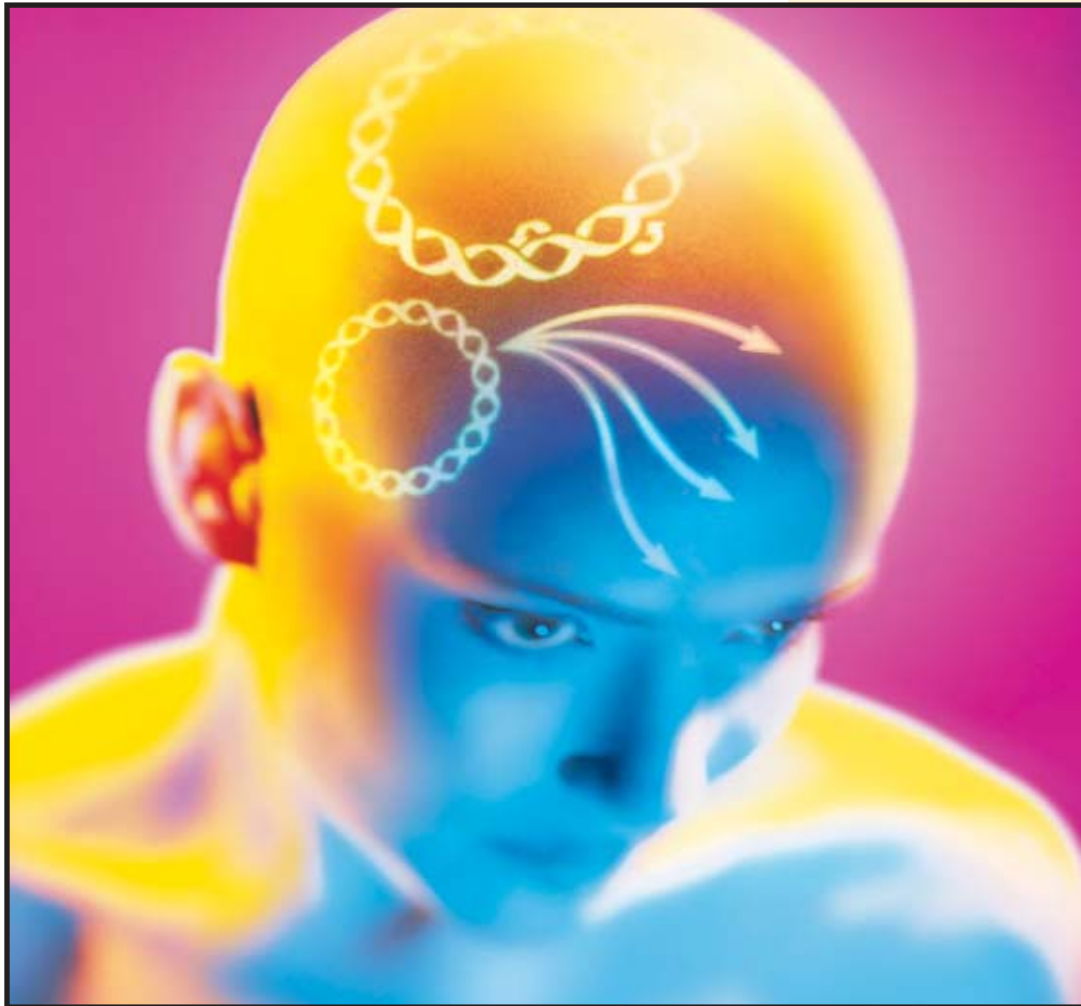


Expressions

A newsletter for gene cloning, expression, and analysis



**The Future of Cloning is Here.
You May Proceed.**

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Express Your Gene in Multiple Systems Without Subcloning

4

High-Performance Protein Separation

12

Better Results in PCR Cloning

15

Improve Eukaryotic Protein Yields in *E. coli*

Express in Multiple Systems with Just One Cloning Step

The Echo™ Cloning System

The Future of Cloning

“Clone your gene in five minutes, recombine it into as many expression vectors as you want, and get the expression results you need.”

It is often necessary to express your gene of interest in multiple expression systems in order to find the one that best meets your criteria for post-translational modifications, yield, and functionality. Limited resources may force you to eliminate several promising expression systems because of the time-consuming task of subcloning your gene into multiple expression vectors. The new Echo™ Cloning System eliminates the need to develop multiple subcloning strategies and allows you—with just one cloning step—to express your gene in as many expression systems as you choose.

Fastest Cloning, Least Effort. The Echo™ Cloning System, based on the univector system described by Liu *et al.* (1), represents the fastest way to get from gene cloning to gene analysis. A single cloning step into the pUni/V5-His-TOPO® donor vector is followed by a rapid recombination event into any Echo™-adapted expression vector. You can recombine your gene of interest into as many expression vectors as you choose. With the Echo™ Cloning System you can:

- Eliminate repetitive cloning—clone and sequence only once to save hours of time
- Recombine into multiple expression vectors simultaneously—easily test expression in more than one system with a minimum investment of time and effort
- Get the expression results you need—choose the expression system best suited to each protein you express

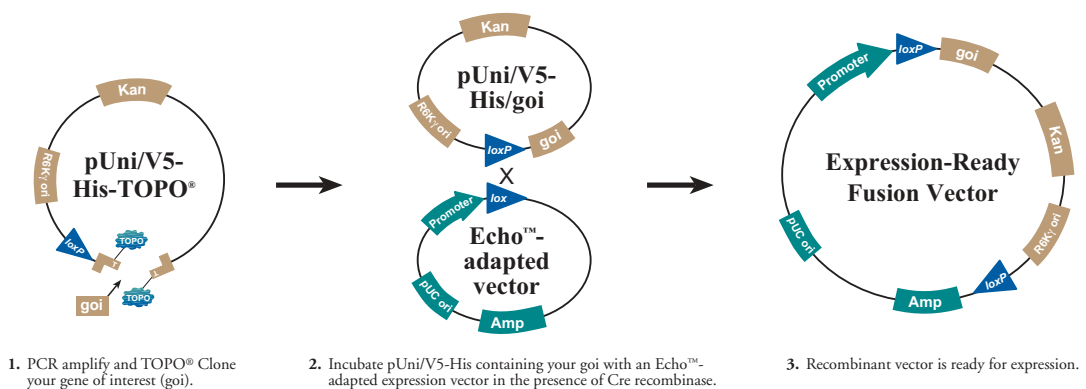
Universal Donor. The pUni/V5-His-TOPO® vector allows you to create a universal donor construct that can be used to recombine your gene into any number of expression vectors. pUni/V5-His-TOPO® is provided linearized with the ends covalently bound to topoisomerase I. This allows efficient, 5-minute, bench-top ligation of your *Taq*-amplified PCR product without the need for ligase or special primers (Figure 1, step 1). pUni/V5-His-TOPO® contains the following features for optimal recombination and selection:

- *loxP* site to allow recombination with any Echo™-adapted acceptor vector
- R6K γ origin of replication to allow positive selection of recombinants by supporting growth only in *E. coli* that contain the *pir* gene (*i.e.* PIR1)
- Neomycin resistance gene for kanamycin selection in *E. coli*

Following selection with kanamycin and sequencing, your pUni/V5-His-TOPO® construct is ready for recombination into any Echo™-adapted expression vector (Figure 1, step 2). Once recombined, pUni/V5-His-TOPO®'s additional features, including regulatory sequences and a C-terminal fusion tag, allow optimized expression, rapid detection, and efficient purification of the recombinant protein.

No More Subcloning. The Echo™ Cloning System eliminates the time and effort of creating individual subcloning strategies. Each Echo™-adapted expression vector contains a *lox* site to allow Cre recombi-

Figure 1 - Rapid Cloning of your Gene of Interest Using the Echo™ Cloning System



The Echo™ Cloning System, continued.

nase-mediated recombination with your pUni/V5-His donor construct (Figure 1, step 2). This results in a fusion vector that contains your gene downstream of a promoter and in the correct orientation for expression. Transformation into TOP10 *E. coli* (which do not carry the *pir* gene) and selection with kanamycin allow growth of only recombined clones. There's no need for individual subcloning strategies so you'll save hours of time.

Cloning Without Limits. A variety of Echo™-adapted expression vectors are currently available for expression and characterization of your gene in the most advanced bacterial, yeast, insect, and mammalian systems (Table 1). Whether you want constitutive or inducible expression, an N-terminal fusion tag, or no tag, there is an Echo™-adapted vector to fit your needs. In addition, you can easily Echo™-adapt your own vectors for use in the Echo™ Cloning System. With the Echo™ Cloning System, there are no limits.

Kits That Fit. To provide you with the kit that fits your needs, the Echo™ Cloning System is available in several configurations. The Echo™ Cloning and Expression Kits contain everything you need to clone your gene and recombine it into an Echo™-adapted expression vector including the pUni/V5-His TOPO TA Cloning® Kit, PIR1 One Shot® *E. coli*, an Echo™-adapted expression vector of your choice, Cre recombinase, and TOP10 One Shot® *E. coli*. Echo™ Expression Vector Kits, which include the Echo™-adapted vector of your choice plus reagents for recombination, are also available. A comprehensive ordering table is available on the web at www.invitrogen.com/catalog_echo.html.

