

Expressions

A newsletter for gene cloning and expression



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Free Mammalian Expression Poster. Check out the back cover

Powerful Inducible Expression from the CMV Promoter

T-REx™ System

Figure 1 - T-REx™ TetR-Expressing Vector

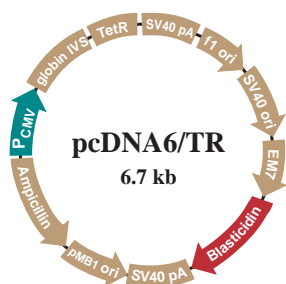
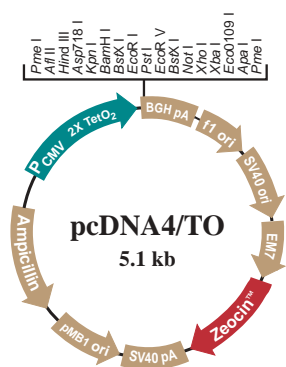


Figure 2 - T-REx™ Inducible Expression Vector



New Vectors

Check Invitrogen's web site for T-REx™ inducible expression vectors that contain epitope tags for rapid detection of recombinant proteins.

Until now, regulated mammalian expression systems have relied on modified promoters and transcriptional activators to modulate expression. Invitrogen's new T-REx™ (Tetracycline-Regulated Expression) System is the first to take advantage of the complete human cytomegalovirus (CMV) promoter to give you:

- High levels of induced expression
- Tight repression for low basal levels
- Expression independent of viral transactivating domains that may harm the cell and lower expression levels

The Complete CMV Promoter. The complete CMV enhancer/promoter is widely recognized for its ability to drive expression in a wide variety of mammalian cells. Other inducible systems use the weaker, minimal CMV promoter to drive expression, resulting in lower yields of recombinant protein. Only the T-REx™ System gives you the power of the complete CMV enhancer/promoter so that you can achieve high levels of induced expression.

The Mechanism of Regulation. In the T-REx™ System, recombinant protein expression is regulated by a simple tetracycline-dependent derepression mechanism (1,2). Expression is actively repressed by the Tet repressor (TetR) protein, which is constitutively expressed from the vector pcDNA6/TR (Figure 1). TetR homodimers tightly bind two tetracycline operator sites (TetO₂) located within the CMV promoter of the inducible expression vector, pcDNA4/TO (Figure 2), to regulate expression of the gene of interest. When TetR is bound to the TetO₂ sites, transcription is effectively repressed. In the presence of tetracycline, the TetR protein releases the TetO₂ sites. This derepression allows transcription, and hence protein expression, to proceed.

Stable Cell Lines—No Waiting. To help you analyze your protein in mammalian cells as quickly and as efficiently as possible, Invitrogen offers three cell lines—293 (T-REx™-293), HeLa (T-REx™-HeLa), and U2OS (T-REx™-U2OS)—each of which stably expresses the TetR repressor protein. For high-level inducible expression, you simply

transfect one of the T-REx™ cell lines with a pcDNA4/TO construct containing your gene of interest and induce with tetracycline. There's no need for you to spend any time or effort generating cell lines that stably express the TetR protein. To ensure the quality of your results, each T-REx™ cell line is functionally tested by transiently transfecting it with pcDNA4/TO/*lacZ*, inducing expression with tetracycline, and analyzing β-galactosidase expression (Figure 3).

Figure 3 - Induced Expression in the T-REx™ Cell Lines



TetR-expressing T-REx™ cell lines were transiently transfected with pcDNA4/TO/*lacZ*. Twenty-four hours posttransfection 1 μg/ml of tetracycline was added. β-galactosidase expression was assayed 24 hours postinduction by western blotting with an Anti-β-galactosidase antibody. U = uninduced, I = induced

Get the Power. T-REx™ is available as a complete system containing pcDNA6/TR, pcDNA4/TO, sequencing primers, selection agents, and tetracycline so you don't have to spend time and money preparing, ordering, and testing reagents. A core kit is also available that contains only the vectors and sequencing primers. Get the power of regulated expression from the complete CMV promoter. Order the T-REx™ System today.

Product	Quantity	Cat. no.	Price
T-REx™ Complete System	1 kit	K1020-01	\$695
T-REx™ Core System	1 kit	K1020-02	\$490
pcDNA4/TO	20 μg	V1020-20	\$275
pcDNA6/TR	20 μg	V1025-20	\$225
T-REx™-293 Cells	3 x 10 ⁶	R710-07	\$395
T-REx™-HeLa Cells	3 x 10 ⁶	R714-07	\$395
T-REx™-U2OS Cells	3 x 10 ⁶	R712-07	\$395
Tetracycline	5 g	Q100-19	\$16
Blasticidin	50 mg	R210-01	\$130
Zeocin™	1 g	R250-01	\$160

References:

1. Yao, F. et al. (1998) *Human Gene Therapy* 9: 1939-1950.
2. Yao, F. and Eriksson, E. (1999) *Human Gene Therapy* 10: 419-427.



One-Step Cloning into High-Level Mammalian Expression Vectors

Eukaryotic and pEF6/V5-His TOPO TA Cloning® Kits

Expressing properly processed, functional recombinant protein often requires using mammalian cells as the host. Two new TOPO TA Cloning® Kits make cloning into a mammalian expression vector faster and more efficient than ever before. Now you can save hours of time by cloning your PCR-amplified sequence and proceeding directly to high-level mammalian expression without subcloning.

High-Level Expression. The pcDNA3.1/V5-His-TOPO® and pEF6/V5-His-TOPO® vectors allow you to clone a *Taq*-amplified PCR product directly into a mammalian expression vector. Each vector carries a strong promoter for high yields of recombinant protein.

