



# expressions

a newsletter for gene cloning, expression, and analysis **volume 8 issue 2** april 2001

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Quickly print  
your own custom  
microarrays with  
spot-ready DNA



Quantitative  
gene analysis  
is now in sight

# Analyze expression of every transcript in a cell

**S**AGE™ (Serial Analysis of Gene Expression) is a powerful technique that provides quantitative gene expression profiles of every transcript in a cell. It is especially useful for quantifying low-abundance transcripts and detecting variations in abundance between cell populations. The I-SAGE™ Kit makes it convenient to construct high-quality SAGE™ libraries in your laboratory so you can elucidate complex expression patterns.

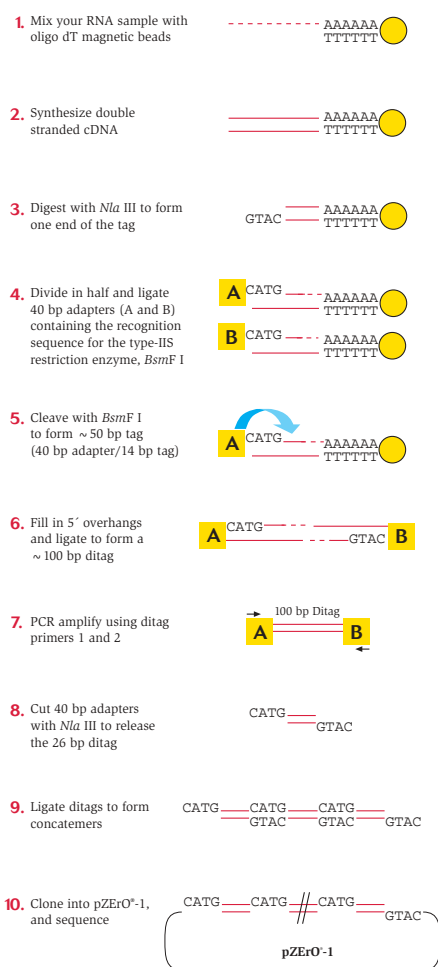
## beyond arrays

SAGE™ technology presents a unique approach to analyzing genome-wide expression. Unlike DNA arrays, SAGE™ is not limited by the selection of genes on a slide or filter. There are no hybridization or probe labeling procedures which can contribute to high background and limit the detection of low-abundance genes. SAGE™ provides quantitative and qualitative data of potentially every transcript—known or unknown—in a particular tissue or cell type without prior knowledge of sequence information. The I-SAGE™ Kit makes it convenient for you to take advantage of the powerful SAGE™ technology.

## powerful method

The I-SAGE™ Kit is designed to construct high-quality SAGE™ libraries. The protocol uses familiar molecular biology techniques to increase the efficiency of key enzymatic steps and reduce the number of purification steps compared to the original published SAGE™ protocol (1). A SAGE™ library is constructed by isolating a defined 14 bp region called a “tag” from each transcript, rather than the entire sequence. This is enough sequence to uniquely identify each mRNA transcript. Once the tags are made, they are serially ligated into concatemers (figure 1, step 9), cloned (20-40 tags per clone) (figure 1, step 10), and sequenced. There is

figure 1 - the I-SAGE™ protocol



See [www.invitrogen.com/SAGE](http://www.invitrogen.com/SAGE) for details.  
 † For more information on pZErO<sup>-1</sup>, visit [www.invitrogen.com](http://www.invitrogen.com)

no PCR bias during ditag amplification because all PCR templates are short and uniform in size (100 bp) (figure 1, step 7). You can use as little as 5 µg total RNA or 50-100 ng polyA<sup>+</sup> RNA to conserve precious starting material (2-4). The resulting library quantitatively represents every transcript expressed in a cell.

## simple serial analysis

Once concatenated tags are cloned and sequenced, they can be analyzed with the easy-to-use SAGE™ analysis software. SAGE™ software extracts and digitally enumerates the tag sequence of every transcript from the raw sequence files (1). To assign identification, extracted tags are referenced to the public SAGEmap expression database ([www.ncbi.nlm.nih.gov/SAGE](http://www.ncbi.nlm.nih.gov/SAGE)) and matched to known genes (5). SAGE™ experimental results can be directly compared to existing libraries shared on the SAGEmap database. From this comparison you'll be able to identify novel genes—without constructing additional libraries.

## reproducibility across experiments

To demonstrate that the I-SAGE™ Kit maintains the fidelity of the original SAGE™ procedure, we compared it to other SAGE™ library preparations. Expression of three housekeeping genes in the human lung carcinoma cell line (A549, ATCC #CCL185) was

continued on page 3

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**table 1** - assessment of expression among SAGE™ library preparations

Tag sequence	UniGene description	Expression (%)			
		I-SAGE™ Kit	I-SAGE™ β-test kit	SAGE™ lib1	SAGE™ lib2
TGTGTTGAGA	EF-1α/Hs.1811654	1.12	1.10	1.26	1.21
GCTTTATTG	β-actin/Hs. 180952	0.06	0.08	0.04	0.06
TACCATCAAT	GAPDH/Hs. 16947	0.43	0.51	0.48	0.40

Gene expression was evaluated in four libraries: I-SAGE™ Kit (made with the final I-SAGE™ Kit), I-SAGE™ β-test kit (made with the I-SAGE™ β-test kit), SAGE™ lib1 and SAGE™ lib2 (made from separate samples generated using conventional SAGE™ methodology by an independent laboratory). The genes analyzed were: elongation factor-1 (EF-1α), β-actin, and glyceraldehydes-3-phosphate dehydrogenase (GAPDH).

used for evaluation purposes. The quantified expression levels of the housekeeping genes were relatively consistent among the four SAGE™ libraries analyzed, demonstrating the quality preparation achieved with the I-SAGE™ Kit (table 1)\*.

### convenient kit

The I-SAGE™ Kit provides you with a complete set of pre-qualified, performance-guaranteed reagents for the convenient preparation of SAGE™ libraries. There's no need for you to gather, prepare, and test your own components. Each kit comes with nine modules that include reagents for cDNA synthesis, restriction digests, ditag formation and amplification, and cloning. Each module is thoroughly tested so you can be assured of constructing high-quality SAGE™ libraries in your lab.

### discover SAGE™

SAGE™ technology allows you to get comprehensive and quantitative gene expression results for every transcript in a cell. The I-SAGE™ Kit not only makes it convenient to use the SAGE™ technology, it also ensures you prepare a high-quality library. Call Invitrogen and inquire about the I-SAGE™ Kit today.

Product	Reactions	Cat. no.	Price
I-SAGE™ Kit			
without magnetic stand	5 libraries	T5000-01	\$3,100
with magnetic stand	5 libraries	T5001-01	\$3,380

\* Slight differences in expression between the libraries were expected since independent samples from the same cell line can reflect varied growth conditions.

#### References:

1. Velculescu, V.E. *et al.* (1995) *Science* **270**: 484-487.
2. Velculescu, V.E. *et al.* (1995) *Trends in Genetics* **16**: 423-425.
3. St. Croix, B. *et al.* (2000) *Science* **289**: 1197-1202.
4. Virlon, B. *et al.* (1999) *Proc. Natl. Acad. Science USA* **96**: 15286-15294.
5. Lash, A.E. *et al.* (2000) *Genome Res.* **10**: 1051-1060.

SAGE™ is a trademark of Genzyme Corporation. Genzyme is a registered trademark and service mark of Genzyme Corporation. The I-SAGE™ Kit is based on technology owned and licensed by Genzyme Corporation under U.S. Patent No. 5,695,937 and patents pending. For research purposes only. Inquiries regarding licenses should be made to Genzyme.



## The *most convenient* DNA electrophoresis

**D**NA electrophoresis is often used to verify size and check the integrity and quality of DNA samples. E-Gels® are bufferless, pre-cast agarose gels that include everything you need for convenient DNA electrophoresis. All you supply is your sample.

### convenient electrophoresis

Each E-Gel® is a self-contained DNA electrophoresis system that includes agarose, electrodes, ethidium bromide, and ion-exchange matrices all packaged inside a UV-transparent cassette. DNA electrophoresis has never been more convenient. Just read what one researcher had to say:

*"I don't have to melt the agarose, deal with ethidium bromide, or worry about the agarose boiling over in my microwave and having to clean up the mess. I don't have to baby-sit the gel anymore. I just turn it on and 35 minutes later, it's done. E-Gels® are always there when I need them."*

C. Pfannkoch, Celera Genomics

### get E-Gels® and get started

For the most convenient electrophoresis, get E-Gels®. They are available in 0.8%, 1.2%, 2%, and 4% agarose to meet your every need. Order your E-Gels® and get started today.

Product†	Quantity	Cat. no.	Price
0.8% E-Gel® Starter Pak	1 kit	G5000-08	\$80
1.2% E-Gel® Starter Pak	1 kit	G5000-01	\$80
2% E-Gel® Starter Pak	1 kit	G5000-02	\$80
4% E-Gel® Starter Pak	1 kit	G5000-04	\$100

† Each Starter Pak includes 9 E-Gels®, an E-Gel® Base, and a manual. E-Gels® and the E-Gel® Base can be purchased separately. Please see our web site at [www.invitrogen.com](http://www.invitrogen.com) or contact our Technical Service Representatives at 800 955 6288, ext. 2, for complete ordering information.