The Future of Cloning is Here. The Echo™ Cloning System is the future of cloning. Now there's no more time-consuming subcloning strategies or restriction digests, no more repetitive cloning and sequencing. Clone your gene in five minutes, recombine it into as many expression vectors as you want, and get the expression results you need. The next time you need to express a protein, think about the future. Then call Invitrogen and order an Echo™ Cloning System.

Table 1 - Echo™-Adapted Expression Vectors

Echo™-Adapted Vector	Promoter	Advantage
<i>E. coli</i>		
pBAD/Thio-E	<i>araBAD</i>	High-level, tightly-regulated expression
pCR®T7-E	T7	High-level, inducible expression
pRSET-E	T7	High-level, inducible expression with an N-terminal purification and detection tag
Yeast		
pYES2.1-E	<i>GAL1</i>	High-level, regulated expression in <i>Saccharomyces cerevisiae</i>
pYC2-E	<i>GAL1</i>	Regulated expression in <i>Saccharomyces cerevisiae</i> from a low-copy plasmid
Insect		
pIB-E	OpMNPV IE2	Stable, non-lytic expression in insect cell lines
pBlueBac4.5-E	Polyhedrin	High-level expression with the MaxBac® 2.0 Baculovirus Expression System
Mammalian		
pcDNA3.1-E	CMV	Strong, constitutive expression
pcDNA4/HisMax-E	CMV	Increased expression from the CMV promoter using the QBI SP163 enhancer; N-terminal fusion tag
pIND-E	HSP	Tightly-regulated expression in the Ecdysone-Inducible Mammalian Expression System
pcDNA4/TO-E	CMV/TetO ₂ Hybrid	High-level induced expression in the T-REx™ System

Product	Quantity	Cat. no.	Price
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Echo™ Cloning and Expression Kits

E. coli

pBAD/Thio-E	1 kit	ET100-10C	\$320
pCR®T7-E	1 kit	ET110-10C	\$307
pRSET-E	1 kit	ET111-10C	\$320

Yeast

pYES2.1-E	1 kit	ET200-10C	\$317
pYC2-E	1 kit	ET210-10C	\$317

Insect

pIB-E	1 kit	ET320-10C	\$317
pBlueBac4.5-E	1 kit	ET310-10C	\$307

Mammalian

pcDNA3.1®-E	1 kit	ET400-10C	\$307
pcDNA4/HisMax®-E	1 kit	ET401-10C	\$343
pIND-E	1 kit	ET450-10C	\$336
pcDNA4/TO®-E	1 kit	ET460-10C	\$336

pUni/V5-His TOPO TA Cloning® Kit

	10 rxns	ET001-10	\$161
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One Shot® Chemically Competent *E. coli*

TOP10	10 rxns	C4040-10	\$121
PIR1	10 rxns	C1010-10	\$121
Cre Recombinase	10 rxns	R100-10	\$75

Reference:

1. Liu, Q. *et al.* (1998) *Curr. Bio.* **8**: 1300-1309.

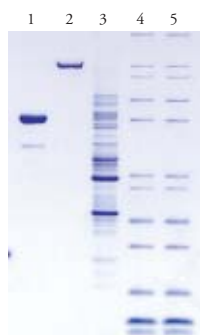
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High Performance Protein Separation for True Sample Analysis

The NuPAGE® Electrophoresis System

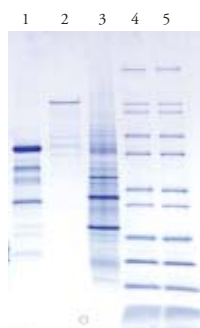
Figure 1 - Demonstrated True Results with NuPAGE® Gels

NuPAGE® 4-12% Bis-Tris Gel



Sample Prep:
NuPAGE LDS
Sample Buffer,
70° C for
10 minutes

Other 4-20% Tris-Glycine Gel



Sample Prep:
Laemmli Sample
Buffer, Boil for
5 minutes

Samples were prepared as indicated and then run on either a NuPAGE® 4-12% Bis-Tris Gel or another manufacturer's 4-20% Tris-Glycine Gel. Note the extra bands and smearing seen in lanes 1-3 on the Tris-Glycine gel.

Sample Loading:

Lane 1: 5 µg of reduced Catalase
Lane 2: 0.4 µg of reduced β-Galactosidase
Lane 3: 7 µg of reduced Lyme Antigen
Lane 4: Mark12™ Standard
Lane 5: Mark12™ Standard

The NuPAGE® Electrophoresis System is a high-performance, pre-cast polyacrylamide mini-gel system designed to optimize protein separation analysis. Traditional Laemmli-style gels and sample preparation methods can actually modify proteins, altering your results. NuPAGE® gels operate in a patented neutral pH environment that overcomes these problems. This minimizes protein modifications and gives you the most reliable results.

Neutral pH Means Fewer Protein Modifications.

The high operating pH (pH 9.5) of Laemmli-style gels actually promotes sulfhydryl and amino alkylation by free, unpolymerized acrylamide. These reactions can change protein mass ratios as determined by spectrometry analysis and can block Edman degradation sequencing, resulting in poor analysis results. In addition, pH environments >7.0 lead to base hydrolysis of amide groups, yielding inaccurate amino acid analysis and lowering sequencing yields. The neutral pH (pH 7.0) operating environment of the NuPAGE® Bis-Tris gel minimizes these protein modifications. The result is that the protein you see on a NuPAGE® gel is the protein you put on the gel, not a product of your sample interacting with the separation medium.

NuPAGE® Gives True Results.

Traditional sample preparation methods can also alter your protein separation analysis. Laemmli-style sample buffers contain Tris base that catalyzes cleavage of aspartic acid-proline (asp-pro) bonds. Heating accelerates this asp-pro bond hydrolysis leading to protein degradation. This means that the traditional method of boiling samples prior to loading actually promotes protein degradation *before* you've even loaded it on the gel. Sample preparation with NuPAGE® LDS Sample Buffer requires warming samples to 70°C for 10 minutes, a much gentler way to treat your protein. In addition, the pH of the NuPAGE® LDS Sample Buffer (pH 8.5) provides an efficient environment for protein denaturation and reduction while avoiding asp-pro bond cleavage. You'll see true separation results every time with NuPAGE® gels (Figure 1).

Long Shelf Life for Big Savings. The neutral pH chemistry of the NuPAGE® system guarantees that

gels are stable for 12 months from the date you receive them—the longest shelf life of any pre-cast gel. This eliminates money wasted by discarding out-of-date gels. NuPAGE® Bis-Tris gels can be stored at any temperature between +4°C and +25°C, so you'll save valuable refrigerator space. You'll save money and be able to keep your gels right where you need them—on your bench top.

Reduce Your Protein Separation Anxieties. The NuPAGE® Electrophoresis System revolutionizes SDS-PAGE by minimizing the occurrence of protein modifications inherent in the traditional Laemmli-style Tris-HCl gel system. The NuPAGE® patented neutral pH environment gives you better protein separation results and takes the worry out of gel expiration dates and storage. Reduce your separation anxieties, order the NuPAGE® System today.

Product	Quantity*	Cat. no.	Price/gel
NuPAGE® 10% Bis-Tris Gel			
1.0 mm, 10 well	1 gel	NP0301	\$9.25
1.0 mm, 12 well	1 gel	NP0302	\$9.25
1.0 mm, 15 well	1 gel	NP0303	\$9.25
1.0 mm, 1 well	1 gel	NP0304	\$9.25
1.0 mm, 2D well	1 gel	NP0306	\$9.25
1.0 mm, 9 well	1 gel	NP0307	\$9.25
1.5 mm, 10 well	1 gel	NP0315	\$9.25
1.5 mm, 2D well	1 gel	NP0317	\$9.25
NuPAGE® 4-12% Bis-Tris Gel			
1.0 mm, 10 well	1 gel	NP0321	\$9.25
1.0 mm, 12 well	1 gel	NP0322	\$9.25
1.0 mm, 15 well	1 gel	NP0323	\$9.25
1.0 mm, 1 well	1 gel	NP0324	\$9.25
1.0 mm, 2D well	1 gel	NP0326	\$9.25
1.0 mm, 9 well	1 gel	NP0327	\$9.25
1.0 mm, IPG well	1 gel	NP0330	\$9.25
1.5 mm, 10 well	1 gel	NP0335	\$9.25
1.5 mm, 2D well	1 gel	NP0337	\$9.25
NuPAGE® 12% Bis-Tris Gel			
1.0mm, 10 well	1 gel	NP0341	\$9.25
1.0mm, 12 well	1 gel	NP0342	\$9.25
1.0mm, 15 well	1 gel	NP0343	\$9.25
1.0mm, 1 well	1 gel	NP0344	\$9.25
1.0mm, 2D well	1 gel	NP0346	\$9.25

* NuPAGE® gels are sold in boxes of 10.

High-Resolution 2D Analysis

ZOOM™ Gels

ZOOM™ Gels are conveniently designed to provide you with the highest resolution in 2D analysis. All gels fully accommodate 7 cm immobilized pH gradient (IPG) strips from any supplier. This means:

- No sample loss—there's no need to trim the ends of the strip
- Clearer separation—your IEF can run to completion

With ZOOM™ Gels you can perform full isoelectric focusing separation for great results.