- **CMV Promoter.** The human cytomegalovirus (CMV) immediate-early enhancer/promoter present in pcDNA3.1/V5-His-TOPO® (Figure 1) offers constitutive, high-level expression of recombinant proteins in several cell lines including CHO, HEK-293, CV-1, and HeLa (1,2).
- **EF-1 α Promoter.** The human elongation factor-1 α (EF-1 α) subunit promoter contained in pEF6/V5-His-TOPO® (Figure 2) expresses high levels of protein across a broad range of mammalian species and cell types (3). Because EF-1 α is a non-viral promoter, it is ideal for use in cell lines that down-regulate viral promoters.

Select, Detect, and Purify. In addition to a powerful promoter for high-level expression, pcDNA3.1/V5-His-TOPO® and pEF6/V5-His-TOPO® include the following unique features for selecting stable cell lines and detecting and purifying fusion proteins:

- Neomycin (pcDNA3.1/V5-His-TOPO®) or blasticidin (pEF6/V5-His-TOPO®) resistance gene for generating stable cell lines
- C-terminal V5 epitope for rapid, low-background detection of fusion proteins with an Anti-V5 Antibody
- C-terminal polyhistidine (6xHis) for simple purification of fusion proteins with ProBond™ resin

Ready-To-Go Vectors. The pcDNA3.1/V5-His-TOPO® and pEF6/V5-His-TOPO® mammalian expression vectors use the unique TOPO TA Cloning® technology to allow quick and efficient cloning of *Taq*-amplified PCR products. Each vector is provided linearized and activated with topoisomerase I. This allows PCR products to be ligated with high efficiency in just 5 minutes at room temperature (4). To clone in frame with the C-terminal fusion tag, you simply need to design the appropriate PCR primers. There's no subcloning required. With the TOPO TA Cloning® mammalian expression vectors, you'll save hours of time by going directly from cloning to high-level expression.

Everything You Need. The Eukaryotic and pEF6/V5-His TOPO TA Cloning® Kits are provided with everything you need to clone your *Taq*-amplified PCR product into a high-level mammalian expression vector. Each kit includes either the pcDNA3.1/V5-His-TOPO® or pEF6/V5-His-TOPO® vector, PCR buffer, dNTPs, 6X TOPO Cloning® Stop Solution, sequencing primers, TOP10 One Shot™ Competent Cells, and cloning and expression controls. For rapid cloning and high-level mammalian expression, order your Eukaryotic or pEF6/V5-His TOPO TA Cloning® Kit today.

Product	Quantity	Cat. no.	Price
Eukaryotic TOPO TA Cloning® Kit			
with the pcDNA3.1/V5-His-TOPO® vector	20 reactions	K4800-01	\$345
pEF6/V5-His TOPO TA Cloning® Kit			
20 reactions	20 reactions	K9610-20	\$345
TOP10 One Shot™ Cells			
20 reactions	20 reactions	C4040-03	\$171
Blasticidin	50 mg	R210-01	\$130
Anti-V5 Antibody	50 μ l*	R960-25	\$135
Anti-V5-HRP Antibody	50 μ l*	R961-25	\$160
ProBond™ Resin	50 ml	R801-01	\$285

* Sufficient antibody is supplied for 25 western blots.

References:

1. Foecking, M. and Hofstetter, H. (1986) *Gene* 45: 101-105.
2. Kronman, C. et al. (1992) *Gene* 121: 295-304.
3. Mizushima, S. and Nagata, S. (1990) *Nuc. Acids Res.* 18: 5322.
4. Shuman, S. (1994) *J. Biol. Chem.* 269: 32678-32684.

“With the TOPO TA Cloning® mammalian expression vectors, you'll save hours of time by going directly from cloning to high-level expression.”

Figure 1 - pcDNA3.1/V5-His-TOPO®

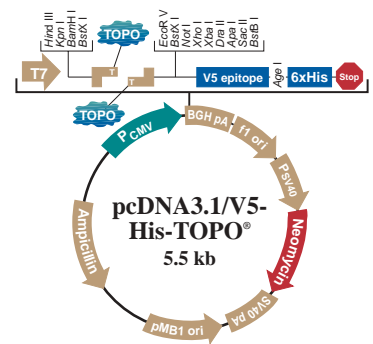
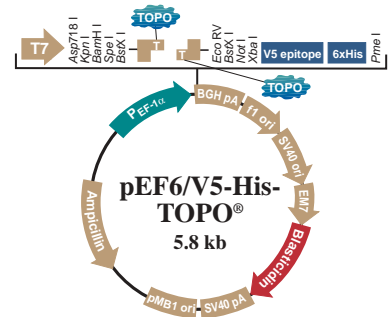


Figure 2 - pEF6/V5-His-TOPO®



TOPO® Represents covalently-bound topoisomerase I

Unique Strains for Specialized Cloning

INV110 and TOP10/P3 One Shot™ Chemically Competent E. coli

Propagating DNA for digestion with methylation-sensitive restriction enzymes, or growing plasmids that carry *supF* selection, require the use of specific *E. coli* strains to ensure your success. Invitrogen offers competent *E. coli* for these specialized cloning needs. The strains, INV110 and TOP10/P3, are supplied in the single-use One Shot™ format for the ultimate in transformation convenience.™

Table 1 - Common *dam*- and *dcm*-Methylation-Sensitive Enzymes and their Sites

A. Enzymes

<i>dam</i>	<i>dcm</i>
<i>Cla</i> I	<i>Apa</i> I
<i>Mbo</i> I	<i>Bsl</i> I
<i>Xba</i> I	<i>Stu</i> I

Please see your restriction enzyme manufacturer's catalog for a complete list of *dam*- and *dcm*-methylation-sensitive restriction enzymes.

