CLONTECHniques

NEW PRODUCTS AND APPLICATIONS

October 2001
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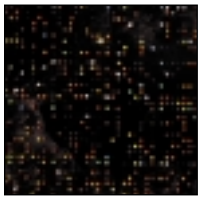

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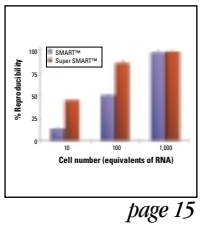

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ABOUT THE COVER

The cover shows an illustration designed for our Atlas™ Glass. The illustration was inspired by the work of computer artist Rick Berry.

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CLONTECHniques

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Atlas™ Glass Mouse 3.8 I Microarray

Now with 3,800 mouse genes

- **Superior signal strength and specificity**
- **Optimized for high-throughput—hybridize and wash up to 20 slides simultaneously**

The **Atlas™ Glass Mouse 3.8 I Microarray** is the newest addition to our Atlas Arrays collection. This array consists of 3,800 well-characterized mouse genes printed on our DNA-Ready™ Type II slides (#7881-1, -2), which are optimized for high-efficiency nucleic acid binding while minimizing spot-to-spot leakage (Figure 1). Each mouse gene is represented by a quality tested 80-base oligonucleotide, which combines the high hybridization efficiency of a cDNA fragment with a short oligo's ability to distinguish homologous genes.

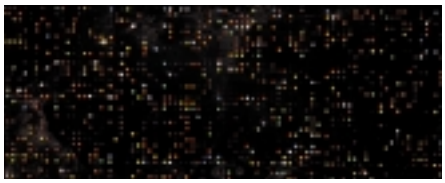

Figure 1. A section of Atlas™ Glass Mouse 3.8 I Microarray hybridized with Cy3-labeled probe generated with 10 µg of Mouse Brain Total RNA (#64024-1). Pseudocolors reflect relative hybridization intensity.

Hybridize 20 slides at one time with the DISCOVERY System from Ventana

Perform automated, high-throughput microarray hybridizations using the DISCOVERY System from Ventana (Figure 2). This revolutionary system minimizes some of the inconsistent and labor-intensive procedural steps, greatly increasing the reproducibility and reliability of the hybridizations. The ChipMap Kit reagents are optimized to work with Atlas Arrays, providing researchers with exceptional confidence in their microarrays. The superior content of our Atlas Microarrays in combination with the high-throughput nature of the DISCOVERY System will allow collection of the highest quality gene expression data in the shortest time.

Figure 2. The Ventana DISCOVERY system with optimized reagents for use with our Atlas™ Glass Microarrays is capable of hybridizing up to 20 slides at one time. For more information on the DISCOVERY System, visit **www.ventanadiscovery.com**.

Atlas™ Plastic Mouse 5K Microarray

Access more than 5,000 mouse genes on a single array

- **Revolutionary surface combines the benefits of both macro- & microarrays**
- **Contains Calibration Standards for direct comparison across lots**
- **Reusable & no special equipment required**

Now you can obtain expression data from all currently named, well-characterized mouse genes on our revolutionary plastic support with the **Atlas™ Plastic Mouse 5K Microarray**. Like glass, these plastic micro-arrays have the benefit of low nonspecific background and ease of handling. Like nylon, plastic can be stripped and reprobed, and requires no special equipment for imaging, just a standard phosphorimager or x-ray film. The plastic surface also allows high-density printing, since each spot is uniform and discrete. All these factors add up to easy, accurate analysis for expression of more than 5,000 mouse genes (Figure 1).

Figure 1. More than 99 percent of all genes printed on the Atlas™ Plastic Mouse 5K Microarray are guaranteed to produce a strong, specific signal. The array was hybridized using a mixture of 5,002 ^{33}P-labeled antisense oligonucleotides corresponding to all printed genes. A portion of the array is shown here.

Product (# genes)	Arrays	Cat. #
Atlas Glass Mouse 3.8 I Microarray (3,800)	2	7907-1
Atlas Plastic Mouse 5K Microarray (5,000)	2	7906-1

Please see Atlas™ legal statement on page 4.

For a complete listing of human, mouse, and rat Atlas™ Arrays and accessory products, visit the Atlas™ Products home page at **www.clontech.com**.

Confirmation of Differentially Expressed Genes in Diabetic Muscle Found Using Atlas™ Plastic Microarrays

Ildana Valisheva & Alexander Munishkin, Ph.D.
Gene Cloning and Analysis Group
BD Biosciences Clontech

We used the Atlas™ Plastic Human 8K Microarray to compare gene expression between normal and diabetic skeletal muscles. We detected more than 300 up-regulated genes and more than 200 down-regulated genes in diabetic tissue. To validate this differential expression, we performed semi-quantitative RT-PCR on 50 randomly selected genes and confirmed the differential expression for 90 percent of the genes with 3-fold differences.

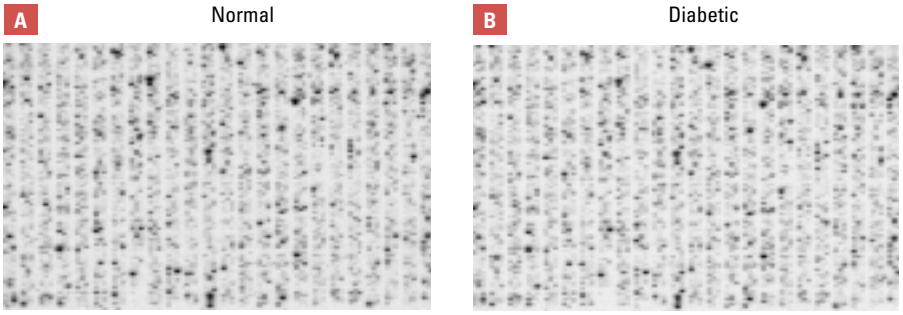

Figure 1. Expression profiling of normal and diabetic human skeletal muscle using Atlas™ Plastic Human 8K Microarrays. Total RNA (10 µg) from normal (**Panel A**) and diabetic (**Panel B**) human skeletal muscle tissues were isolated and labeled with ^{33}P using the Atlas Pure Total RNA Labeling System (#K1038-1). The probes were hybridized to separate Atlas Plastic Arrays according to the User Manual. The Plastic Arrays were washed using a high salt buffer (2X SSC, 0.1% SDS) then by a low salt buffer (0.1X SSC, 0.1% SDS) at 58°C for 10 minutes, and followed by a room temperature rinse using 0.1X SSC. The hybridized arrays were exposed to Fuji ^{33}P screens for 5 days and scanned on the Storm 860 Phosphorimager from Molecular Dynamics at 50-micron resolution. We analyzed the images using AtlasImage 2.01 and the global sum normalization method.

Previously, nylon membranes and glass slides were the only formats available for gene expression profiling. While radioactive detection combined with nylon membranes is the most sensitive and well-established method of expression profiling, limitations in signal resolution restrict the maximal printing density. As a result, it is not feasible to print more than a few thousand genes on a standard nylon membrane. Glass slides using fluorescent detection provide the resolution necessary to print at a far greater density; however, the use of these slides requires specialized reagents and equipment that are not commonly found in molecular biology laboratories. Atlas™ Plastic Microarrays combine the benefits of conventional hybridization and detection techniques with high gene density on a plastic format.

Like glass slides, the plastic format is nonporous, which decreases nonspecific binding and results in a clean background with little washing. While the plastic format can be stripped and reprobed several times like the nylon membranes, the rigid plastic maintains its original configuration, thus reducing the time required to align the grid for image analysis. AtlasImage™ 2.01, with its auto-alignment features, makes image analysis easy with Plastic Arrays.

Profile 8,300 human genes

We hybridized ^{33}P-labeled total RNA from normal and diabetic muscles to duplicate Atlas Plastic 8K Human Microarrays (Figure 1) and analyzed the phosphorimages using AtlasImage 2.01. Of the 8,300 genes surveyed, we found that more than 500 genes were differentially regulated more than 2 fold between the two samples. Diabetic tissues had 359 up-regulated and 210 down-regulated genes (Table I).

RT-PCR confirmation of differential expression

We used RT-PCR to confirm the profiling results obtained using the plastic format. We randomly selected 50 differentially expressed genes and analyzed their expression using RT-PCR. Of the genes that exhibited signal intensity differences of 3 fold or greater, we confirmed 92 percent by RT-PCR. Limitations of the semi-quantitative RT-PCR method could not allow us to reliably confirm differential expression of genes with signal intensity ratios of 2 or less. We found that 83 percent of the differentially expressed genes with signal intensity ratios ranging from 1–1.5 fold did not demonstrate differential expression using RT-PCR. Table I displays the results for 14 of the 50 genes with expression differences ranging from 1.6–95 fold.

Table I: Partial list of differentially expressed genes

Down-regulated genes in diabetic skeletal muscle	GenBank Acc.#	Fold Difference	Confirmed
carbonic anhydrase III, muscle specific	NM_005181	23	+
ATPase, Ca^{++} transporting, plasma membrane 2	L20977	2	+
calpain 3, (p94)	NM_000070	2	+
Up-regulated genes in diabetic skeletal muscle	**GenBank Acc.#**	**Fold Difference**	**Confirmed**
colipase, pancreatic	NM_001832	95	+
DnaJ (Hsp40) homolog, subfamily B, member 1	D49547	93	+
unactivated progesterone receptor, 23 kD	NM_006601	21.5	+
heat shock 70 kD protein 1A	M11717	19.5	+
heat shock 90 kD protein 1, alpha	X07270	15.2	+
dynein, cytoplasmic, light polypeptide	NM_003746	11.4	+
stress-induced-phosphoprotein 1 (Hsp70/Hsp90-organizin)	NM_006819	7.8	+
X-box binding protein 1	NM_005080	5.8	+
prothymosin, alpha (gene sequence 28)	NM26708	3.67	+
pM5 protein	NM_014287	2.84	+
proteasome (prosome, macropain) subunit, beta type, 7	NM_002799	1.6	+

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Confirmation of Differentially Expressed Genes in Diabetic Muscle Found Using Atlas™ Plastic Microarrays

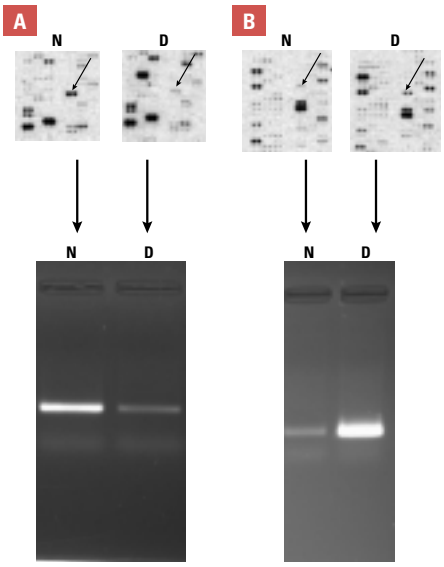

Figure 2. RT-PCR analysis and the corresponding array picture for two genes listed in Table I. We performed RT-PCR using the Advantage™ RT-for-PCR Kit (#K1402-1) and Advantage™ 2 PCR Kit (#K1910-y) on 50 randomly selected genes that showed differential expression using the Atlas Plastic Human 8K Microarray. We used the RT-PCR protocol in the Custom Atlas Primers User Manual (#PT3270-1), but substituted random N15 primers for the oligo(dT) primers in the first strand synthesis steps. We removed aliquots of PCR products after every 2 cycles and electrophoresed on an agarose gel. **Panel A.** The carbonic anhydrase III (CAIII) gene is down regulated in diabetic muscle. **Panel B.** The heat shock protein *hsp86* gene is up regulated in diabetic muscle. D=Diabetic, N=Normal.

Figure 2 shows RT-PCR results and expression array data for two of these up-regulated genes, carbonic anhydrase and the heat shock protein *hsp86*. In diabetic muscle tissue, carbonic anhydrase III (CAIII) was down regulated. Carbonic anhydrases are a class of metalloenzymes that catalyze the reversible hydration of carbon dioxide. The expression of the CAIII gene is strictly tissue specific, CAIII is present at high levels in skeletal muscle and at much lower levels in cardiac and smooth muscle. The *hsp86* gene was observed to be up regulated in the diabetic muscle tissue. Currently, the biological significance of these differentially expressed genes and the other genes listed in Table I is unknown.

In conclusion, RT-PCR validated the expression results we obtained using Atlas Plastic Human 8K Microarray and demonstrated that the plastic microarray format provides accurate, semi-quantitative expression results. The Plastic Arrays combine affordable hybridization techniques with high gene densities for reliable gene expression profiling.

Product	Size	Cat. #
Advantage 2 PCR Kit	30 rxns	K1910-y
AtlasImage 2.01	CD-ROM	V1213-1
Atlas Plastic Human 8K Microarray	2 arrays	7905-1
Atlas Pure Total RNA Labeling System	each	K1038-1
Advantage RT-for PCR Kit	25 rxns	K1402-1

Print you own Plastic Arrays!

Now you can print onto the same plastic that we use for our Atlas™ Plastic Human and Mouse Microarrays. The Atlas Plastic Printing Kit (#K1846-1) contains 10 plastic films (8 x 12 cm), 2 ml of 8X printing buffer, and a User Manual. Plastic is the ideal surface for printing long oligonucleotides or RNA when the detection method is radioactivity or chemiluminescence. BD Biosciences Clontech tested dozens of plastics and buffers before assembling this kit—now you can benefit from our experience.

Creator™ System Overview

The fastest path to high-throughput functional analysis

- **Quickly transfer your gene of interest into any expression vector**
- **Useful with large inserts or genes, and plasmids**
- **Virtually no background**
- **And...no commercial licensing fees**

The simplicity of the Creator System lies in the Cre-*lox*P recombinase which rapidly transfers your gene of interest into multiple expression vectors, allowing you to move quickly from gene discovery to functional studies. With Creator, it is possible to generate dozens of recombinant plasmids in one day, thus enabling high-throughput functional studies or validation of drug targets. Because it eliminates the need for subcloning, the Creator System makes it possible for you to analyze the expression of multiple genes simultaneously.

Our system uses highly efficient Cre-*loxP* recombination to shuttle your gene between a specialized Donor Vector and any number of expression Acceptor Vectors tailored to fit our specific assay or expression requirements (1). This system does not require restriction enzymes or ligation steps to generate the appropriate recombinant expression constructs. Any gene cloned into a Donor Vector is easily transferred *in vitro* into any Creator Acceptor Vector using our Cre Recombinase. The reaction takes only 15 minutes, and is both precise and directional; thus ensuring your gene retains both orientation and reading frame. Unlike the Echo Cloning System from Invitrogen, only the insert-containing DNA fragment located between the two-*loxP* sites in the Donor Vector is transferred into the Acceptor Vector, so your recombinant expression constructs are never excessively large. In addition, our system typically shows little or no background, so it is highly amenable to high-throughput production of expression vectors for large-scale screening experiments.