High-Performance Gels. ZOOM™ Gels with IPG wells are available in NuPAGE® 4-12% Bis-Tris or NOVEX® 4-20% Tris-Glycine formats. Pre-cast and ready to run, ZOOM™ Gels will save you hours of preparation time and give reproducible 2D separations time after time. Figure 1 demonstrates the high resolution obtained with ZOOM™ Gels.

Figure 1 - High Resolution using NuPAGE® 4-12% Bis-Tris ZOOM™ Gels



Rat kidney lysate was first subjected to IEF using a 7 cm IPG strip (pH 3-10). The strip was then placed into the IPG well of the NuPAGE® 4-12% Bis-Tris ZOOM™ Gel and the sample separated in the second dimension. After a 35 minute electrophoresis run, the gel was stained using the SilverXpress™ Silver Staining Kit.

Mini-Gels with Maxi Results. The perfectly-sized ZOOM™ Gels allow you to use the IPG strip of your choice, eliminate sample loss, and be assured of high resolution 2D separation. Kick your research into high gear and order ZOOM™ Gels today.

Product	Quantity*	Cat. no.	Price/gel
NuPAGE® Bis-Tris 4-12% ZOOM™ Gel			
1.0 mm, IPG Well	1 gel	NP0330	\$9.25
NOVEX® Tris-Glycine 4-20% ZOOM™ Gel			
1.0 mm, IPG Well	1 gel	EC60261	\$9.25

* Gels are sold in boxes of 10.

Increase Specific Sensitivity in Western Blots

WesternBreeze™ Immunodetection Kits

Western blotting detection is an effective technique for visualizing the protein of interest with a selective antibody. WesternBreeze™ Immunodetection Kits are complete, pre-optimized western blotting kits designed to increase specific sensitivity when detecting proteins immobilized on nitrocellulose or PVDF membranes.

Pre-Optimization is the Key. Every reagent in the WesternBreeze™ Kits has been optimized to ensure low background and highly-specific protein detection. Each kit includes blocker, primary antibody diluent, secondary antibody solution, and substrate for western blotting detection. All solutions have been optimized to work together at specific and predetermined concentrations. There's no need for you to spend time and effort preparing and testing your own solutions. With the WesternBreeze™ Kits, all of the work has been done so you save time and effort and get great results.

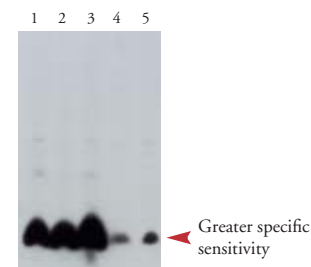
Highly Specific and Sensitive. To prove that the WesternBreeze™ Kits increase specific sensitivity, western blots detected with either the WesternBreeze™ Kit (Figure 1, Blot A) or another commercially-available detection kit (Figure 1, Blot B) were compared. The results clearly demonstrate that greater specific sensitivity and less non-specific binding was achieved with the WesternBreeze™ Kit. For maximum specific sensitivity in western blotting, order the WesternBreeze™ Kit today.

Product	Quantity*	Cat. no.	Price
WesternBreeze™ Chromogenic Detection Kit			
Anti-Mouse	1 kit	WB7103	\$199
Anti-Rabbit	1 kit	WB7105	\$199
Anti-Goat	1 kit	WB7107	\$199
WesternBreeze™ Chemiluminescent Detection Kit			
Anti-Mouse	1 kit	WB7104	\$249
Anti-Rabbit	1 kit	WB7106	\$249
Anti-Goat	1 kit	WB7108	\$249

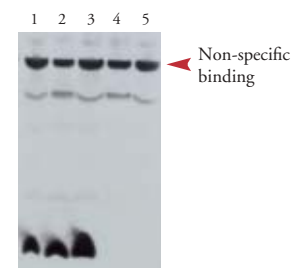
* Each kit supplies sufficient reagents for detection of 20 mini-blots.

Figure 1 - Greater Specific Sensitivity with the WesternBreeze™ Kit**

Blot A: WesternBreeze™ Kit



Blot B: Competitor's Kit



Fifty micrograms of cell lysates from *E. coli* expressing a 6xHis-tagged, partially soluble zinc finger peptide were run on duplicate 16% Tris-Glycine SDS-PAGE gels and transferred to nitrocellulose membranes. Each blot was probed with a rabbit polyclonal antibody to the 6xHis tag (1:2,000 dilution) and developed with either the WesternBreeze™ Chemiluminescent Detection, Anti-Rabbit Kit (Blot A) or a competitor's kit (Blot B). Blots were exposed to the same piece of film for 30 seconds.

Lane 1: Whole cell lysates from *E. coli* induced with IPTG for 1 hour
 Lane 2: Whole cell lysates from *E. coli* induced with IPTG for 2 hours
 Lane 3: Whole cell lysates from *E. coli* induced with IPTG for 4 hours
 Lane 4: Soluble lysates; 2 hour induction
 Lane 5: Soluble lysates; 4 hour induction

** Courtesy of Aaron McCarty and Stephen Smale of Howard Hughes Medical Institute/UCLA.

Ready-to-Use Standards for All Your Protein Electrophoresis Needs

Protein Standards

Invitrogen's complete line of molecular weight protein standards offers maximum convenience for all your protein electrophoresis needs. Whether you need to approximate molecular weight, verify western transfer efficiency, or determine isoelectric points, we have a standard that will fit your needs (Table 1).

Ready-to-Use Standards. For fast and easy usage, Invitrogen's protein standards are supplied ready to use straight from the vial. There's no need to mix, reduce, or heat before using so you'll save time.

Table 1 - Selecting the Right Protein Standard

Application	MultiMark®	SeeBlue® Plus2	SeeBlue®	Mark12™	SERVA® IEF Marker 3-10
SDS-PAGE	✓	✓	✓	✓	n/a
IEF Gels	n/a	n/a	n/a	n/a	Best!
Immediate Band Identification	Best!	✓	✓	(bands visible only after staining)	(bands visible only after staining)
Sharp Bands	✓	Best!	Best!	✓	✓
MW Estimation	✓	✓	✓	Best!	pI Estimation
Monitor Migration During Electrophoresis	✓	✓	✓	n/a	n/a
Silver Staining	✓	✓	✓	Best!	✓
MW/pI Range	4-250 kDa	4-250 kDa	4-250 kDa	2.5-200 kDa	pI 3.5-10.7

Immediate Band Identification. For immediate band identification on SDS-PAGE gels and western transfers, the MultiMark® Multi-Colored Protein Standard shows our true colors. MultiMark® lets you quickly visualize molecular weight ranges from 4 to 250 kDa during electrophoresis, and evaluate western transfer efficiency at a glance (Figure 1A).

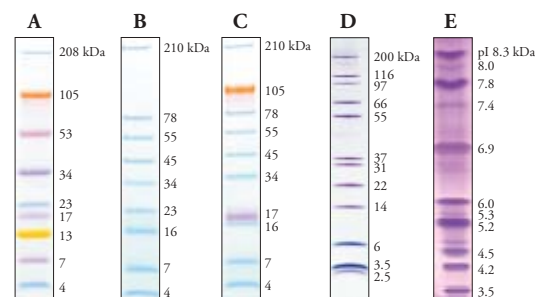
Sharp, Consistently-Stained Bands. For SDS-PAGE applications requiring the sharpest bands, SeeBlue® and SeeBlue® Plus2 Pre-Stained Standards are your best choices. SeeBlue® contains nine individual protein bands, all consistently stained blue. Our newest standard, SeeBlue® Plus2 contains eight blue protein bands plus two additional colored bands for easy analysis (Figures 1B and 1C).

Accurate Molecular Weight Estimation. When you need the best approximation of true molecular weight in SDS-PAGE, use the Mark12™ Unstained Standard. The Mark12™ Standard contains 12 unstained proteins ranging from 2.5 to 200 kDa.

Each polypeptide resolves into a sharp, tight band that is easily visualized after electrophoresis with Coomassie®, silver, or other protein stains (Figure 1D).

Flexible Isoelectric Focusing. The SERVA® IEF Marker 3-10 is ideal for accurately determining isoelectric points of unknown proteins. It contains nine clearly identifiable proteins (13 isoforms) in the range of pI 3.5 to 10.7. It is flexible enough to be used on vertical or horizontal IEF gels under either native or denaturing conditions (Figure 1E).

Figure 1 - Ready-to-Use Standards for Protein Electrophoresis



A: MultiMark® on a NOVEX® 10-20% Tricine Gel
 B: SeeBlue® on a NOVEX® 10-20% Tricine Gel
 C: SeeBlue® Plus2 on a NOVEX® 10-20% Tricine Gel
 D: Mark12™ on a NOVEX® 10-20% Tricine Gel
 E: SERVA® IEF Marker 3-10 on a NOVEX® 3-10 IEF Gel

Convenient, One-Stop Shopping. Now you can obtain all of your protein standards with just one easy phone call. Invitrogen's complete line of ready-to-use protein standards allows you to easily visualize the progress of your electrophoresis, quickly judge western transfer efficiency, effectively approximate molecular weights, and accurately determine isoelectric points. Order your Protein Standard today.