## Rapid, simplified purification of recombinant proteins in bacteria

The PurePro™ *Caulobacter* Expression System is designed for high-level expression and rapid, simple purification of small- to medium-sized (<450 amino acids) recombinant proteins. These characteristics make the system ideal for producing protein or peptide antigens for use in antibody production.

### ? What is *Caulobacter* and how does it differ from *E. coli*?

*Caulobacter crescentus* is a well-characterized gram-negative bacterium commonly found in freshwater environments. It is covered by a crystalline protein surface layer (S-layer) composed of the hydrophilic structural protein, RsaA (1,2). RsaA and RsaA-fusion proteins are transported to the cell surface using an efficient ABC transporter-mediated Type I secretion mechanism (3). When the RsaA transmembrane anchor domain is removed, fusion proteins are secreted directly into the medium. In contrast, *E. coli* usually secretes protein into the periplasmic space, requiring many steps for release. *Caulobacter* eliminates these time-consuming procedures, resulting in a faster, easier purification process.

figure 1 - pCX-TOPO® vector

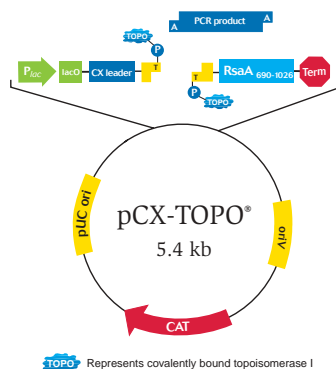
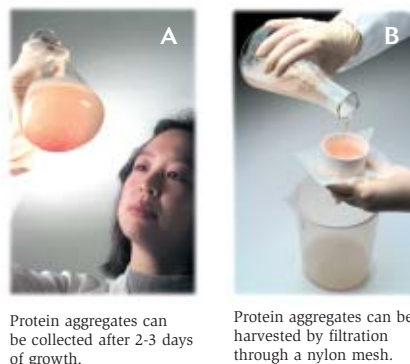


figure 2 - harvesting RsaA fusion protein aggregates from *Caulobacter*



Protein aggregates can be collected after 2-3 days of growth.

Protein aggregates can be harvested by filtration through a nylon mesh.

### ? How do I express protein with the PurePro™ *Caulobacter* Expression System?

To express your gene of interest, first clone it into the pCX-TOPO® vector (figure 1) using the effective, 5-minute TOPO® Cloning method. pCX-TOPO® contains a *lac* promoter for high-level, constitutive expression and an N-terminal CX leader sequence for optimal translational efficiency in *Caulobacter*. The vector also contains a portion of the *rsaA* gene on the C-terminus for secretion and aggregation of RsaA-fusion proteins in the medium. After cloning, simply transform your construct into B5 BAC *Caulobacter* cells. Within 2-3 days, aggregated fusion proteins will accumulate in the medium (figure 2A).

### ? How does the PurePro™ System simplify protein purification?

With PurePro™ there is no need to lyse bacteria, prepare inclusion bodies, or use purification columns. The RsaA-fusion protein is secreted directly into the medium where the RsaA portion of the fusion causes it to form a highly hydrated aggregate. To isolate the fusion protein, simply filter the medium through nylon mesh and rinse with deionized water (figure 2B). The protein aggregate captured in the mesh is typically over 90% pure.

### ? What can I do with the protein aggregate?

RsaA fusion proteins have been successfully used as antigens to generate antibodies. The RsaA secretion signal is an ideal fusion partner because it has minimal effect on the antigenicity of fusion proteins tested.

### simplify your purification

The PurePro™ *Caulobacter* Expression System contains everything you need to clone, transform, express, and purify your protein. For simplified purification of recombinant proteins, order the PurePro™ *Caulobacter* Expression System today.

Product	Quantity	Cat. no.	Price
PurePro™ <i>Caulobacter</i> Expression System	1 kit	K600-01	\$510

#### References:

1. Smit, J. et al. (1981) *J. Bacteriol.* **146**: 1135-1150.
2. Smit, J. et al. (1992) *J. Bacteriol.* **174**: 6527-6538.
3. Awram, P., and Smit, J. (1998) *J. Bacteriol.* **180**: 3062-3069.

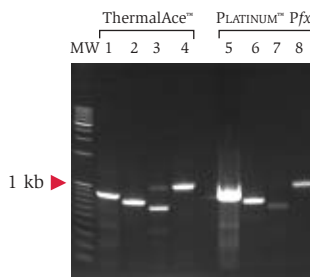
# High-fidelity, high-yield RACE PCR with efficient cloning and sequencing

The GeneRacer™ Kit ensures that you amplify only full-length transcripts in your RACE experiments. To further improve your results and save you time, the GeneRacer™ Kit now includes the Zero Blunt® TOPO® PCR Cloning Kit for Sequencing for fast cloning and streamlined sequencing of your RACE PCR products.

## improve your RACE results

The GeneRacer™ protocol works at the RNA level to eliminate truncated transcripts and ensure that you only amplify the 5' ends of full-length mRNA in your RACE experiments. This advanced GeneRacer™ method often results in a single PCR product, eliminating time spent sorting through a pool of truncated products. You'll get efficient RACE results so you can get started with your downstream experiments faster.

**figure 1** - 5' RACE PCR products using PLATINUM™ Pfx and ThermalAce™



RACE-ready cDNA was generated from 3.5 µg of HeLa total RNA using the GeneRacer™ Kit with SUPERSCRIPT™ II RT. PCR reactions were assembled according to the protocol using 1 µl of the RT reaction as template and ThermalAce™ or Pfx. PCR program was as follows: 94°C for 2 min, 35 cycles of 94°C for 30 seconds, 65°C for 30 seconds, 72°C for 1 minute, followed by 72°C for 10 minutes.

Lanes 1,5: β-actin (1.8 kb)  
Lanes 2,6: eIF4E (translation initiation factor 4E, 1.8 kb)  
Lanes 3,7: SMAP (thyroid hormone receptor coactivating protein, 3.1 kb)  
Lanes 4,8: ARF4 (ADP-ribosylation factor 4, 1.5 kb)  
MW: Mixed 1 kb and 100 bp DNA ladder

## accurate RACE PCR

High-fidelity PCR ensures the 5' end RACE sequence you amplify is correct. Using a proofreading enzyme that also delivers higher yields, such as PLATINUM™ Pfx or ThermalAce™, ensures that you generate sufficient quantities of your RACE PCR products for cloning. To illustrate the performance you can expect when using Pfx and ThermalAce™, we tested various templates ranging in size from 1.5-3.1 kb (figure 1). The single bands from one round of PCR demonstrate the accuracy of the reaction while the intensity of the bands indicates the high yield capability of each polymerase.

## fast and easy blunt-end cloning

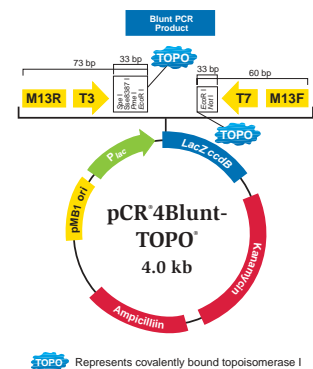
To save you time cloning blunt-end RACE PCR products, the GeneRacer™ Kit includes the Zero Blunt® TOPO® PCR Cloning Kit for Sequencing. This kit includes the pCR®4-Blunt vector (figure 2) which has been specially designed for:

- Fast, five-minute cloning–vector is provided linearized and activated with topoisomerase I
- Producing > 90% recombinants–ligation using topoisomerase is highly efficient
- Streamlined sequencing–T7, T3, M13 (–20) forward, and M13 reverse primer sites are located as close as 33 bp to the PCR insertion site

## RACE to the finish

The GeneRacer™ Kit uses an advanced technique to eliminate truncated transcripts and

**figure 2** - pCR®4Blunt-TOPO® vector



amplify only the 5' ends of full-length PCR products, providing efficient RACE results. The fast cloning and streamlined sequencing provided by the Zero Blunt® TOPO® PCR Cloning Kit for Sequencing save you time. Call Invitrogen and order today.

Product	Quantity	Cat. no.	Price
GeneRacer™ Kit			
with SUPERSCRIPT™ II RT and Zero Blunt® TOPO® PCR Cloning Kit for Sequencing	1 kit	L1502-02	\$395
with AMV RT and Zero Blunt® TOPO® PCR Cloning Kit for Sequencing	1 kit	L1500-02	\$395
PLATINUM™ Pfx DNA Polymerase <sup>4,5,14</sup>			
	100 units	11708-013	\$85
	250 units	11708-021	\$200
	500 units	11708-039	\$375
ThermalAce™ DNA Polymerase			
	200 units	E0200	\$165
	1,000 units	E1000	\$600

<sup>4,5,14</sup> These products are subject to the Limited Label License indicated by the superscript number. Please refer to the Life Technologies web site ([www.lifetech.com](http://www.lifetech.com)) or catalog for more information.

# New Destination Vectors *expand your gene expression options*



The fastest, most comprehensive route to functional gene analysis and expression is through the GATEWAY™ Cloning Technology. Four new GATEWAY™ Destination Vectors expand your gene expression options and make the system more flexible than ever.

