensive list of all array genes. Both lists include the quantified gene spot intensities and a classification of genes as up- or down-regulated with fold changes in expression indicated (Figure 1). Further annotations indicate genes with particularly weak signals that should be carefully scrutinized as well as the parameters used in the analysis. In addition to the spreadsheet report, we provide you with the AtlasImage pseudocolor picture of the array comparison (Figure 1).

Results are guaranteed for all Atlas Custom Services, provided RNA samples pass our quality tests. Results are typically delivered in 4–6 weeks. Atlas Custom Services request form are available at: **www.clontech.com/atlas/custom/**

References

1. Atlas SMART Probe Amplification Kit (July 2000) *CLONTECHniques* **XV**(3):2–3.
2. Livesey, F., *et al.* (2000) *Curr. Biol.* **2000**(10):301–310.
3. Spirin, K., *et al.* (1999) *Invest. Ophthalmol. Vis. Sci.* **40**:3108–3115.
4. Endege, W., *et al.* (1999) *BioTechniques* **26**:542–550.
5. Chenchik, A., *et al.* (1998) In *Gene Cloning and Analysis by RT-PCR*, Eds. Siebert, P. & Larrick, J. (BioTechniques Books, MA), pp. 305–319.
6. Vernon, S. D., *et al.* (2000) *J. Mol. Diagnostics* **2**:124–127.

See page 12 for a complete listing of Atlas™ Arrays.

Custom Atlas™ Hybridization & Analysis...continued

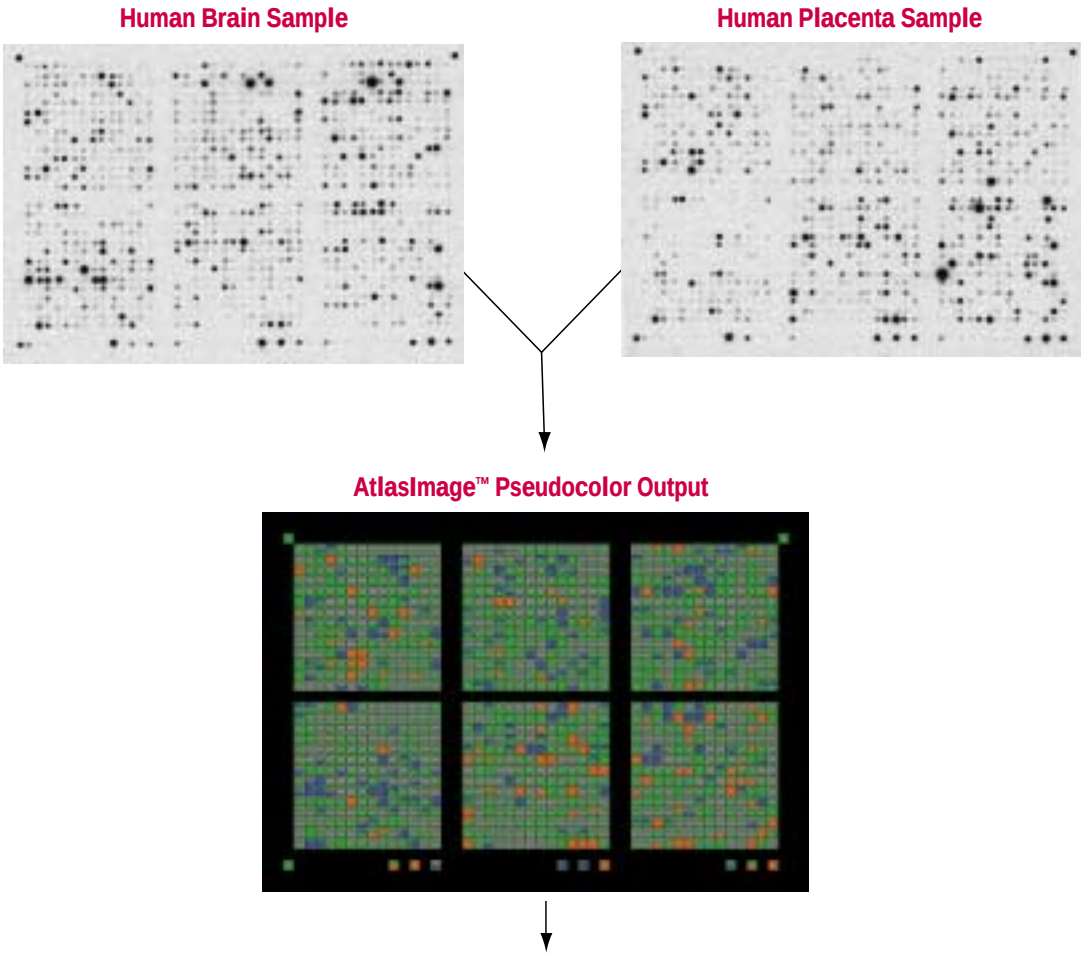

Figure 1. Comprehensive array analysis data supplied with Custom Atlas™ Services. For each Custom Atlas Hybridization & Analysis order, we provide high-quality ^{33}P phosphorimages. The images shown here are for the Atlas Human 1.2 Array (#7850-1). Also included is a pseudocolor picture of the AtlasImage array comparison which indicates up-regulated genes (red) and down-regulated genes (blue). This image depicts changes in expression as determined by both the *ratio* of intensity values between arrays (upper half of boxes) and the *difference* in intensity values (lower half of boxes). Complete AtlasImage data is also provided in a spreadsheet which clearly marks up- and down-regulated genes and indicates other experimental parameters.

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Atlas™ Glass Total RNA Isolation Kit

For isolation of highly pure total RNA

- **Only kit optimized for use with Atlas™ Glass Microarrays**
- **Ultra-pure RNA for accurate results**

The **Atlas™ Glass Total RNA Isolation Kit** yields high-quality total RNA that is virtually free of genomic DNA, nucleases, and other impurities. Specifically tested for compatibility with CLONTECH's Atlas™ Glass Microarrays, this kit produces RNA ready for either fluorescent or ^{33}P labeling. The Atlas Glass Total RNA Isolation Kit utilizes a guanidinium thiocyanate/phenol extraction, ensuring the highest purity of RNA for your experiments.

Total RNA isolated using other commercially available kits often becomes degraded because of residual RNase contamination, especially when working with RNase-rich tissues such as pancreas, spleen, and liver. Samples may also contain genomic DNA impurities, which can cause inaccurate results and high levels of nonspecific background in array hybridization experiments. Only Atlas Glass RNA is reliably free of genomic DNA and protein impurities, including RNases.

Optimized reagents provided

The Atlas Glass Total RNA Isolation Kit includes all the necessary reagents for isolation of high-quality total RNA and a complete User Manual.

Atlas™ Product Ordering Information

Custom Atlas Analysis Services	Size	Cat. #
Custom Atlas Hybridization & Analysis	each	CS2000
Custom Atlas SMART Probe Amplification	each	CS2004
Custom AtlasImage Analysis	each	CS2002

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

Atlas Glass Accessory Products	Size	Cat. #
NEW Atlas Glass Total RNA Isolation Kit	each	K1036-1
Atlas Glass Fluorescent Labeling Kit	10 rxns	K1037-1
Atlas Glass Hybridization Chamber	each	7899-1
DNA-Ready Type I Slides	5 25	7880-1 7880-2
DNA-Ready Type II Slides	5 25	7881-1 7881-2
GlassHyb Hybridization Solution	50 ml	8016-1
Atlas Glass Approved DMSO	500 µl	7898-1

Atlas Human Arrays (# membranes)	Genes per array	Cat. #
1.2 Array (4)	1,176	7850-1
1.2 Array II (4)	1,176	7852-1
1.2 Array III (4)	1,176	7855-1

Atlas Human Arrays (# membranes)	Genes per array	Cat. #
Cancer 1.2 Array (4)	1,176	7851-1
Apoptosis Array (2)	205	7743-1
Cancer cDNA Expression Array (2)	588	7742-1
Cardiovascular Array (2)	588	7734-1
cDNA Expression Array (2)	588	7740-1
Cell Cycle Array (2)	111	7748-1
Cell Interaction Array (2)	265	7746-1
Cytokine/Receptor Array (2)	268	7744-1
Hematology/ Immunology Array (2)	406	7737-1
Neurobiology Array (2)	588	7736-1
Oncogene/Tumor Suppressor Array (2)	190	7745-1
Stress Array (2)	234	7747-1
Toxicology Array II (2)	588	7733-1

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

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

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

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

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

[a] Used with the SMART PCR cDNA Synthesis Kit. (#K1052-1)

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

Creator™ System Overview

Simplify cloning—multiply possibilities

- **Quick, directional cloning into the widest range of expression vectors**
- **Convenient & simple selection scheme**
- **Most efficient recombinase**
- **No commercial license required**

In April, CLONTECH introduced the Creator™ Gene Cloning & Expression System†, marking the beginning of a new approach to gene expression. Now CLONTECH introduces new premade donor vector libraries and complete RevTet, and PROTet Systems, making Creator the most extensive and versatile rapid gene transfer system available.