Description	Quantity	Cat. no.	Price
MultiMark® Multi-Colored Standard	500 µl	LC5725	\$105
SeeBlue® Plus2 Pre-Stained Standard	500 µl	LC5925	\$99
SeeBlue® Pre-Stained Standard	500 µl	LC5625	\$99
Mark12™ Unstained Standard	1 ml	LC5677	\$85
SERVA® IEF Marker 3-10	500 µl	39212-01	\$145

SAVE 15%

From now until March 31, 2000, save 15% off the price of these standards when you order two or more. Give us a call at 1-800-955-6288 and mention PROM182 when placing your order to receive your discount.

Expression-Ready Mouse Clones Save Time

GeneStorm® Expression-Ready Mouse Clones

GeneStorm® is a collection of full-length, partially-sequenced,[†] expression-tested open reading frames (ORFs) designed to save you time. The collection has now been expanded to include mouse ORFs. GeneStorm® Mouse Clones allow comparative analysis of human clones when studying diseases, the effects of over-expression, or validating gene function.

Save Time and Effort. The GeneStorm® Mouse Clones are cloned into the widely-used pcDNA3.1/GS® mammalian vector and expression-tested to save you time. This state-of-the-art vector provides high-level, CMV-driven expression, rapid selection in *E. coli* and mammalian cells with Zeocin™, and convenient detection and simplified purification with the C-terminal V5 epitope and polyhistidine (6xHis) tag. Each clone is transfected into Chinese hamster ovary (CHO) cells and expression is confirmed by western blot using the Anti-V5 Antibody. You'll save valuable time and effort because all of the cloning and testing has been done for you.

Added Value. Each GeneStorm® Mouse Clone is supplied lyophilized and comes with high-quality reagents so you can easily get started. You'll get One Shot® TOP10 Chemically Competent *E. coli* ready for transformation, the Anti-V5-HRP Antibody to detect expression, and a pcDNA3.1/GS® negative control vector. Extra reagents mean added value.

Find It Online. Find your GeneStorm® Mouse Clones of interest online at www.invitrogen.com/genestorm. At the GeneStorm® web site, you can easily search the database with a few clicks and find your clones. Order your GeneStorm® Mouse Clone today and you can start your experiments tomorrow.

Description	Quantity	Order no.	Price*
pcDNA3.1/GS® Mouse Clone	50 ng	M-(GenBank #)M	\$650

[†] Partially sequenced to confirm insert identity. GeneStorm® Clones are not guaranteed to exactly match GenBank sequence and may differ by one or more bases.
* Check web site for special multi-clone pricing.

XCell SureLock™ Makes Electrophoresis Easier

XCell SureLock™ Mini-Cell

The XCell SureLock™ Mini-Cell is the easiest, most convenient mini-vertical electrophoresis unit available. It is specially designed for use with all NOVEX® mini-gels and can be easily converted to run QuickPoint® nucleic acid gels. This means that one unit can meet all of your electrophoresis needs.

Easy Set-Up. The XCell SureLock™ Mini-Cell is easy to set up. There are no screws, clamps, or other awkward devices needed. Instead, a simple gel tension wedge holds the gels in place. The set-up procedure is as easy as 1-2-3 and takes just 15 seconds (Figure 1). The easy set-up ensures leak-free and trouble-free electrophoresis from run to run.

It Works for Multiple Applications. The XCell SureLock™ Mini-Cell is highly convenient for a variety of applications. The conversion kits allow you to switch between running NOVEX® mini-gels or QuickPoint® gels. In addition, the XCell SureLock™ Mini-Cell can easily be used for blotting by inserting the XCell II™ Blot Module into the unit in place of the gel/buffer core assembly. One unit does it all so there's no need to purchase and store three different units.

Convenient Packages Available. For the easiest, most convenient way to perform electrophoresis, choose the XCell SureLock™ Mini-Cell. With the convenient SureLock System, you can run mini-gels, QuickPoint® gels, and do western transfers. To simplify your electrophoresis, call Invitrogen today.

Product	Cat. no.	Price
XCell SureLock™ Mini-Cell	EI0001	\$350
XCell SureLock™ and XCell II™ Blot Module	EI0002	\$590
XCell SureLock™ for QuickPoint® Gel	EI0201	\$425
QuickPoint® Conversion Kit	EI0220	\$310
NOVEX® Mini-Gel Conversion Kit	EI0025	\$235
XCell II™ Blot Module	EI9051	\$240
SureLock System*	EI0030	\$815

* SureLock System includes the XCell SureLock™ Mini-Cell, XCell II™ Blot module, and the QuickPoint® Conversion Kit.

Figure 1 - Easy Set-up of the XCell SureLock™ Mini-Cell

Step One



Place one or two pre-cast gels on the buffer core.

Step Two



Drop buffer core and gel(s) into the lower buffer chamber of the XCell SureLock™ Mini-Cell.

Step Three

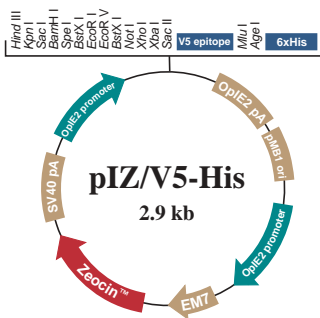


Lock the gel tension wedge, load samples, fill buffer chambers and you're ready to run.

Non-Viral, Stable Expression of hIL6 in Insect Cells

M. Galleno, J. Rogers, and S. Downing, Viral Expression Team, Invitrogen Corporation, Carlsbad, CA

Figure 1 - pIZ/V5-His Vector



Insect cells are often chosen for protein production because they grow quickly, do not require CO₂, and perform posttranslational modifications similar to mammalian cells. In the past, expression in insect cells has been commonly done with baculovirus expression systems. However, baculovirus methods require tedious, time-consuming viral production and titering steps. The InsectSelect™ System uses simple, integrative plasmids to stably express your protein of interest. Here we demonstrate the use of the InsectSelect™ System for non-viral expression of human interleukin 6 (hIL6) in insect cells.

Methods.

Cell culture. Sf21 and Sf9 cells were grown in complete TMN-FH + 10% fetal bovine serum (FBS). *Drosophila* S2 cells were grown in serum-free medium. All cells were grown as 125 ml suspension cultures in 250 ml spinner flasks to the following densities: Sf9 and Sf21—2.2 x 10⁶ cells/ml and S2 cells—6 x 10⁶ cells/ml.

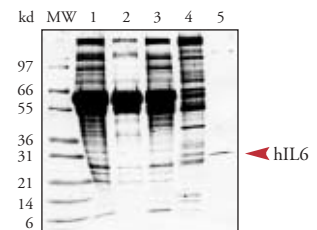
Cloning and Selection of Cell Lines. cDNA encoding hIL6 was cloned into the pIZ/V5-His vector (Figure 1) using traditional restriction digest methods. pIZ/V5-His uses the OpIE2 promoter to constitutively express the gene of interest in insect cells. pIZ/V5-His/hIL6 was introduced into Sf9 and Sf21 cells using Insectin-Plus™ lipids. S2 cells were transfected using calcium phosphate. Twenty-four hours posttransfection, the medium was removed and replaced with 125 ml medium containing 400 µg/ml Zeocin™. Cells were kept under selective pressure for one (Sf9, Sf21) to four (S2) weeks.

Expression and Purification. Zeocin™-resistant cells were spun down in a Sorvall centrifuge with a GSA rotor at 4000 rpm for 10 minutes at +4°C and the cell culture supernatant harvested. A small aliquot of each supernatant was reserved for western blotting. The remaining supernatant was dialyzed overnight at +4°C in ProBond™ Native Binding Buffer (200 mM sodium phosphate, 500 mM sodium chloride, pH 7.8). The supernatants were subsequently concentrated using Centricon filters (Millipore) and loaded onto a 2 ml ProBond™ nickel-chelating resin

column. Columns were washed and protein eluted as described in Invitrogen's Xpress™ Purification Manual. Eluted protein was analyzed by SDS-PAGE and quantified by Bradford assay.

Results. Sf9, Sf21, and S2 cells demonstrated expression of hIL6 from pIZ/V5-His. In addition, all of these cell lines recognized the native hIL6 signal and secreted the protein out of the cells into the medium. Total protein content of purified samples was determined using a Bradford assay (Table 1). Purified hIL6 protein was estimated to be 80% pure as determined by SDS-PAGE analysis (Figure 2).

Figure 2 - Expression and Purification of hIL6 in Sf9 Cells



pIZ/V5-His/hIL6 was transfected into Sf9 cells. The Sf9 cell lysate and purified supernatant were analyzed by SDS-PAGE. Cells were selected on 400 µg/ml Zeocin™, passaged ten times, and then split 1:2. Three days after the split, cells were harvested. The lysate and purified supernatant were analyzed by SDS-PAGE.