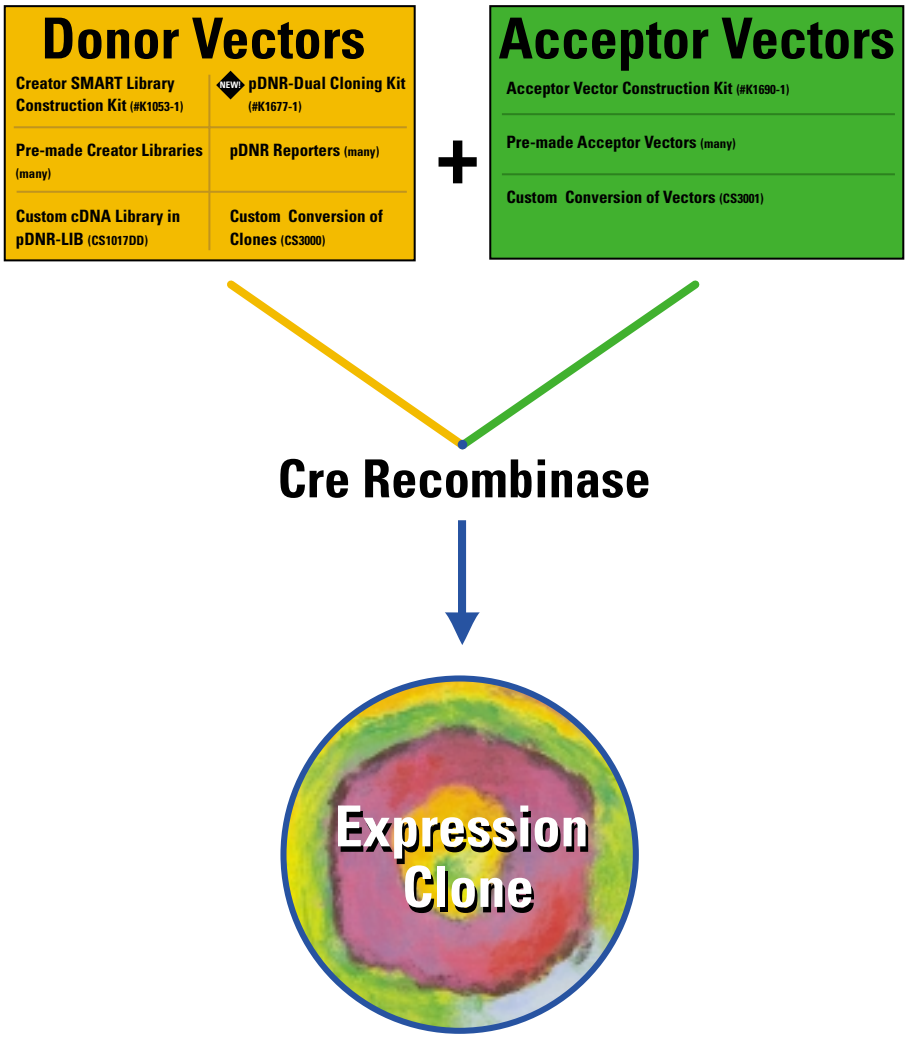

Figure 1. Overview of the Creator™ System. Combine your insert-containing Donor Vector with any Acceptor Vector and Cre Recombinase to generate the Expression Clone of your choice in only 15 minutes.

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Creator™ System Overview...continued

Table I: Creator™ Quick Reference

pDNR Cloning Kit now includes	• Improved backbone gives little or no background • Improved buffer increases transformation efficiency in electrocompetent cells
pDNR-1r Donor Vector	• Makes expression constructs with 5′ tags to your gene of interest
pDNR-Dual Cloning Kit	• Makes expression constructs with 5′ or 3′ tags to your gene of interest
Pre-made Acceptor Vectors	• Contain assay-specific vectors for yeast two-hybrid analysis, tet-inducible protein expression, retroviral expression, bacterial expression, IRES and fluorescent protein expression
Acceptor Vector Construction Kit	• 161-bp cassette converts your expression vector into a Creator Acceptor Vector (compatible with 5′ tags only)
Custom Access Services	
Conversion of Clones	• Our Creator experts make your clones compatible with the Creator System
Conversion of Vectors	• Our Creator experts make your vectors compatible with the Creator System

Analyze your gene using any functional assay

We have a wide variety of Acceptor Vectors that let you generate recombinant expression constructs spanning a range of functional studies, including yeast two-hybrid analysis, inducible expression, retroviral expression, and fusion to enhanced green fluorescent protein (EGFP). In addition, we offer the Creator™ Acceptor Vector Construction Kit, which provides you with the necessary reagents to make any expression vector compatible with the Creator System. If you prefer, we can convert your expression vectors to be Creator compatible with our Creator Access Service: Conversion of Vectors (#CS3001).

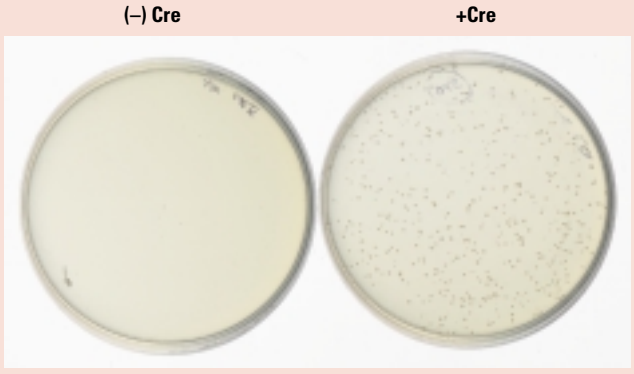
References

1. Creator™ System Overview (October 2000) *CLONTECHniques* **XV**(4):13.

Table II: Features and Benefits of the Creator™ System

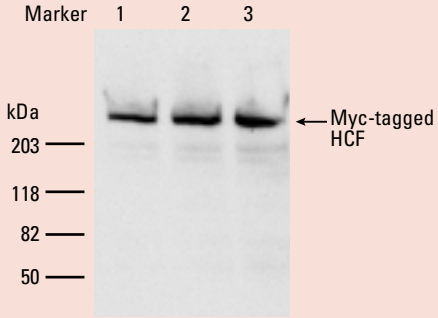
Features	Benefits
In vitro Cre-*lox*P-mediated gene transfer	• Needs no restriction enzymes • More versatility in cloning • Save days or weeks over traditional cloning • No limitations on size of insert or Acceptor Vectors
Transfer of only the DNA fragment between *lox*P sites in Donor Vector	• No limitation on size or kind of vectors used with the Creator System—even use with retroviral vectors
New and improved Cre buffer and backbone of the Donor Vector	• Little or no background and high recombination efficiency
161-bp Creator cassette	• Easy conversion of any vector into a Creator Vector
Custom Creator Access Services	• Our experts convert your clones and vectors into the Creator System
No licensing fees	• Open platform with no limitations

Little or no background



Two plates showing typical results from a Cre transfer reaction. Recombinase reactions, done in either the presence or absence of Cre recombinase, were plated on chloramphenicol/sucrose plates and allowed to grow overnight.

Expression of large inserts



Western blot showing the expression of full-length HCF (220 kDa) tagged with the c-Myc epitope. Three independent clones of HCF in pLP-CMV-myc were tested following transfer from a Donor Vector containing the 8 kb HCF cDNA.

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Creator™ pDNR–Dual Cloning Kit

New Creator™ vectors generate N- or C-terminal tagged fusion proteins

- **Generate expression clones with either N- or C-terminal tags**
- **No added sequence between your protein and the C-terminal tag**
- **Improved vector backbone**

The newest addition to our Creator™ System is the **pDNR-Dual Cloning Kit**. This kit contains a novel Donor Vector, pDNR-Dual, which lets you generate fusion proteins with either N- or C-terminal tags. Like our standard Creator™ Cloning and Expression System, you can express these proteins in both prokaryotic and eukaryotic systems. By integrating the ability to generate C-terminal fusions with the speed and efficiency of our Creator System, we now provide you with unlimited potential for designing biological and biochemical assays to understand your gene's function.

How our new system works

C-terminal tagging with the Creator System is made possible by the insertion of splicing donor (SD) and splicing acceptor (SA) sites into Donor and pLPS Acceptor Vectors, respectively (Figure 1). The SD site in pDNR-Dual is located downstream of the multiple cloning site (MCS). We have also incorporated our 6xHN (histidine affinity) tag downstream of the SD site. The SA site in the Acceptor Vector is located between the 3′ tag and the *loxP* site. When the SD and SA sites are brought together in the same construct through Cre recombinase, they create an artificial intron that is removed by eukaryotic cellular machinery. The splicing reaction removes the chloramphenicol resistance gene and the 6xHN tag, and fuses the 3′ end of your gene with the new tag.

Table I: Tag either end of your protein

	5′ Tags	3′ Tags
Eukaryotes	any tag	any tag
Prokaryotes	any tag	6xHN
Acceptor Vector	pLP	pLPS
Donor Vector	pDNR/pDNR-Dual	pDNR-Dual

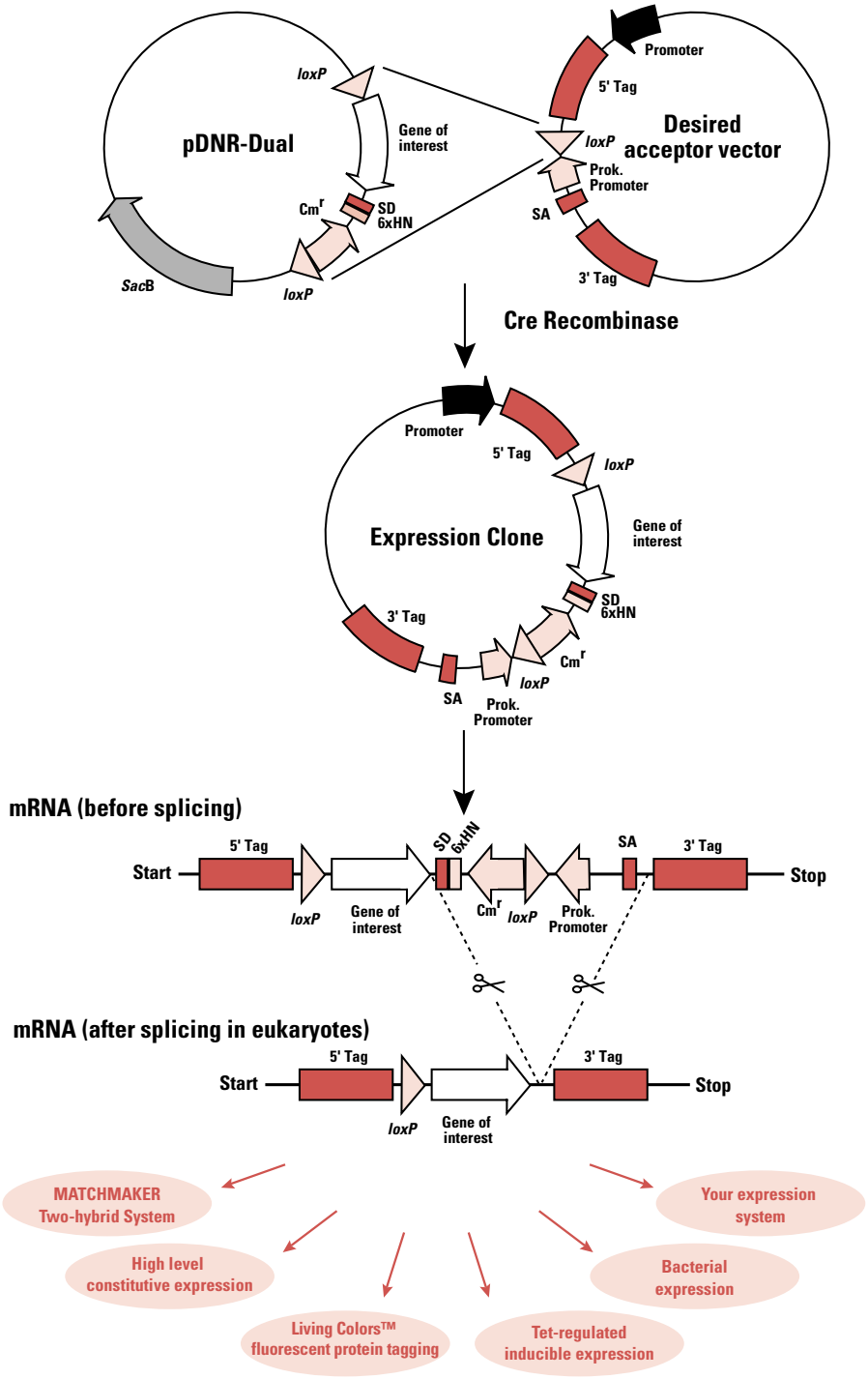

Figure 1. pDNR-Dual generates expression clones with either 5′ or 3′ tags to your gene of interest.

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Creator™ pDNR–Dual Cloning Kit...continued

Generate C-terminal tags in eukaryotes

The artificial intron is removed via intron splicing when the recombinant construct is expressed in eukaryotes. The resulting mRNA will have the tag of your choice (e.g., Enhanced Green Fluorescent Protein, EGFP) located 3′ to your gene of interest with no additional sequences. The splicing of the artificial intron is highly efficient in eukaryotes, as shown in Figure 2, resulting in expression equivalent to those of traditional cloning.

Generate C-terminal tags in prokaryotes

To generate a C-terminal tagged protein, simply clone your gene with the 3′ end in frame with the 6xHN tag and with no intervening stop codons into the pDNR-Dual Vector. After gene transfer into a prokaryotic pLP Acceptor Vector using Cre recombinase, the resulting recombinant expression construct will have the C-terminal 6xHN tag. Such proteins are easily purified using our TALON™ Metal Affinity Resin. To prevent your gene from having the 6xHN tag when using our pLP Acceptor Vectors, simply insert a stop codon between the gene and the 6xHN tag.

The addition of the 6xHN tag to the pDNR-Dual Vector provides an added benefit. You can use the pDNR-Dual Vector to directly express your gene of interest by *in vitro* transcription/translation through the T7 promoter located upstream from the MCS. Such proteins, translated *in vitro*, will carry the 6xHN tag, thus enabling easy purification with our TALON resins.