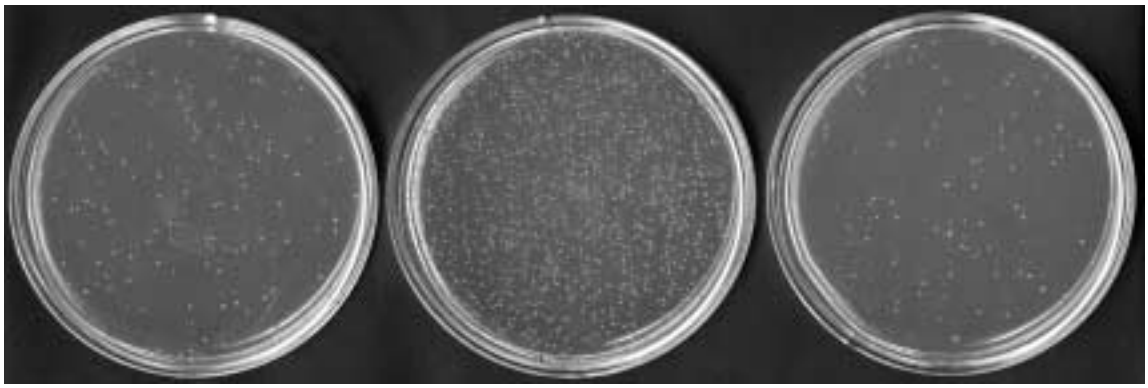
Features of the Creator™ Gene Cloning & Expression System

- Cre-*loxP* recombination reaction takes just 15 minutes
- Only insert is transferred during recombination
- No cofactors or specialized host strains required
- No preference by Cre for supercoiled or linear DNA
- No commercial license required for use
- Mammalian and bacterial inducible expression available
- N-terminal tags compatible and available
- Two-hybrid compatible and available
- Fluorescent tags compatible and available
- IRES and retroviral compatible and available

The Creator Gene Cloning & Expression System offers the best combination of features for rapid functional analysis. The Creator System utilizes Cre-*loxP* recombination to transfer a target gene from a single donor vector into any of a variety of expression acceptor vectors. Unlike other recombination-based cloning systems, Creator transfers only your insert of interest from donor to acceptor vectors. Other systems fuse donor and acceptor plasmids, resulting in bulky constructs that may be too large for certain applications. With the Creator System, even size-sensitive bicistronic and retroviral vectors can be used. Also, unlike other systems, Creator requires no commercial licensing for use—you can pursue your research goals without restriction.

Creator does not require specialized competent cells. You can transform any standard bacterial strain after incubating the donor and acceptor vectors with Cre recombinase. Other systems require specialized host strains, the inclusion of specific cofactors, or both. With our carefully designed selection strategy, you harvest recombinants simply by plating on medium containing chloramphenicol and sucrose. Cells transformed with the parental donor vector or insert-lacking acceptor vector are eliminated by sensitivity to sucrose and chloramphenicol, respectively. Only cells carrying functional, recombinant acceptor vector will survive on the selection medium.

CLONTECH's Cre recombinase has the highest efficiency of any commercially available enzyme, with more than 95% of resulting colonies containing the correct construct. The higher efficiency also results in more colonies per reaction (Figure 1), ensuring that you will get a correct clone every time. CLONTECH's Cre has no preference for linear or supercoiled DNA, so no special DNA treatment is needed for recombination.



No Cre **CLONTECH's Cre** **Competitor's Cre**

Figure 1. CLONTECH's Cre outperfoms the competition. Recombination reactions with donor and acceptor vectors were set up without recombinase, with CLONTECH's recombinase, or with recombinase from a competitor. The reactions were used to transform *E. coli*, which were then plated on selective medium.

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Creator™ SMART™ cDNA Libraries

High-quality, full-length cDNA libraries in a transfer-ready donor vector

- **Generated for the NIH—now available to you!**
- **Save time & resources—transfer clones directly into expression vectors**
- **Made using high-quality RNA & SMART™ technology**

CLONTECH introduces **Creator™ SMART™ cDNA Libraries**—the ultimate resource for gene cloning and expression. Creator SMART cDNA Libraries make characterizing your full-length cDNA clones easy. Once you've isolated clones using standard library screening techniques, transfer them directly into a variety of acceptor expression vectors—with no subcloning (Figure 1). Constructed in our novel donor vector, pDNR-LIB, these libraries are compatible with all of our acceptor expression vectors.

Pretransformed for fast results

Creator SMART cDNA Libraries are pretransformed into a strain of *E. coli* specially prepared to accept plasmids containing large inserts. Libraries may be screened by conventional colony hybridization or by other screening approaches. The pDNR-LIB Vector contains M13 forward and reverse primer sites flanking the MCS, allowing you to quickly screen inserts and eliminate duplicate clones using the supplied primers. For optimal LD-PCR amplification, we recommend our Advantage™ 2 PCR Kit (#K1910-1, -y).

Creator SMART cDNA Libraries can also be screened with our ClonCapture™ cDNA Selection Kit (#K1056-1). Simply grow up cells from an aliquot of the library, prepare supercoiled DNA, and screen. The ClonCapture RecA-mediated enrichment procedure eliminates the need for conventional library screening, saving time and money.

Advanced library construction technology

The Creator SMART cDNA Libraries are generated using our patented SMART technology (1, 2). SMART, which stands for **S**witching **M**echanism **a**t the 5' end of the **R**NA **T**ranscript, ensures high representation of full-length cDNAs. We use the SMART IV™

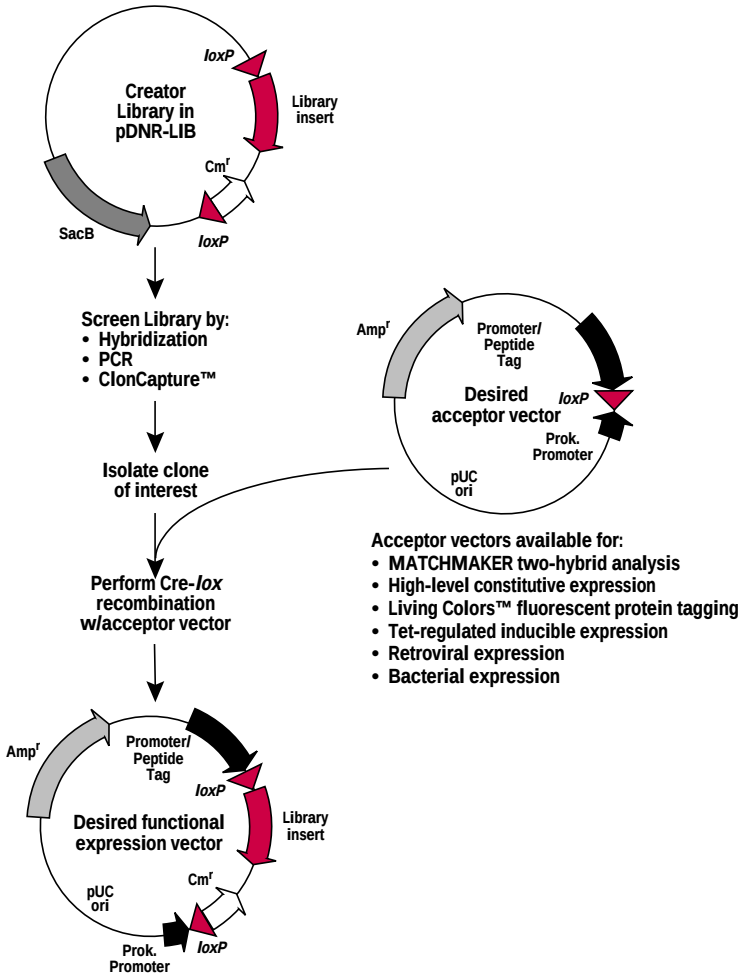

Figure 1. Generate functional expression constructs directly from your library clone.