Lane 1: Sf9 lysate
Lane 2: Flow through
Lane 3: Wash (10 mM imidazole)
Lane 4: Elution 1 (100 mM imidazole)
Lane 5: Elution 2 (500 mM imidazole)

Discussion. Using the InsectSelect™ System we were able to simultaneously express hIL6 in three different cell lines without time-consuming viral production procedures. In addition, the protein was efficiently secreted into, and easily purified from, the culture medium. InsectSelect™ is ideal for non-viral, stable expression in insect cells.

Table 1 - Protein Yields from Purification of hIL6

Cell Line	Amount of Protein in Purified Sample (in 125 ml)
Sf9 cells	1.39 mg
Sf21 cells	0.48 mg
<i>Drosophila</i> S2 cells	3.52 mg

Product	Quantity	Cat. no.	Price
InsectSelect™ System			
with Sf9 cells	1 kit	K800-01	\$730
pIZ/V5-His Vector Kit	1 kit	V8000-01	\$335
Zeocin™	1 g	R250-01	\$175
ProBond™ Resin	50 ml	R801-01	\$315

New Method for Fast and Easy Subcloning of BAC Clones

TOPO® Shotgun Subcloning Kit

Bacterial artificial chromosome (BAC) vectors are often used in genome research because they are capable of accepting large inserts—up to 150 kb. To sequence an insert, the BAC clone is sheared into small DNA fragments which are used to construct a library in a vector suitable for sequencing. This “shotgun” cloning procedure requires several laborious steps and takes days to complete. The new TOPO® Shotgun Subcloning Kit simplifies this shotgun cloning process by offering:

- an easy and effective method for shearing BAC clones and blunting the resulting fragments
- a novel topoisomerase I-activated vector for rapid ligation
- streamlined sequence analysis to save you time

Fast Shearing and Blunting Protocol. When preparing BAC clones for effective subcloning and sequencing, it’s important that the DNA be sheared to ~2-3 kb. Traditional shearing requires time-consuming sonication procedures, a French press, or partial restriction digestion. The TOPO® Shotgun Subcloning Kit uses a nebulizer—a small plastic device used to atomize liquids—and compressed air to shear BAC DNA to the ideal size in just 30 seconds. Once the BAC DNA is sheared, the ends are made blunt with T4 DNA polymerase and Klenow so it can be readily ligated into the pCR®4Blunt-TOPO® vector. The entire procedure is rapid and effective—yielding 2-3 kb blunt-end BAC DNA in less than 1 hour (Figure 1). This saves 2-3 hours over traditional shearing methods.

Faster Library Construction. The pCR®4Blunt-TOPO® vector included in the TOPO® Shotgun Cloning Kit is provided linearized, blunt-ended, and activated with topoisomerase I to readily accept the sheared, blunt-ended BAC DNA (Figure 2). To use, simply add the sheared and blunted BAC DNA to the vector, incubate on your bench top for five minutes, and transform competent cells (1). The topoisomerase I-activated pCR®4Blunt-TOPO® vector eliminates overnight ligations and speeds up library construction. You’ll spend more time analyzing clones and less time making libraries.

Novel Vector Eliminates Background. The TOPO® Shotgun Subcloning Kit offers low background so you don’t waste any time on non-recombinant clones. That’s because pCR®4Blunt-TOPO® includes the *ccdB* (control of cell death) gene in the multiple cloning site for positive selection. If the vector ligates to itself without an insert, the CcdB protein is expressed and the transformed cells will die. However, when an insert is ligated into the vector, expression of the *ccdB* gene is disrupted and transformed cells will grow (2). With the positive selection mechanism of *ccdB*, you won’t waste time sequencing background clones because the only recombinant colonies will be on your plate.

Streamlined Sequence Analysis. In addition to fast cloning and low background, the pCR®4Blunt-TOPO® vector offers a minimized multiple cloning site to make sequencing your BAC clones more efficient. Sequencing primer sites are located as close as 33 base pairs from the cloning site so you’ll read more of your insert and less of the vector. When you’re sequencing hundreds of clones, the proximity of primer sites can save you hours of time.

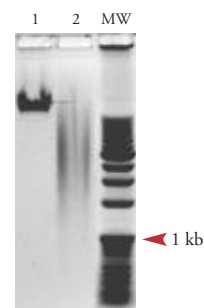
Reagents for Five Complete Libraries. The TOPO® Shotgun Subcloning Kit provides enough reagents to make five libraries. Each kit includes linearized and topoisomerase I-activated pCR®4Blunt-TOPO® vector, five nebulizers for shearing BAC DNA, reagents for blunting and cloning, and TOP10 One Shot® Competent *E. coli* for transformation. If you’ve been looking for a way to simplify the subcloning and analysis of BAC DNA, the TOPO® Shotgun Subcloning Kit is your answer. Call and order today.

Description	Reactions	Cat. no.	Price
TOPO® Shotgun Subcloning Kit with TOP10 One Shot® Chemically Competent <i>E. coli</i>	5	K7000-01	\$399
TOPO® Shotgun Subcloning Kit with TOP10 One Shot® Electrocomp™ <i>E. coli</i>	5	K7050-01	\$399

References:

1. Shuman, S. (1994) *J. Biol. Chem.* **269**: 32678-32684.
2. Bernard, P. et al. (1995) *J. Mol. Biol.* **254**: 534-541.

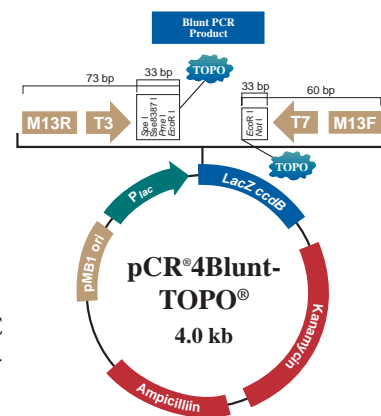
Figure 1 - DNA Sheared with a Nebulizer



Fifty micrograms of purified, high molecular weight *E. coli* genomic DNA was sheared on ice in 2 ml TE containing 20% glycerol using a nebulizer. The DNA was subjected to 9-10 psi of compressed air for 30 seconds.

Lane 1: unsheared DNA
Lane 2: DNA sheared for 30 seconds

Figure 2 - pCR®4Blunt-TOPO® Vector



 Represents covalently-bound topoisomerase I

Features of pCR®4Blunt-TOPO® include:

- Flanking *EcoR* I sites to simplify excision of cloned products
- Unique *Sse8387* I site in the multiple cloning site to simplify performing nested deletions
- T7, T3, M13(-20) forward, and M13 reverse sequencing primer sites

The Ultimate Convenience in *E. coli* Medium Preparation

imMedia™

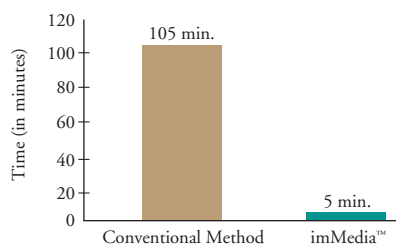
It's late. You're the last one in lab. Before you leave you need to plate a few transformations, but you've run out plates. Don't panic. Now there's imMedia™. imMedia™ offers the ultimate convenience by allowing you to prepare low-salt LB media in just 5 minutes in your microwave oven.

Time-Saving Convenience. imMedia™ is presterilized *E. coli* growth medium that contains everything you need in a single convenient pouch (Table 1). With imMedia™ there's:

- No measuring and mixing of medium components
- No autoclaving
- No waiting for the medium to cool before adding antibiotics, IPTG, or X-gal

Simply mix imMedia™ with water and heat in a microwave oven. In less than 5 minutes, you can prepare 200 ml of liquid medium or 8-10 agar plates. With imMedia™ you'll save hours of medium preparation time (Figure 1) and get great results.

Figure 1 - Comparison of Time Needed to Prepare Agar Plates



The Highest Quality. To ensure robust performance, our quality control team tests each lot of imMedia™ for sterility using non-autoclaved flasks and non-sterile, ionized water. *E. coli* strains carrying the appropriate antibiotic resistance marker are used to ensure that imMedia™ provides the same growth support and selection efficiency as conventionally-prepared LB medium. For imMedia™ Blue products, which contain IPTG and X-gal, our test requires that blue colonies be easily recognized within 16 hours of incubation at 37°C (Figure 2). These high standards guarantee that you'll get great results.

Figure 2 - TOP10 *E. coli* Growth on imMedia™ Plates



TOP10 *E. coli* were plated on imMedia™ Agar plates. The plates were incubated at 37°C for 16 hours.

- A:** TOP10 *E. coli* grown on an imMedia™ Amp Agar plate.
B: Amp^R TOP10 *E. coli* grown on an imMedia™ Amp Agar plate.
C: Amp^RlacZ⁺ TOP10 *E. coli* grown on an imMedia™ Amp Blue plate.

Pick Your Pack Today. imMedia™ offers the ultimate convenience for preparing *E. coli* media. There's no weighing, mixing, autoclaving, or waiting. Start preparing your medium the imMedia™ way. Use Table 1 to pick your favorite type, then give Invitrogen a call and order today.