Generate N-terminal tagged proteins as usual

Just like the original pDNR vectors, pDNR-Dual can be used with any of our standard pLP Acceptor Vectors to generate expression clones with tags located 5′ to your gene of interest, so your resulting proteins will have the N-terminal tag of your choice. Once you have inserted your gene into the pDNR-Dual Vector, use the Cre-*lox*P reaction to transfer the gene into any Acceptor Vector carrying a 5′ tag.

See page 11 for ordering information.

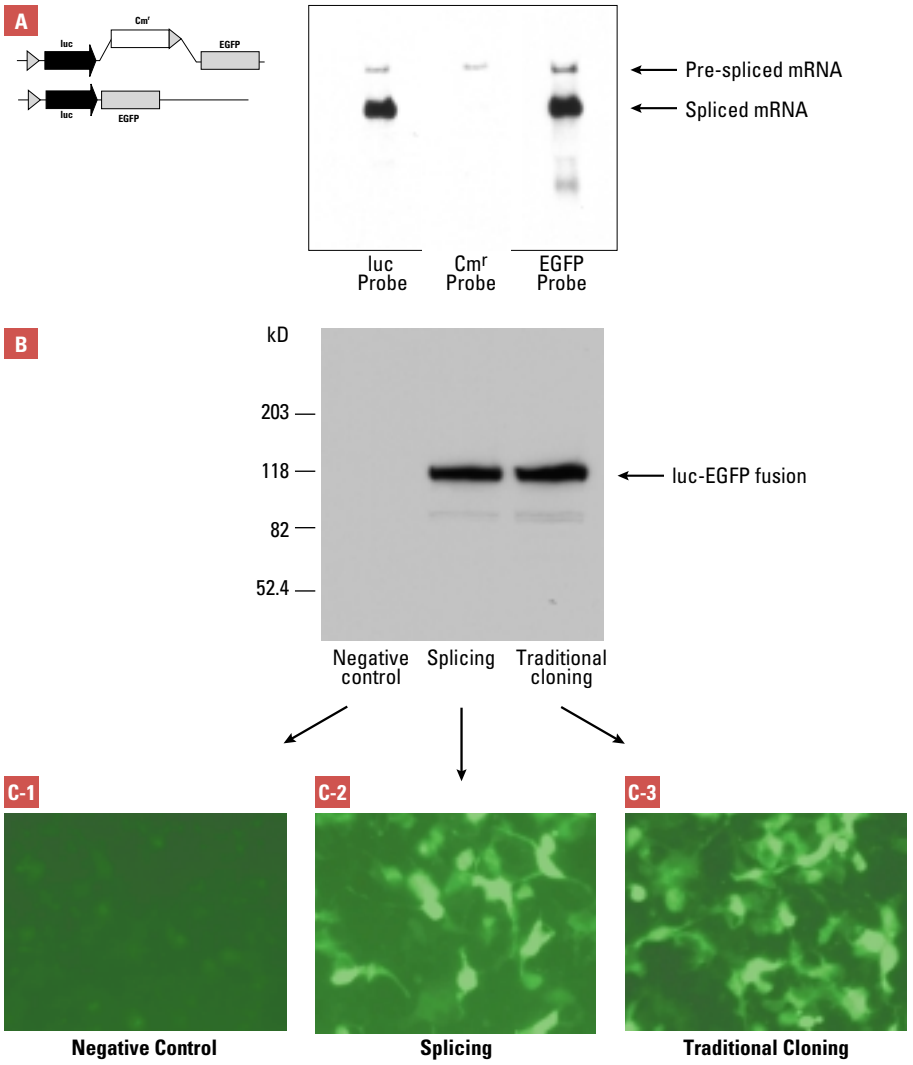

Figure 2. Generation of 3′ tags via splicing mechanism is as efficient as tagging via traditional cloning. Panel A. Northern blot made with total RNA shows a majority of the messages were spliced (99 percent by densitometry). Schematic to left of blot shows the structure of the different RNA species. **Panel B.** A Western blot was probed with an anti-luciferase antibody to visualize the expression of luciferase-EGFP fusion proteins made with Creator-based intron splicing or with traditional cloning methods. **Panel C.** Fluorescence photomicrographs taken of the same cells used to make the protein extracts for Panel B. These show that the fluorescence signal from the C-terminal EGFP is the same whether the fusion protein was generated by splicing or by traditional cloning. Luc=luciferase.

Table II: Unique features of pDNR-Dual Cloning

Splice Donor Site	5 bp	• Located directly downstream of multiple cloning site in pDNR-Dual • Removed via intron splicing
Splice Acceptor Site	50 bp	• Located between 3′ tag and the *loxP* site in pLPS Acceptor Vectors • Removed with prokaryotic promoter, *loxP* site, and Cm^r open reading frame via intron splicing
6xHN tag	36 bp	• Located directly downstream of Splice Donor Site in pDNR-Dual • Removed via intron splicing in eukaryotes • Retained, when in frame, in prokaryotes

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Creator™ SMART™ Library Construction Kit

Combining the best of both worlds

- **Unique SMART™ cDNA synthesis results in full-length cDNA**
- **No adaptor ligation required for cDNA cloning**
- **Swiftly transfer your library inserts into expression vectors for use functional studies**

We have united our SMART™ and Creator™ technologies in our **Creator™ SMART™ cDNA Library Construction Kit** to bring you the fastest and easiest method for accurate and representative library construction. SMART technology is the best way to synthesize full-length cDNA from very small quantities of RNA. Creator allows you to shuttle full-length inserts between different expression vectors.

Generate full-length cDNA libraries

Our SMART (**S**witching **M**echanism **A**t the 5′ end of **R**NA **T**ranscript) technology allows you start with only 2 µg of total RNA to generate a library. This patented method uses a SMART Oligo and reverse transcriptase to make a universal priming site on the 5′end of the newly synthesized first-strand cDNA (Figure 1). Second-strand synthesis is easily accomplished using primer extension or PCR with the universal primers. We have also introduced the rare restriction site for *Sfi* I into these universal primers so it is easy to directionally insert the cDNA into the Creator pDNR-LIB Library Vector.

The fastest way to functional studies

Our Creator pDNR-LIB Vector was designed for easy handling in bacteria and effortless transfer of your gene of interest from the library vector to any Creator-compatible expression vector. Once you have identified positive clones, simply combine each clone with the expression vectors of your choice and Cre recombinase to generate expression clones containing your genes.

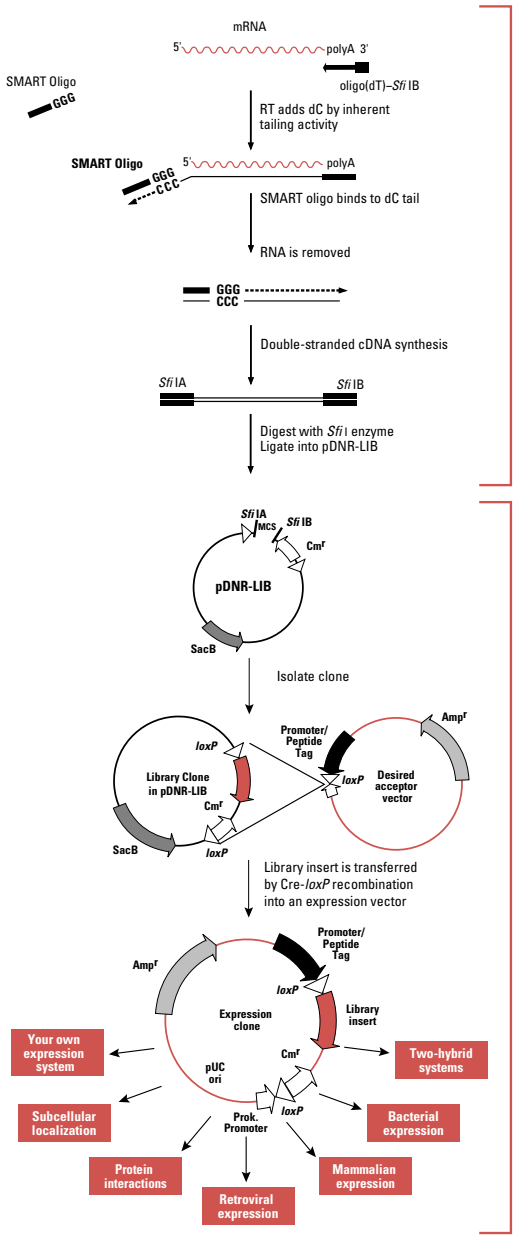

Figure 1. How our Creator™ SMART™ cDNA Library Construction Kit works.

The foundation for the Mammalian Gene Collection Project

The Creator SMART cDNA Library Construction Kit was used to make the Creator SMART cDNA Libraries, which serve as the foundation of 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 human and other mammalian sources.

We also offer premade and custom made libraries. See page 11 for a complete list of our premade Creator SMART Libraries. Please contact your Sales Territory Manager for information on our Custom Creator SMART cDNA Library Services.

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Creator™ Acceptor Vector Construction Kit

Make your vectors Creator™-compatible

- **161-bp cassette converts your expression vectors into Acceptor Vectors**
- **Works with any multiple cloning site in any vector**
- **Requires only minimal modification to existing vectors**

Our **Creator™ Acceptor Vector Construction Kit** provides everything you need to turn any plasmid into a Creator™-compatible Acceptor Vector (Figure 1). With this technology, you have unlimited possibilities to design functional assays, beyond those specified by our premade Creator Acceptor Vectors.

Adapt any vector to the Creator™ System

We provide a 1-kb template that contains the 161-bp cassette needed to adapt your expression vector to the Creator System. This cassette includes a *loxP* sequence and a promoter that is identical to the one found in our Acceptor Vectors. You design primers using our guidelines to incorporate restriction enzyme sites that are compatible with sites in the MCS of your vector. Then, amplify our template with your primers to make a fragment containing a *loxP* site, promoter, and the restriction enzyme sites specific for your vector. Simply ligate the digested fragment to your digested vector, and you have made your Creator-compatible expression Acceptor Vector.

This straightforward procedure is easily completed in a single day, letting you quickly move on to transferring your gene of interest into the expression vector you need. Combine your newly constructed expression Acceptor Vector with your insert-containing pDNR clone and our Cre recombinase to transfer your gene in only 15 minutes. Now, you are ready to perform the desired functional assay using your newly constructed expression clone with the appropriate tag located 5′ to your gene of interest.

See page 11 for ordering information.

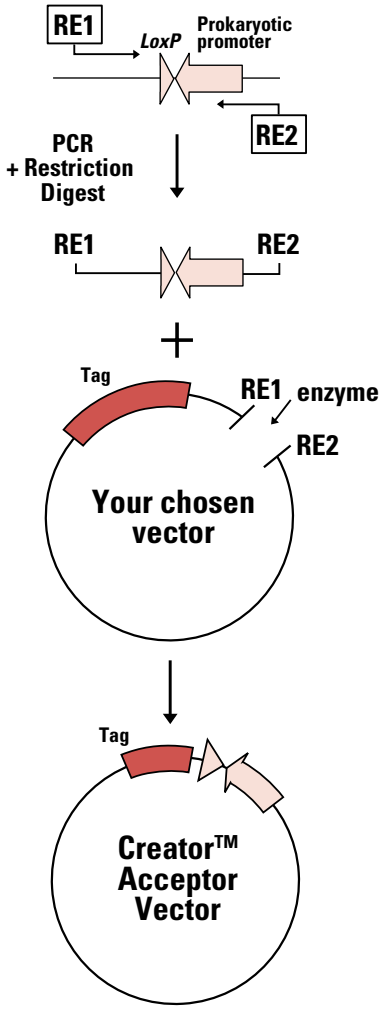

Figure 1. Easily generate Creator™-compatible Acceptor Vectors from any expression vector you choose.

Table I: Custom Creator™ Access Services

Service	Description	What you get	Turn-around Time
Conversion of Clones (CS#3000)	• Establishment of your collection of Creator clones in the pDNR Vector—ready for manipulation of gene expression	• Up to four plasmid isolates per clone in pDNR-LIB • Primers • CD-ROM of sequencing data	• 6–8 weeks
Conversion of Vectors (CS#3001)	• Generation of Creator-compatible Acceptor Vectors from your expression vectors	• Converted vector • CD-ROM of 5′ sequencing data • Primers	• 6–8 weeks

Please visit **www.clontech.com** for more information, order forms and optional confidentiality agreement pertaining to our Custom Services.

TEL: 800-662-2566 (CLON) • 650-424-8222 • www.clontech.com
E-MAIL: products@clontech.com • orders@clontech.com

Creator™ Products Ordering Information

Notices to Purchaser for Creator™ products

Advantage™ products are covered by U.S. Patent #5,436,149.

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

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For-Profit research institutes or entities that wish to use this product in non-commercial or commercial applications are required to obtain a license from Aurora Biosciences Corporation. For license information contact: Court Turner at 858-404-8416 or Fax 858-404-6743 or www.aurorabio.com. Please contact BD Biosciences Clontech directly for any other assistance, including purchasing and technical support.

All companies and institutions purchasing Living Colors™ products will be included in a quarterly report to Aurora Biosciences Corporation, as required by the BD Biosciences Clontech/Aurora license agreement.

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

Practice of the two-hybrid system is covered by U.S. Patents #5,283,173 and #5,468,614 assigned to the Research Foundation of the State University of New York. Purchase of any BD Biosciences 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. BD Biosciences Clontech is required by its licensing agreement to submit a report of all purchasers of two-hybrid reagents to SUNY Stony Brook. Please contact Barbara A. Sawitsky of SUNY Stony Brook for license information (Tel: 516-632-4163; Fax: 516-632-9839).

The PROtet Vectors are the subjects of pending patent applications.

Use of the *Sfi* I cloning strategy is licensed under U.S. Patent #5,595,895.