Libraries developed for the NIH—now available to you!

CLONTECH's Creator Libraries were developed to serve as the foundation for the new Mammalian Gene Collection (MGC) project, a joint effort of the National Institutes of Health (NIH) and the National Cancer Institute (NCI). This project aims to provide researchers with a full set of inexpensive, full-length clones and sequences from humans and other mammalian sources. Using a full-length sequencing pipeline, the project plans to produce 20,000 highly accurate full-length sequences per year. These sequences will be analyzed by a bioinformatics group, and then made available to the research community through GenBank and the MGC website. By providing the biomedical research community with inexpensive, full-length clones in a rapid-cloning format, the MGC project hopes to accelerate the functional analysis of genes identified by mammalian genome projects.

Creator™ SMART™ cDNA Libraries...continued

Oligonucleotide and long-distance PCR (LD-PCR) or primer extension to generate full-length, double-stranded cDNA. After synthesizing and digesting the cDNAs with *Sfi* I, we enrich for large, full-length clones by size fractionation on a low-melting-point agarose gel. Finally, we excise cDNAs greater than 1.0 kb and directionally clone them into our Creator cloning vector, pDNR-LIB (Figure 2).

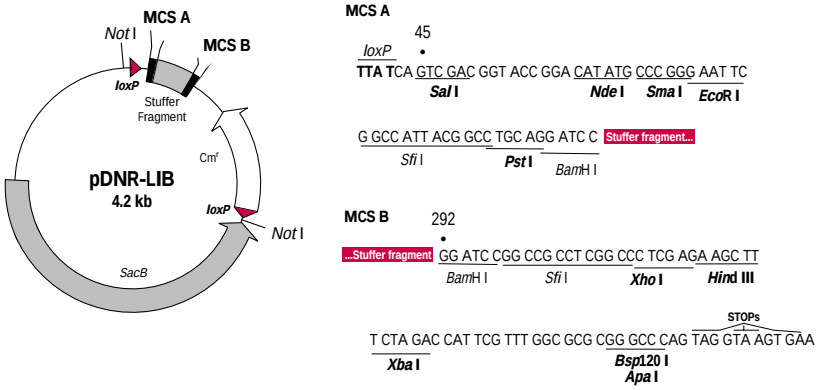

Figure 2. Map and MCS of the Creator Library cloning vector, pDNR-LIB. Unique restriction sites are shown in bold.

Higher number of inserts than conventional libraries

The pDNR-LIB Vector is specially designed to ensure that a high percentage of Creator Library clones contain inserts, typically 93% or greater (Figure 3). The key to this high recombination rate is the carefully designed stuffer fragment. This inserted fragment makes it possible to distinguish between completely digested vector and partially digested vector during gel purification. Only completely digested vector is extracted and used for library construction, ensuring that virtually every clone contains an insert.

Avoid complicated digestion, ligation & selection

The pDNR-LIB donor vector contains two *loxP* sites, which flank the 5' end of the MCS and the 5' end of the open reading frame for the chloramphenicol resistance gene (Cm^r). Creator acceptor vectors contain a single *loxP* site, followed by a bacterial promoter, which drives expression of the chloramphenicol marker after Cre-*loxP*-mediated recombination.

To transfer your gene from the donor vector, pDNR-LIB, into any acceptor vector using the Creator System, simply combine your donor vector clone and an acceptor vector with purified Cre recombinase. Cre binds to the *loxP* sites on both the donor vector and the acceptor vector, cleaves the DNA, and covalently attaches itself to the DNA. Then Cre catalyzes strand exchange and ligation of the DNA so that the gene is transferred from pDNR-LIB into the acceptor expression vector (Figure 1).

Obtaining recombinant expression vectors is simple—just use the recombination reaction to transform any appropriate strain of *E. coli* and plate on medium containing chloramphenicol and sucrose. Cells transformed with the parental donor vector or insert-lacking acceptor vector are eliminated by sensitivity to sucrose and chloramphenicol, respectively. Only cells carrying functional, recombinant acceptor vector will survive on the selection medium. The gene of interest, once transferred, will become linked to the specific expression elements for which the acceptor vector was designed.

Transfer is directional & precise

No sequence is added or lost during the Cre-*loxP* recombination reaction—only the sequence that lies between the two *loxP* sites of the donor vector is transferred to the acceptor vector. This is demonstrated by the results shown in Figure 4. In this experiment, a randomly selected Creator library clone, C5, was transferred to the pLP-CMV-Myc Acceptor Vector. Figure 4A shows an *Sfi* I digestion of the original C5 library clone, pDNR-LIB-C5, and three independent clones of the resulting recombinant acceptor expression vector, pLP-CMV-Myc-C5. Figure 4B shows the same constructs digested with *Not* I. In the donor vectors, pDNR-LIB and pDNR-LIB-C5, the two *Not* I sites lie on either side of the region bounded by the *loxP* sites, whereas the two *Sfi* I sites lie just within the bounded region (see Figure 2; MCS). As shown, digestion of the donor vector library clone, pDNR-LIB-C5, with either enzyme will excise the C5 insert. The parental acceptor vector, pLP-CMV-Myc lacks both *Not* I and *Sfi* I sites—digestion by either enzyme leaves the vector uncut.

Digestion of the recombinant pLP-CMV-Myc-C5 clones with the two restriction enzymes yields different results. Digestion by *Not* I leaves

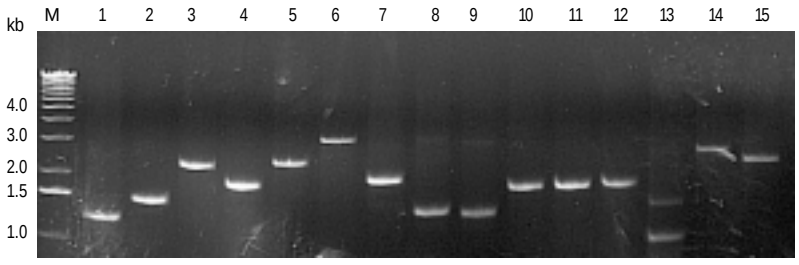

Figure 3. More than 93% of Creator Library clones contain an insert. Fifteen clones were selected randomly from the Human Liver Creator SMART cDNA Library (#HL9502DD) and screened for insert by LD-PCR. Lane M: DNA size markers.

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Creator™ SMART™ cDNA Libraries...continued