Table 1 - imMedia™ Components and Ordering Information

imMedia™ Type	Components					Ordering Information	
	Low-Salt LB Media Components	Agar	Antibiotic	IPTG and X-gal	Heat Stabilizers	Quantity (Pouches*)	Cat. no. Price
imMedia™ Amp Liquid	✓		ampicillin		✓	20 Q600-20 \$130	
imMedia™ Kan Liquid	✓		kanamycin		✓	20 Q610-20 \$130	
imMedia™ Zeo Liquid	✓		Zeocin™		✓	20 Q620-20 \$210	
imMedia™ Amp Agar	✓	✓	ampicillin		✓	20 Q601-20 \$155	
imMedia™ Kan Agar	✓	✓	kanamycin		✓	20 Q611-20 \$155	
imMedia™ Zeo Agar	✓	✓	Zeocin™		✓	20 Q621-20 \$260	
imMedia™ Amp Blue	✓	✓	ampicillin	✓	✓	20 Q602-20 \$210	
imMedia™ Kan Blue	✓	✓	kanamycin	✓	✓	20 Q612-20 \$210	

* Each imMedia™ pouch contains sufficient reagents to prepare 200 ml of liquid media or 8-10 agar plates using standard 100 mm dishes.

Inducible Expression of Native hAE1 in the Ecdysone-Inducible Mammalian Expression System

Richard T. Timmer and Robert B. Gunn, Department of Physiology, Emory University, Atlanta, GA

The human anion exchange protein 1 (hAE1) plays a key role in anion transport and buffering red blood cell pH during carbon dioxide (CO₂) exchange in the peripheral and lung capillaries. During the process of CO₂ exchange, CO₂ enters red blood cells (RBC) by simple diffusion where it is hydrated into cellular bicarbonate (HCO₃⁻). Bicarbonate exits the cell via hAE1 in exchange for extracellular chloride (Cl⁻) ions. In order to accurately study the activity and kinetics of hAE1, it is necessary to express the protein in cell lines that do not have native hAE1. However, when expressed in non-RBC type cells, functional hAE1 attempts to bring the cell line to electrochemical equilibrium. This acidifies the cytoplasm and kills cells that have normal negative membrane potentials. For this reason, previous attempts to establish permanent non-RBC cell lines that express sufficient amounts of hAE1 for experimental studies have been unsuccessful. Here we demonstrate that the Ecdysone-Inducible Mammalian Expression System overcomes these expression difficulties and expresses ample amounts of hAE1 for structural and functional analyses.

Methods. hAE1 cDNA was cloned into the pIND Ecdysone-Inducible Expression Vector to generate pIND-hAE1. After confirming that the cDNA was in the correct orientation, pIND-hAE1 was transfected into EcR-293 cells (HEK-293 cells that stably express the ecdysone receptor from the pVgRXR regulatory vector) using a standard calcium phosphate precipitation method. Stable recombinant cells were generated by growth in MEM medium containing Hanks' salts, L-glutamine, 5% FCS, 2.5 mM Zeocin[™] and 400 µg/ml G418. Clonal cell populations were isolated and tested for their ability to express hAE1 upon induction with 2.5 µM muristerone A for 48 hours. Expression was assayed by western blot analysis using an anti-hAE1 antibody. hAE1-expressing cell populations were isolated and used in further expression experiments with varying expression conditions. Functional analysis was performed by measuring the efflux of ³⁶Cl⁻ tracer ions from cells attached in 12-well plates or the influx

of ³⁵SO₄²⁻ tracer ions from cells attached in 24-well plates as a function of time.

Results. We were able to produce sufficient amounts of hAE1 for subsequent use in experimental studies without harming the HEK-293 cells. The level of hAE1 induction was measured over various time-points and muristerone A dosages. Results show that the amount of hAE1 protein produced increased as the cells were exposed to higher dosages of muristerone A. This increase was also observed as the cells were exposed to muristerone A for a longer period of time (Figure 1). Uninduced cells showed no detectable expression of hAE1. Induced cells showed normal morphology and remained adherent. However, they did exhibit a slower doubling time than uninduced EcR-293/hAE1 or nontransfected HEK-293 cells. Functional analysis revealed that uninduced cell lines showed minimal ³⁶Cl⁻ tracer efflux activity. Induced cells showed a flux that was approximately 40 times the background flux in non-induced cells. Transport of ³⁶Cl⁻ and ³⁵SO₄²⁻ by induced hAE1 protein exhibited the same characteristics of hAE1-mediated transport in RBCs (1).

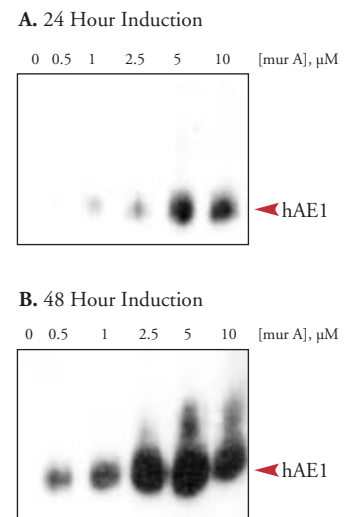
Discussion. Due to the tight regulation of the Ecdysone System, we were able to generate a non-RBC cell line capable of expressing functional hAE1. Sufficient protein was expressed to enable us to track the kinetic characteristics of hAE1 without any deleterious effects on the host cell. The Ecdysone-Inducible Mammalian Expression System and the EcR-293/hAE1 cell line will be useful as a model for future monitoring of the function of mutant anion exchangers.

Product	Quantity	Cat. no.	Price
Ecdysone-Inducible Mammalian Expression System	1 kit	K1001-01	\$500
EcR-293	1 x 10 ⁶ cells	R650-07	\$415
Muristerone A	1 mg	H100-01	\$170
Zeocin [™]	1 g	R250-01	\$175

Reference:

1. Timmer, R.T. and Gunn, R.B. (1999) *Am. J. Physiol.* **276**: C66-75.

Figure 1 - Time- and Dose-Dependent Induction of hAE1 in EcR-293 cells



EcR-293/hAE1 cells were induced with varying amounts of muristerone A for either 24 or 48 hours, as indicated. One microgram of whole cell lysates with equal amounts of total protein were run on an SDS gel and then electroblotted to nitrocellulose paper. Protein was detected with an anti-hAE1 antibody.

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Better Results in PCR Cloning

TOPO TA Cloning® Kits

The TOPO TA Cloning® protocol has been improved to yield two to three times more colonies in less time than the original procedure. A new ligation procedure and faster transformation method have been incorporated to give you better results.

Improved Results. The five-minute TOPO® Cloning procedure revolutionized PCR cloning by making it faster and more efficient. DNA ligase and overnight ligations were replaced by vectors covalently-bound to topoisomerase I (Figure 1) and a five-minute ligation in water. TOPO® Cloning saves an entire day and results in ≥95% recombinants. Recent data from Invitrogen's PCR Cloning Experts show a two- to three-fold increase in the total number of colonies when the TOPO® Cloning reaction is performed in NaCl and MgCl₂ rather than water (Figure 2). This means better results in all of your TOPO® Cloning.

Five-Minute Transformation. The new TOPO® Cloning procedure includes a streamlined transformation protocol to save you time. We've reduced the ice incubation step from 30 minutes to just 5 minutes. This faster protocol saves you almost half an hour when compared to the original protocol—and still yields great transformation results.

Improve your Cloning. All TOPO TA Cloning® Kits come with prepared vector, PCR reagents (except a thermostable polymerase), salt solution, One Shot® Competent *E. coli*, and controls. Call and order your TOPO TA Cloning® Kit today.

Product	Reactions	Cat. no.	Price
TOPO TA Cloning® Kit with TOP10 One Shot® Chemically Competent Cells	20	K4500-01	\$315
TOPO TA Cloning® Kit Dual Promoter with TOP10 One Shot® Chemically Competent Cells	20	K4600-01	\$330

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

TOPO TA Cloning® is covered under one or more of U.S. Patents 5,487,993; 5,766,891; 5,827,657 and corresponding foreign patents. Other patents pending. For basic research only.

Maximize Your Productivity

Custom Services

Maximizing productivity in your laboratory, whether academic or commercial, is always a top priority. This becomes challenging when resources such as time, facilities, staff, and expertise are limited. The Custom Services Specialists at Invitrogen can help you overcome these limitations. By outsourcing your research projects to Invitrogen's Custom Services Scientists, you can limit your risk and maximize your productivity.

Fully-Integrated Capabilities. Invitrogen's Custom Services provide complete, fully-integrated molecular biology and protein expression services. Table 1 summarizes some of the laboratory services available. From project initiation to completion, our staff of experts will work with you to meet your goals.

Table 1 - Custom Services Available

Services	Details	Advantage
Subcloning	Primer design PCR gene from appropriate sample Cloning into vector of choice Sequencing	Fast service—eliminates the tedious tasks of finding, cloning, and sequencing your gene of interest
Baculovirus Expression	Cloning Transfection Plaque purification Expression	High-quality protein—provides posttranslational modifications rivaling native protein
<i>Pichia</i> Expression	Cloning Transfection Expression Scale up	Large quantities—provides cost-effective scale-up production at industrial-scale levels

Services on Demand. The principal benefit of outsourcing research projects to Invitrogen's Custom Services is the access to facilities and expertise on demand. Outsourcing reduces the need to train staff, maintain equipment, pay licensing fees, and take on unnecessary risk so you save time and resources. To learn more about how Invitrogen can maximize your laboratory's productivity, contact our Custom Services Representative at 800-955-6288, ext. 265 today.