## use more expression systems—faster

The GATEWAY™ Cloning Technology accelerates your research by allowing you to quickly clone a gene of interest into multiple expression systems for analysis. Using GATEWAY™, you clone your gene of interest once into a donor or entry vector and then use a simple recombination reaction to move your gene into a limitless number of expression vectors. The reading frame and integrity of your gene are always preserved so you can test multiple vector systems for analysis or protein production without additional cloning, subcloning, or sequence validation. You'll save hours of time for each vector you use.

## new vectors expand your options

Four new GATEWAY™ Destination Vectors further expand your GATEWAY™ expression and analysis options. pcDNA-DEST40 allows high-level expression in mammalian cells with convenient detection and purification. The GFP fusion vector, pcDNA-DEST47, expresses your protein fused to the green fluorescent protein (GFP) for non-invasive protein detection. pMT-DEST48 is designed for inducible expression in the *Drosophila* Expression System (DES®). DES® combines insect expression with simple plasmid transfection for easy protein production. And pYES-DEST52 offers tightly regulated, high-

level expression in *S. cerevisiae*. Table 1 highlights the benefits of each new vector.

## great expression results

The new GATEWAY™ Destination Vectors offer the same great expression results that you've achieved for years from the parent vectors. That's because GATEWAY™-adapted vectors are always tested for expression in side-by-side comparisons with their parent vector to ensure equivalent expression (data available at [www.invitrogen.com](http://www.invitrogen.com)).

## expand your gene analysis research

There's no faster or more flexible cloning and expression technology than GATEWAY™. Four new GATEWAY™ Destination Vectors give you even more options for protein production and analysis. Call and order today.

table 1 - new GATEWAY™ Destination Vectors

Host	Destination Vector	Parent Vector*	Benefits
Mammalian	pcDNA-DEST40	pcDNA3.1 <sup>+</sup> /V5-His	<ul style="list-style-type: none"> <li>• Full-length CMV promoter for high-level expression</li> <li>• C-terminal V5 epitope and 6xHis tag for convenient detection and purification of your fusion proteins</li> </ul>
	pcDNA-DEST47	pcDNA3.1 <sup>+</sup> /CT-GFP	<ul style="list-style-type: none"> <li>• C-terminal GFP fusion for non-invasive, <i>in vivo</i> detection</li> <li>• Full-length CMV promoter for high-level expression</li> </ul>
<i>Drosophila</i> S2 cells	pMT-DEST48	pMT/V5-His	<ul style="list-style-type: none"> <li>• <i>Drosophila</i> metallothionein (MT) promoter for inducible expression in S2 cells</li> <li>• C-terminal V5 epitope and 6xHis tag for convenient detection and purification of your fusion proteins</li> </ul>
Yeast	pYES2-DEST52	pYES/CT	<ul style="list-style-type: none"> <li>• Promoter and enhancer sequences from the <i>GAL1</i> gene for tightly regulated, high-level expression in <i>S. cerevisiae</i></li> <li>• C-terminal V5-His tag for convenient protein detection and purification</li> </ul>

\* For information on the parent vectors, check our web site at [www.invitrogen.com](http://www.invitrogen.com)

Product	Quantity	Cat. no.	Price
pcDNA-DEST40	6 µg	12274-015	\$110
pcDNA-DEST47	6 µg	12288-010	\$140
pMT-DEST48	6 µg	12282-018	\$95
pYES-DEST52	6 µg	12286-019	\$95



## learn more about GATEWAY™

For the latest information on the GATEWAY™ Cloning Technology and new GATEWAY™-compatible vectors, visit our web site at [www.invitrogen.com](http://www.invitrogen.com).

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## CONCERT™ 96 Plasmid Purification System

# Fast and simple *high-throughput DNA* isolation without alkaline lysis

Now you can perform high-throughput plasmid DNA purification without alkaline lysis and cell harvesting. With the CONCERT™ 96 Plasmid Purification System you'll eliminate these tedious procedures and get reliable results—fast.

## fast purification, less work

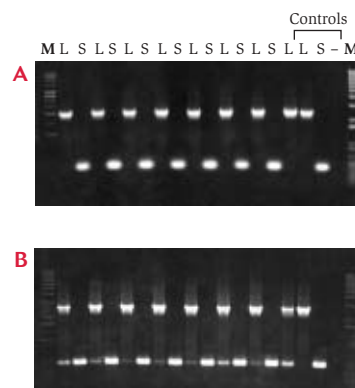
The CONCERT™ 96 Plasmid Purification System is a unique centrifugation-based system that allows you to simultaneously purify multiple plasmid DNA samples in a 96-well format. This innovative, solid-phase technology eliminates the numerous reagents and mixing steps associated with alkaline lysis, saving you time and labor. Now you can directly load bacterial cultures onto the purification matrix. There are no cell harvesting and resuspension steps. The streamlined CONCERT™ 96 protocol (figure 1) requires no mixing, no transfer of viscous material, and fewer pipetting steps—and it can be completed in as little as 45 minutes. Plasmid DNA purified with CONCERT™ 96 is ideal for automated sequencing, restriction enzyme digestions, and PCR including applications for microarrays.

## high-performance system

In addition to time savings, the CONCERT™ 96 Plasmid Purification System offers:

- High yields—purify up to five micrograms per 200 ml sample of high-copy number plasmid DNA up to 28 kb in size
- High-purity preparations—there are no mixing and filtration steps, reducing the risk of well-to-well cross contamination seen in vacuum-based purification protocols (figure 2)
- Reliable results—sequencing of samples purified using CONCERT™ 96 demonstrates increased read lengths and superior data quality (figure 3).

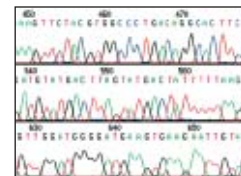
figure 2 - analysis of cross-contamination



Plasmid DNA was isolated from clones carrying two different sized inserts (L-2.4 kb; S-400 bp). Clones were grown and purified in adjacent wells. DNA samples were diluted 1:800 and 1 µl was used as template in PCR with primers flanking the cloning site.

Panel A: Results from plasmids isolated using CONCERT™ 96.  
Panel B: Results from plasmids isolated using a commercially available vacuum-based 96-well method. Clone S amplifies preferentially when there are a mixture of clones in a DNA sample.  
M: 1 Kb Plus DNA Ladder

figure 3 - high-quality sequencing data



Electropherogram of a rat BAC subclone purified using the CONCERT™ 96 System. Five percent of the template DNA was used in a 1/4X BigDye Terminator sequencing reaction followed by isopropanol precipitation. One-tenth of the sequencing reaction was loaded onto a capillary for analysis.

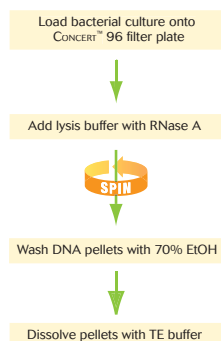
## economical format

Unlike other high-throughput systems, CONCERT™ 96 allows you to process fewer than 96 samples at a time. This means you can use only the number of wells you need and store the filter plate for later use. You have the freedom to perform smaller experiments without wasting money on unused reagents.

## try CONCERT™ 96 today

The CONCERT™ 96 Plasmid Purification System includes all the components you need for quick and hassle-free high-throughput plasmid DNA isolation. Order today.

figure 1 - schematic of the CONCERT™ 96 Plasmid Purification System protocol



Product	Quantity	Cat. no.	Price
CONCERT™ 96 Plasmid Purification System*	1 kit	12263-018	\$365

For large volume needs, individual components are sold separately. Contact Customer Service for ordering information at 800 955 6288.

\* This product is subject to Limited Label License No. 27. Please refer to the Life Technologies web site ([www.lifetech.com](http://www.lifetech.com)) or catalog for details.

# New microwave procedure *speeds up protein staining*

When ultra-fast staining results are critical in characterizing your proteins in polyacrylamide gels, try SimplyBlue™ SafeStain. Using the SimplyBlue™ microwave procedure, you'll achieve sensitive staining results in just 12 minutes—that's faster than any other commercially available protein stain.

## microwave protocol saves time

SimplyBlue™ SafeStain is a uniquely formulated Coomassie® G-250 stain specifically designed to give you fast, sensitive staining results. SimplyBlue™ is ready-to-use, eliminating preparation time and effort. The new microwave procedure (table 1) makes staining even faster. Following this procedure, you will achieve sensitive staining results in just 12 minutes. You'll save hours over other methods.

## sensitive staining in the fast lane

Until now, fast protein staining has produced lower sensitivity results. But with the SimplyBlue™ microwave staining procedure, you'll detect as little as 20 ng in 12 minutes (figure 1A, lane 4). Other commercially available stains require as much as three

table 1 - fast microwave procedure using SimplyBlue™ SafeStain

Step	Procedure	Elapsed Time	Sensitivity
1	Place the gel in 100 ml of ultrapure water and microwave on High (950 to 1100 watts) for 1 minute.	1 minute	—
2	Gently shake the gel on an orbital shaker or rocker for 1 minute. Discard wash.	2 minutes	—
3	Repeat steps 1 and 2 two more times.	6 minutes	—
4	Add 20 ml of SimplyBlue™ SafeStain and microwave on High for 45 seconds to 1 minute.	7 minutes	—
5	Shake the gel on an orbital shaker or rocker for 5 minutes. Discard stain.	12 minutes	20 ng BSA
6 <sup>†</sup>	Wash the gel in 100 ml of ultrapure water on an orbital shaker or rocker for 10 minutes.	22 minutes	10 ng BSA
7 <sup>†</sup>	Add 20 ml of 20% NaCl and shake gel on an orbital shaker or rocker for at least 5 minutes.	27 minutes	5 ng BSA

<sup>†</sup> Steps 6 and 7 are only necessary if higher sensitivity results are desired.