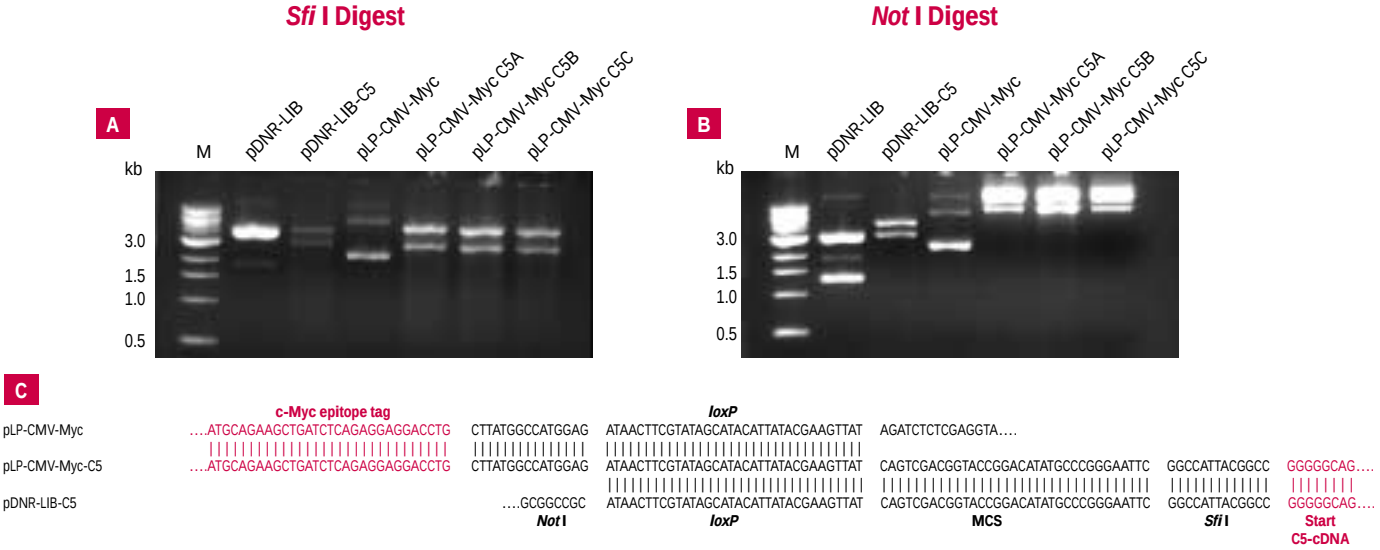

Figure 4. Clones are conserved during recombination and transfer. A randomly selected library clone was isolated and its insert transferred to the pLP-CMV-Myc Acceptor Vector by Cre-*loxP* recombination. The original library clone and three independent acceptor vector clones were digested with either *Sfi* I (**Panel A**) or *Not* I (**Panel B**). Only the sequence between the *loxP* sites in the donor vector is transferred to the acceptor vector, as shown by the transfer of the *Sfi* I sites, but not the *Not* I sites, to the acceptor vector. Also shown are *Sfi* I and *Not* I digestions of insert-lacking pDNR-LIB and pLP-CMV-Myc as controls. **Panel C** shows the precision of the sequence transfer. Lane M: DNA size markers.

the recombinant acceptor vectors uncut, but digestion with *Sfi* I excises the C5 insert. This is because the *Sfi* I sites were transferred to the acceptor vector with the C5 clone. The *Not* I sites, lying outside the *loxP*-bounded region, were not transferred.

The precision of the transfer can also be seen in the sequence alignment of the parental and recombinant constructs (Figure 4C). If the coding sequence for the clone of interest is in frame with the upstream *loxP* site in the donor vector with no intervening stop codons, it will automatically be in frame with any peptide in the acceptor vector. Therefore, you only need to determine the correct reading frame once, and your target gene will always be transferred in the correct reading frame and with proper orientation.

Creator™ Libraries available from a wide range of tissues

Creator SMART cDNA Libraries are currently available from 15 normal and tumor human tissues. We are always expanding our line, so please check **libraries.clontech.com** for a complete listing.

All libraries are provided at a minimum titer of 10^8 cfu/ml; however most libraries have titers of 10^9–10^{11} cfu/ml. This quantity is sufficient for several thousand screenings.

See page 19 for a current list of Creator Libraries and ordering information.

Custom Creator™ Libraries

CLONTECH also offers Custom Creator Library construction services. Starting with RNA, tissue, or cells that you provide, we construct a high-quality cDNA library in our Creator donor vector, pDNR-LIB using our patented SMART™ technology. Using the SMART method, we generate high yields of cDNA with complete 5'-end sequences, providing the greatest chance of identifying full-length clones. Like our premade Creator SMART cDNA Libraries, our Custom Creator Libraries are ready for recombination. Simply screen your library by the method of your choice, and then transfer your clone of interest into any of our many acceptor expression vectors.

The turnaround time for custom libraries is about six weeks if you send poly A^+ as starting material or about eight weeks for total RNA, cells, or tissue. The library price does not include RNA isolation. For poly A^+ or total RNA, please provide a gel photo and OD readings for each sample. Please visit **libraries.clontech.com** or ask your local representative for the custom library ordering form.

New Creator™-Compatible Expression Systems

Wide range of acceptor expression vectors available

- **Creator™—the universal platform for investigating gene function**
- **Make multiple constructs in one day**

In one easy step the Creator™ Cloning System lets you transfer a gene into a wide range of expression vectors, providing swift access to multiple functional studies. You can produce vectors for discovering novel protein interactions, experimenting with tetracycline-regulated and retroviral expression, tagging with fluorescent proteins, and further characterizing your protein of interest—in less than half an hour.

By uniting our wide array of expression products, the Creator System provides access to many strategies for gene function analysis. Our MATCHMAKER Vectors**,** pLP-GBKT7 and pLP-GADT7, enable you to discover novel protein interactions with yeast two-hybrid analysis, while our Living Colors™ Vectors, pLP-EGFP-C1, pLP-ECFP-C1 and pLP-EYFP-C1, allow you to create C-terminal fusions of your gene to a fluorescent protein. With Living Colors, you can track protein localization in live cells and in real time without cofactors or detection kits.

The IRES Bicistronic Expression Vectors, pLP-IRESneo and pLP-IRES2-EGFP, utilize an IRES (internal ribosomal entry site) sequence and a constitutive CMV promoter to produce a bicistronic message for high protein expression in mammalian cells. You can also use retroviral-delivery for constitutive expression of your gene with our pLP-LNCX Vector**.**

For dose-dependent inducible expression of your gene of interest, use pLP-TRE2, which employs the technology of CLONTECH's Tet Systems. Our new **Creator™-Compatible PROTet™ 6xHN System** lets you also perform Tet-regulated expression in bacterial systems. For regulated mammalian expression in a retroviral-based system, our pLP-RevTRE Vector is available separately or as part of our new **Creator™-Compatible RevTet™ Systems**.

Table I: Creator™ Acceptor Vectors

Acceptor Vector	Promoter/Features	Functional Application
MATCHMAKER Vectors		
pLP-GADT7	ADH1/GAL4 activation domain	Express fusions to GAL4 AD to study protein interactions by two-hybrid screening
pLP-GBKT7	ADH1/GAL4 DNA-binding domain	Express fusions to GAL4 DNA-binding domain to study protein interactions by two-hybrid screening
Constitutive Mammalian Expression Vectors		
pLP-CMV-Myc	CMV/Myc tag	Express proteins identified by two-hybrid screening and purify using c-Myc tag
Living Colors™ Vectors		
pLP-EGFP-C1	CMV/C-terminal fusions to EGFP (green)	Express fusion to EGFP (enhanced green fluorescent protein) to study where protein of interest is localized in live cells; no dyes or cofactors required
pLP-ECFP-C1	CMV/C-terminal fusions to ECFP (cyan)	Express fusion to ECFP (enhanced cyan fluorescent protein) to study where protein of interest is localized in live cells; no dyes or cofactors required
pLP-EYFP-C1	CMV/C-terminal fusions to EYFP (yellow)	Express fusion to EYFP (enhanced yellow fluorescent protein) to study where protein of interest is localized in live cells; no dyes or cofactors required
Bicistronic Vectors		
pLP-IRESneo	CMV/IRES, neo selection marker	Constitutive mammalian expression with single transcript for both gene of interest and neo selection marker
pLP-IRES2-EGFP	CMV/IRES, EGFP selection marker	Constitutive mammalian expression with single transcript for both gene of interest and EGFP selection via cell sorting (FACS)
Tetracycline-Regulated Expression Vectors		
pLP-TRE2	Inducible tet-responsive promoter in mammalian expression vector	High-level, regulated mammalian expression
pLP-RevTRE	Inducible tet-responsive promoter in retroviral expression vector with hyg selection	High-level, regulated retroviral expression in mammalian cells
Retroviral Vectors		
pLP-LNCX	CMV in retroviral expression vector with neo selection	Constitutive retroviral expression
Prokaryotic Expression Vectors		
pLP-PROTet-6xHN	Inducible tet-responsive promoter in bacterial expression vector	High-level, regulated bacterial expression

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New Creator™-Compatible Expression Systems...*continued*

Creator™-Compatible PROTet™ 6xHN Bacterial Gene Expression System

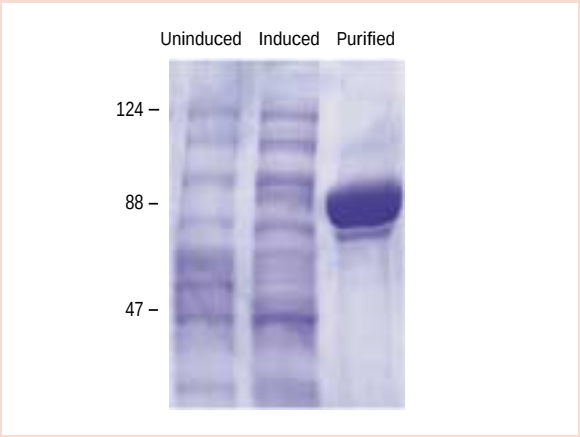


Figure 1. 6xHN-tagged inducible expression from pLP-PROTet-6xHN. The Creator System was used to transfer luciferase into a pPROTet acceptor vector, pLP-PROTet-6xHN. Luciferase expression was induced by the addition of anhydrotetracycline. Tagged protein was purified using TALON Resin. The figure shows analysis of inducible luciferase expression in a representative clone.