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

For Tet-based Expression Products

Use of the Tetracycline controllable expression systems (the "Tet Technology") is covered by a series of patents including U.S. patents #5,464,758 and #5,814,618, which are proprietary to Abbott Laboratories. Academic research institutions are granted an automatic license with the purchase of this product to use the Tet Technology only for internal, academic research purposes, which license specifically excludes the right to sell, or otherwise transfer, the Tet Technology or its component parts to third parties. In accepting this license, all users acknowledge that the Tet Technology is experimental in nature. Abbott makes no warranties, express or implied or of any kind, and hereby disclaims any warranties, representations, or guarantees of any kinds as to the Tet Technology, patents, or products. All others are invited to request a license from Abbott 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 Abbott. For license information, please contact:

US office: Abbott Bioresearch Center
100 Research Drive,
Worcester, MA 01605-4314, U.S.A.,
Fax: 508-755-8506

Creator Products	Size	Cat.#
Creator pDNR-Dual Cloning Kit	each	K1677-1 ◆
Creator pDNR Cloning Kit	each	K1670-1
Creator SMART cDNA Library Construction Kit	each	K1053-1
Creator Acceptor Vector Construction Kit	5 rxns	K1690-1
pDNR-LIB Vector	20 µg	6339-1
pDNR-LacZ Donor Reporter Vector	20 µg	6358-1
pDNR-SEAP Donor Reporter Vector	20 µg	6359-1
pDNR-d2EGFP Donor Reporter Vector	20 µg	6356-1
pDNR-EGFP Donor Reporter Vector	20 µg	6357-1
Cre Recombinase	20 rxns	8480-1

Creator-Compatible Expression Systems	Size	Cat.#
Creator-Compatible RevTet-Off Retroviral Gene Expression System	each	K1674-1
Creator-Compatible RevTet-On Retroviral Gene Expression System	each	K1675-1
Creator-Compatible PROTet-6xHN Bacterial Expression System	each	K1676-1

Human Creator SMART cDNA Libraries	Vector	Cat. #
Acute Mylogenous Leukemia	pDNR-LIB	HL9506DD
Bladder Carcinoma	pDNR-LIB	HL9505DD
Bone Marrow	pDNR-LIB	HL9525DD
Brain	pDNR-LIB	HL9500DD
Chronic Mylogenous Leukemia	pDNR-LIB	HL9507DD
Fetal Brain	pDNR-LIB	HL9520DD
Heart	pDNR-LIB	HL9501DD
Fetal Kidney	pDNR-LIB	HL9522DD
Fetal Liver	pDNR-LIB	HL9521DD
Fetal Lung	pDNR-LIB	HL9524DD
Glioblastoma	pDNR-LIB	HL9509DD
Hypernephroma	pDNR-LIB	HL9510DD
Kidney	pDNR-LIB	HL9517DD
Liver	pDNR-LIB	HL9502DD
Lung	pDNR-LIB	HL9515DD
Lung Mucoepidermoid Carcinoma	pDNR-LIB	HL9511DD
Mammary Gland	pDNR-LIB	HL9523DD
Melanoma	pDNR-LIB	HL9514DD
Neuroectodermal	pDNR-LIB	HL9508DD

Human Creator SMART cDNA Libraries	Vector	Cat. #
Pancreas	pDNR-LIB	HL9518DD
Placenta	pDNR-LIB	HL9516DD
Prostate	pDNR-LIB	HL9519DD
Prostate Adenocarcinoma	pDNR-LIB	HL9512DD
Skeletal Muscle	pDNR-LIB	HL9503DD
Testis	pDNR-LIB	HL9504DD
Testis Embryonal Carcinoma	pDNR-LIB	HL9513DD

Custom Creator Services	Size	Cat.#
Custom Creator cDNA Library in pDNR-LIB	each	CS1017DD
Creator Access Services: Conversion of Clones	each	CS3000
Creator Access Services: Conversion of Vectors	each	CS3001

Acceptor Vectors	Size	Cat.#
pLP-GADT7 AD Acceptor Vector	20 µg	6349-1
pLP-GBKT7 DNA-BD Acceptor Vector	20 µg	6350-1
pLP-EGFP-C1 Acceptor Vector	20 µg	6342-1
pLP-ECFP-C1 Acceptor Vector	20 µg	6343-1
pLP-EYFP-C1 Acceptor Vector	20 µg	6341-1
pLP-IRESneo Acceptor Vector	20 µg	6346-1
pLP-IRES2-EGFP Acceptor Vector	20 µg	6345-1
pLP-TRE2 Acceptor Vector	20 µg	6348-1
pLP-RevTRE Acceptor Vector	20 µg	6347-1
pLP-LNCX Acceptor Vector	20 µg	6344-1
pLP-CMV-Myc Acceptor Vector	20 µg	6351-1
pLP-CMV-HA Acceptor Vector	20 µg	6362-1 ◆
pLP-PROTet-6xHN Acceptor Vector	20 µg	6352-1
pLPS-3'EGFP Acceptor Vector**	20 µg	6360-1 ◆

** pLPS Vector is only compatible with pDNR-Dual Kit.

For additional information visit the Creator™ Products home page at **www.clontech.com**.

◆ = NEW!

SMART™ RACE cDNA Amplification Kit

The smartest choice for cloning complete cDNAs with 5′- and 3′-RACE

- **Fast & easy—one-step RT-PCR RACE reaction**
- **Sensitive—only 50 ng of total RNA required**
- **Reliable—no more hassles with adaptor ligation**
- **Creator™-compatible—newly modified for seamless use with the best cloning & expression system available**

The **SMART™ RACE cDNA Amplification Kit** lets you perform rapid amplification of cDNA ends (RACE) for both the 5′ and 3′ cDNA termini in one kit. SMART technology allows you to isolate the complete 5′ sequence, including the transcriptional start site, of your target cDNA more consistently than any other method. The SMART RACE Kit eliminates the need for problematic adaptor ligation steps and unreliable enzymes such as tobacco acid pyrophosphatase (TAP). Additionally, you can use first-strand cDNA directly for RACE PCR, making this method less complex and much faster than other methods (1).

The efficiency and reliability of SMART RACE is made possible by our versatile enzyme collection. The kit includes trial sizes of both the Advantage™ 2 PCR Kit and PowerScript™ Reverse Transcriptase. Paired together, Advantage and Powerscript are the best choice for long distance cDNA synthesis and amplification. These enzymes provide you with the highest sensitivity and yields, while at the same time reduce the background of RACE reactions. As a result, you can use either poly A$^+$ or total RNA as starting material for constructing full-length cDNAs, from even the rarest of transcripts (Figure 1).

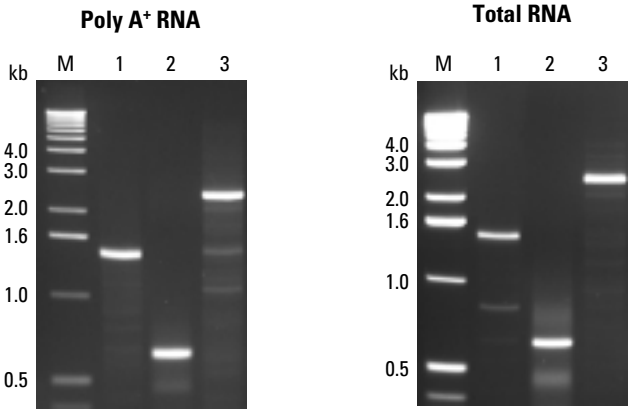

Figure 1. The SMART™ RACE Kit successfully amplifies 5′-cDNA ends from both poly A$^+$ and total RNA. Both poly A$^+$ and total RNA from human placenta were used in 5′-RACE reactions. Lane 1: interferon-γ receptor. Lane 2: 23-kDa highly basic protein. Lane 3: transferrin receptor. Lane M: 1-kb size markers.

SMART™ method for cDNA synthesis

Our patented SMART (**S**witching **M**echanism **A**t the 5′-end of **R**NA **T**ranscript) technology maximizes the probability of generating anchored, full-length cDNA in reverse transcription reactions because of the combination of our unique SMART IIa Oligonucleotide—newly modified for seamless use with the Creator™ System—and PowerScript Reverse Transcriptase. When PowerScript reaches the end of an RNA template, it exhibits a tailing activity that adds 3–5 residues (predominantly dC) to the 3′-end of the first-strand cDNA. This overhang is harnessed by the SMART Oligo, whose terminal stretch of guanine nucleotides anneals to the (dC)-rich cDNA tail and serves as an extended template for the reverse transcriptase. After PowerScript switches templates from the mRNA molecule to the SMART Oligo, a complete cDNA copy of the original RNA is synthesized with the additional SMART sequence at the end. Because the (dC) tailing activity of PowerScript is only efficient if the enzyme has reached the end of the RNA template, complete first-strand cDNAs will typically have the SMART sequence added. This selective anchoring ensures that the maximal 5′-sequence will be amplified during RACE PCR (Table I; Figure 2).

Because the 5′-elongation benefits of SMART technology are only relevant for 5′-RACE, the SMART RACE Kit includes a protocol for the synthesis of two separate cDNA populations: 5′-RACE-ready cDNA and 3′-RACE-ready cDNA. The cDNA for 5′-RACE is synthesized utilizing the SMART Oligo as described above; the 3′-RACE cDNA is synthesized using a traditional reverse transcription procedure, but with a modified oligo(dT) primer.

No more adaptor ligation or second-strand cDNA synthesis

The SMART IIa Oligo eliminates the need for troublesome adaptor ligation steps. This simplification is possible because of the incorporation of the SMART sequence into the 5′-RACE-ready cDNA via the SMART Oligo and into the 3′-RACE-ready cDNA via the modified oligo(dT) primer. This SMART sequence is then used as a primer binding site in both the 5′- and 3′-RACE PCR reactions. Because the same priming site is used in both RACE syntheses, a universal primer can be used in conjunction with your gene-specific primers (GSPs) to generate 5′- and 3′-RACE fragments.

When the 5′-RACE-ready cDNA is made, the SMART sequence is incorporated only at the 5′ end of the complete first-strand syntheses. As a result, the 5′-RACE PCR product contains a high proportion of cDNAs with full-length 5′-sequences. The elimination of adaptor ligation allows you to use first-strand cDNA directly in PCR reactions, without the need for time-consuming second-strand synthesis and DNA purification steps. Consequently, 5′- and 3′-RACE are much faster and easier, with fewer steps and enzymatic treatments during which numerous problems can occur.

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SMART RACE...continued

Table I: Additional 5'-RACE sequence obtained using SMART™ technology

Human gene	Size of mRNA (kb)	Additional sequence (bp)*	Matches genomic sequence	Includes transcription start site
Transferrin receptor	5.0	+25	yes	yes
Smooth muscle γ-actin	1.3	+31	yes	yes
Vascular smooth muscle α-actin	1.3	+17	yes	yes
p53	2.6	+4	yes	yes
Interferon-γ receptor	2.1	+14	yes	yes
Interferon-α receptor	2.8	+17	yes	yes

*Compared to the longest cDNA sequence available in GenBank

High specificity and sensitivity with touchdown PCR

RACE PCR often requires a greater amount of high-quality poly A$^+$ RNA than is available from limited tissue supplies. This limitation forces many researchers to use total RNA, and as a consequence, undesired background bands can appear. To avoid this problem, the SMART RACE Kit is optimized for touchdown PCR (2, 3). Touchdown PCR uses GSPs that have a melting temperature (T_m) several degrees higher than that of the universal primers. During the first several rounds of RACE PCR, the annealing temperature is set at the T_m of the GSP. As a result, only gene-specific synthesis primed by the GSP occurs. This process enriches the template cDNA for the target gene in the early rounds of PCR. During the remaining cycles, the annealing temperature is lowered to the T_m of the universal primer, allowing specific amplification of the gene-of-interest. The touchdown protocol's specificity and sensitivity allows you to amplify extremely rare transcripts—using either poly A$^+$ or total RNA with equal success. Thus, as little as 50 ng of total RNA can be used in SMART RACE.

The flexibility of SMART™ RACE

The SMART RACE Kit can be used to construct a full-length cDNA with knowledge of as few as 25 nucleotides of your target gene's sequence. This minimal requirement makes SMART RACE ideal for characterizing genes identified through such diverse methods as cDNA subtraction, differential display, RNA fingerprinting, ESTs, and library screening. The SMART RACE Kit is also Creator™-compatible, so it can be used to prepare cDNA for expression and functional analysis with any of our Creator Gene Cloning & Expression System products (See page 5 for a description of the Creator™ System).

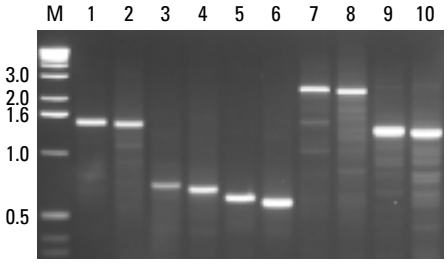

Figure 2. SMART™ RACE produces longer 5′-RACE products than the competitors. Starting with human placenta poly A$^+$ RNA, several cDNAs were amplified by 5′-RACE using either the SMART RACE Kit (odd lanes) or a competitor's RACE Kit (even lanes). For each target gene, the SMART RACE product is longer than the competitor's kit's product. Lanes 1 & 2: interferon-γ receptor. Lanes 3 & 4: ribosomal protein S9. Lanes 5 & 6: 23-kDa highly basic protein. Lanes 7 & 8: transferrin receptor. Lanes 9 & 10: interferon-α receptor. Lane M: 1-kb size markers.

Product	Size	Cat. #
SMART RACE cDNA Amplification Kit	7 cDNA & 30 PCR rxns	K1811-1

The SMART™ RACE cDNA Amplification Kit comes with free sample of PowerScript™ Reverse Transcriptase and a free trial-size Advantage™ 2 PCR Kit, sufficient for 30 PCR reactions.

Notice to Purchaser

Advantage™ 2 Products are covered by U.S. Patent #5,436,149.

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

The SMART™ Technology is covered by US Patents # 5,962,271 and 5,962,272.

Components

- **SMART IIa™ Oligonucleotide**
- **5′- & 3′-RACE cDNA Synthesis Primers**
- **5X First-Strand Buffer**
- **dNTP Mix**
- **DTT**
- **Control Human Placenta Total RNA**
- **Deionized H_2O**
- **Universal Primer Mix**
- **Nested Universal Primer 2**
- **Control 5′- & 3′-RACE TFR Primers**
- **Tricine-EDTA Buffer**
- **Advantage™ 2 PCR Kit** (trial size)
- **PowerScript™ Reverse Transcriptase** (sample)
- **NucleoTrap® Gel Extraction Kit**
- **User Manual** (PT3269-1)
- **Protocol-at-a-Glance** (PT3269-2)

Related Products

Advantage™ 2 PCR Kit (#K1910-y, -1)

Advantage™-HF 2 PCR Kit (#K1914-y, -1)

Advantage™-GC 2 PCR Kit (#K1913-y, -1)

PowerScript™ Reverse Transcriptase (#8460-1, -2)

References

1. Frohman, M.A. (1993) *Methods Enzymol.* **218**:340–358.
2. Don, R.H., *et al.* (1991) *Nucleic Acids Res.* **19**:4008.
3. Roux, K.H. (1995) *PCR Methods Appl.* **4**:5185–5194.

For additional information visit the SMART™ Product home page at **www.clontech.com**.