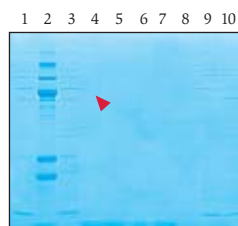
**Note:** The times and volumes used in this procedure are for 1.0 mm thick mini-gels.

hours staining time to achieve this same sensitivity. Using SimplyBlue™, you'll achieve

the most sensitive staining results in the least amount of time.

figure 1 - results obtained using the SimplyBlue™ SafeStain microwave procedure

### A. 12 minutes



Immediately after 12 minutes (table 1, step 5), 20 ng reduced BSA is visible (as indicated by the arrow in lane 4).

### B. 27 minutes



Immediately after 27 minutes (table 1, step 7), 5 ng of reduced BSA is visible (as indicated by the arrow in lane 6).

Lanes 1,3,9,10: 5 µl Mark12™ Standard  
Lane 2: 6 µg protein mix  
Lane 4: 20 ng reduced BSA  
Lane 5: 10 ng reduced BSA  
Lane 6: 5 ng reduced BSA  
Lane 7: 2.5 ng reduced BSA  
Lane 8: blank

Samples were run on a NuPAGE® Novex Bis-Tris 4-12% Gel and then stained using the SimplyBlue™ microwave procedure. Total time for the entire staining procedure is indicated above each gel.

## speed up your staining today

Only SimplyBlue™ gives you the speed and sensitivity you require for ultra-fast Coomassie staining results. Start staining in the fast lane. Order SimplyBlue™ today.

Product	Reactions	Cat. no.	Price
SimplyBlue™ SafeStain	1 L*	LC6060	\$75
	3.5 L (w/pump)	LC6065	\$195

\* Sufficient reagent is supplied to stain 50 mini-gels.

# Take the fastest route from PCR to pET expression

The new pET Directional TOPO® Expression Kits are specifically designed for five-minute, directional cloning into a pET vector for high-level expression in *E. coli*. You'll eliminate time-consuming subcloning steps, save hours of time, and get great expression.

## How do I save time with the pET Directional TOPO® Expression Kits?

The pET Directional TOPO® Expression Kits take advantage of Directional TOPO® Cloning technology to reduce time spent on tedious cloning and screening steps. Your cloning is complete in five minutes and results in >90% of recombinant clones in the correct orientation for expression. To use, simply mix your PCR product with a pET Directional TOPO® vector, incubate five minutes, and transform *E. coli*. There's no need for ligase, restriction enzymes, or long incubations, saving you valuable time. Because >90% of the inserts will be in the proper orientation for expression, screening time is greatly reduced. You'll perform fewer minipreps and proceed quickly to the expression part of your experiment.

## What features do the pET Directional TOPO® vectors offer?

In addition to being ready for Directional TOPO® Cloning, the pET Directional TOPO® vectors offer the IPTG-inducible T7 *lac* promoter for the same regulated, high-level expression you've relied on for years from pET vectors. In addition, these vectors carry fusion tags for convenient protein detection and purification (table 1).

## What level of expression can I expect from the pET Directional TOPO® vectors?

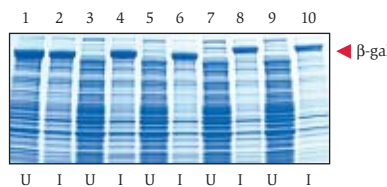
pET vectors are well-known for high-level *E. coli* expression. Expression levels achieved using the Directional TOPO®-adapt-

table 1 - features of the pET Directional TOPO® vectors

Vectors	Fusion Tags	Advantage
pET100/D-TOPO®	N-terminal Xpress™, 6xHis	Cleavable detection and purification tag
pET101/D-TOPO®	C-terminal V5, 6xHis	Convenient detection and purification
pET102/D-TOPO®	N-terminal Xpress™, Thioredoxin	Cleavable thioredoxin tag enhances protein translation and solubility
	C-terminal V5, 6xHis	Convenient detection and purification

ed pET/D-TOPO® vectors are equivalent to those seen with their non-TOPO® counterparts (figure 1).

figure 1 - expression in Directional TOPO®-adapted pET vectors



The *lacZ* gene was Directionally TOPO® Cloned into pET100/D-TOPO®, pET101/D-TOPO®, and pET102/D-TOPO® and cloned by restriction digest and ligation into the non-TOPO® pET15b and pET32a vectors. Constructs were transformed into BL21 Star™(DE3) *E. coli*. A single colony from each transformation was used to inoculate 1 ml LB medium supplemented with 100 µg/ml ampicillin. Induction with 1 mM IPTG was performed at OD<sub>600</sub> = 0.5. Two and one-half hours postinduction, cultures were harvested by centrifugation. Pellets were resuspended in 300 µl sample buffer. Ten microliters of each sample was analyzed on a 4-20% Novex® Tris-Glycine gel.

Lanes 1 and 2: pET15b/*lacZ*  
 Lanes 3 and 4: pET101/D-TOPO®/*lacZ*  
 Lanes 5 and 6: pET100/D-TOPO®/*lacZ*  
 Lanes 7 and 8: pET102/D-TOPO®/*lacZ*  
 Lanes 9 and 10: pET32a/*lacZ*  
 U = Uninduced I = Induced

## What do the pET Directional TOPO® Expression Kits include?

The pET Directional TOPO® Expression Kits contain PCR reagents, linearized and topoisomerase-activated pET/D-TOPO® vector, One Shot® TOP10 Chemically Competent *E. coli* for high-efficiency transformations and BL21 Star™ (DE3) One Shot® Chemically Competent *E. coli* for maximized expression. All components are optimized to work together, ensuring you the best results in your T7 expression experiments.

## get to pET expression faster

Save cloning and screening time in your next pET expression experiment without sacrificing results. Order a pET Directional TOPO® Expression Kit from Invitrogen today.

Product	Reactions	Cat. no.	Price
pET Directional TOPO® Expression Kit			
with pET100/D-TOPO® vector	20 rxns	K100-01	\$400
with pET101/D-TOPO® vector	20 rxns	K101-01	\$400
with pET102/D-TOPO® vector	20 rxns	K102-01	\$400

# Save time performing inducible mammalian expression studies with multiple genes

Performing functional studies that require regulating gene transcription from a stable cell line can be a tedious and time-consuming task—especially when working with multiple genes. The Flp-In™ T-REx™ expression vectors combine two advanced technologies to save you weeks of time generating multiple stable cell lines for your inducible expression experiments.

## time-saving integration studies

Inducible mammalian systems have been extensively used to study protein function in stable cell lines. Unfortunately, these systems are not set up to easily study multiple genes. For each gene, single clones need to be isolated, tested for expression, and expanded. The new Flp-In™ T-REx™ expression vectors use site-specific recombination to integrate an inducible vector into a pre-determined locus in the genome. This eliminates the need for clonal isolation and testing, saving you weeks of time.

## powerful vectors

Two Flp-In™ T-REx™ vectors are available—pcDNA5/FRT/TO® and pcDNA5/FRT/TO-TOPO® (figure 1). Each vector contains:

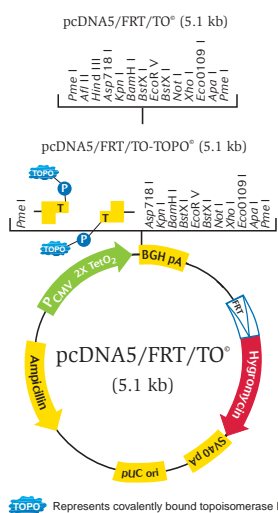
- A FRT site for efficient targeted integration† into a Flp-In™ T-REx™ Cell Line\*
- A hybrid CMV/TetO<sub>2</sub> promoter for tetracycline-regulated expression† of your gene
- The hygromycin resistance gene for selection of stable clones

In addition, pcDNA5/FRT/TO-TOPO® is topoisomerase-activated to enable five-minute ligation of PCR products. No matter which vector you choose, you'll save time with site-specific stable integration and achieve tightly regulated expression.

## how it works

To rapidly generate multiple stable cell lines, clone your genes of interest into a Flp-In™ T-REx™ vector and co-transfect the construct

figure 1 - Flp-In™ T-REx™ expression vectors

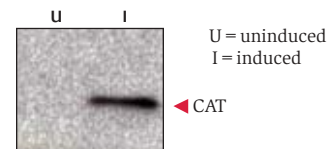


with a source of Flp recombinase into the Flp-In™ T-REx™-293 Cell Line\*. This cell line contains a single genomic FRT site for targeting integration and stably expresses the TetR protein for regulating expression. Integration of the Flp-In™ T-REx™ expression vector results in a population of hygromycin-resistant clones that have integrated the expression construct into the same genomic locus. These cells are isogenic and can be passaged *en masse*, eliminating the time and effort of isolating and expanding individual clones—an especially large task when studying multiple genes.