Figure 1 - pCR®II-TOPO® and pCR®2.1-TOPO® Vectors

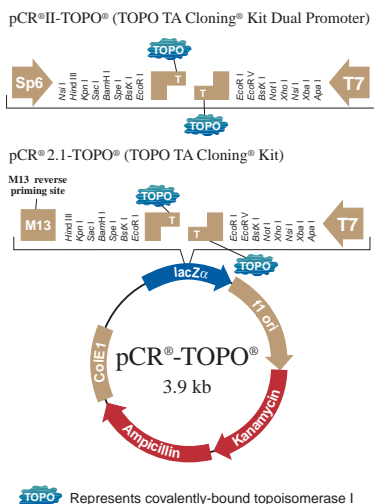
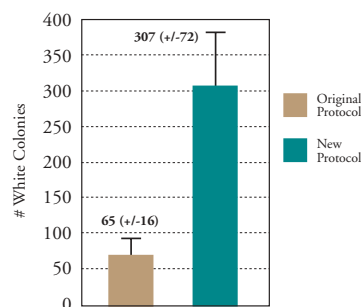


Figure 2 - Comparison of Results using Different TOPO® Cloning Protocols



1 µl of a 750 bp PCR product and either 3 µl H₂O (original protocol) or 3 µl of 300 mM NaCl, 15 mM MgCl₂ solution (new protocol) were added to 1 µl of pCR®2.1-TOPO®. Reactions were incubated at room temperature for 5 minutes then 2 µl of each was used to transform 50 µl of TOP10 One Shot® Chemically Competent *E. coli*. 10 µl of each reaction was plated on LB/Kanamycin/X-gal plates and incubated overnight at 37°C. Bars represent an average of three separate reactions.

Inducible Mammalian Expression with the Lowest Basal Levels is Now Even Easier

GeneSwitch™ Cell Lines

The GeneSwitch™ System is an inducible mammalian expression system that offers the absolute lowest levels of basal expression. This means that even the most toxic proteins can be expressed. To save you time and effort when using the GeneSwitch™ System, two ready-to-use GeneSwitch™ Cell Lines are now available.

Tight Transcriptional Control. GeneSwitch™ uses a two plasmid system to provide inducible expression with low background levels. The expression plasmid, pGene/V5-His (Figure 1), carries a specialized hybrid promoter composed of six Gal4 binding sites and the E1b TATA box. This hybrid promoter is transcriptionally silent in the absence of additional factors so there's virtually no basal expression. The second plasmid used in the system, pSwitch, expresses the GeneSwitch™ regulatory protein. This protein has a tripartite structure made up of the Gal4 DNA binding domain, a truncated human progesterone receptor ligand binding domain (hPR-LBD), and the NFκB activation domain (AD). When mifepristone, the inducer, is added, it binds to the hPR-LBD region of the GeneSwitch™ protein and causes the molecule to undergo a conformational change. In the new conformation, the Gal4 binding domain is

able to bind the Gal4 binding sites on pGene/V5-His. The NFκB AD then activates transcription of the gene of interest (Figure 1).

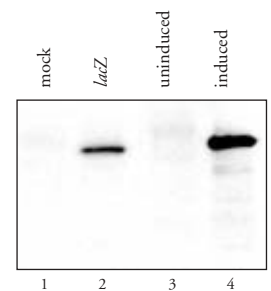
Accelerate Discovery. To save you weeks of time and effort when using the GeneSwitch™ System, the GeneSwitch™-3T3 and GeneSwitch™-CHO cell lines are now available. Each cell line has been stably transfected with the pSwitch plasmid and functionally tested for use in the GeneSwitch™ System. This means that all you have to do to take advantage of the low basal expression of the GeneSwitch™ System is clone your gene of interest into the pGene/V5-His vector and transfect into pre-tested GeneSwitch™ Cell Lines.

Just Flip the Switch. The GeneSwitch™ Cell Lines not only save you time, but also offer great results. Each cell line is extensively tested to ensure low basal expression and high inducibility with mifepristone. To demonstrate the tight control in these cell lines, the *lacZ* gene was cloned into pGene/V5-His and transfected into the GeneSwitch™-3T3 Cell Line. As shown in Figure 2, β-galactosidase expression is undetectable in the absence of mifepristone. However, in the presence of mifepristone there is significant expression.

Get Results. The GeneSwitch™ System offers the lowest basal levels of of any inducible system. Using the GeneSwitch™ Cell Lines will save you weeks of time and effort. With your choice of GeneSwitch™-3T3 or GeneSwitch™-CHO Cell Lines, there's no better way to get started. Order today.

Product	Quantity	Cat. no.	Price
GeneSwitch™-3T3 Cell Line	3 x 10 ⁶ cells	R770-07	\$415
GeneSwitch™-CHO Cell Line	3 x 10 ⁶ cells	R772-07	\$415
GeneSwitch™ Complete Kit	1 kit	K1060-01	\$750
GeneSwitch™ Core Kit	1 kit	K1060-02	\$545

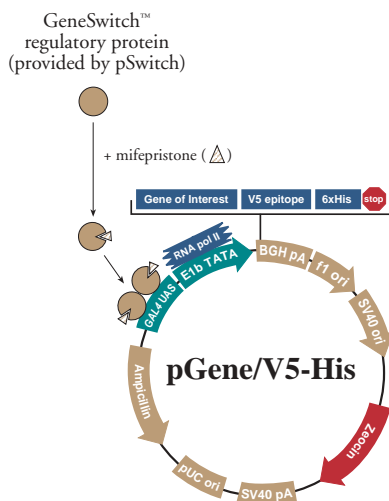
Figure 2 - Tight Control of *lacZ* Expression in GeneSwitch™-3T3 Cells



The *lacZ* gene was cloned into pGene/V5-His to generate pGene/V5-His/*lacZ*. Four micrograms of this construct was transfected by calcium phosphate into 1 x 10⁵ GeneSwitch™-3T3 cells. Expression was induced with 10⁻⁸ M mifepristone for 24 hours. Cells were harvested and 20 μg of cell lysate loaded onto a gel, transferred to nitrocellulose, and detected with the Anti-V5 Antibody.

Lane 1: Mock transfected GeneSwitch™-3T3 cells
 Lane 2: *lacZ* control
 Lane 3: Uninduced GeneSwitch™-3T3/pGene/V5-His/*lacZ* cells
 Lane 4: Induced GeneSwitch™-3T3/pGene/V5-His/*lacZ* cells

Figure 1 - GeneSwitch™ Mechanism



High-Level Expression of Recombinant ADP-Ribosyl Cyclase in *Pichia pastoris*

Cyrus Munshi and Hon Cheung Lee, Department of Pharmacology, University of Minnesota, Minneapolis, MN

“The 300 mg/L yield in *Pichia pastoris* shows a vast improvement over the 1 mg/L previously obtained in *E. coli*.”

Several mechanisms exist for mediating calcium release from intracellular stores. One enzyme, ADP-ribosyl cyclase, catalyzes the synthesis of two structurally and functionally distinct calcium signaling molecules, cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate (1). The former is known to be important in regulating the calcium-induced calcium-release mechanism in cells. Because of this role, it is important to understand ADP-ribosyl cyclase’s novel catalytic mechanism. In order to perform adequate structural and functional studies, large quantities of ADP-ribosyl cyclase protein are required. Earlier expression attempts in a bacterial system yielded only 1 mg/L. Here we describe the use of the *Pichia pastoris* Expression System to express ADP-ribosyl cyclase to levels as high as 300 mg/L (2).

Construction of an ADP-Ribosyl Cyclase Clone.

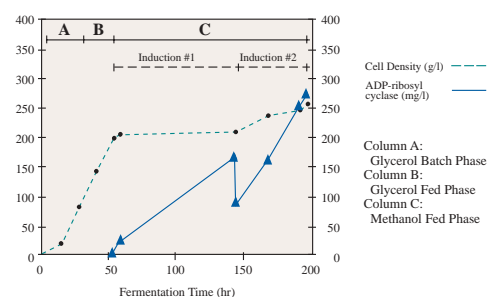
cDNA encoding ADP-ribosyl cyclase was PCR amplified and cloned into pPICZ α (Figure 1) to generate pPICZ α /cyclase. pPICZ α uses the AOX1 promoter and the α -factor secretion signal for high-level, secreted expression. To simplify growth medium requirements, we generated a His⁺ cell line by transforming the histidine-deficient GS115 *Pichia pastoris* cell line with the pPIC9 vector, which carries a wild-type *HIS4* gene. This His⁺ GS115 cell line was transfected with pPICZ α /cyclase.

Transformants were selected on YPDS medium containing 100 μ g/ml Zeocin[™]. Transformants were tested for their ability to cyclize nicotinamide guanosine dinucleotide (NGD⁺) and the clone with the highest activity was selected for fermentation.