B. Methylation Sites

Methylase	Methylation Site
<i>dam</i>	G ^m ATC
<i>dcm</i>	C ^m C(A/T)GG

Additional vector sequence may be required for enzyme recognition. For enzymes that recognize palindromic sequences, both ends of the sequence must be checked for the possibility of overlapping methylation (i.e. *Cla*I, *GATC*_{gat} and *atcGATC*).

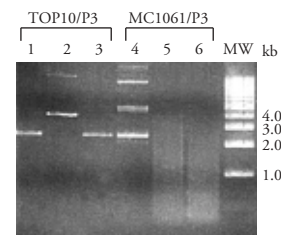
***dam/dcm* Methylation-Free DNA.** INV110 is the ideal strain to use when your cloning strategy calls for restriction with a *dam*- or *dcm*-sensitive restriction enzyme* (Table 1). The INV110 strain is a significant improvement over JM110, the strain historically used for generating *dam*- or *dcm*-methylation-free DNA. INV110 carries an *endA1* mutation, which eliminates the non-specific endonuclease Endonuclease I, for improved plasmid DNA preparation. In addition, INV110 *E. coli* feature:

- Blue/white screening of recombinant clones carrying the alpha fragment of β-galactosidase (*lacZ*ΔM15)
- Reduced cleavage of foreign methylated DNA for improved cloning of genomic DNA (Δ(*mrr-mcr*BC))

Cleaner DNA from *supF*-Containing Plasmids.

If you are using MC1061/P3 cells to isolate pCDM8, pcDNA I, pcDNA1.1, or any other *supF*-containing plasmid, then you know it can be difficult to obtain high yields of clean plasmid. TOP10/P3 *E. coli* eliminate this challenge. The TOP10/P3 strain carries the P3 plasmid, which is essential for selection of *supF*-containing plasmids. It also carries a mutated *endA1* gene to eliminate the non-specific endonuclease Endonuclease I. Plasmid isolated from TOP10/P3 is clean and intact compared to DNA purified from M1061/P3 (Figure 1) and is ready for downstream applications like mammalian transfection. In addition, TOP10/P3 cells have the *recA1* genotype for reduced occurrence of recombination of your gene of interest.

Figure 1 - Comparison of Plasmid DNA Isolated from TOP10/P3 and MC1061/P3



Plasmid was isolated from overnight cultures of pcDNA1.1-transformed TOP10/P3 and MC1061/P3 cells using the TENS method. Purified plasmid DNA was incubated for 1 hour at 37°C in restriction enzyme buffer with or without 1 unit of *Xho*I (NEB). Following incubation, the plasmid DNA was analyzed on a 1% agarose gel.

Lanes 1 and 4: Supercoiled pcDNA1.1

Lanes 2 and 5: *Xho*I digested pcDNA1.1 (4.0 kb)

Lanes 3 and 6: pcDNA1.1 incubated with restriction enzyme buffer (no *Xho*I)

The One Shot™ Advantage. Both the INV110 and TOP10/P3 strains are provided in the convenient One Shot™ format. One Shot™ Chemically Competent *E. coli* are supplied in 50 μl, single-use aliquots. This means you never have to put your cells through efficiency-zapping freeze-thaw cycles or waste money by throwing away unused cells.

Unique Strains for Special Needs. If you have specialized cloning needs, we have a strain for you. For improved quality of DNA for restriction with methylation-sensitive enzymes, order an INV110 One Shot™ Kit. For clean, intact plasmids that require *supF* selection, try the TOP10/P3 One Shot™ Kit. Order today.

Product	Transformations	Efficiency†	Cat. no.	Price
INV110	20	1 x 10 ⁶	C7171-03	\$168
TOP10/P3**	20	1 x 10 ⁸	C5050-03	\$168

† cfu/μg supercoiled pUC18 DNA

* *dam/dcm* strains tend to have a higher recombination frequency. They should only be used to propagate plasmids for subsequent digestion with *dam/dcm*-sensitive enzymes.

** TOP10/P3 transformation efficiency using pcDNA1.1 is 1 x 10⁷-5 x 10⁷ cfu/μg supercoiled DNA. Therefore, we recommend using MC1061/P3 (catalog no. C663-03) for transformation of libraries constructed in *supF*-containing plasmids due to its slightly higher transformation efficiency (1 x 10⁸ cfu/μg supercoiled pcDNA1.1). We recommend TOP10/P3 for propagation of DNA for use in mammalian transfection.

Fast Preparation of *E. coli* Growth Medium

imMedia™

imMedia™ is the first and only premixed *E. coli* growth medium that's specially formulated to eliminate autoclaving. It can be prepared in less than 5 minutes by heating in a microwave oven (Figure 1) and includes everything you need to prepare low-salt LB medium or agar plates—even antibiotics, IPTG, and X-gal. The following are some commonly-asked questions about imMedia™.

Q. What is in imMedia™?

A. imMedia™ is supplied in individual ready-to-use pouches. Each pouch contains low-salt LB medium components, with or without agar, and antibiotic (ampicillin, kanamycin, or Zeocin™). The imMedia™ Blue products also include IPTG and X-gal for blue/white colony screening. To maintain the activities of heat-sensitive media components (i.e. antibiotics, IPTG, and X-gal) during and after microwave heating, imMedia™ contains special heat stabilizers (patent pending). Table 1 lists the effective concentrations of the key components in prepared imMedia™.

Q. Is imMedia™ sterile?

A. Yes. The components in each imMedia™ pouch are presterilized by irradiation. This allows you to prepare sterile medium without autoclaving.