## regulated results

To demonstrate the tightly regulated expression you'll get using the Flp-In™

figure 2 - expression in the Flp-In™ T-REx™-293 Cell Line



The Flp-In™ T-REx™-293 Cell Line was transfected with pcDNA5/FRT/TO\*/CAT and pOG44, a source of Flp recombinase, followed by selection with 0.1 mg/ml hygromycin for 18 days. Post-selection, cells were induced with 1 µg/ml tetracycline or remained uninduced. Cell lysates were harvested 24 hours post-induction and 10 µg of total protein was loaded on an SDS-PAGE gel. Western blot analysis was performed using the Anti-V5-HRP Antibody.

T-REx™ vectors, we transfected the pre-made Flp-In™ T-REx™-293 Cell Line with pcDNA5/FRT/TO\*/CAT. As shown in figure 2, protein is expressed only upon induction with tetracycline.

## get the advantage now

For rapid generation of multiple stable cell lines with the ability to regulate expression, get Flp-In™ T-REx™. Order today.

Product	Quantity	Cat. no.	Price
Flp-In™ T-REx™ Core Kit	1 kit	K6500-01	\$799
pcDNA5/FRT/TO*	20 µg	V6520-20	\$315
pcDNA5/FRT/TO* TOPO* TA Expression Kit	20 rxns	K6510-20	\$365
Flp-In™ T-REx™-293 Cell Line	3 x 10 <sup>6</sup> cells	R780-07	\$550

† For information on the Flp-In™ and T-REx™ technologies, check out our web site at [www.invitrogen.com](http://www.invitrogen.com).

\* The Flp-In™ T-REx™-293 Cell Line is available from Invitrogen or you can create your own Flp-In™ T-REx™ cell line. For details, call Technical Service at 800 955 6288, ext. 2.

## OLIGOFECTAMINE™ Reagent

# Ensure delivery and *functional activity* of your antisense oligonucleotides

When it comes to transfecting antisense oligos, delivery doesn't assure function. OLIGOFECTAMINE™ Reagent demonstrates efficient delivery of antisense oligos with high specific activity and low non-specific effects. Expect superior transfection with specific effects on your target with OLIGOFECTAMINE™ Reagent.

## optimized for efficient delivery

Typical problems encountered when transfecting antisense oligos include delivery without functional activity, high non-specific effects, and cytotoxicity. OLIGOFECTAMINE™ Reagent is a proprietary formulation that overcomes these hurdles. This non-toxic lipid reagent is designed specifically for the transfection of oligonucleotides into eukaryotic cells.

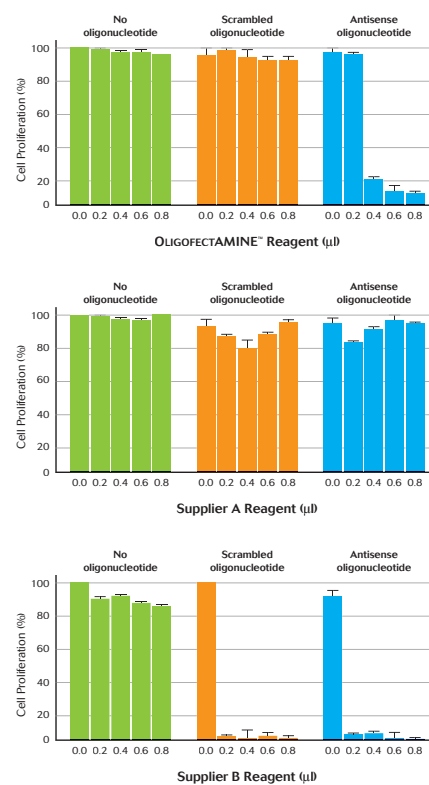
## rely on the proof of a functional assay

Manufacturers of other transfection reagents claim high-efficiency transfection of antisense oligos without mention of functional activity once inside the cell. OLIGOFECTAMINE™ Reagent is the only transfection reagent proven to transfect functionally active antisense oligos. To demonstrate, HeLa cells were transfected with a *c-myc* phosphorothioate antisense oligonucleotide using OLIGOFECTAMINE™. Results show high specific activity and low non-specific effects of the targeted antisense oligo (figure 1). For antisense applications, the functional delivery provided by OLIGOFECTAMINE™ ensures great results.

## flexible use

OLIGOFECTAMINE™ has been successfully used to transfect a broad range of cell lines including CHO, Embryonic Kidney 293, NIH 3T3, and HeLa cells. In addition,

figure 1 - transfection of a *c-myc* antisense oligonucleotide into HeLa cells



HeLa cells were transfected with no oligonucleotide (■), 200 nM of a scrambled oligonucleotide (■), or 200 nM of a phosphorothioate-modified antisense oligonucleotide (■) using OLIGOFECTAMINE™ Reagent, Supplier A Reagent, or Supplier B Reagent. Oligonucleotides were HPLC-purified. The antisense oligonucleotide was directed against *c-myc* and is expected to decrease cell proliferation. Results are expressed as a mean ± SEM for N=8 for at least three separate experiments.

OLIGOFECTAMINE™ works for both nuclear and cytoplasmic targets. You won't have the hassle of purchasing and storing numerous oligo transfection reagents for different cell types and targets. For your added convenience, OLIGOFECTAMINE™ has been optimized for use in high-throughput applications.

## easy and economical

OLIGOFECTAMINE™ is easy to use. Simply dilute the reagent, mix with your oligonucleotide, and add to cells. To assist you with your experimental design, the included protocol provides starting points for optimizing antisense applications. OLIGOFECTAMINE™ Reagent also allows you to use nanomolar amounts of antisense oligos compared to micromolar amounts used in some protocols. One milliliter of OLIGOFECTAMINE™ is sufficient for 1,250-2,500 transfections in 96-well plates or 500-1,000 transfections in 24-well plates. A small amount of reagent can go a long way—that means significant cost savings for you.

## you got it in—make sure it functions

For successful transfection of your antisense oligonucleotides with demonstrated functional activity, order OLIGOFECTAMINE™ today.

Product	Reactions	Cat. no.	Price
OLIGOFECTAMINE™ Reagent*	1 ml	12252-011	\$190

\* This product is subject to Limited Label License No. 12. Please refer to the Life Technologies web site ([www.lifetech.com](http://www.lifetech.com)) or catalog for details.

# TOPO® Cloning Kits with DH5α™-T1<sup>R</sup> *E. coli* guarantee your *cloning results*

**T**OPO® Cloning Kits make cloning PCR products faster and more successful. With yields of ≥95% recombinants via a simple five-minute, room-temperature ligation, it's the most effective PCR cloning technology available. DH5α™ *E. coli* strains consistently provide superior transformation results. Now TOPO® Cloning Kits are available with the latest DH5α™ strain to guarantee your great cloning results.

## TOPO® means results

TOPO® Cloning revolutionized cloning by replacing DNA ligase with topoisomerase I. This enables five-minute, bench-top ligations yielding ≥95% recombinants. All TOPO® Cloning vectors are supplied linearized with topoisomerase I covalently bound to each 3' phosphate, making them ready for rapid ligation. TOPO TA Cloning® vectors contain 3'-T overhangs for ligating PCR products amplified by *Taq* polymerase (figure 1). Zero Blunt® TOPO® vectors have blunt ends to ligate the blunt-end PCR products produced by proofreading polymerases. Whichever enzyme you use for your PCR, there's a TOPO® vector available that will clone the resulting products quickly and reliably.

## protect your valuable samples with DH5α™-T1<sup>R</sup>

For years scientists have chosen DH5α™ *E. coli* for their transformation needs. Now, DH5α™ is available with T1 phage resistance. The DH5α™-T1<sup>R</sup> *E. coli* strain offers

**table 1** - features of the DH5α™ *E. coli* strain

<i>tonA</i>	Guards against T1 and T5 phage infection, preventing loss of precious DNA clones
<i>endA1</i>	Cleaner preparations of DNA and better results in downstream applications due to the elimination of non-specific digestion by Endonuclease I
<i>recA1</i>	Reduced occurrence of unwanted recombination in cloned DNA
<i>hsdR</i>	Efficient transformation of unmethylated DNA from PCR applications
<i>lacZΔM15</i>	Blue/white color screening of recombinant clones carrying the alpha fragment of β-galactosidase

the same great features as DH5α™ (table 1) with the addition of the *tonA* genotype, which confers T1 phage resistance. This reduces the risk of losing your precious samples and incurring down time due to phage contamination. DH5α™-T1<sup>R</sup> *E. coli* are provided at an efficiency of 1 x 10<sup>9</sup> cfu/μg supercoiled pUC19 in the convenient One Shot® format. Supplied as 50 μl, single-use aliquots, One Shot® cells eliminate efficiency-zapping freeze-thaw cycles and money wasted on unused cells.

## get it together

For the best cloning and transformation results, plus protection from T1 phage con-

tamination, get a TOPO® Cloning Kit with DH5α™-T1<sup>R</sup> Chemically Competent *E. coli*. Call and order today.