A Super SMART™ Protocol Increases Sensitivity and Reproducibility of Gene Expression Data

Christine Glidewell-Kenney, Brad Scherer, Ph.D., and Alex Chenchik, Ph.D.
Custom Atlas™ Array Hybridization and Analysis Service
BD Biosciences Clontech

We describe a modified SMART™ protocol that can be used to perform global gene expression profiling from as little as 100 cells or approximately 2 ng of total RNA. Our Super SMART protocol provides a 30 percent increase in reproducibility and a 10-fold increase in sensitivity when compared to our traditional SMART protocol using less than 1,000 cells or 20 ng of RNA. This experiment illustrates that a few simple modifications to the SMART protocol can be used to facilitate the analysis of global gene expression when working with extremely limiting amounts of RNA.

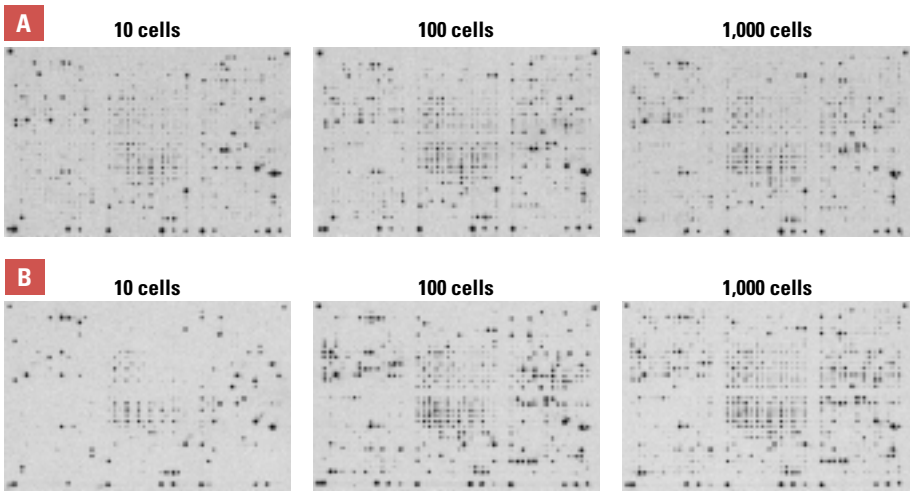

Figure 1. Super SMART™ results in higher sensitivity and better representation of medium and low abundance genes using the total RNA equivalent of only 10 cells. We isolated total RNA from serially diluted, frozen pellets of 10, 100, 1,000, and 100,000 HeLa cells (n= 3 for each group of cells) using a modified NucleoSpin® RNA II protocol. To eliminate variability related to RNA content, we pooled cell pellets for each group prior to RNA isolation in the Super SMART protocol. We used the Super SMART protocol (as outlined in Table 1) to perform reverse transcription and PCR on these RNA samples. Due to downstream volume constraints, we used 100,000 cells to isolate RNA for the SMART protocol and then, serially diluted the RNA to the equivalent of 10, 100, and 1,000 cells. We used the standard SMART protocol to perform reverse transcription and PCR on these RNA samples. We purified both the Super SMART and SMART PCR products using NucleoSpin Extraction Kit (#K3051-1). Samples were labeled using Klenow and hybridized to the Atlas™ Human 1.2 Arrays (#7850-1). We exposed all arrays to a phosphorimaging screen for 10 days and scanned at 100-micron resolution using a Storm 860 phosphorimager from Molecular Dynamics. We aligned phosphorimages to the Human 1.2 Array template using AtlasImage™ 2.0 software (#V1212) and normalized each array to a single array in each group using the global sum normalization method (AtlasImage™ User Manual). **Panel A.** Phosphorimages of Atlas Arrays hybridized with cDNA generated using the Super SMART protocol. **Panel B.** Phosphorimages of Atlas Arrays hybridized with cDNA generated using the standard SMART protocol.

Generally, our Atlas™ membrane array expression profiling technologies require 2 µg of total RNA for direct labeling, and ideally, 50 µg of total RNA for labeling when streptavidin magnetic beads are used for poly A$^+$ enrichment. Our SMART™ (**S**witching **M**echanism **A**t the 5′ end of **R**NA **T**ranscripts) protocol is cDNA amplification procedure that requires 20-50 ng of total RNA, equivalent to approximately 1,000 cells, to produce sufficient quantities of full-length, double stranded (ds) cDNA, while maintaining the original representation of RNA in the samples (1; see pages 12–13 for a description of the SMART protocol). In this report, we demonstrated that a few simple modifications to the established SMART protocol made it possible to decrease this minimum requirement to as little as 100 cell equivalents or ~2 ng of total RNA to generate reproducible gene expression data. This 'Super SMART' protocol extends the possible applications of SMART by allowing samples, such as laser-capture microscopy samples, with extremely small amounts of RNA to be used for gene expression profiling.

Table I: Comparison of Protocols

Standard SMART	Super SMART
50–1,000 ng total RNA volume ≤ 3.5 µl	**2–20 ng total RNA volume ≤50 µl**
SMART first-strand cDNA synthesis **Volume = 10 µl 1.5 hours**	SMART first-strand cDNA synthesis **Volume = 106 µl 1.5 hours**
Dilute 1:5 with TE Volume = 50 µl	**Purify with NucleoSpin® column**
Use 10 µl cDNA for SMART PCR amplification 100-µl reaction cycle optimization and scale-up	**Use 79 µl cDNA** for SMART PCR amplification 100-µl reaction cycle optimization and scale up
Purify PCR products with NucleoSpin®	Purify PCR products with NucleoSpin®
Yields **1 to 2 µg** ds DNA	Yields **0.8 to 1.7 µg** ds DNA

Note: Differences between protocols appear in **bold**.

Super SMART™—simple modifications of the SMART protocol

We modified the traditional SMART protocol by increasing the reaction volumes and performing an additional column purification step. With this protocol, we used only 2 ng of total RNA compared to the 50 ng required for use in the standard SMART protocol—a 25-fold reduction in the sample size (Table I). We also included a purification step after first-strand synthesis that enabled us to use the entire volume of the purified single-stranded cDNA for a single SMART PCR amplification. These modifications produced yields of ds cDNA ranging from 0.8–1.7 µg.

Increased sensitivity with Super SMART™

Figure 1 shows representative phosphorimages for each group used in both the Super SMART and standard SMART protocols. The arrays hybridized with cDNA equivalents from 10 cells prepared using the Super SMART method showed a pattern more similar to the pattern with 1000 cells than the standard SMART method, especially for signals of medium and low abundance genes. This shows that quality hybridizations can be achieved with 10 cells

A Super SMART™ Protocol Increases Sensitivity and Reproducibility of Gene Expression Data...*continued*

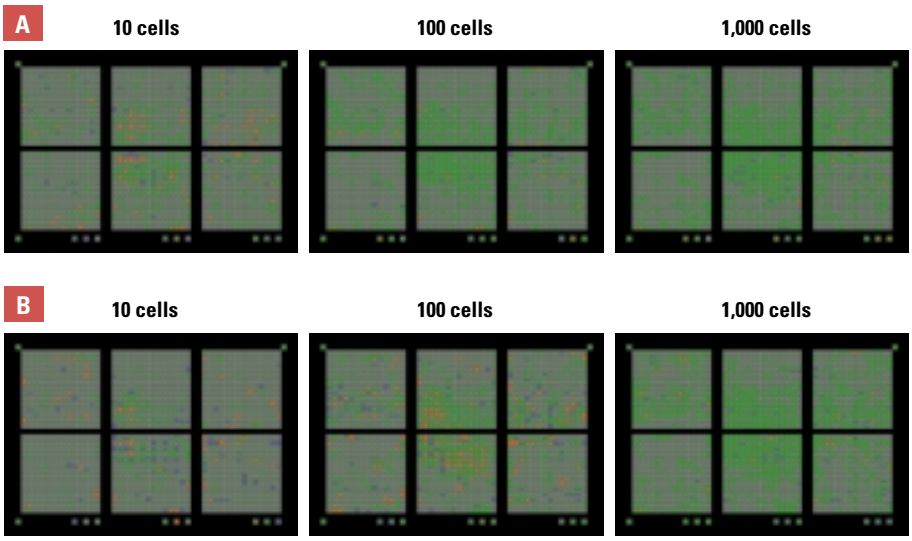

Figure 2. The Super SMART™ protocol produced reproducible expression profiling using cDNA generated from the RNA equivalent of 100 cells analyzed with conservative stringency settings in AtlasImage™ 2.0 software. AtlasImage generates graphical representations of array data. These pseudocolor images usually indicate differentially expressed genes, however, we used replicates of the same RNA sample for each group in this experiment. Thus, any differences, shown by either blue or red color in the images, indicated non-reproducible data, *i.e.* differences in cDNA populations as a result of either the Super SMART or SMART protocols, while green indicated no differences in the cDNA populations. We demonstrated that the Super SMART protocol gave about 30 percent better reproducibility when using the RNA equivalent of 10 and 100 cells. Representative pseudocolor images generated from Atlas Arrays hybridized with cDNA prepared using our Super SMART (**Panel A**) or SMART protocols (**Panel B**).

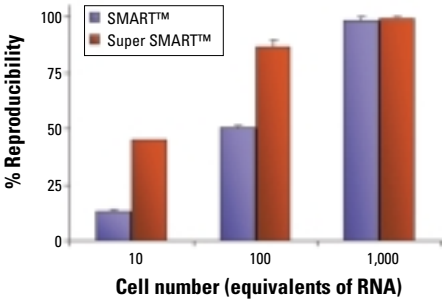

Figure 3. Comparison of the reproducibility of both the Super SMART™ and the SMART™ protocols using the equivalent of 10, 100, or 1,000 cells. Vertical brackets represent SEM (Standard Error of the Mean).

using Super SMART versus 100 cells with standard SMART, thus providing a 10-fold increase in sensitivity.

Increased reproducibility with Super SMART™

To determine the reproducibility of the Super SMART and SMART protocols, we performed three pair-wise comparisons between replicates for each sample group shown in Figure 1 using AtlasImage 2.0 software. This program generates pseudocolor images illustrating differential gene expression. Blue coordinates denoted down-regulated genes, red coordinates denoted up-regulated genes and green coordinates denoted no change in gene expression. For our experiments, the presence of blue or red colors in these images indicated significant differences in apparent expression for identically prepared samples, *i.e.* differences in gene expression between replicates of the Super SMART or SMART protocols and not as a result of differences between the protocols (Figure 2). We classified such data as non-reproducible and calculated a percent reproducibility for each group of cell equivalents of RNA for both protocols (Figure 3).

Although Super SMART results had 30 percent greater reproducibility than SMART, expression results for both protocols were below 50 percent reproducible when starting with only 10 cell equivalents. At 100 cells, Super SMART showed better reproducibility than SMART and approached 90 percent reproducibility when using conservative threshold values (ratio = 3x, difference = 4x background). Less conservative stringency demonstrated an adverse effect on the percent reproducibility (data not shown). We observed few differences between the reproducibility of Super SMART and SMART when using cDNA equivalents of 1000 cells. Both methods generated reproducible data at this level, even with liberal stringency threshold values (ratio = 2, difference = 2X background).

Use smaller amounts of cells with Super SMART™

Thus, for reproducible expression data from samples of low cell number, we recommend using at least the equivalent of 100 cells with our Super SMART protocol for cDNA preparation and also using conservative stringency values for expression analysis in AtlasImage. We demonstrated that the Super SMART protocol maintained the original RNA representation and provided quality hybridization data. Thus, the sensitivity of the Super SMART protocol allowed the detection of medium and low abundance genes when using the cDNA equivalents of only 10 cells.

Note: Our current SMART PCR cDNA Synthesis Kit (#K1052-1) does not include sufficient quantities of reagents to complete the Super SMART protocol.

Reference

1. Zhu, Y. Y., *et al.,* (2001) *BioTechniques.* **30**:892–897.

ApoAlert™ Apoptosis Detection Products

Comprehensive line of easy-to-use kits for detecting different stages in apoptosis

- **Perform rapid and sensitive assays**
- **Easily adapted to high-throughput analysis**
- **Now study the link between nitric oxide production and apoptosis**

Apoptosis, or programmed cell death, is a highly ordered, genetically controlled process that plays a fundamental role in normal biological processes as well as in disease states. BD Biosciences Clontech offers a full line of ApoAlert™ apoptosis detection products. These products allow you to use a variety of different techniques to measure indicators of cell death at different stages during apoptosis (Figures 1). These techniques include monitoring changes in mitochondrial transmembrane potential, detecting phosphatidylserine translocation, and measuring the activation of caspases. Our latest kit, the **ApoAlert™ Nitric Oxide/Annexin V-PE Dual Sensor Kit**, provides a simple method for measuring nitric oxide in apoptotic cells—one of the newest apoptosis detection methods.

Detect apoptosis by measuring nitric oxide synthesis

The ApoAlert Nitric Oxide/Annexin V-PE Dual Sensor Kit offers a fluorescence-based method for correlating nitric oxide (NO) synthesis with one of the key events in apoptosis—the exposure of phosphatidylserine (PS) on the plasma membrane. The kit lets you monitor NO synthesis before, during, and after the onset of apoptosis. Our method uses a membrane-permeable dye that fluoresces bright green upon binding NO (1). The NO-sensing dye readily diffuses into living cells where it serves as a direct reporter of nitric oxide synthase activity.

The kit also contains annexin V-phycoerythrin (PE), a protein-fluorophore conjugate with a strong, specific affinity for PS. Annexin V binds PS when it flips from the inner to the outer leaflet of the plasma membrane. Cells reaching this critical stage of apoptosis fluoresce bright red because of the phycoerythrin label joined to annexin V. Because of their distinct emission spectra, the NO-sensing dye and annexin V-PE can be used together to follow NO production and PS externalization in the same cell. With flow cytometry, the green and red signals are clearly distinguished on separate channels so you can follow both processes simultaneously (Figure 2).

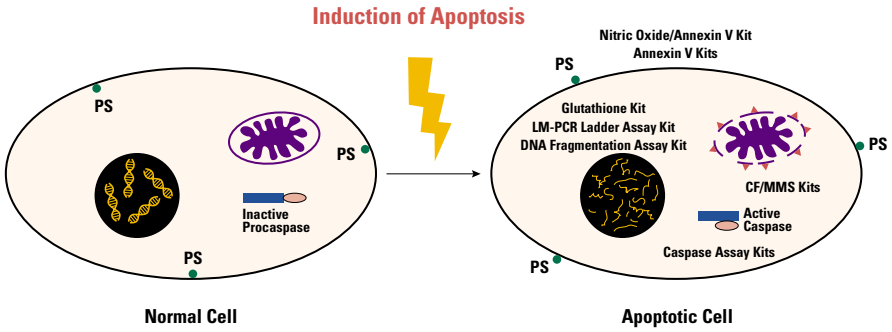

Figure 1. ApoAlert™ Kits detect apoptotic events occurring in different areas of the cell, including the mitochondria, the plasma membrane, and the cytosol. CF= Cell fractionation MMS = Mitochondrial membrane sensor. PS = Phosphatidylserine.