The Creator™-Compatible PROTet™6xHN System combines the superb expression features of the PRO™ System and the superior protein purification of TALON™ Metal Affinity Resins with the ease of the Creator Cloning System. Linking PROTet with Creator is a powerful strategy for expressing many proteins in *E.coli* under the tight control of a tet-inducible promoter. PROTet is particularly suited for expressing toxic proteins. The pLP-PROTet-6xHN Vector includes a 6xHN affinity tag to allow easy and efficient protein purification with our TALON Metal Affinity Resins. TALON Resin has an extremely high affinity for 6xHN-tagged proteins and other polyhistidine-tagged proteins, but does not bind nontagged proteins, allowing superior protein purification with fewer washes.

Creator™-Compatible RevTet™ Retroviral Gene Expression Systems

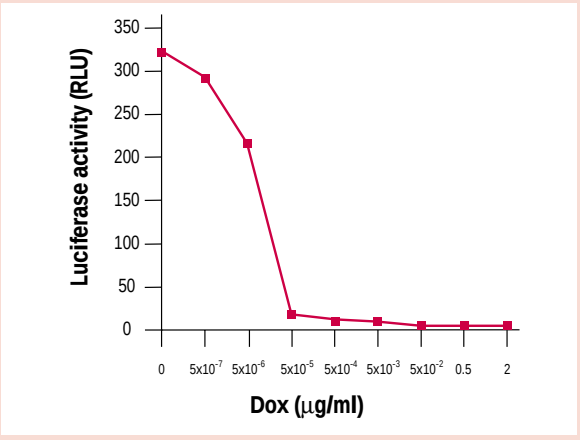


Figure 2. High induction with pLP-RevTRE-Luc. HeLa Tet-Off cells were infected with VSVG-pseudotyped pLP-RevTRE-Luc virus and selected in 200 µg/ml hygromycin for 10 days. Surviving clones were pooled and grown in a range of doxycycline (Dox) concentrations for 48 hr, and then assayed for luciferase activity. RLU=relative light units.

CLONTECH's new Creator™-Compatible RevTet™ Systems add the ease of Creator cloning to the elegant, tightly controlled expression provided by our retroviral RevTet-Off™ and RevTet-On™ Systems. In the new Creator-Compatible Systems, the pRevTRE vector has been replaced by the Creator acceptor vector, pLP-RevTRE, allowing for rapid generation of retroviral expression constructs. As in our conventional RevTet Systems, the RevTet Vectors establish a Tet-regulated expression system more efficiently than plasmid-based methods, especially when dealing with difficult-to-transfect cell types. Regulation of gene expression in the Creator-Compatible RevTet Systems is highly specific and offers a wide range of inducibility. Each Creator-Compatible RevTet System is a complete gene expression system containing retroviral expression vectors and the RetroPack™ PT67 Packaging Cell Line (#K1060-D) for the safe, efficient production of infectious, replication-incompetent retrovirus.

Creator™ Systems Ordering Information

Creator™ SMART™ cDNA Libraries

Human Poly A+ RNA Source	Vector	Size	Cat. #
Human Brain	pDNR-LIB	2 x 1 ml	HL9500DD
Human Heart	pDNR-LIB	2 x 1 ml	HL9501DD
Human Liver	pDNR-LIB	2 x 1 ml	HL9502DD
Human Skeletal Muscle	pDNR-LIB	2 x 1 ml	HL9503DD
Human Testis	pDNR-LIB	2 x 1 ml	HL9504DD
Human Bladder Carcinoma	pDNR-LIB	2 x 1 ml	HL9505DD
Human Acute Mylogenous Leukemia	pDNR-LIB	2 x 1 ml	HL9506DD
Human Chronic Mylogenous Leukemia	pDNR-LIB	2 x 1 ml	HL9507DD
Human Primitive Brain Neuroectodermal	pDNR-LIB	2 x 1 ml	HL9508DD
Human Glioblastoma	pDNR-LIB	2 x 1 ml	HL9509DD
Human Hypernephroma	pDNR-LIB	2 x 1 ml	HL9510DD
Human Lung Mucoepidermoid Carcinoma	pDNR-LIB	2 x 1 ml	HL9511DD
Human Prostate Adenocarcinoma	pDNR-LIB	2 x 1 ml	HL9512DD
Human Testis Embryonal Carcinoma	pDNR-LIB	2 x 1 ml	HL9513DD
Human Melanoma	pDNR-LIB	2 x 1 ml	HL9514DD

Product	Size	Cat. #	
Creator-Compatible PROTet 6xHN Bacterial Expression System	each	K1676-1	NEW!
Creator-Compatible RevTet-Off System	each	K1674-1	NEW!
Creator-Compatible RevTet-On System	each	K1675-1	NEW!
Cre Recombinase	20 rxns	8480-1	
Creator pDNR-1 Cloning Kit	each	K1670-1	
Creator pDNR-2 Cloning Kit	each	K1671-1	
Creator pDNR-3 Cloning Kit	each	K1672-1	
pLP-CMV-Myc Acceptor	20 µg	6351-1	NEW!
pLP-PROTet-6xHN Acceptor	20 µg	6352-1	NEW!
pLP-GADT7 AD Acceptor	20 µg	6349-1	
pLP-GBKT7 DNA-BD Acceptor	20 µg	6350-1	
pLP-EGFP-C1 Acceptor	20 µg	6342-1	
pLP-ECFP-C1 Acceptor	20 µg	6343-1	
pLP-EYFP-C1 Acceptor	20 µg	6341-1	
pLP-IRESneo Acceptor	20 µg	6346-1	
pLP-IRES2-EGFP Acceptor	20 µg	6345-1	
pLP-TRE2 Acceptor	20 µg	6348-1	
pLP-RevTRE Acceptor	20 µg	6347-1	
pLP-LNCX Acceptor	20 µg	6344-1	

Creator™ SMART™ cDNA Library Components

- **Creator™ Library Culture**
- **M13 Forward Primer**
- **M13 Reverse Primer**
- **Vector Information Packet** (PT3508-5)
- **Complete User Manual** (PT3534-1)

Related Products

- **Tet-Off™ Gene Expression System** (#K1620-1)
- **Tet-On™ Gene Expression System** (#K1621-1)
- **Tet-Off™ & Tet-On™ Cell Lines** (many)
- **Retro-X™ System** (#K1060-1)
- **Retroviral Packaging Cell Lines** (many)

References

1. Barnes, W. M. (1994) *Proc. Natl. Acad. Sci. USA* **91**:2216–2220.
2. Chang S., *et al.* (1994) *Proc. Natl. Acad. Sci. USA* **91**:5695–5699.
3. Estojak, J., *et al.* (1995) *Mol. Cell. Biol.* **15**: 5820–5829.
4. Aho, S., *et al.* (1997) *Anal. Biochem.* **253**:270–272.