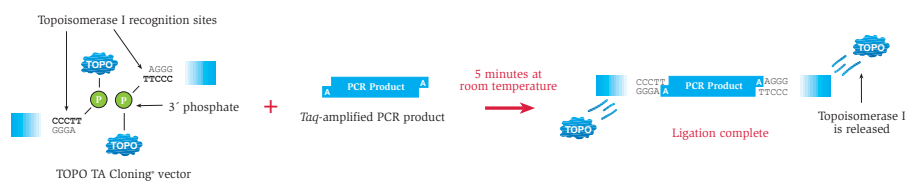
Product*	Quantity	Cat. no.	Price
TOPO TA Cloning® Kit	20 rxns	K4520-01	\$321
	40 rxns	K4520-40	\$589
TOPO TA Cloning® Kit Dual Promoter	20 rxns	K4620-01	\$336
	40 rxns	K4620-40	\$617
	TOPO TA Cloning® Kit for Sequencing	20 rxns	K4595-01
	40 rxns	K4595-40	\$617
Zero Blunt® TOPO® PCR Cloning Kit	20 rxns	K2820-20	\$352
	40 rxns	K2820-40	\$647
Zero Blunt® TOPO® PCR Cloning Kit for Sequencing	20 rxns	K2895-20	\$352
	40 rxns	K2895-40	\$647

\* All of the above kits are supplied with DH5α™-T1<sup>R</sup> One Shot® Chemically Competent *E. coli*. For information on the TOPO® Cloning vectors supplied in each kit or for the genotype of DH5α™-T1<sup>R</sup> strain, please visit our web site at [www.invitrogen.com](http://www.invitrogen.com) or contact our Technical Service Representatives.

## Reference:

Shuman, S. (1994) *J. Biol. Chem.* **269**: 32678-32684.

**figure 1** - TOPO TA Cloning® of a *Taq*-amplified PCR product



# Use BAC-TO-BAC® for the *fastest method* of baculovirus production

The BAC-TO-BAC® Baculovirus Expression System provides the fastest method for producing recombinant baculovirus. Using BAC-TO-BAC®, you can express your protein of interest in as little as 9 days—that's weeks faster than any other system.

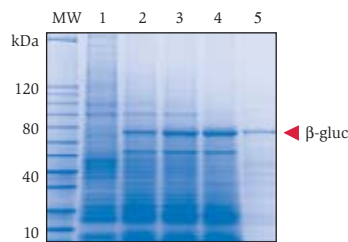
## get eukaryotic protein faster

For years scientists have used recombinant baculovirus to express large quantities of eukaryotic protein in insect cells. The BAC-TO-BAC® System for rapid baculovirus production provides significant advantages over other systems. It uses a unique Bacmid shuttle vector to produce a high titer of pure, recombinant baculovirus particles in the very first insect cell transfection plate. Traditional baculovirus systems yield a low-titer ( $10^2$  pfu/ml) viral supernatant that must be purified and amplified before it can be used for expression. Using BAC-TO-BAC® technology, your initial insect cell transfection generates viral titers as high as  $10^7$ . You can use these titers directly and start testing for protein expression by day nine of your experiment. This saves you up to two weeks over traditional baculovirus methods.

## efficient viral generation

BAC-TO-BAC® provides the most efficient method for generating recombinant virus. To use, transform pFASTBAC™, the BAC-TO-BAC® expression vector, containing your gene of interest into DH10BAC™ *E. coli*. This strain of *E. coli* contains a specialized Bacmid that recombines with your construct via site-spe-

**figure 2** - expression with the BAC-TO-BAC® Baculovirus Expression System



Sf9, Sf21, and High Five™ cells were infected at an MOI of 10 with GUS-recombinant virus. Samples were analyzed by SDS-PAGE. 25 µg protein was loaded into each lane (48 h post-infection).

MW: 10 kD protein ladder  
Lane 1: uninfected Sf9  
Lane 2: Sf9 infected cells  
Lane 3: Sf21 infected cells  
Lane 4: High Five infected cells  
Lane 5: 2 µg purified β-glucuronidase.

cific transposition to create a recombinant expression Bacmid. When the recombinant Bacmid is transfected into insect cells, pure, recombinant viral particles are produced (figure 1). This generates a ready-to-use viral stock of approximately  $10^7$  pfu/ml—that's higher than any other method.

## high expression levels

pFASTBAC™ uses the strong polyhedrin promoter to generate high levels of expression

in various insect cell lines. To demonstrate, β-glucuronidase was expressed in Sf9, Sf21, and High Five™ cells using the BAC-TO-BAC® System. Figure 2 shows the high expression levels achieved in all three cell lines.

## faster expression

You'll save weeks of time and get great results in your baculovirus expression when you take advantage of the BAC-TO-BAC® System. The BAC-TO-BAC® Baculovirus Expression System comes as a complete kit including the pFASTBAC™ vector and ready-to-use DH10BAC™ competent *E. coli* for cloning, CELLFECTIN™ reagent for efficient transfection, controls to ensure your success, and a comprehensive manual. Order today.

Product	Quantity	Cat. no.	Price
BAC-TO-BAC® Baculovirus Expression System	1 kit	10359-016	\$525
pFASTBAC™	10 µg	10360-014	\$231
DH10BAC™ Competent <i>E. coli</i>	0.5 ml	10361-012	\$161

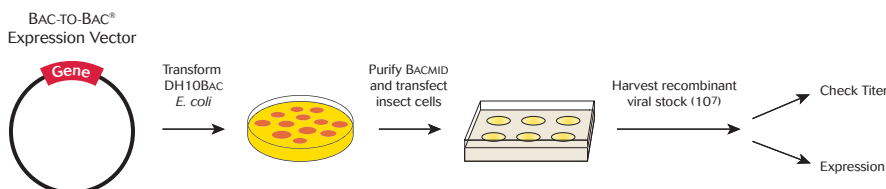


## the power of GATEWAY™

The GATEWAY™ Cloning Technology provides a rapid, efficient route to protein expression in multiple systems. Three BAC-TO-BAC® Expression Vectors have been adapted for use with GATEWAY™ Technology, allowing you quick and flexible access to baculovirus expression. For more information, please visit our web site at [www.invitrogen.com](http://www.invitrogen.com).

These products are sold under license from Monsanto for research purposes only. No license for commercial use is included. Requests for commercial manufacture or use should be directed to the Office of the Director, Mail Zone 02A, Monsanto Corporate Research, 800 N. Lindbergh, St Louis, MO.

**figure 1** - virus generation with BAC-TO-BAC® technology



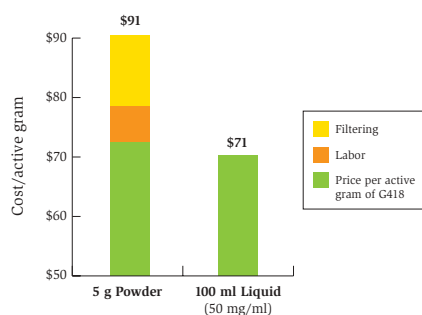
## Liquid GENETICIN® solution *offers savings* and reliable selection

For over twenty years GENETICIN® has been the antibiotic of choice for selecting and maintaining transformed eukaryotic cells in culture. To make using GENETICIN® simple and convenient, it is available in a ready-to-use liquid format. This format offers reliable, potent selection while saving you time and money.

### ready-to-use solution saves time and money

Liquid GENETICIN® is a ready-to-use G418 solution supplied at a 50 mg/ml concentration. You'll save time and money because there's no need to weigh out messy powders or solubilize and filter your preparations. In addition, your experiments won't suffer from errors or contaminants introduced during stock solution preparation. In the end you'll save hours of time and about 15% of the cost using liquid GENETICIN® compared to the powdered antibiotic (figure 1).

**figure 1** - actual cost of GENETICIN® antibiotic powder versus liquid



### high quality ensures success

During the manufacturing process, liquid GENETICIN® solution is stringently tested to ensure successful selection in your experiments. The high-quality solution you receive will provide:

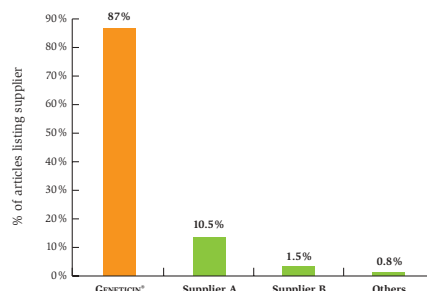
- Higher potency than powder—100% activity vs. 75% activity
- Consistent active concentration—every lot is supplied at 50 mg/ml
- Great results—functional testing in cell culture assay ensures performance and validates label potency

Liquid GENETICIN® is guaranteed active for two years. Expiration dates are clearly labeled on all bottles so you can perform your selection with confidence.

### researchers agree on GENETICIN®

Scientists who demand quality, potency, consistency, and economy have relied on GENETICIN® for years. Hundreds of papers published in national and international journals prove the reliability of GENETICIN® (figure 2). When you use GENETICIN®, you'll experience the same successful selection and maintenance in your protocols.

**figure 2** - G418 antibiotic cited in the literature



### be selective

Now you can save time and money and be selective with your antibiotic. For reliable, potent selection, order ready-to-use liquid GENETICIN® solution today.

Product*	Quantity	Cat. no.	Price
GENETICIN® Selective Antibiotic Liquid, 50 mg/ml (G418 Sulfate)	20 ml	10131-035	\$98
	100 ml	10131-027	\$355
GENETICIN® Selective Antibiotic Powder	1 g	11811-023	\$71
	5 g	11811-031	\$266

### Use GENETICIN® with GATEWAY™ Cloning

GENETICIN® works great with the new pcDNA-DEST40 and pcDNA-DEST47 GATEWAY™ Destination Vectors. For more information on these vectors and other new developments in the GATEWAY™ Cloning Technology, see the article on [page 6](#).