Q. Do I have to use sterilized water and flasks to prepare imMedia™?

A. No. While sterilized water and flasks are preferred in all medium preparation, we use deionized water and clean, washed flasks in our quality control experiments to test the sterility of imMedia™. The presterilized imMedia™ components, microwaving process, and presence of antibiotic ensure that the medium maintains sterility during overnight incubation.

Q. How do you test the quality of imMedia™?

A. To ensure the consistency and quality of imMedia™, each lot is tested for sterility, *E. coli* growth support, and selection efficiency using *E. coli* strains that carry the appropriate antibiotic

resistance marker. imMedia™ must display the same growth support and selection efficiency as conventional LB medium. imMedia™ Blue products are further tested to ensure efficient blue color development. Colonies grown on agar plates prepared with imMedia™ Blue medium must develop a deep blue color within 16 hours of incubation at 37°C. With imMedia™ you not only get your media fast, you can count on quality results as well.

Q. What is the shelf life of imMedia™?

A. imMedia™ is provided in sealed pouches and should be stored at room temperature. When stored properly, the stability and quality of imMedia™ is guaranteed for six months. Compared to prepared liquid medium and agar plates that need to be used within 2-3 weeks, imMedia™ gives you the flexibility to prepare fresh medium when you need it.

Try it Today. imMedia™ saves you time because there's no weighing, mixing, autoclaving, or waiting. When you need *E. coli* growth medium, and you need it fast, you can rely on imMedia™. Call and order imMedia™ today.

Product	Pouches†	Cat. no.	Price
<i>For the preparation of liquid medium</i>			
imMedia™ Amp Liquid	20	Q600-20	\$127
imMedia™ Kan Liquid	20	Q610-20	\$127
imMedia™ Zeo Liquid	20	Q620-20	\$200
<i>For the preparation of agar plates</i>			
imMedia™ Amp Agar	20	Q601-20	\$150
imMedia™ Kan Agar	20	Q611-20	\$150
imMedia™ Zeo Agar	20	Q621-20	\$250
<i>For the preparation of agar plates with IPTG and X-gal</i>			
imMedia™ Amp Blue	20	Q602-20	\$200
imMedia™ Kan Blue	20	Q612-20	\$200

† Each pouch contains reagents to prepare 200 ml of liquid media or 8-10 100 mm agar plates.

Figure 1 – Preparation of imMedia™ Agar Plates



Mix the imMedia™ pouch contents with 200 ml water.



Microwave on MEDIUM setting for 2-3 minutes. Then mix the solution and reheat for 30 seconds.



Pour 8-10 agar plates.

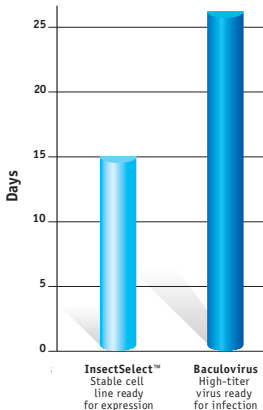
Table 1 - Effective Concentration of imMedia™ Components

Component	Effective Conc. (µg/ml)
Ampicillin	100
Kanamycin	50
Zeocin™	25
IPTG	100
X-gal	100

Rapid Expression in Insect Cells

InsectSelect™ System

Figure 1 - Time Comparison of InsectSelect™ and Baculovirus Expression Systems



Rapidly produce recombinant proteins in insect cells with the new non-lytic InsectSelect™ System. Using InsectSelect™ you'll get continuous, high-level expression in insect cells from a strong viral promoter and save significant time as compared to baculovirus expression systems.

Rapid Expression. InsectSelect™ is a simple, plasmid-based system designed for expression in the same insect cell lines used by baculovirus expression systems. Comparing the InsectSelect™ and baculovirus expression systems reveals that InsectSelect™ saves you significant time (Figure 1). With InsectSelect™ protein can be expressed transiently in just three days. In addition, stable expression cell lines can be generated in just a couple of weeks. Baculovirus expression systems require at least four weeks for recombinant protein expression. With InsectSelect™ you can produce protein more rapidly than with baculovirus systems.

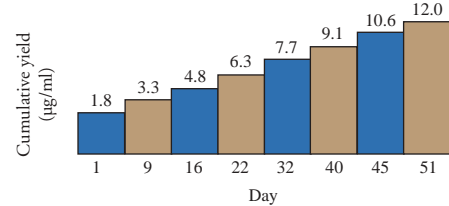
Versatile Expression Vector. In the InsectSelect™ System, a single vector is used for both protein expression and the generation of stable expression cell lines (1-3). The vector, pIZ/V5-His (Figure 2), provides the immediate-early promoter, *OpIE2*, from the *Orgyia pseudotsugata* (*Op*) baculovirus for continuous expression of the gene of interest. This promoter is active in both lepidopteran and dipteran cell lines (1) so you can choose the best host for expression of your protein. To facilitate expression and analysis, pIZ/V5-His offers:

- The Zeocin™ resistance gene for efficient selection of stably transfected cell lines
- A large multiple cloning site to simplify sub-cloning your gene of interest
- A C-terminal V5 epitope for simple detection
- A C-terminal 6xHis sequence for rapid purification

Continuous Expression. Continuous expression with the InsectSelect™ System means that significant quantities of recombinant protein can be collected over time. For example, human IL6 (hIL6) was secreted from High Five™ cells stably transfected with pIZT/V5-His/IL6. Samples of the culture medium were harvested at regular intervals and assayed for

hIL6. With each sample having a concentration of 1.4 to 1.8 µg/ml hIL6, the cumulative yield was 12 µg/ml over the 51 day period (Figure 3).