New Tet Vectors: pTRE2pur & pTRE2hyg

Create a stable Tet-regulated expression system without cotransfection

- **Less work in establishing Tet-inducible gene expression systems**
- **Eliminates need for cotransfection with pTK-Hyg**
- **Two selection options: hygromycin or puromycin**

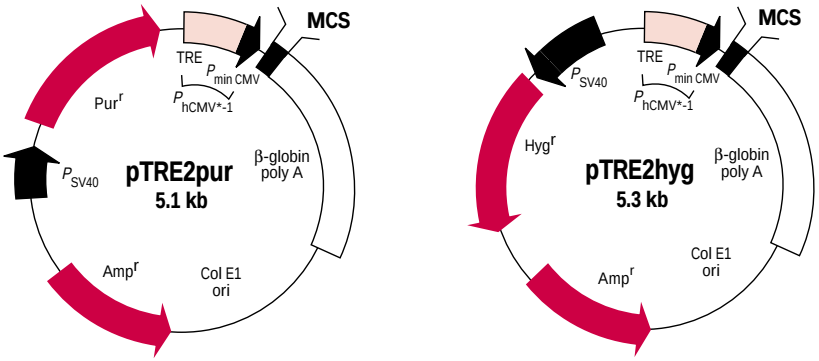


Figure 1. Maps of pTRE2pur and pTRE2hyg Vectors. MCS: *Bam*H I, *Pvu* II, *Mlu* I, *Nhe* I, *Not* I, *Cla* I, *Sal* I, *Acc* I & *Eco*R V.

CLONTECH adds two new response vectors, **pTRE2hyg** and **pTRE2pur,** to our growing line of Tet-Off™ and Tet-On™ System vectors. These two vectors include the selection marker for hygromycin (hyg) or puromycin (pur) in the backbone (Figure 1), allowing you to directly select for cells that have been transfected with your response construct. These vectors reduce the work needed to establish double-stable Tet expression cell lines by producing a higher percentage of resistant clones that carry your gene of interest.

Precise control of gene expression

The Tet-Off and Tet-On Systems provide precise regulation of gene expression that is both reversible and quantitative. Using tetracycline or doxycycline (Tc or Dox), you can control the quantity and timing of target gene expression in a wide variety of cell types. This dynamic response is more than just an on/off switch—it allows you to fine-tune the expression level of a given gene. These features allow the study of proteins whose constitutive expression may alter normal function of the host cell.

The transcription of your gene of interest is controlled by the regulator plasmid, pTet-Off™ or pTet-On™. In the Tet-Off System, the tetracycline-controlled transactivator(tTA) binds the response element (TRE) and activates transcription in the absence of Tc or Dox. In the Tet-On System, the transactivator contains mutations that allow it to bind the TRE in the presence of Dox. With either Tet System, your gene of interest is ready for expression after it is subcloned into a response vector such as pTRE2hyg or pTRE2pur. Following transfection, the dose response offers a wide range of possibilities for studying gene expression in your system.

Simple set-up of Tet-Off™ & Tet-On™ Systems

These complete response vectors can be used with the Tet-Off and Tet-On Systems as well as with premade Tet Cell Lines. Previous protocols required cotransfection of the pTK-Hyg Vector when transfecting cell lines with pTRE response vectors in order to select for transfected cells. The new vectors eliminate effort by allowing you to directly screen for the target construct using only one vector.

A comprehensive vector series

CLONTECH offers a comprehensive set of vectors for use with the Tet-Off and Tet-On Expression Systems (see table, facing page). The regulatory vectors pTet-Off and pTet-On provide the essential regulatory elements for the Tc-regulated expression of your target gene. In addition to pTRE2hyg and pTRE2pur, our family of Tet system response vectors includes the basic pTRE2 Vector. You can also express your gene fused to a destabilized green fluorescent protein (GFP) using pTRE-d2EGFP.

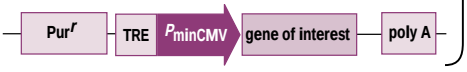
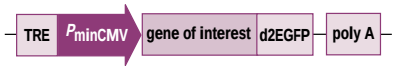
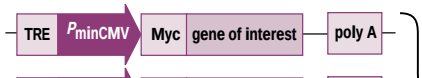
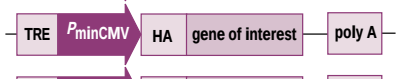
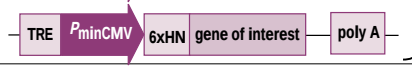
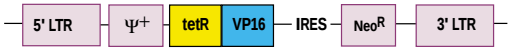
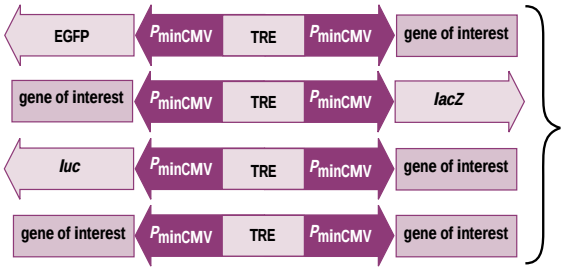
We also offer the pTRE-HA, pTRE-Myc, and pTRE-6xHN response vectors for monitoring your protein by immunohistochemistry. pTRE-6xHN allows you to purify the desired protein using TALON™ Resin or nickel IMAC columns.

The RevTet™ System offers the additional advantage of infecting hard-to-transfect cell lines and quickly establishing stable cell lines. The retroviral RevTet System is also best for use with genes prone to rearrangement and is ideal for establishing transgenic mice. For additional information, please refer to **tet.clontech.com.**

Related Products

- **Tet-Off™ Gene Expression System** (#K1620-1)
- **Tet-On™ Gene Expression System** (#K1621-1)
- **RevTet-Off™ Expression System** (#K1626-1)
- **RevTet-On™ Expression System** (#K1627-1)
- **Doxycycline** (#8643-1)
- **Tet System Approved Fetal Bovine Serum** (#8630-1)

Tet Vector Ordering Information

Name	Diagram	Applications	Cat. #
Basic Vectors			
pTet-Off		Regulator vector for use in Tet-Off System	K1620-A
pTet-On		Regulator vector for use in Tet-On System	K1621-A
pTRE2		Response vectors encoding the Tet Response Element (TRE) for use in either Tet-Off or Tet-On	6241-1
pTRE2hyg			6255-1
pTRE2pur			6254-1
Accessory Vectors			
pTet-tTS		For tighter control of transient gene expression in Tet-On Systems	6248-1
ptTA-2, 3, 4		Minimal domain vectors Can be used with Tet-Off System to minimize VP16 toxicity	K1625-1
pTRE-d2EGFP		Reporter or control vector for either Tet-Off or Tet-On	6242-1
Tagged Vectors			
pTRE-Myc		Response vectors for use in either Tet-Off or Tet-On System	6247-1
pTRE-HA		Used for screening with antibodies or for purification	6249-1
pTRE-6xHN			6246-1
RevTet™ Basic Vectors			
pRevTet-Off		Regulator vector for use in RevTet-Off System	6140-1
pRevTet-On		Regulator vector for use in RevTet-On System	6159-1
pRevTRE		Response vector for use in either RevTet-Off or RevTet-On System	6137-1
RevTet™ Accessory Vectors			
pRevTet-Off-IN		Can be used for quickly establishing a Tet-Off cell line	6134-1
Bidirectional Tet Vectors			
pBI-EGFP		Response vectors for monitoring expression of a target gene via expression of a coregulated reporter or for regulating two genes simultaneously	6154-1
pBI-G			6150-1
pBI-L			6151-1
pBI			6152-1

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Infinity™ Human Mammary Epithelial Cell Line

Telomerase-immortalized normal human breast epithelial cells

- **Extended lifespan**
- **Normal phenotype & genotype**
- **More useful than transformed breast cell lines**

CLONTECH introduces the third member of our Infinity™ family of telomerase-immortalized cell lines: the **hTERT-HME1** human mammary epithelial cell line, which joins hTERT-RPE1 (retinal pigment epithelium; 1) and hTERT-BJ1 (foreskin fibroblast; 2). Infinity Cell Lines stably express human telomerase reverse transcriptase (hTERT), which preserves telomere length and allows the cells to divide indefinitely while retaining normal function and phenotype (3, 4).