# Ready-to-use western blotting kits provide *faster protein detection*

**M**ost commercially available western blotting detection systems require you to spend a substantial amount of time optimizing reagents and protocols. The pre-optimized WesternBreeze® Immunodetection Kits eliminate tedious optimization steps and allow you to quickly and easily obtain western blot results. You'll save time and effort on every western blot.

## ? How can WesternBreeze® Immunodetection Kits speed up my protein detection?

WesternBreeze® is the only complete, pre-optimized western blotting system designed to obtain sensitive results in minimal time. Unlike other blotting kits, each WesternBreeze® Kit includes all the reagents you need for blotting and detection with your own primary antibody. All kit reagents have been optimized at a predetermined concentration and tested to work together to ensure great results. There's no need for you to spend time and effort gathering, preparing, and testing your own solutions. With WesternBreeze®, all of the optimization work has been done for you, saving you hours of time and effort.

## ? How much time can I save with WesternBreeze®?

The fast and simple WesternBreeze® protocol allows you to get great western blot results in less than three hours. The time required for all major steps—including blocking, primary antibody and secondary antibody incubation—is much less than that

needed in conventional protocols (table 1). In addition, the WesternBreeze® ready-to-use and easy-to-dilute reagents minimize the time spent preparing solutions. All together, you'll save up to 17 hours with WesternBreeze® Kits over other western blot detection methods.

## ? How can I visualize the signals achieved with WesternBreeze®?

The alkaline phosphatase (AP)-based WesternBreeze® Immunodetection Kits enable you to easily visualize your protein bands using either chemiluminescent light emission or colorimetric stain. The WesternBreeze® Chemiluminescent Kits apply a ready-to-use CDP-Star® substrate that is catalyzed by AP to produce light signals at the specific protein bands. These signals can be captured by X-ray film (figure 1A), CCD-camera image systems, and most luminometers. The WesternBreeze® Chromogenic Kits use a ready-to-use BCIP/NBT\* substrate that is catalyzed by AP to form a stable purple color at the specific bands directly on the membrane (figure 1B). With WesternBreeze®, you'll be able to easily

obtain western blot results by film, image system, or colorimetric stain, whichever is suitable for your laboratory setting.

**figure 1** - sensitive signals achieved with the WesternBreeze® Kit



One nanogram of human IgG developed with the WesternBreeze® Chemiluminescent (A) and Chromogenic (B) Kits.

## fast and easy protein detection

You can save time and effort in both optimizing solutions and obtaining final results with the WesternBreeze® Immunodetection Kits. Six kits are currently available so you can choose the one that best fits your needs. To experience fast protein detection with minimal effort, call Invitrogen and place your order today.

Product	Quantity†	Cat. no.	Price
WesternBreeze® Chromogenic Detection Kit			
Anti-Mouse	1 kit	WB7103	\$205
Anti-Rabbit	1 kit	WB7105	\$205
Anti-Goat	1 kit	WB7107	\$205
WesternBreeze® Chemiluminescent Detection Kit			
Anti-Mouse	1 kit	WB7104	\$255
Anti-Rabbit	1 kit	WB7106	\$255
Anti-Goat	1 kit	WB7108	\$255

\* BCIP/NBT is a compound of 5-bromo-4-chloro-3-indolyl-1-phosphate/nitro blue tetrazolium.

† Each kit supplies sufficient reagents for detection of 20 mini-blots.

CDP-Star® is a registered trademark of Tropix, Inc.

**table 1** - time comparison of the WesternBreeze® and conventional western blotting protocols

Major Steps	WesternBreeze® Protocol	Conventional Protocol
Prepare solutions	5 minutes	30 minutes
Incubate membrane in blocking solution	30 minutes	1 to 15 hours
Incubate membrane in primary antibody	1 hour	1 to 2 hours
Incubate membrane in secondary antibody	30 minutes	1 to 2 hours
<b>Total time</b>	<b>&lt; 3 hours</b>	<b>4 to 20 hours</b>

## High-quality microarrays ensure accurate analysis results

Screen thousands of genes in a single experiment with ResGen™ GeneFilters® microarrays from Invitrogen. Each array is spotted with thousands of carefully selected human, rat, or mouse cDNA clones, or yeast ORFs. Each microarray must meet stringent quality standards, ensuring that you obtain accurate downstream results in your gene expression studies.

### choice of quality microarrays

GeneFilters® microarrays offer a convenient method for parallel assessment of gene expression. Each microarray consists of thousands of human, mouse, or rat genes, or yeast ORFs spotted onto a positively charged nylon membrane. All cDNA clones are amplified and sequence-verified before spotting, ensuring the production of a high-quality membrane. A wide selection of ready-to-use GeneFilters® enables you to choose the one that best meets your needs (table 1).

### quality ensures success

The quality of the cDNA clones used to generate a microarray is a vital factor in the success of your gene expression analysis. The GeneFilters® microarrays are spotted with extensively characterized sequences to minimize redundancy and represent as many unique transcripts as possible. This ensures a high degree of reliability of your expression results. Clones are carefully propagated to eliminate phage and other laboratory con-

taminants so you can be assured that the differential expression detected in your experiments is truly due to transcriptional variations. In addition, the first 1000 base pairs (on average) from the 3' untranslated region (UTR) are included in the cDNAs spotted on GeneFilters® microarrays to minimize false positives due to non-specific cross-hybridization. Successful hybridization of a GeneFilters® microarray probed with radiolabeled cDNA is demonstrated in figure 1. With the high-quality GeneFilters® microarrays, you can be assured of accurate analysis results each time.

### affordable microarrays

In addition to providing successful results, GeneFilters® microarrays are affordable. Screening is done using standard hybridization methods, such as radiolabeling, making microarray technology accessible to any lab. A test membrane is included with each GeneFilters® microarray purchase so that the experimental parameters can be optimized

**figure 1** - GeneFilters® microarray screened with labeled HeLa cDNA



5 µg of total HeLa RNA was reverse transcribed and labeled with <sup>33</sup>P-CTP during amplification. The labeled cDNA was purified away from unincorporated radionucleotides using the S.N.A.P.™ purification columns (GeneFilters® probe labeling kit). The probe was hybridized to a GeneFilters® microarray.

before actual testing begins. The GeneFilters® microarrays can also be stripped and reused up to five times. With GeneFilters® microarrays you will obtain good analysis results with minimum expenditure.

### detect expression with accuracy

Take advantage of quality ResGen™ GeneFilters® microarrays for accurate gene expression analysis results. To obtain more information, check out our web site at [www.resgen.com](http://www.resgen.com) or call 800 533 4363.

**table 1** - the GeneFilters® microarray collection

Name	Description
Human GeneFilters® Releases I to VII	Contains 5,184 clones, including named genes and ESTs, per membrane
Human Named Genes GeneFilters® Release I	Over 4,000 genes of known function
Human Tissue-Specific Microarrays	Clones isolated from Prostate, Ovary, Breast, Colon, Bone, Neuro, and Skin tissues
Rat GeneFilters® Releases I to III	Contains 5,184 clones, including named genes and ESTs, per membrane
Mouse GeneFilters® Release I	Over 5,000 mouse genes and ESTs
Yeast GeneFilters® Microarrays	Contains 6,144 yeast ORFs spotted on two nylon filters

GeneFilters® Type	Quantity	Price
Mammalian (Human, Mouse, Rat)	1 filter	\$980
Human Tissue-Specific or Named Genes	1 filter	\$1,460
Yeast ORFs*	1 set	\$1,345

\* The yeast ORF GeneFilters® microarray is a set of two filters.

## Flexible analysis of any microarray with Pathways™ 3 software

**A**nalysis of multiple microarrays requires the use of varied techniques to extract important gene expression data. You can do this using multiple software packages or you can use Invitrogen's all-inclusive ResGen™ Pathways™ 3 software. Pathways™ 3 software is an extensive and flexible tool designed to produce accurate results in all of your microarray analysis experiments.

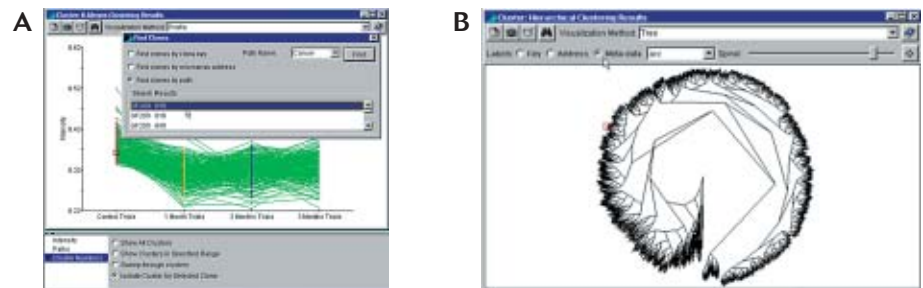
### comprehensive analysis

Pathways™ 3 software is a comprehensive software package that facilitates image analysis, data analysis, filtering, and visualization of microarray images. The basic software includes several normalization algorithms that correct for global intensity shifts across multiple experiments. Several clustering algorithms are provided along with a variety of cluster distance and cluster visualization programs (figure 1). Pathways™ 3 also allows the addition of new sampling, normalization, and clustering techniques to suit your particular needs (table 1). The highly modular format of Pathways™ 3 provides maximum flexibility so you can use the analysis techniques that are best suited for your experiments.