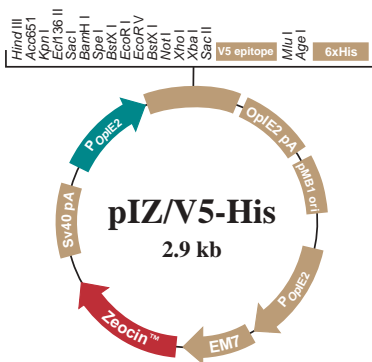
Figure 3 - Continuous Expression of Secreted hIL6 in High Five™ Cells



High Five™ cells stably transfected with pIZT/V5-His/IL6 were maintained in High Five™ Serum-Free medium without Zeocin™ at cell densities between 0.8 and 4 x 10⁶ cells/ml. To quantitate hIL6 expression, 1 ml samples of the culture supernatant were assayed at regular intervals by ELISA.

Why Wait? The InsectSelect™ System allows you to quickly express a host of proteins in insect cells. The complete system includes pIZ/V5-His, an expression control, a sequencing primer, Insectin-Plus™ lipid transfection reagent, Zeocin™ culture medium, and your choice of Sf9 or High Five™ cells. If you already have cultured insect cells in your lab, the expression vectors may be purchased separately. For fast expression in insect cells, nothing beats InsectSelect™. Order your kit today.

Figure 2 - InsectSelect™ Vector



Product	Quantity	Cat. no.	Price
InsectSelect™ System			
with High Five™ Cells	1 kit	K805-01	\$745
with Sf9 Cells	1 kit	K800-01	\$695
pIZ/V5-His Vector Kit	1 kit	V8000-01	\$325
Zeocin™	1 g	R250-01	\$160
Insectin-Plus™	1 kit	K2695-01	\$225
High Five™ Cells, frozen			
3 x 10 ⁶ cells		B855-02	\$380
Sf9 Cells, frozen	10 ⁷ cells	B825-01	\$150
Anti-V5 Antibody	50 µl*	R960-25	\$135
Anti-V5-HRP Antibody			
50 µl*		R961-25	\$160

* Sufficient antibody is supplied for 25 western blots.

References:

1. Pfeifer, T.A. *et al.* (1997) *Gene* **188**: 183-190.
2. Hegedus, D.D. (1998) *Gene* **207**: 241-249.
3. Hegedus, D.D. (1999) *Prot. Purif. Exp.* **15**: 296-307.

High-Level Expression in Transgenic Mouse Milk

pBC1 Milk Expression Vector

The pBC1 Milk Expression Vector is designed for high-level protein production in the milk of transgenic animals. pBC1 allows you to quickly produce milligram quantities of secreted proteins in mouse milk. Expression in mouse milk provides an indication of the level of production that can be achieved in larger transgenic animals, saving you significant time and effort.

Rapid Feasibility Studies. pBC1 uses genomic DNA sequences from the goat β -casein gene to limit expression of inserted genes to the mammary epithelial tissue of transgenic mice, goats, rabbits, and cows. Expression of the recombinant protein in mouse milk is generally predictive of the expression level in transgenic goats (Table 1). Being able to use transgenic mouse expression data to predict the production levels in large herd animals saves you months of effort and tremendous expense.

Table 1 - Expression Levels of Various Proteins in Mice and Goats Using the Goat β -casein Promoter

Protein	Animal	Expression (mg/ml)
Longer acting tPA	Mouse	6
	Goat	6
Antithrombin III	Mouse	10
	Goat	14
α 1-Protease inhibitor	Mouse	35
	Goat	20
Anti-cancer MAb	Mouse	10
	Goat	10

High Yield in Mammalian Hosts. The pBC1 Milk Expression Vector is a powerful tool for the production of extraordinary quantities of recombinant proteins in a mammalian host. The vector is available in a kit that includes the pBC1 vector, reagents for the analysis of transgenic mice, and a detailed manual. For the production of large quantities of secreted proteins in a mammalian host, use the pBC1 Milk Expression Vector. Contact Invitrogen today for more information about this powerful technology.

Description	Quantity	Cat. no	Price
pBC1 Milk Expression Vector	1 kit	K270-01*	\$695

* Distribution is limited to North America and Europe.

Secretion Simplifies Protein Purification

pSecTag2 and pSecTag2/Hygro

One way to simplify the purification of recombinant proteins in mammalian cells is to direct expression out of the cell via the secretory pathway. To allow you to effectively secrete your protein, the pSecTag2 and pSecTag2/Hygro vectors contain an efficient secretion signal in addition to elements for high-level protein expression.

Efficient Secretion. The pSecTag2 and pSecTag2/Hygro vectors carry the secretion signal from the V-J2-C region of the mouse Ig κ -chain to allow proteins to be transported through the secretory pathway into the medium (Figure 1). The majority of the host cell proteins are absent in the medium, making it easier to purify your protein.

All the Right Features. For high-level expression and efficient analysis of your recombinant protein, the pSecTag2 and pSecTag2/Hygro vectors (Figure 2) offer several advantageous features including:

- The human cytomegalovirus (CMV) enhancer/promoter for high-level expression
- The Zeocin™ or hygromycin resistance gene for establishing stable cell lines
- A C-terminal *c-myc* epitope for rapid detection with an Anti-*myc* Antibody
- A C-terminal polyhistidine (6xHis) sequence for simple purification on ProBond™ resin

In addition, each vector is provided in three reading frames to simplify cloning in frame with the N-terminal secretion signal.

Simplify Purification. The pSecTag2 and pSecTag2/Hygro vectors contain features for high-level expression and efficient secretion of your protein. To simplify your protein purification, call and order your vector today.

Product	Quantity	Cat. no.	Price
pSecTag2 A, B, & C	20 μ g ea.	V900-20	\$320
pSecTag2/Hygro A, B, & C	20 μ g ea.	V910-20	\$320
Anti- <i>myc</i> Antibody	50 μ l*	R950-25	\$135
Anti- <i>myc</i> -HRP Antibody	50 μ l*	R951-25	\$160

* Sufficient antibody is supplied for 25 westerns.