Fermentation. Fermentation was carried out in a Bioflo III bioreactor equipped with a five-liter fermentation vessel. A starting culture was grown to OD₅₉₅=10 and used to inoculate the fermenter. The culture was allowed to grow until the glycerol was completely depleted (Figure 2, Column A). A continuous feed of 50% glycerol at a rate of 9.0 ml/h/L to 18.2 ml/h/L was added until the culture density reached 200 g/L (Figure 2, Column B). Expression was induced by the addition of 100% methanol at 3.0 ml/h/L. Protein was detected as early as five hours postinduction (Figure 2, Column C,

Induction #1). After 100 hours, the protein was estimated to be 180 mg/L. Two liters of the fermentation broth were removed and centrifuged at 1,500 x g for 20 minutes. Yeast cells were resuspended in two liters of fresh medium and reintroduced into the fermenter. The methanol feed was increased to 4.2 ml/h/L for an additional 53 hours (Figure 2, Column C, Induction #2). Total protein was again measured and estimated to be 300 mg/L. SDS-PAGE analysis revealed that ADP-ribosyl cyclase comprised 90-95% of the total secreted protein.

Figure 2 - ADP-Ribosyl Cyclase Fermentation Timeline



Analyzing Cyclase Activity. Recombinant ADP-ribosyl cyclase was tested for activity using a NGD⁺ cyclization assay (3). Cyclization activity indicated that at least 94% of the total protein in the media was functional recombinant cyclase.

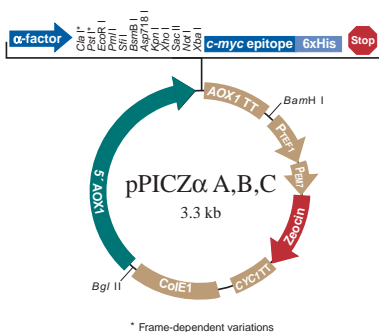
Discussion. Here we demonstrate high-level expression of functional ADP-ribosyl cyclase in the *Pichia pastoris* Expression System. The 300 mg/L yield shows a vast improvement over the 1 mg/L previously obtained in *E. coli*. This high yield will facilitate the production of various mutant forms of the cyclase protein for functional analysis.

Product	Quantity	Cat. no.	Price
EasySelect [™] <i>Pichia</i> Expression Kit (with pPICZ α)	1 kit	K1740-01	\$725
pPICZ α	20 μ g	V195-20	\$350
Zeocin [™]	1 g	R250-01	\$175

References:

- Lee, H.C. (1997) *Physiol. Rev.* 77: 1133-1164.
- Munshi, C. and Lee, H. C. (1997) *Prot. Exp. and Purif.* 11: 104-110.
- Graeff, R.M. et al. (1994) *J. Biol. Chem.* 269: 30260-30267.

Figure 1 - pPICZ α Vector



Enhanced Expression of Eukaryotic Proteins in *E. coli*

pTrcHis TOPO TA Cloning[®] Kits

Eukaryotic proteins are frequently expressed at low levels in *E. coli* because of inefficient translation initiation of heterologous mRNA sequences (1,2). Invitrogen offers several *trc* promoter-based expression vectors specifically designed to circumvent this problem. Two state-of-the-art *trc* expression vectors, pTrcHis-TOPO[®] and pTrcHis2-TOPO[®], combine the ease of TOPO Cloning[®] with enhanced eukaryotic protein translation to improve protein yields in *E. coli*.

The Key to Efficient Translation. Poor translation initiation of eukaryotic proteins in *E. coli* is often caused by secondary structure formation between the mRNA sequences in the protein coding region and the 5' untranslated region (3). Both pTrcHis-TOPO[®] and pTrcHis2-TOPO[®] (Figure 1) incorporate an AT-rich minicistron element that minimizes unfavorable secondary structure formation (4). This minicistron is the key to efficient translation and improved eukaryotic protein yields in *E. coli*.

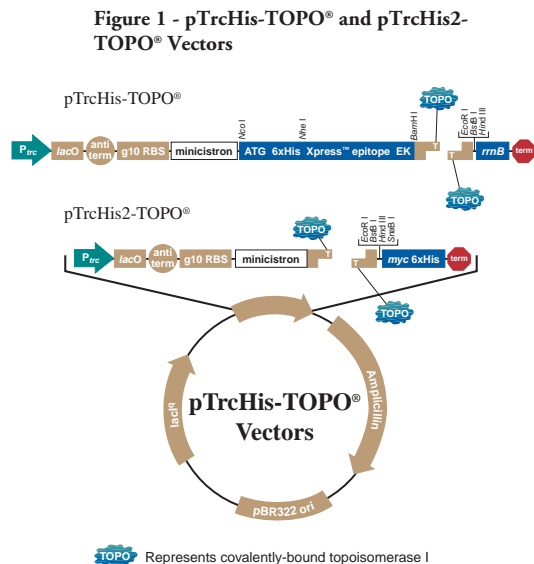


Figure 1 - pTrcHis-TOPO[®] and pTrcHis2-TOPO[®] Vectors

minicistron element. The western blot in Figure 2 shows a dramatic increase in the yield of hVEGF-B in the presence of the minicistron.

State-of-the-Art Expression Tools. The pTrcHis-TOPO[®] and pTrcHis2-TOPO[®] vectors carry the popular *trc* promoter derived from the *trp* and *lac* promoters. Both vectors are supplied linearized and activated with topoisomerase I for 5-minute cloning of *Taq*-amplified PCR fragments. You'll save an entire day because there's no overnight ligation and no additional subcloning required. You'll also get great results—>85% recombinants. In addition, pTrcHis-TOPO[®] and pTrcHis2-TOPO[®] encode an N- or C-terminal fusion tag, respectively, to facilitate downstream protein detection and purification (Table 1).

Table 1 - Fusion Tags in the pTrcHis-TOPO[®] Vectors

Vector	Fusion Tag	Tag Position	Tag Cleavage
pTrcHis-TOPO [®]	Xpress™ 6xHis	N-term	Enterokinase
pTrcHis2-TOPO [®]	myc 6xHis	C-term	—

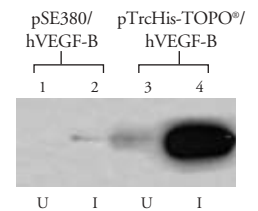
Convenient Kits. The pTrcHis and pTrcHis2 TOPO TA Cloning[®] Kits come complete with all the reagents you need for PCR cloning and gene expression. Each kit includes a topoisomerase I-activated expression vector, PCR reagents (except *Taq* polymerase), primers, a positive control vector, and TOP10 One Shot[®] Chemically Competent *E. coli*. Start increasing your yield of eukaryotic proteins in *E. coli*. Order a pTrcHis TOPO TA Cloning[®] Kit today.

Description	Reactions	Cat. no.	Price
pTrcHis TOPO TA Cloning [®] Kit	20	K4410-01	\$360
pTrcHis2 TOPO TA Cloning [®] Kit	20	K4400-01	\$360

References:

1. Varadarajan, R. *et al.* (1985) *Proc. Natl. Acad. Sci. USA* **82**: 5681-5684.
2. Nagai, K. *et al.* (1984) *Nature* **309**: 810-812.
3. Berkhout, B. *et al.* (1985) *Nuc. Acid Res.* **13**: 6955-6956.
4. Schoner, B. E. *et al.* (1986) *Proc. Natl. Acad. Sci. USA* **83**: 8506-8510.

Figure 2 - The Effect of the Minicistron on hVEGF-B Expression in *E. coli*



TOP10 *E. coli* were transformed with pSE380/hVEGF-B or pTrcHis-TOPO[®]/hVEGF-B. Positive transformants were grown to OD₆₀₀=0.6 and induced for 4 hours with 1 mM IPTG. *E. coli* cell lysates harvested from 1 ml cultures were analyzed by SDS-PAGE and western blot. The blot was probed with the Anti-Xpress™-HRP Antibody (1:5,000) and detected using chemiluminescence.

Lane 1: pSE380/hVEGF-B uninduced
 Lane 2: pSE380/hVEGF-B induced
 Lane 3: pTrcHis-TOPO[®]/hVEGF-B uninduced
 Lane 4: pTrcHis-TOPO[®]/hVEGF-B induced

Proven Results. To demonstrate the effectiveness of the minicistron element in improving yields of eukaryotic protein, we compared the expression levels of human vascular endothelial growth factor B (hVEGF-B) from *trc* promoter-based vectors with (pTrcHis-TOPO[®]) and without (pSE380) the



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Research Genetics to Join Invitrogen

Invitrogen's intent to acquire Research Genetics was announced on December 8, 1999. Invitrogen is pursuing this merger in order to provide you with a more diverse set of tools to accelerate genomics research. Research Genetics is a leading supplier of services and products that simplify and improve functional genomics and gene-based drug discovery research. Product lines include custom services for DNA and peptide synthesis, antibody production, library screening, genotyping, and sequencing. In addition, Research Genetics offers DNA microarrays and custom software for microarray data analysis, PCR primers for specific gene amplification, genomic and cDNA libraries, and MapPairs® microsatellite markers. This merger will enable Invitrogen to continue providing you with innovative products and services designed to make your research faster, easier, and more efficient. For more information, check out the letter of intent to merge on our web site at www.invitrogen.com. ■

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