### adaptable for better results

Pathways™ 3 software performs complex microarray analyses using advanced techniques. It is designed for use with GeneFilters® microarrays and can easily accommodate other formats. Newer sam-

figure 1 - examples of clustering results



- A. The cluster profile plot shows the cluster variables (e.g. image intensity) over the specified conditions. Clusters containing clones of interest can be investigated in further studies.  
 B. The hyperbolic viewer shows the clustering results in a hierarchical tree that can be magnified to find individual clones of interest.

pling, normalization, and statistical methods can be added as needed. This enables you to adapt Pathways™ 3 to perform the techniques you want for achieving the best analysis results.

### Pathways™ 3 updates keep you current

To keep you in stride with quickly evolving microarray analysis technology, Pathways™ 3

provides easy data and program updates. An integrated web browser directly links to UniGene, GenBank, and other public databases for information on clones selected on the screen. The latest updates on microarray data, such as UniGene clustering information and plug-ins, are just a click away via the internet.

### get the results you need

The flexible ResGen™ Pathways™ 3 software offers more options and techniques for every aspect of microarray analyses. You can perform advanced analysis with the software as it is supplied and extend it to fit your research needs. Call 800 533 4363 for more information.

table 1 - a sample of the program modules available in Pathways™ 3 software

Functions	Choice of methods	Benefits
Clustering	Algorithms—Hierarchical, KMeans Variables—Intensities, Ratios Distance calculators—Euclidian, Squared radius Visualization—Clustergram, Tree, Table	Clustering analysis made reliable and easy with choice of plug-ins
Normalization	Mean of all spots except controls, Mean of controls, Chen (YC), Path	Choice of four techniques so that you can pick the one that best suits your microarrays
Data Analysis	Ratio analysis, Difference analysis, Outlier analysis, Statistical analysis (unrelated t-test)	Options for analysis of different types of experiments gives better results

Product	Quantity	Cat. no.**
Pathways™ 3 software	1*	PW3

\* Includes complete software on CD, users' manual, and one-year technical support subscription.  
 \*\* Call for pricing.

# Print your own microarrays *immediately* with spot-ready DNA

Replace the time-consuming tasks of searching for and preparing clones for printing microarrays with ResGen™ MyArray™ DNA from Invitrogen. Just find the clones you want in our easy-to-use online database and order. MyArray™ DNA comes ready to spot so you can print your microarrays immediately.

## spotting made easy

ResGen™ MyArray™ DNA is spot-ready DNA for use in printing your own microarrays. With MyArray™ DNA, you won't spend time hunting for clones or amplifying DNA, saving you valuable research time. Simply search our comprehensive clone resource for the genes you are interested in and submit your order. It's all done online. You will receive the ready-to-spot purified PCR product of each clone so you can begin printing your microarrays immediately.

## comprehensive clone resource

Get the relevant cDNA clones you need from our comprehensive clone resource. You can choose from (table 1):

- The human or mouse clones in the I.M.A.G.E. collection
- The human or mouse clones in our sequence-verified collection. The sequence-verified collection contains high-quality clones from one cDNA chosen from each UniGene cluster. Clones are re-transformed to eliminate database error and phage contamination
- Our pre-established sequence-verified clone sets, including named human genes

With a comprehensive collection to search, you're sure to find relevant genes that will deliver the results you need\*.

## straightforward searching

Assembling the clones you need is a straightforward process with our on line cMiner

**table 1** - human and mouse cDNA clones available for ResGen™ MyArray™ DNA\*

Source	Human	Mouse
I.M.A.G.E.	> 3 million clones	> 1.5 million clones
Sequence-verified	> 45,000 clones	5,184 clones
Pre-established sequence-verified sets	9 sets each with 5,184 clones	n/a
Pre-established sequenced-verified named sets	1 set with 4,000 clones, 1 set with 3,000 clones	n/a
Brain molecular anatomy project	n/a	> 11,000 clones

query function. cMiner is a powerful tool that allows you to perform highly specific searches of our databases. Using cMiner, you can include or exclude clones from particular tissues. You can even choose the clone end sequence you need (3', 5', or both). The query function will return a description of the clusters or clones matching your query. Once you have selected the clones you want, simply place your order online.

## assured quality

To ensure optimal gene representation, MyArray™ DNA undergoes strict quality control analysis. Each PCR product is run on a gel to demonstrate product integrity. You will receive a CD containing electronic gel images and an annotated data file with current information on your clones.

## print your microarrays immediately

When you order ResGen™ MyArray™, your DNA will be shipped to you in 2-4 weeks in a convenient 96-well format so you can print immediately. You will receive 1-3 µg of purified PCR product at a fixed volume

in the buffer of your choice. You can also choose to receive a glycerol stock\*\* of each clone for fast confirmation of your microarray screening results. Why wait? Order ResGen™ MyArray™ DNA today. Call us at 800 533 4363 or visit our web site at [www.resgen.com/products/MyArrayDNA.php3](http://www.resgen.com/products/MyArrayDNA.php3).

Product	Cat. no.	Price
MyArray™ DNA		
PCR product only	MAD1000	\$18/clone†
PCR product plus glycerol stock	MAD2000	\$23/clone†
Pre-established sequence-verified set	inquire	\$8/clone
Pre-established sequence-verified named set	inquire	\$9/clone

\* Please inquire about our rat, yeast, *Drosophila*, *C. elegans*, zebrafish, and *Xenopus* clone collections.

\*\* Additional charge for glycerol stock.

† Pricing is based on a minimum order of 384 clones, charged in units of 96 thereafter.

## Save time in gene discovery and analysis with premade RNA-based products

Whether you are studying cancer genetics or working on gene discovery, you can count on the ResGen™ Discovery Line™ from Invitrogen to accelerate your research. Using the pre-made RNA-based Discovery Line™ products, you will eliminate tedious preparation procedures and save several weeks of valuable research time.

### time-saving tools

Discovery Line™ is an RNA-based product line made using high-quality RNA extracted from documented human normal, fetal, and tumor tissue sources. All products are supplied ready to use, eliminating time-consuming preparation steps. The Discovery Line™ products include:

- **Discovery Line™ mRNA and Total RNA**—high-quality RNA ready for use in a variety of applications including RACE, RT-PCR, and microarray analysis
- **Northern Territory™ RNA Blots**—hybridization-ready northern blots for gene expression analysis
- **Gene Pool™ cDNA**—PCR-ready first-strand cDNA for gene amplification and expression analysis

### eliminate time-consuming steps

Obtaining high-quality RNA for your experiments involves the time-consuming procedures of acquiring tissue, extracting RNA, and purifying samples. When you use the Discovery Line™ products, you eliminate these tedious steps. In the time it would normally take to isolate RNA, prepare blots, or perform RT-PCR, you can have your results (figure 1). With the time you save, you will get further in your experiments faster.

### quality takes you further

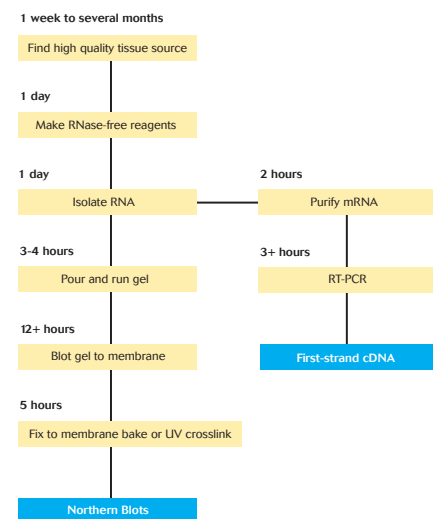
To ensure success in all your applications, stringent procedures are followed to maintain an RNase-free environment during the

**figure 1** - time comparison of using Discovery Line™ or conventional methods used

#### The Discovery Line™ way

1 day  
Purchase Northern blots, mRNA, Total RNA, cDNA and start your research

#### The old way



manufacturing of all Discovery Line™ products. Total RNA integrity is determined by electrophoresis to ensure that it is intact and full-length. Gene Pool™ first-strand cDNA is verified by PCR to ensure that it is full-length. The blotting process is optimized for minimal degradation of RNA, ensuring successful analysis with the Northern Territory™ blots. With this tight quality control, you will get results on your first try.

### get Discovery Line™ and save time

Ready-to-use human RNA-based Discovery Line™ products are the fastest way to start gene expression analysis. You don't need to spend time isolating RNA, preparing northern blots, or generating first-strand cDNA for your research. For a complete list of the variety of tissue sources available and, ordering information check out our web site at [www.resgen.com](http://www.resgen.com). A quick visit could save you weeks of time.

Product*	Quantity	Price
<b>Discovery Line™ RNA</b>		
mRNA		
Normal	5 µg	\$387
Fetal	5 µg	\$387
Tumor	5 µg	\$710
Total RNA		
Normal	250 µg	\$360
Disease	150 µg	\$482
<b>Gene Pool™ cDNA</b>		
Normal	40 µl	\$403†
Fetal	40 µl	\$403†
Tumor	40 µl	\$546
<b>Northern Territory™ Blots</b>		
mRNA blots		
Normal	1 blot	\$641
Fetal	1 blot	\$641
Tumor	1 blot	\$1,230
Total RNA Blots		
Normal	1 blot	\$535
Fetal	1 blot	\$618
Tumor	1 blot	\$1,230

\* For a complete list of tissue sources and catalog numbers, visit our web site at [www.resgen.com](http://www.resgen.com)

† Additional charges apply for special order tissues.

# EXPRESSIONS



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