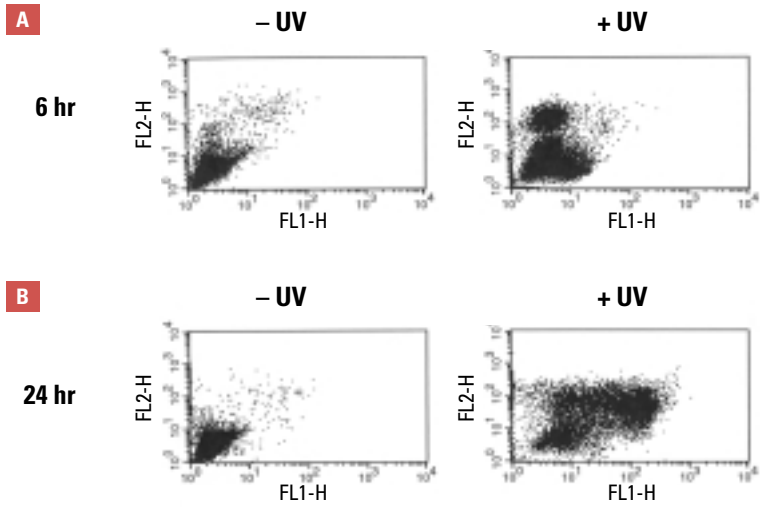

Figure 2. Using flow cytometry to follow nitric oxide production and phosphatidylserine externalization in HeLa cells. Cells were incubated with the NO Sensor Dye (5 µM) for 30 min, exposed to UV light for 5 min, and then incubated at 37°C for the indicated times. Cells were then resuspended in Annexin V Binding Buffer containing annexin V-PE and incubated at room temperature for 15 min before scanning with flow cytometry. Green and red fluorescent emissions were read using the FL-1 and FL-2 channels, respectively. The analysis shows that nitric oxide synthesis and phosphatidylserine externalization do not occur at the same time in the same cells (**Panel A** & **Panel B**). With flow cytometry, these subpopulations can be separated and further characterized with a third marker of apoptosis (see Related Products).

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E-MAIL: products@clontech.com • orders@clontech.com

ApoAlert™ Apoptosis Detection Products...*continued*

Wide range of apoptosis detection methods

Our other kits also provide simple, convenient methods for detecting apoptosis from its earliest to its most advanced stages. All of our assay systems are rapid, sensitive, and several can be easily modified for high-throughput applications.

The **ApoAlert™ Glutathione Detection Kit** is a quantitative assay that detects a decrease in cytosolic glutathione levels, an early indicator of apoptosis in Jurkat cells. In this simple assay, you induce apoptosis and incubate cell lysates with a fluorescent dye that has a high affinity for glutathione. The unbound dye is almost nonfluorescent, while the glutathione-bound dye fluoresces blue. Fluorescence can be easily detected using a fluorometer. This assay can be conveniently adapted to a 96-well, high-throughput format.

ApoAlert™ Caspase-8 Assay Kits let you use crude cell lysates to detect one of the earliest events in apoptosis—the activation of the caspase-8 protease (2, 3). Because caspase-8 activation is one of the initial events of apoptosis, these kits can detect apoptosis earlier than assays that measure membrane blebbing, chromatin condensation, and nuclear breakdown. The caspase-8 assay is highly sensitive, straightforward, and can be performed in microtiter plates for high-throughput analysis. We offer a caspase-8 colorimetric assay kit and a caspase-8 fluorimetric assay kit, so you can choose the detection method you prefer.

With the **ApoAlert™ Caspase-9/6 Colorimetric Fluorescent Assay Kit**, you can investigate the role of the mitochondria in apoptosis. Cells exposed to apoptotic stimuli release cytochrome c from the mitochondria into the cytosol. Once in the cytosol, cytochrome c becomes part of a complex that cleaves inactive caspase-9 to generate the active caspase-9 (4). Activated caspase-9 then initiates the proteolytic activities of other downstream caspases, such as caspase-6, causing the systematic disintegration of the cell. With the caspase-9/6 assay kit, you can quickly detect caspase activation by assaying for the cleavage of a fluorescent substrate. Both caspase-9 and caspase-6 cleave the substrate, which fluoresces green.

The **ApoAlert™ Mitochondrial Membrane Sensor Kit** enables you to detect changes *in vivo* in mitochondrial membrane potential during the early stages of apoptosis. The kit contains MitoSensor™ dye, which fluoresces differently in apoptotic and nonapoptotic cells. In healthy cells, MitoSensor is taken up in the mitochondria, where it forms aggregates that exhibit red fluorescence. In apoptotic cells, MitoSensor cannot enter the mitochondria because of altered mitochondrial membrane potentials. As a result, the dye remains in a monomeric form in the cytoplasm, where it exhibits green fluorescence. Results can be easily visualized using flow cytometry or microscopy.

The **ApoAlert™ Cell Fractionation Kit** provides an efficient way to isolate a highly enriched mitochondrial fraction from the cytosol of apoptotic and nonapoptotic cells. This kit uses just two standard centrifugation steps to separate the mitochondria-enriched fraction from the cytosol. When an apoptotic stimulus triggers the releases of cytochrome c from the mitochondria into the cytosol, it initiates the caspase cascade. The antibodies included in this kit allow you to distinguish between mitochondria-enriched fractions and the cytosol, revealing whether or not cytochrome c was released from the mitochondria and apoptosis was initiated.

Product	Size	Cat. #
ApoAlert Nitric Oxide/Annexin V-PE Dual Sensor Kit	25 assays	K2013-1
	100 assays	K2013-2
ApoAlert Caspase-8 Colorimetric Assay Kit	25 assays	K2029-1
	200 assays	K2029-2
ApoAlert Caspase-8 Fluorescent Assay Kit	25 assays	K2028-1
	100 assays	K2028-2
ApoAlert Caspase-9/6 Fluorescent Assay Kit	25 assays	K2015-2
	100 assays	K2015-1
ApoAlert Mitochondrial Membrane Sensor Kit	100 assays	K2017-1
ApoAlert Glutathione Detection Kit	25 assays	K2014-1
	100 assays	K2014-2
ApoAlert Cell Fractionation Kit	100 assays	K2016-1

Related Products

- **ApoAlert™ Caspase-3 Fluorescent Assay Kit** (#K2026-1, -2)
- **ApoAlert™ Caspase-3 Colorimetric Assay Kit** (#K2027-1)
- **ApoAlert™ Annexin V-EGFP Apoptosis Kit** (#K2019-1, -2)
- **ApoAlert™ Annexin V-FITC Apoptosis Kit** (#K2025-1, -2)
- **ApoAlert™ LM-PCR Ladder Assay Kit** (#K2021-1)
- **ApoAlert™ DNA Fragmentation Assay Kit** (#K2024-1, -2)
- **PARP Monoclonal Antibody** (#8192-1)
- **Apoptosis inducing agents and inhibitors** (many)

References

1. Nakatsubo, N., *et al.* (1998) *FEBS Letters* **427**:263–266.
2. Boldin, M. P., *et al.* (1996) *Cell* **85**:803–815.
3. Muzio, M., *et al.* (1996) *Cell* **85**:817–827.
4. Peng, L., *et al.* (1997) *Cell* **91**:479–489.
5. Zhang, C., *et al.* (1996) *J. Immunol.* **157**:3980–3987.
6. Koester, S. K., *et al.* (1997) *Cytometry* **29**:306–312.

For additional information visit the ApoAlert™ Products home page at **www.clontech.com**.

Reporter Systems & Detection Kits

Both compatible with many Mercury™ Pathway Profiling Systems

- **No cell lysis required—simple assay for secreted alkaline phosphatase (SEAP)**
- **Choose between fluorescent or chemiluminescent SEAP detection methods**
- **Linear over a 10^4-fold range**
- **Detect 0.1 pg of SEAP with our chemiluminescent assay**

BD Biosciences Clontech offers three reporter detection kits for quickly and easily determining reporter expression. The **Great EscAPe™ SEAP Chemiluminescence** and **Fluorescence Detection Kits** provide simple, nonradioactive methods for detecting SEAP (secreted alkaline phosphatase) activity. The **Luciferase Reporter Assay Kit** lets you quickly quantify luciferase activity using a firefly luciferase substrate. These kits are perfect companions for our Mercury Pathway Profiling SEAP or Luciferase Systems to monitor the activation of signal transduction pathways (Figure 1). We also offer the **Great EscAPe™ SEAP Reporter System 3** for using SEAP in a wide range of reporter-based assays.

Measure reporter activity without lysing cells

SEAP offers several advantages over other known transcription reporters. Cells transfected with SEAP constructs secrete SEAP enzyme into the culture medium in proportion to RNA levels of SEAP in transfected cells. Because SEAP is secreted into the culture medium, you can easily study gene expression kinetics by repeatedly sampling the culture medium. There's no need to lyse cells. After assaying for SEAP activity, you can continue to study the cells using other methods, such as Northern blots, RNase protection assays, or Western blots.

Another important advantage of SEAP is that background signals due to endogenous alkaline phosphatases are nearly absent. Unlike most endogenous alkaline phosphatases, SEAP is extremely heat stable and resistant to the inhibitor L-homoarginine. Thus, you can eliminate endogenous alkaline phosphatase activity by preheating the culture medium sample at 65°C and assaying in the presence of L-homoarginine.

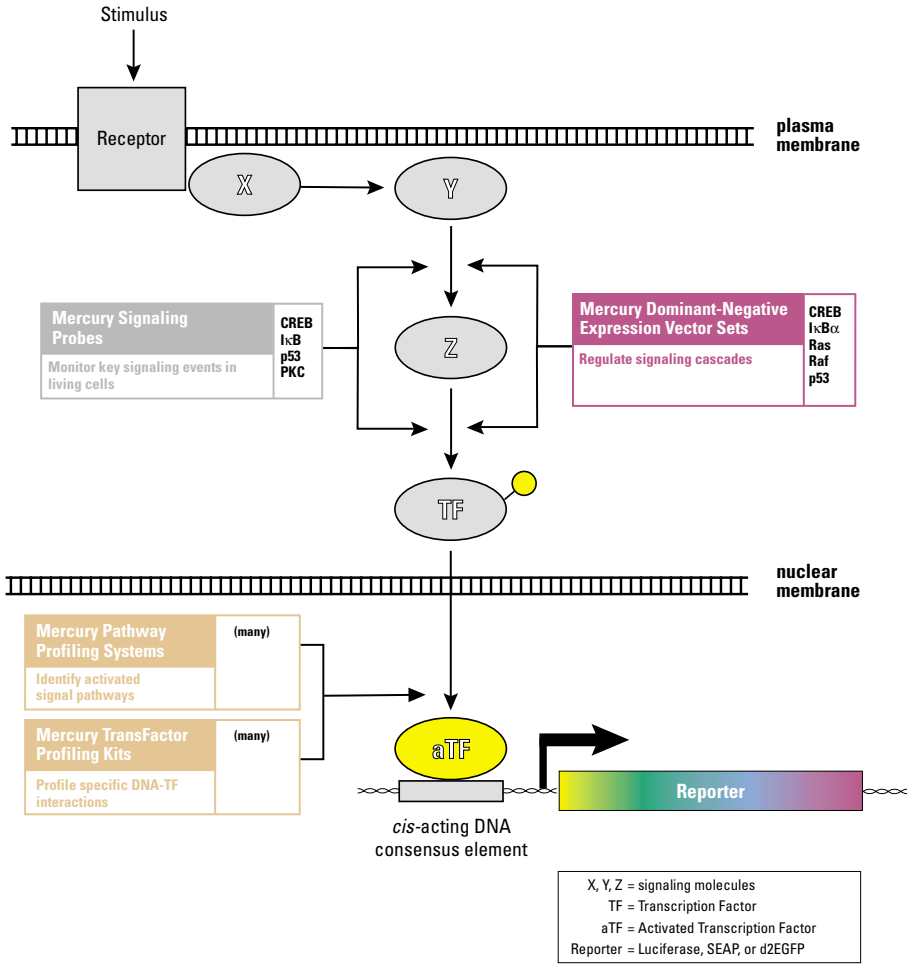

Figure 1. Measuring reporter activity is a key component to investigating signal transduction pathways using the Mercury™ product line. Colored boxes indicate Mercury products compatible with our SEAP and/or Luciferase Detection Kits.

Choose your SEAP detection method

We offer two different SEAP detection kits: one for fluorescence detection and one for chemiluminescence detection. The chemiluminescence assay can detect as little as 0.1 pg of SEAP protein. The sensitivity of the fluorescence assay is comparable to that of firefly luciferase assays. Both the chemiluminescence and the fluorescence assays are linear over a 10^4-fold range of enzyme concentrations (Figure 2).

Luciferase—a proven reporter system

In addition to detection kits for SEAP, we also offer a luciferase reporter assay detection kit. Firefly luciferase is a reporter commonly used to quantify gene expression in a diverse range of organisms, and across a wide range of assay conditions. The Luciferase Reporter Assay Kit quickly detects luciferase activity with high sensitivity. Luciferase interacts with its substrate luciferin to produce light emission peaking at 562 nm, which can be read with any standard luminometer. The kit includes a firefly luciferase substrate formulation and cell lysis buffer to release the luciferase.

Reporter Systems & Detection Kits...continued

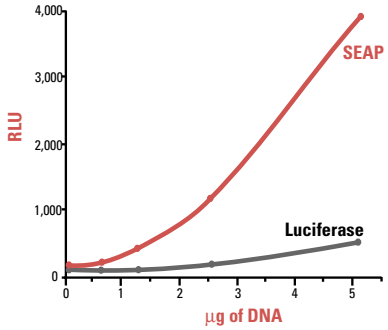

Figure 2. Comparison of the sensitivity of SEAP and firefly luciferase. Parallel cultures of BHK cells were transiently transfected with the indicated amounts of either pSEAP2-Control Vector or a similar vector expressing luciferase. After 24 hr, SEAP activity was assayed in the appropriate culture media using the Great EscAPe Chemiluminescent Assay. Similarly, after 24 hr, cell lysates were prepared from the luciferase cultures, and luciferase activity was assayed. RLU = relative light units.

SEAP and luciferase—complimentary to our Mercury™ Pathway Profiling Systems

The Mercury Pathway Profiling Systems let you assess the activation of signal transduction pathways *in vivo* (Figure 1). These systems are sets of vectors that each contain a distinct *cis*-acting DNA consensus element upstream of a reporter gene. Depending on the pathway profiling system you choose, the reporter gene may encode SEAP or luciferase. Expression of the reporter gene means that a transactivator, or transcription factor, present in the cell has bound the enhancer element in the Mercury Vector, indicating that a particular signaling pathway has been activated. Mercury Pathway Profiling Systems are designed for both broad-spectrum and specific profiling. Many Mercury Vectors are also available separately.