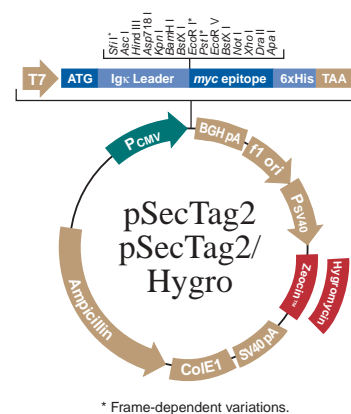
Figure 1 - Secreted Expression of PSA from pSecTag2/Hygro



Ten micrograms of pSecTag2/Hygro/PSA or pHook™-3/PSA was transfected into 2×10^6 SKBR-3 cells. pHook™-3 creates a fusion to the *c-myc* epitope, but does not secrete the protein. Ninety-six hours post-transfection, 20 μ l of medium from each transfection was separated by SDS-PAGE. The gel was blotted and probed with the Anti-*myc*-HRP antibody.

Lane 1: Medium from cells transfected with pSecTag2/Hygro/PSA
Lane 2: Medium from cells transfected with pHook™-3/PSA

Figure 2 - pSecTag2 and pSecTag2/Hygro



Convenient Inducible Mammalian Expression System

Ecdysone-Inducible Mammalian Expression System

The Ecdysone-Inducible Mammalian Expression System gives you precise control of recombinant gene expression in mammalian cells. The system meets even the most stringent requirements for tightly-regulated expression. A potent, new inducing agent, the availability of the regulatory vector pVgRXR separately, and a variety of inducible expression vectors make the Ecdysone System convenient to use. It's a great time to take control of your inducible expression experiments.

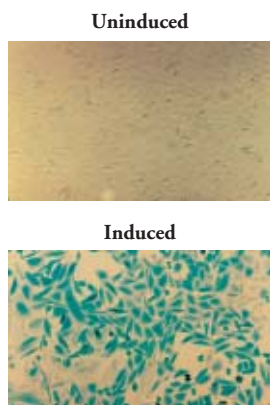
GS™-E: A New, Potent Inducer. To make the Ecdysone-Inducible Mammalian Expression System more versatile, GS™-E, a new, potent inducing agent is now available. GS™-E is capable of inducing expression to levels that are equivalent to those achieved with ponasterone A and muristerone A. GS™-E has been shown to be non-mutagenic and cause no developmental or reproductive effects in mammalian cells (1). This means GS™-E will not exert pleiotropic effects on your cells. Figure 1 demonstrates induced expression of β-galactosidase in CHO cells.

More Convenient. In response to requests by many Ecdysone users, the pVgRXR regulatory vector (Figure 2) is now available separately. pVgRXR constitutively expresses the heterodimeric ecdysone receptor, which controls expression in the Ecdysone System. Having pVgRXR available separately means you don't have to spend time transforming pVgRXR into *E. coli*, growing up the bacterial culture, and performing tedious plasmid purification procedures. The convenience of having pVgRXR on hand can save you almost three days of time and effort.

Powerful Vectors. The Ecdysone-Inducible Mammalian Expression System is available with a variety of pIND-based inducible expression vectors that make the system highly versatile. Each powerful vector carries the ecdysone-responsive promoter along with features for selection, purification, detection, rapid cloning, or increased levels of expression. This allows you to express your recombinant protein in a form that best fits your experimental goals. Table 1 provides a list of available pIND-based inducible expression vectors and their applications.

Take Control Today. The Ecdysone-Inducible Mammalian Expression System offers tightly-regulated expression of recombinant proteins. The system has been used to successfully express a variety of toxic proteins (contact Invitrogen for a list of references). A new, potent inducing agent, the availability of the pVgRXR vector, and the large selection of pIND-based expression vectors make the Ecdysone System convenient to use. Take control of your inducible expression. Call Invitrogen and order your Ecdysone-Inducible Mammalian Expression System today.

Figure 1 - Induction of β-galactosidase with GS™-E



2 x 10⁵ CHO cells stably transfected with pVgRXR and pIND(SP1)/lacZ were induced with 10 μM GS™-E. Twenty-four hours postinduction, cells were stained with the β-Gal Staining Kit (Invitrogen).

Figure 2 - The Regulatory Vector



Table 1 - Ecdysone-Inducible Expression Vectors

Vector	Application
pIND, pIND(SP1)*	Selection of stable cell lines with neomycin
pIND/V5-His, pIND(SP1)/V5-His*	Rapid purification and detection of recombinant fusion proteins
pIND/Hygro, pIND(SP1)/Hygro*	Selection of stable cell lines with hygromycin
pIND/V5-His-TOPO®	5-minute, efficient ligation of PCR products; rapid purification and detection of recombinant proteins

* The presence of the SP1 transcriptional enhancer can increase expression levels two to five fold.

Product	Quantity	Cat. no.	Price
Ecdysone-Inducible Mammalian Expression System with pIND	1 kit	K1001-01	\$475
with pIND(SP1)	1 kit	K1001-02	\$495
with pIND/Hygro	1 kit	K1002-01	\$495
with pIND(SP1)/Hygro	1 kit	K1002-02	\$495
with pIND/V5-His A, B, & C	1 kit	K1003-01	\$545
with pIND(SP1)/V5-His A, B, & C	1 kit	K1003-02	\$545
with the pIND TOPO TA Cloning® Kit	1 kit	K1004-01	\$620
pVgRXR	20 μg	V730-20	\$230
GS™-E	1 mg	H102-01	\$39
Ponasterone A	1 mg	H101-01	\$37

Reference:
1. Dhadialla, T.S. *et al.* (1998) *Ann. Rev. Entomol.* 43: 545-569.