Infinity Cell Lines provide a uniform, stable, and perpetual source of untransformed cells for a wide range of cellular studies, including any assays or gene expression studies requiring long-term growth of cells. The unlimited lifespan of these cells also makes them valuable for studies of aging and the progression of cancer and other diseases. hTERT-HME1 cells are particularly useful for sorting out the stages of breast cancer development. With a long-living normal breast cell line, you can observe changes over time, and can introduce genetic alterations without the complications of a transformed cell line.

Opens a new door in breast cancer research

One of the most commonly used cell lines for breast cancer research is MCF7, a breast tumor line. MCF7, while extremely useful for some purposes, is plagued by the problems of a transformed cell line, such as anchorage independence, aneuploidy, and expression of high levels of the estrogen receptor. Because the cells are altered, experimental findings using MCF7 may not accurately reflect normal cell behavior.

Infinity HME1 Cells overcome these limitations, providing both the extended lifespan of transformed cells, and the appearance and behavior of normal primary cells. Infinity Cell Lines take advantage of the discovery that primary cells expressing telomerase become immortalized (3–8). As shown in Figure 1, the primary HME cells are hTERT-negative, whereas hTERT-HME1 cells have telomerase activity similar to that found in transformed cells.

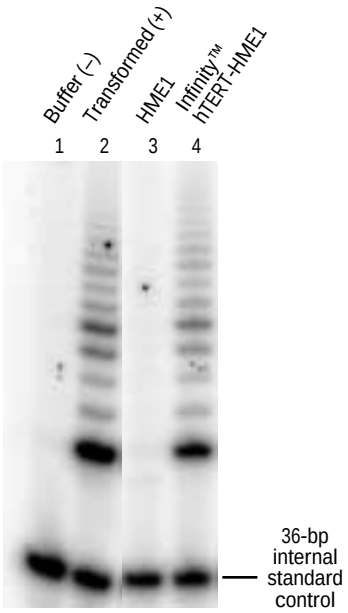

Figure 1. Telomerase activity of hTERT-HME1 cells. hTERT-HME1 cells were analyzed for telomerase activity using TRAP (telomerase repeat amplification protocol). Lane 1: lysis buffer (negative control) Lane 2: MDA-MB-321 (transformed cell line for positive control). Lane 3: HME1 cells. Lane 4: hTERT-HME1 cells. Data provided courtesy of Dr. Jerry Shay; University of Texas Southwestern Medical Center; Dallas, TX.

hTERT expression releases Infinity Cells from senescence, compared to unmodified primary cells, which senesce and die after a few dozen population doublings. In addition, the telomeres of the Infinity Cells' chromosomes have been shown to be maintained or extended in comparison to the parental cells that do not express exogenous telomerase (3, 7).

Table I summarizes the differences between primary breast cells, hTERT-HME1 cells, and MCF7 breast cancer cells. With the addition of hTERT, the doubling time of HME cells is as low as MCF7, but the hTERT cells are estrogen receptor-negative. The hTERT cells have an average telomere length between that of the other two cell lines, retain requirements for defined growth medium, and do not lose anchorage dependence.

The hTERT-HME1 Cell Line is suitable for any cell-based studies, but the cells are particularly useful for understanding the response to and production of growth factors, factors controlling expression of mammary-specific properties, and the progressive events leading to malignancy. hTERT-HME1 cells have the additional advantage of growing in defined, serum-free medium and are therefore more amenable to cellular studies, especially to unravelling the role of particular oncogenes and tumor suppressors in growth control.

Infinity™ Cells look & act like normal cells

CLONTECH extensively tests all Infinity Cell Lines to ensure that, despite continued passaging, they maintain a normal phenotype and are genetically stable. Even after hundreds of population doublings, Infinity cells adhere to tissue culture surfaces, are inhibited by contact and serum deprivation, respond normally to G1 and G2 phase blockers and spindle inhibitors, do not grow in soft agar or form tumors in nude mice, and do not acquire abnormal karyotypes (4–8).

Now you can choose from three types of telomerase-immortalized cell lines—fibroblast and two types of epithelial—to replace primary or transformed cell lines in long-term studies. For biochemical and physiological studies of cell growth, or for creating genetically modified cell lines, Infinity Cell Lines are the ideal choice.

Infinity™ Cells Undergo Rigorous QC Testing

CLONTECH's extensive quality control testing ensures that Infinity Cells...

- Are viable after freezing; ≥ 50% viability after 48 hours in culture
- Maintain a primary cell morphology and function
- Are free of bacteria, fungi, and *Mycoplasma*
- Test negative for HIV, hepatitis B, and hepatitis C
- Are contact inhibited
- Actively express telomerase

New Infinity™ Cell Line...continued

Table I: Comparison of human mammary epithelial cells (HME) ± hTERT to MCF7 breast adenocarcinoma cells

	Primary HME (9)	Infinity™ hTERT-HME1	MCF7
Source	Normal tissue	Normal tissue	Breast adenocarcinoma
Morphology	Epithelial	Epithelial	Epithelial
Karyotype	Normal, diploid	Normal, diploid	Aneuploid
Growth media	Defined	Defined	Serum
Doubling time (hours)	56	39	39
Estrogen receptor	Negative	Negative	Positive
p16	Negative	Negative	Negative
p53	+/+	+/+	+/+
Telomerase activity	Negative	Positive	Positive
Average telomere length (kb)	4.5	5.6	7.0
Anchorage-independent growth	No	No	Yes

Product	Size	Cat. #
hTERT-HME1 Cell Line	1 ml	C4002-1
hTERT-BJ1 Cell Line	1 ml	C4001-1
hTERT-RPE1 Cell Line	1 ml	C4000-1

References

1. Telomerase-Immortalized hTERT-RPE1 Cell Line (July 1999) *CLONTECHniques* **XIV**(3):2–3.
2. Infinity Telomerase-Immortalized Cell Lines (January 2000) *CLONTECHniques* **XV**(1):12–13.
3. Bodnar, A. G., *et al.* (1998) *Science* **279**:349–352.
4. Jiang, X. R., *et al.* (1999) *Nature Genet.* **21**:111–114.
5. Vaziri, H. & Benchimol, S. (1998) *Curr. Biol.* **8**: 279–282.
6. Wang, J., *et al.* (1998) *Genes Devel.* **12**:1769–1774.
7. Yang, J., *et al.* (1999) *J. Biol. Chem.* **274**:26141–26148.
8. Morales, C. P., *et al.* (1999) *Nature Genet.* **21**:115–118.
9. Shay, S. W., *et al.* (1993) *Oncogene* **8**:1407–1413.

Notice to Purchaser

Telomerase-immortalized cell lines are provided under at least one of the following: UK Patent GB 2 317 891 B, Swiss Patent 689 672, and Published PCT Applications WO98/14592 and WO98/14593. Additional patent rights are pending worldwide. These cells are sold to purchase under a restricted use license.

A license is required for users at commercial companies. For more information, please contact the Geron Corporation, Attention: Director, Corporate Development, at (650) 473-7734, Fax (650) 473-7701, or e-mail to corpdev@geron.com.

Employment Opportunities

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

Our continued success has created the following opportunities:

- **Research Scientist I/II/III**
- **Production Staff**
- **Sales Territory Manager**
- **MarCom Writer/Editor**
- **Technical Writer**

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Living Colors™ Endosome Localization Vectors

See endosomes labeled with green, cyan, or yellow fluorescent protein

- **Watch endosomal vesicle trafficking in living cells in real time**
- **Monitor endocytosis of labeled receptors by co-localization**
- **Combine with other subcellular localization vectors for dual or triple labeling**

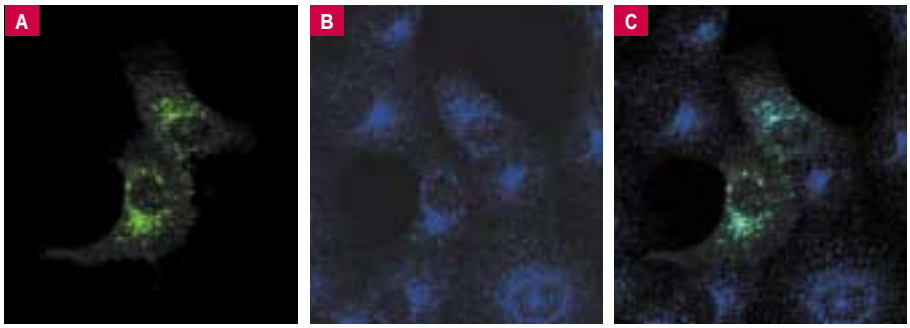

Figure 1. The Living Colors™ Endo Vectors express fluorescent proteins targeted specifically to endosomes. Vero cells were transiently transfected with pEGFP-Endo, then fixed and immunostained for the early endosomal marker EEA1 using Cy5-conjugated secondary antibody. **Panel A** shows the EGFP fluorescence, **Panel B** shows EEA1 immunostaining, **Panel C** is an overlay of images A and B, showing the co-localization of RhoB-EGFP and EEA1, which indicates the localization of RhoB-EGFP to endosomes. Micrographs courtesy of Dr. Harry Mellor, Department of Biochemistry, University of Bristol, UK.