Mercury™ TransFactor Kits

Our new Mercury™ TransFactor Kits (Figure 1) are the perfect match to both the Mercury Profiling Kits and the Great EscAPe SEAP Reporter Assay. These kits profile DNA-transcription factor interactions.

Great EscAPe SEAP Reporter System 3

The Great EscAPe SEAP Reporter System 3 is designed for incorporating SEAP as a reporter in a wide variety of experiments. The system includes the pSEAP2-Basic and -Control Vectors and all the reagents necessary for SEAP expression and detection with fluorescent and chemiluminescent methods.

SEAP and the Creator™ Gene Cloning & Expression System

Now, you can use the Great EscAPe SEAP Detection Kits with the Creator™ Gene Cloning & Expression System (page 5). Our Creator Donor Reporter Vectors include pDNR-SEAP, a Donor Vector that lets you use SEAP as a cotransfection marker in any expression system. The Creator System uses a Cre-*lox*P recombination method to reduce time-consuming vector construction to a simple 15-minute reaction. In this system, Cre recombinase mediates the transfer of the SEAP gene from the Donor Vector into all of the expression Acceptor Vectors you want. These newly constructed clones are ready for many applications, including transfection efficiency controls, markers for FACS sorting, or quantifiers of gene expression via induction. In all of these applications, SEAP activity can be measured using the Great EscAPe SEAP Detection Kits.

Luminescent β-galactosidase Reporter System

The Luminescent β-galactosidase Reporter System 3 provides reagents for sensitive chemiluminescent assay, as well as cloning vectors for the detection of β-galactosidase activity. The vectors can also be used to normalize transfection efficiencies across a range of transfection. For researchers who already have suitable β-galactosidase vectors, the β-galactosidase Detection Kit II can be used to detect β-galactosidase activity in any experiments that use *lacZ* as a reporter.

Notice to Purchaser

Certain aspects of the cis-acting reporter construct products are the subject of pending U.S. patents.

Product	Size	Cat. #
Great EscAPe SEAP Chemiluminescence Detection Kit	50 rxns	K2041-3
	300 rxns	K2041-1
Great EscAPe SEAP Fluorescence Detection Kit	300 rxns	K2043-1
Great EscAPe SEAP Reporter System 3	each	K2054-1
Luciferase Reporter Assay Kit	100 rxns	K2039-1
pDNR-SEAP Donor Reporter Vector	20 µg	6359-1
Luminescent β-gal Reporter System 3	each	K2055-1
Luminescent β-gal Detection Kit II	300 rxns	K2048-1

β-gal Reporter System 3 Components

- **pβgal-Basic Vector**
- **pβgal-Control Vector**
- **Reaction Buffer**
- **Reaction Substrate**
- **Positive Control β-galactosidase**
- **User Manual** (PT2106-1)
- **Protocol-at-a-Glance** (PT2106-2)

Great EscAPe Reporter System 3 Components

- **pSEAP2-Basic Vector**
- **pSEAP2-Control Vector**
- **CSPD Chemiluminescent Substrate**
- **Chemiluminescent Enhancer**
- **MUP Fluorescent Substrate**
- **Assay Buffer**
- **Dilution Buffer**
- **Positive Control Placental Alkaline Phosphatase**
- **User Manual** (PT3057-1)
- **Protocol-at-a-Glance** (PT3057-2)

Luciferase Reporter Assay Kit Components

- **Substrate A**
- **Substrate B**
- **3X Cell Lysis Buffer**
- **User Manual** (PT3392-1)

Related Products

- **Mercury™ Pathway Profiling SEAP Systems** (many)
- **Mercury™ Pathway Profiling Luciferase Systems** (many)
- **Mercury™ Vectors** (many)
- **Mercury™ TransFactor Kits** (many)
- **TransFactor Extraction Kit** (#K2064-1)

Clontech PCR-Select™ Products

Create subtracted libraries and identify novel, differentially expressed genes

- **Patented PCR method to produce subtracted libraries**
- **Requires minimal amount of RNA with SMART™ cDNA amplification**
- **Custom subtraction and analysis now available**

Clontech PCR-Select™ is a powerful PCR-based method that allows you to compare gene expression between two experimental samples. The Suppression Subtractive Hybridization (SSH) method allows you to identify unknown genes that are uniquely expressed in one experimental sample, but not in another.

Subtraction Method

The tester cDNA is the pool of cDNA from which differentially expressed genes will be identified. The driver cDNA population is the control pool that will be used to remove or subtract out common sequences.

Figure 1 depicts an overview of the PCR-Select method. cDNA is synthesized using a conventional method, or by the SMART cDNA synthesis method when the available quantity of RNA is limited. The cDNA is cut into shorter fragments and two types of adaptors are ligated to the tester cDNA from two pools of tester cDNA in order to increase subtractive efficiency. After adaptor ligation, two rounds of hybridization are performed. Finally, two rounds of PCR are performed to selectively amplify differentially expressed sequences.

The first hybridization removes sequences that are common in both the tester and driver populations. The two different pools of tester cDNA are combined in the second hybridization. Only sequences that are unique to the tester have the adaptor combination required for exponential amplification in subsequent PCR steps (Figure 2).

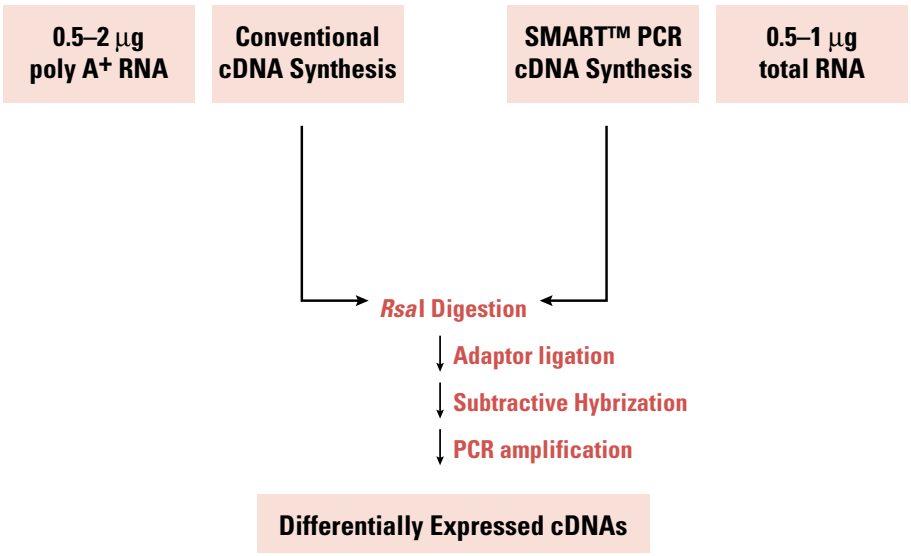

Figure 1. PCR-Select (SSH) cDNA Subtraction.

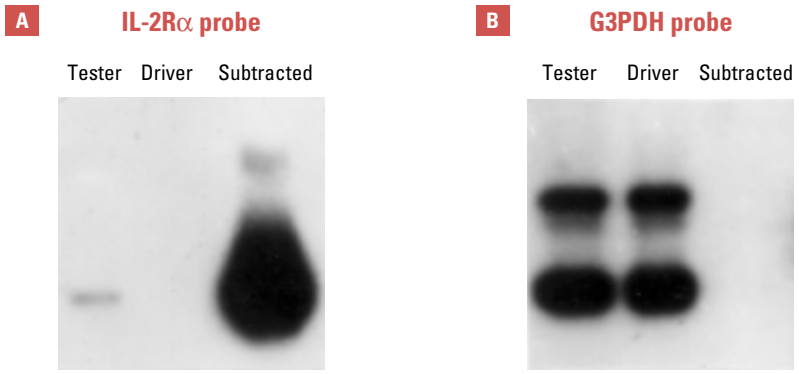

Figure 3. PCR-Select enriches for a differentially expressed transcript and reduces abundance of a common transcript (2). Tester cDNA was prepared from human Jurkat cells incubated with 2 µg/ml PHA and 2 ng/ml PMA for 72 hr. Driver cDNA was prepared from untreated cells. Amplified tester, driver, and subtracted cDNA were electrophoresed on a 1.5 percent agarose gel (0.3 µg per lane), transferred onto nylon filters, and hybridized with either an IL-2Rα probe (**Panel A**) or a G3PDH probe (**Panel B**).

Differentially expressed genes are enriched

Figure 3 shows how the subtraction process enriches of the differentially expressed IL-2Rα gene. The IL-2Rα gene was differentially expressed in the initial tester and driver samples and the process has enriched for the genes that are unique to the tester. Also, Figure 3 illustrates how G3PDH, a common housekeeping gene can be completely subtracted from the final Library.

Screening kit allows rapid screening of subtracted libraries

Our PCR-Select Differential Screening Kit allows you to rapidly screen subtracted libraries. This kit supplies all of the reagents necessary to array and analyze subtracted libraries. This kit greatly reduces false positives and immediately focuses your research on real differentially expressed clones.

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Clontech PCR-Select™ Products...continued

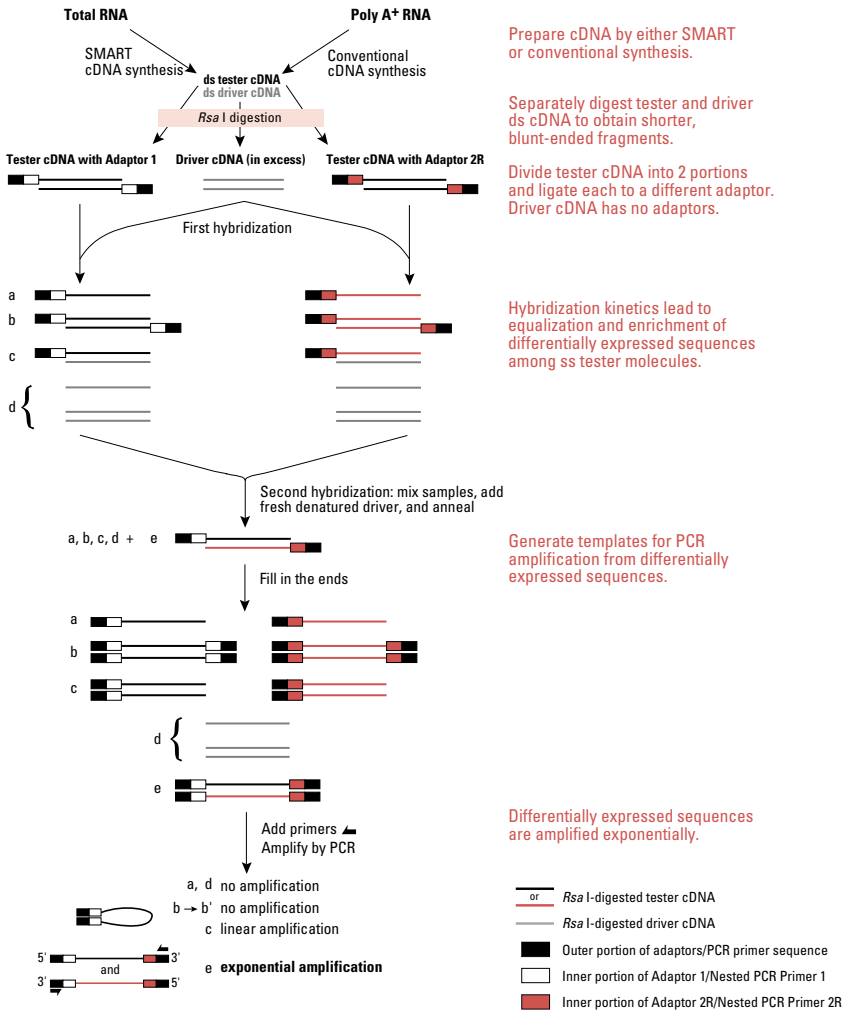


Figure 2. The PCR-Select cDNA subtraction technique.

Product	Size	Cat. #
Clontech PCR-Select cDNA Subtraction Kit	7 rxns	K1804-1
PCR-Select Differential Screening Kit	each	K1808-1
Custom Clontech PCR-Select Subtraction: Level I	each	CS1103
Custom Clontech PCR-Select Subtraction Differential Screening: Level II	each	CS1104
Custom Clontech PCR-Select SMART Amplification	2 amplifications	CS1105

Notice to Purchaser for Clontech PCR-Select™

Clontech PCR-Select™ cDNA Subtraction products are covered by U.S. Patent Nos. 5,565,340 and 5,759,822, as well as pending foreign patent applications. For-profit and not-for-profit purchasers of PCR-Select products are entitled to use the reagents for research, including identification of molecular markers or differentially expressed genes; however, the following uses expressly are prohibited: (1) performing services for third parties; (2) identifying nucleic acid sequences to be included on nucleic acid arrays, blots or in libraries or other cDNA collections which are then sold to third parties; or (3) constructing databases which are then sold to third parties. Further, reproduction, amplification, modification, reformulation or resale of the reagents provided in the PCR-Select products is not permitted. For information on licensing PCR-Select for these purposes, please contact: Product Manager, PCR Applications, Clontech Laboratories, Inc., 1020 East Meadow Circle, Palo Alto, CA, 94303 or call 650-424-8222 x7816.

References

1. Nucleic Acid Hybridization (1985) Ed. James, B. D. & Higins, S. J. (IRL Press Ltd., Oxford).
2. Lukyanov, S. A. *et al.* (1994). *Bioorganic Chem.* (Russian) **20**:701–704.
3. Siebert, P. D. *et al.* (1995). *Nucleic Acids Res.* **23**:1087–1088.

For additional information visit the Clontech PCR-Select™ Products home page at **www.clontech.com**.

Custom Subtraction Services

By allowing our experts in subtraction science to perform your subtraction and analysis experiments, you can save time and be assured that your subtracted libraries contain the largest number of differentially expressed genes.

We offer two levels of Custom PCR-Select Subtraction Services. Our Level I Subtraction Service (#CS1104) includes production of forward and reverse subtracted libraries. We also begin the differential screening process by estimating the percentage of differential expression, by screening 96 clones from both the forward and reverse subtracted libraries to confirm differential expression, and by performing the purification, sequencing, and BLAST search of confirmed differentially expressed clones.