GS™-E is a trademark of Rohm & Haas.

TOPO TA Cloning[®] and Electroporation—Fast, Efficient PCR Cloning

TOPO TA Cloning[®] Kits with TOP10 One Shot[™] Electrocomp[™] Cells

TOPO TA Cloning[®] is the fastest way to clone *Taq*-amplified PCR products. Electroporation provides a convenient method for high-efficiency transformation. Now these two techniques have come together in the new TOPO TA Cloning[®] Kits with TOP10 One Shot[™] Electrocomp[™] Cells. These kits represent the fastest, easiest, and most efficient way to clone PCR products.

5-Minute TOPO TA Cloning[®] Saves Time.

TOPO TA Cloning[®] is the method of choice for cloning *Taq*-amplified PCR products because it's fast, easy, and efficient. In just 5 minutes you can ligate PCR products right on your bench top and get ≥95% recombinants. TOPO[®] Cloning saves you an entire day of cloning time.

TOPO[®] Cloning is Easy. The pCR[®]-TOPO[®] vectors (Figure 1) provided in the TOPO TA Cloning[®] Kits are activated with topoisomerase I. This eliminates the hassles of conventional cloning using ligase, ligation buffer, and ATP. With TOPO TA Cloning[®], there is no need for post-PCR modification or clean-up, no special primers, no vector preparation, and no extra reagents required. The procedure is easy. Simply:

1. Add 1 µl of your *Taq*-amplified PCR reaction and 3 µl of water to 1 µl of the pCR[®]-TOPO[®] vector.
 2. Incubate for 5 minutes on your bench top.
 3. Transform the competent cells provided.
- It's that easy.

Fast and Efficient Transformation. Electroporation is a fast and highly efficient transformation method. Unlike chemical transformation, electroporation does not require a 30-minute incubation on ice. Instead, you simply aliquot cells into a cuvette, add DNA, electroporate, let the cells recover, and plate. In addition, larger constructs are more efficiently transformed with electroporation. To take advantage of these benefits, the TOPO TA Cloning[®] Kits are now available with One Shot[™] TOP10 Electrocomp[™] cells. These cells yield high transformation efficiencies—up to 1x10⁹ cfu/µg supercoiled DNA—so you are sure to get your clone (Table 1).

Table 1 - Results Obtained Using the TOPO TA Cloning[®] Kit with TOP10 One Shot[™] Electrocomp[™] Cells

Sample	Total Number of Colonies	Number of White Colonies	% Recombinants
1	291	284	97.6%
2	277	270	97.5%

A 750 bp *Taq*-amplified PCR product was cloned into pCR[®]2.1-TOPO[®]. TOP10 One Shot[™] Electrocomp[™] cells were electroporated with the ligation product. The number of white and blue colonies that formed on LB agar plates containing kanamycin and X-gal were counted. The percent recombinants represents the number of white colonies/total colonies.

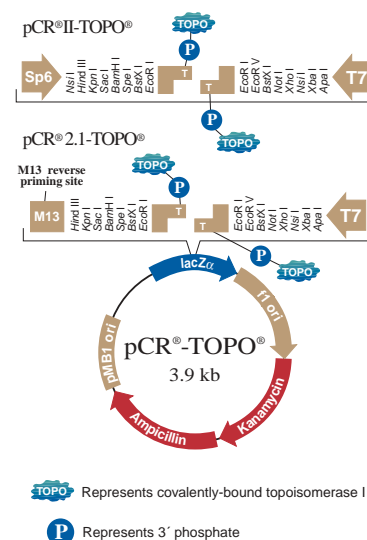
One Shot[™] Convenience. For maximum convenience, the TOP10 Electrocomp[™] cells available with the TOPO TA Cloning[®] Kit are supplied in the One Shot[™] format. The One Shot[™] format provides cells in single-use aliquots so you only thaw the cells you need. This means there are no lost efficiencies caused by freeze-thawing and no money wasted on unused cells. One Shot[™] cells provide the ultimate in transformation convenience and efficiency.[™]

Increase the Efficiency of TOPO TA Cloning[®].

The TOPO TA Cloning[®] Kits are provided as complete kits for fast, consistent, and efficient PCR cloning. The kits include your choice of linearized, topoisomerase I-activated pCR[®]2.1-TOPO[®] or pCR[®]II-TOPO[®] vector, TOP10 One Shot[™] Electrocomp[™] cells, 10X PCR buffer, sterile water, dNTPs, control template and primers, and M13 forward and reverse primers for sequencing or PCR screening. For the fastest, most efficient PCR cloning, try the new TOPO TA Cloning[®] Kits with TOP10 One Shot[™] Electrocomp[™] cells. Order yours today!

Product	Reactions	Cat. no.	Price
TOPO TA Cloning [®] Kit with TOP10 One Shot [™] Electrocomp [™] Cells			
with pCR [®] 2.1-TOPO [®] vector	20	K4560-01	\$305
with pCR [®] II-TOPO [®] vector	20	K4660-01	\$320
TOP10 One Shot [™] Electrocomp [™] Kit	10	C4040-50	\$131
Electroporation Cuvettes			
0.1 cm (white)	50/bag	P410-50	\$133
0.2 cm (blue)	50/bag	P450-50	\$133

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



Special Offer

Try a new TOPO TA Cloning[®] Kit with TOP10 One Shot[™] Electrocomp[™] Cells and get 50% off a bag of 0.1 cm or 0.2 cm Electroporation Cuvettes. Simply mention PROM143 when you order a TOPO TA Cloning[®] Kit with TOP10 One Shot[™] Electrocomp Cells! This offer expires August 31, 1999.