CLONTECH introduces three new Living Colors™ Subcellular Localization Vectors that label endosomes with enhanced green fluorescent protein (EGFP), enhanced cyan fluorescent protein (ECFP), or enhanced yellow fluorescent protein (EYFP). **pEGFP-Endo**, **pECFP-Endo**, and **pEYFP-Endo** express fluorescent proteins fused to the endosome-localized protein RhoB. These vectors allow you to observe movement of the vesicles of the endocytic pathway in living cells by fluorescence microscopy (Figure 1)—either through time-lapse photography or by watching in real time. Visit **www.bch.bris.ac.uk/staff/Mellor/docs/protocols/gfp.html** to see vesicle movements in time lapse (along with a detailed protocol on how to use the marker).

By using these vectors to visualize endosomes, you can monitor the internalization of labeled receptors or ligands by co-localization with the RhoB-fluorescent protein fusion. Additionally, they can be used to see endocytic vesicles in fixed cells. These new vectors can be combined with other subcellular localization vectors (Figure 2; Table I) for dual or triple labeling studies.

RhoB is entirely localized to both early and late endosomes, which makes it a useful marker of the lifespan of these cellular compartments. Figure 1 shows the specificity of the colocalization of EGFP-RhoB with another early endosomal marker, EEA1.

Quick and easy visualization

These vectors work in a wide range of mammalian cell types. Simply transfect the vectors into cells using standard transfection techniques and proceed with your experiment under the microscope. Because RhoB is toxic to cells over time, we recommend using the cells for experiments within 24 hours of transient transfection.

At low to moderate levels of expression, the RhoB-EGFP fusion protein does not have any detectable effect on endosomal trafficking. Endocytic vesicles move on microtubule tracks, and endosomal motion can clearly be seen in the cell with real-time microscopic analysis. For photographing the endosomes, fast shutter speeds (~200 msec) are required to prevent blurring of the images.

These endosome localization vectors also contain a c-Myc epitope. The addition of c-Myc allows you to identify the fusion protein using our c-Myc antibodies (#3800-1 & #3801-1) independently of fluorescence in fixed cells.

Clarify the role of endosomes

RhoB—a GTPase that is a member of the Ras superfamily—has been shown to be localized to early endosomes, recycling endosomes, and multivesicular bodies, but not to mature lysosomes (1).

RhoB regulates receptor traffic through activation of PRK kinases. The RhoB-EGFP fusion protein does not bind PRK completely, which makes it useful for imaging endocytic traffic at low levels of expression. Because of the decreased binding of PRK, we do not recommend using these vectors for studying RhoB function specifically.

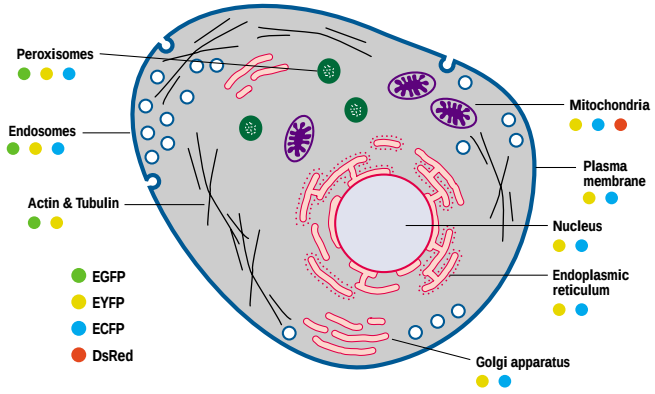

Figure 2. Organelles targeted by Living Colors™ Subcellular Localization Vectors.

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Living Colors™ Vectors...continued

An extensive line of localization vectors

Table I shows our extensive line of subcellular localization vectors for labeling cellular structures with vivid colors. By using multiple vectors labeled with different color variants, you can monitor the location of subcellular structures relative to each other or relative to fluorescently labeled proteins. Also, targeting a fluorescent protein to a specific organelle allows you to mark the structure for destruction using laser ablation microsurgery—providing insights into the function of a given subcellular structure (2, 3).

References

1. Adamson, P., *et al.* (1992) *J. Cell Biol.* **119**:617–627.
2. Khodjakov, A., *et al.* (1997) *Cell Motil. Cytoskeleton* **38**:311–317.
3. Living Colors Subcellular Localization Vectors (April 2000) *CLONTECHniques* **XV**(2):24–25.

Table I: Living Colors™ subcellular localization vectors

Targeted subcellular structure	Color variants available	Localization tag or gene	Potential applications
Endosomes	Green, cyan, yellow	RhoB	• Observe movement of vesicles of endocytic pathway • Monitor endocytosis of labeled receptors or ligands
Mitochondria	Cyan, yellow, red	Targeting sequence from subunit VIII of cytochrome c oxidase	• Study normal & disease state • Track mitochondrial dynamics
Nucleus	Cyan, yellow	SV40 T-antigen NLS*; 3 tandem repeats	• Study nuclear import • Track cell lineage • Monitor cell growth & division
Endoplasmic reticulum	Cyan, yellow	Targeting sequence of calreticulin; KDEL retrieval sequence	• Visualize tubules & cisternae • Track morphology & intracellular distribution
Golgi apparatus	Cyan, yellow	Targeting sequence from human β 1; 4-galactosyltransferase	• Study organelle dynamics • Track morphology & intracellular distribution
Plasma membrane	Green, cyan, yellow	Palmitoylation domain of neuromodulin; farnesylation sequence from Ha-Ras (pEGFP-F)	• Study membrane dynamics & protrusions • Monitor membrane-associated changes during apoptosis
Peroxisome	Green, cyan, yellow	Peroxisomal targeting signal 1 (PST1)	• Monitor movement, segregation, biogenesis & degradation • Study peroxisome purification
Actin filaments	Green, yellow	Human β-actin	• Study cytoskeletal dynamics • Monitor co-localization with associated proteins or organelles
Microtubules	Green, yellow	Human α-tubulin	• Study cytoskeletal dynamics • Monitor co-localization with associated proteins or organelles

* nuclear localization signal

Product	Size	Cat. #
pECFP-Endo Vector	20 µg	6934-1
pEGFP-Endo Vector	20 µg	6935-1
pEYFP-Endo Vector	20 µg	6936-1
pDsRed1-Mito Vector	20 µg	6928-1
pECFP-Mito Vector	20 µg	6903-1
pEYFP-Mito Vector	20 µg	6115-1
pECFP-Nuc Vector	20 µg	6904-1
pEYFP-Nuc Vector	20 µg	6905-1
pECFP-ER Vector	20 µg	6907-1
pEYFP-ER Vector	20 µg	6906-1
pEGFP-F Vector	20 µg	6074-1
pECFP-Golgi Vector	20 µg	6908-1
pEYFP-Golgi Vector	20 µg	6909-1
pECFP-Mem Vector	20 µg	6918-1
pEYFP-Mem Vector	20 µg	6917-1
pECFP-Peroxi Vector	20 µg	6931-1
pEGFP-Peroxi Vector	20 µg	6932-1
pEYFP-Peroxi Vector	20 µg	6933-1
pEGFP-Actin Vector	20 µg	6116-1
pEYFP-Actin Vector	20 µg	6902-1
pEGFP-Tub Vector	20 µg	6117-1
pEYFP-Tub Vector	20 µg	6118-1

For Profit Entities:

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