Our Level II Service (#CS1104) takes the differential screening process one step further by completing the Level I Service, and then screening a total of 480 clones from the forward library. With the Level II Service, we perform a complete analysis of the differentially expressed clones in your test population. From your RNA, we are able to provide you with a complete set of purified and sequenced differentially expressed clones. Allow our scientists to perform the subtraction for you, so you can begin to work on only the genes that are differentially expressed.

Premium RNA™

Highly pure Total and Poly A+ RNA

- **Screen multiple Total RNA samples with new 50 µg size**
- **Ideal for high-throughput RT-PCR, cDNA synthesis, ribonuclease protection assays, and Northern blotting**
- **Broadest selection of RNA samples available**

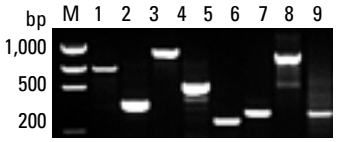
With more than 4,000 references in scientific journals, we have set the industry standard for providing high quality RNA with our **Premium RNA™** products. For more than 13 years, our Premium RNA products have provided easy access to a wide variety of hard-to-obtain tissue sources. Our continued commitment to providing high quality RNA now extends into our **Premium Reserve™ RNA**.

Our Premium Reserve RNA samples are exceptionally rare, high-quality RNA from extremely difficult-to-obtain tissue samples, and are only available in limited quantities for custom packaging. Our inventory for these samples varies so visit **www.clontech.com** to obtain the most up-to-date list.

We also offer Total RNA from 22 different human tissue sources in 50-µg quantities to make your screening assays even easier. With this new size, you now have a reasonably priced alternative for generating accurate screening results. Our new Human Total RNA Master Panel (#K4008-1) features 10 µg of total RNA from 20 different human tissue sources.

Standard for quality

The foundation of our Premium RNA and Premium Reserve RNA is highly purified total and poly A+ RNA. 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. We perform rigorous quality control tests to confirm that each preparation consists of intact, full-length RNA with virtually no genomic DNA. Figure 1 demonstrates that a wide variety of genes are easily amplified by RT-PCR from Human Placenta Total RNA (#64024-1).



Lane	Transcript	Relative abundance	# PCR cycles
1	EGFR3	low	40
2	p53	low	30
3	IFN-8-R	med	25
4	ILGF-1	low	40
5	PDGFB	low	25
6	IFN-8	low	30
7	β-actin	high	30
8	IL-8	low/med	30
9	G3PDH	high	25

Figure 1. RT-PCR using Human Placenta Total RNA demonstrates that a wide variety of genes are easily amplified. Our TITANIUM™ One-Step RT-PCR Kit (#K1403-1, -2) was used to amplify cDNA from 1 µg of Human Placenta Total RNA (#6518-1). RT-PCR products were analyzed via agarose/EtBr gel electrophoresis. Nine different human transcripts were amplified. Lane M: DNA size marker.

Human Poly A+ RNA	Size	Cat. #
Human Adrenal Gland	5 µg	6571-1
Human Bone Marrow	5 µg	6573-1
Human Brain, whole	5 µg	6516-1
Human Brain, amygdala	5 µg	6574-1
Human Brain, caudate nucleus	5 µg	6575-1
Human Brain, cerebellum	5 µg	6543-1
Human Brain, corpus callosum	5 µg	6577-1
Human Brain, hippocampus	5 µg	6578-1
Human Brain, thalamus	5 µg	6582-1
Human Colorectal Adenocarcinoma (SW480); ATCC #CCL228	5 µg	6586-1
Human Fetal Brain	5 µg	6525-1
Human Fetal Kidney	5 µg	6526-1
Human Fetal Liver	5 µg	6527-1
Human Fetal Lung	5 µg	6528-1
Human Heart	5 µg	6533-1
Human HeLa Cell (S3)	5 µg	6522-1
Human Kidney	5 µg	6538-1
Human Leukemia Chronic Myelogenous (K-562); ATCC #CCL243	5 µg	6532-1
Human Leukemia Lymphoblastic, (MOLT-4); ATCC #CRL1582	5 µg	6587-1
Human Leukemia Promyelocytic, (HL-60); ATCC #CCL240	5 µg	6530-1
Human Liver	5 µg	6510-1
Human Lung	5 µg	6524-1
Human Lung Carcinoma (A549); ATCC #CCL185	5 µg	6592-1
Human Lymph Node	5 µg	6594-1
Human Lymphoma Burkitt's, (Daudi); ATCC #CCL213	5 µg	6531-1
Human Lymphoma Burkitt's, (Raji); ATCC #CCL86	5 µg	6588-1
Human Mammary Gland	5 µg	6545-1
Human Melanoma (G361)	5 µg	6591-1
Human Pancreas	5 µg	6539-1
Human Pituitary Gland	5 µg	6584-1
Human Placenta	5 µg	6518-1
Human Prostate	5 µg	6546-1

Premium RNA™ ...continued

Human Poly A+ RNA	Size	Cat. #
Human Salivary Gland	5 µg	6534-1
Human Skeletal Muscle	5 µg	6541-1
Human Small Intestine	5 µg	6547-1
Human Spinal Cord	5 µg	6593-1
Human Spleen	5 µg	6542-1
Human Stomach	5 µg	6548-1
Human Testis	5 µg	6535-1
Human Thymus	5 µg	6536-1
Human Thyroid	5 µg	6570-1
Human Trachea	5 µg	6549-1
Human Uterus	5 µg	6537-1

Mouse Poly A+ RNA	Size	Cat. #
Mouse Brain	5 µg	6616-1
Mouse Heart	5 µg	6611-1
Mouse Kidney	5 µg	6613-1
Mouse Liver	5 µg	6610-1
Mouse Lung	5 µg	6618-1
Mouse Pancreas	5 µg	6615-1
Mouse Skeletal Muscle	5 µg	6617-1
Mouse Smooth Muscle	5 µg	6619-1
Mouse Spleen	5 µg	6614-1
Mouse Testis	5 µg	6612-1

Rat Poly A+ RNA	Size	Cat. #
Rat Brain	5 µg	6712-1
Rat Heart	5 µg	6718-1
Rat Kidney	5 µg	6722-1
Rat Liver	5 µg	6710-1
Rat Lung	5 µg	6720-1
Rat Pancreas	5 µg	6715-1
Rat Retina	5 µg	6724-1
Rat Skeletal Muscle	5 µg	6725-1
Rat Smooth Muscle	5 µg	6726-1
Rat Spleen	5 µg	6716-1
Rat Testis	5 µg	6714-1

Human Total RNA	Size	Cat. #	Price
Human Total RNA Master Panel II	20 x 10 µg	K4008-1	◆
Human Adrenal Gland	50 µg	64096-1	
	250 µg	64016-1	
Human Bone Marrow	50 µg	64106-1	
Human Brain	50 µg	64098-1	
Human Brain, whole	250 µg	64020-1	

Human Total RNA	Size	Cat. #
Human Brain, cerebellum	50 µg	64103-1
	250 µg	64035-1
Human Colon	50 µg	64112-1
	250 µg	64065-1
Human Fetal Brain	50 µg	64094-1
	250 µg	64019-1
Human Fetal Liver	250 µg	64018-1
Human Heart	50 µg	64100-1
	250 µg	64025-1
Human HeLa Cell	250 µg	64021-1
Human Kidney	50 µg	64097-1
	250 µg	64030-1
Human Liver	50 µg	64099-1
	250 µg	64022-1
Human Lung	50 µg	64092-1
	250 µg	64023-1
Human Mammary Gland	250 µg	64037-1
Human Pancreas	250 µg	64031-1
Human Placenta	50 µg	64095-1
	250 µg	64024-1
Human Prostate	50 µg	64108-1
	250 µg	64038-1
Human Salivary Gland	50 µg	64110-1
	250µg	64026-1
Human Skeletal Muscle	50 µg	64102-1
	250 µg	64033-1
Human Small Intestine	50 µg	64105-1
	250 µg	64039-1
Human Spinal Cord	50 µg	64113-1
Human Spleen	50 µg	64093-1
	250 µg	64034-1
Human Stomach	250 µg	64090-1
Human Testis	50 µg	64101-1
	250 µg	64027-1
Human Thymus	50 µg	64107-1
	250 µg	64028-1
Human Thyroid	50 µg	64104-1
Human Trachea	50 µg	64111-1
Human Uterus	50 µg	64109-1
	250 µg	64029-1

Mouse Total RNA	Size	Cat. #
Mouse Brain	250 µg	64040-1
Mouse Bone Marrow	250 µg	64080-1
Mouse 7-day Embryo	250 µg	64046-1
Mouse 11 day Embryo	250 µg	64047-1
Mouse 15-day Embryo	250 µg	64048-1
Mouse 17-day Embryo	250 µg	64049-1
Mouse Eye	250 µg	64051-1
Mouse Heart	250 µg	64041-1

Mouse Total RNA	Size	Cat. #
Mouse Kidney	250 µg	64052-1
Mouse Liver	250 µg	64042-1
Mouse Lung	250 µg	64043-1
Mouse Lymph Node	250 µg	64053-1
Mouse Prostate	250 µg	64054-1
Mouse Salivary Gland	250 µg	64081-1
Mouse Smooth Muscle	250 µg	64055-1
Mouse Spinal Cord	250 µg	64056-1
Mouse Spleen	250 µg	64044-1
Mouse Stomach	250 µg	64057-1
Mouse Testis	250 µg	64045-1
Mouse Thymus	250 µg	64058-1
Mouse Uterus	250 µg	64059-1

Rat Total RNA	Size	Cat. #
Rat Brain	250 µg	64060-1
Rat Colon	250 µg	64066-1
Rat Heart	250 µg	64061-1
Rat Kidney	250 µg	64062-1
Rat Liver	250 µg	64063-1
Rat Lung	250 µg	64064-1

Human Tumor Total RNA	Size	Cat. #
Human Breast Tumor	40 µg	64015-1
Human Cervix Tumor	40 µg	64010-1
Human Colon Tumor	40 µg	64014-1
Human Kidney Tumor	40 µg	64012-1
Human Lung Tumor	40 µg	64013-1
Human Ovary Tumor	40 µg	64011-1
Human Stomach Tumor	40 µg	64009-1
Human Uterus Tumor	40 µg	64008-1

Related Product

TITANIUM™ One-Step RT-PCR Kits (K1403-1, -2)

For additional information visit the Premium RNA Products home page at **www.clontech.com**.

◆ = NEW!

Products for Mutagenesis

Perform controlled random mutagenesis or site-directed mutagenesis

- **Precisely control random mutagenesis**
- **Obtain high efficiency with site-directed mutagenesis**
- **Investigate protein function and create novel proteins**

BD Biosciences Clontech gives you the choice of two different mutagenesis methods. The **Diversify™ PCR Random Mutagenesis Kit** provides a PCR-based method for performing random mutagenesis to create novel proteins or investigate protein function, while the **Transformer™ Site-Directed Mutagenesis Kit** lets you introduce specific mutations into a target gene or region.

Engineer and analyze proteins using random mutagenesis

The Diversify PCR Random Mutagenesis Kit offers a convenient method for engineering and analyzing proteins. PCR-based random mutagenesis lets you make novel proteins and investigate protein function by identifying functional domains or amino acid residues. Unlike conventional PCR-based random mutagenesis methods, this kit allows you to manipulate the conditions for optimal mutagenesis of sequences of 4.0 kb in length and longer. The Diversify Kit also generates all possible types of mutations within a sequence and provides high DNA yield despite the stress of mutagenic conditions.

We used the Diversify kit to create mutants of the DsRed1 fluorescent protein (Figure 1). The DsRed1 gene was subjected to PCR under mutagenic conditions, then sub-cloned, and transformed into *E. coli*. Following colony screening, mutants were identified that had stable orange and green colors. Additionally, a novel mutant was found to change colors from green to red over time (3).

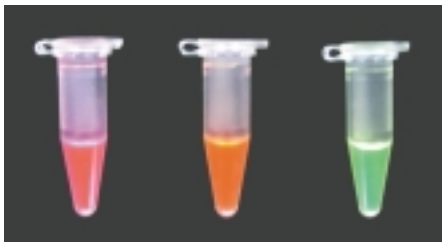


Figure 1. Random mutagenesis of DsRed1 fluorescent protein. The open reading frame of DsRed1 was subjected to random mutagenesis using the Diversify™ PCR Random Mutagenesis Kit. The proteins were expressed in *E. coli*, purified using TALON™ resin, and illuminated under ambient light. Orange (center) and green (right) mutants were identified following random mutagenesis, subcloning, and screening of the mutant library. Wild-type DsRed1 is shown at left.

Efficient site-directed mutagenesis with Transformer™

The Transformer Site-Directed Mutagenesis Kit is a highly efficient system for performing *in vitro* site-directed mutagenesis. Specific mutations (base changes, deletions, or insertions) can be introduced into a target gene or region cloned into virtually any double-stranded plasmid with a unique restriction site and a bacterial selection marker (1). Transformer can also be used to generate unidirectional nested deletions through an alternative procedure (2).

The mismatch-repair deficient *E. coli* strain BMH 71-18 *mutS* is included in the Transformer Kit. This *E. coli* strain is used to propagate the plasmid mutated with the Transformer Kit. Performing two rounds of DNA digestion and transformation ensures that a very high frequency of transformants carry the mutated plasmid, which nearly always contains both mutations—the desired mutation and the selection mutation.

Product	Size	Cat. #
Diversify PCR Random Mutagenesis Kit	30 rxns	K1830-1
Transformer Site-Directed Mutagenesis Kit	each	K1600-1
BMH 71-18 *mutS* Chemically Competent Cells	5 x 0.2 ml	C2010-1

Diversify™ Kit Components

- **TITANIUM™ *Taq* DNA Polymerase**
- **TITANIUM™ *Taq* PCR Buffer**
- **Diversify™ dNTP Mix**
- **dGTP**
- **dNTP Mix**
- **Manganese Sulfate**
- **Purified H_2O**
- **Control PCR Template & Primer Mix**
- ***Taq* I Restriction Enzyme**
- **User Manual** (PT3393-1)
- **Protocol-at-a-Glance** (PT3393-2)

Transformer™ Kit Components

- ***E. coli* BMH 71-18 *mutS* Strain**
- **Annealing Buffer**
- **Synthesis Buffer**
- **T4 DNA Polymerase**
- **T4 DNA Ligase**
- **Control Template pUC19M**
- **2 Control Primers** (mutagenic & selection)
- **Control Restriction Enzyme**
- **User Manual** (PT1130-1)

References

1. Haught, C., *et al.* (1994) *BioTechniques* **16**(1):47–48.
2. Zhu, L. & Holtz. A. (1996) *Methods Mol. Biol.* **57**:119–137.
3. Terskikh, A., *et al.* (2000) *Science* **290**:1585–1588.

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