Double Screening Saves Time and Resources

Dual Bait Hybrid Hunter™ Yeast Two-Hybrid System

Figure 1 - pHybLex/Zeo

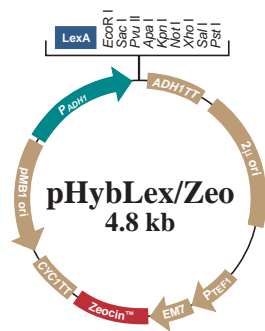


Figure 2 - pHybcl/HK

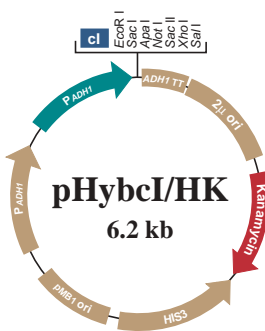
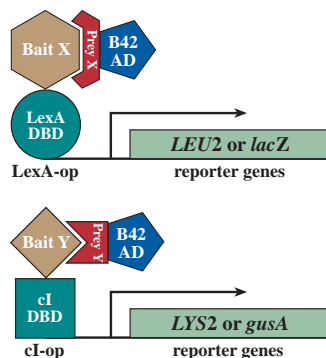


Figure 3 - Dual Bait Hybrid Hunter™ Yeast Two-Hybrid System



The Dual Bait Hybrid Hunter™ Yeast Two-Hybrid System offers significant savings in time and resources by allowing the simultaneous analysis of two bait proteins using a single prey library. This novel system is ideal for:

- Delineation of the protein domains necessary for protein interaction
- Competitive screening of proteins or compounds for disruption of protein interactions

The Key Components. The Dual Bait Hybrid Hunter™ Yeast Two-Hybrid System builds on the power of the original Hybrid Hunter™ LexA-based two-hybrid system (1,2). The Dual Bait Hybrid Hunter™ System uses two unique bait vectors, a specially-designed strain, and a convenient prey vector or library of prey proteins to allow the simultaneous identification of two independent protein-protein interactions (3).

Two Unique Bait Vectors. Two vectors, pHybLex/Zeo and pHybcl/HK, allow you to clone and simultaneously analyze two bait proteins. The gene for the first bait is cloned into the pHybLex/Zeo vector as a fusion to a LexA DNA-binding domain (LexA DBD) (Figure 1). The pHybLex/Zeo vector features:

- Small size (4.8 kb) for easy subcloning and manipulation
- Zeocin™ resistance gene for selection in both *S. cerevisiae* and *E. coli*
- Nine unique restriction sites for simplified cloning of the gene of interest in frame with the LexA DBD

The gene for the second bait is cloned into the pHybcl/HK vector in frame with the lambda cl DNA-binding domain (cl DBD) (Figure 2). The pHybcl/HK vector offers:

- *HIS3* gene for selection and maintenance in *S. cerevisiae*
- Kanamycin gene for selection and maintenance in *E. coli*
- Seven unique restriction sites for simplified cloning in frame with the lambda cl DBD

Specially Designed Strain. In the Dual Bait Hybrid Hunter™ Yeast Two-Hybrid System, two unique sets of reporter genes are engineered into the *Saccharomyces cerevisiae* host, SKY48/pLacGUS, to allow the identification of two distinct protein-protein interactions. The first set of reporters, *lacZ* and *LEU2*, are located downstream of LexA operator sites. The second set of reporter genes, *LYS2* and *gusA*, are downstream of lambda cl operator sites. The *LEU2* and *LYS2* reporters are integrated into the host genome. The *lacZ* and *gusA* reporter genes are supplied on the 2μ ori plasmid pLacGUS. Since the SKY48/pLacGUS strain is pretransformed with pLacGUS, it's ready to use in your dual bait two-hybrid assay.

Convenient Prey Vector and Libraries. The prey vector, pYESTrp2, included in the Dual Bait Hybrid Hunter™ Kit allows you to clone a gene of interest or construct a cDNA library for analysis. Alternatively, a selection of high-quality premade libraries are available constructed in pYESTrp2 or pJG4-5 to allow you to screen for unknown interactors. The pYESTrp2 vector offers several key features to facilitate two-hybrid analysis, including:

- B42 activation domain (AD) for fusion to the prey protein and activation of reporter gene transcription
- SV40 large T antigen nuclear localization signal (NLS) for efficient targeting of the B42-prey fusion to the nucleus
- V5 epitope tag for easy detection of recombinant prey protein expression with an Anti-V5 Antibody
- Small vector size (5.8 kb) for easy subcloning and improved transformation efficiency
- Versatile multiple cloning site with eight unique restriction sites, including sites for unidirectional cDNA library cloning

How the System Works. To screen for protein-protein interactions using the Dual Bait Hybrid Hunter™ Yeast Two-Hybrid System:

1. Transform the two bait vector constructs into the SKY48/pLacGUS *S. cerevisiae* host and verify expression.

continued on next page...

Maximize Your Productivity

Custom Laboratory Services

Dual Bait Hybrid Hunter™ System, continued.

2. Transform a prey gene or library of prey genes cloned into pYESTrp2 into the SKY48/pLacGUS host expressing the baits.

If a bait protein interacts with a prey protein, the B42 AD fused to the prey protein is brought into proximity of the DNA Binding Domain (LexA or cI) and transcription of the downstream reporter genes is activated (Figure 3, page 10). Positive interactors are assayed by growth on leucine-deficient and lysine-deficient medium and confirmed by simple colorimetric assay (Table 1).

Table 1 - Assay of Positive Interactions Using the Dual Bait Hybrid Hunter™ Yeast Two-Hybrid System

Bait Vector	Selective Medium	Colorimetric Confirmation
pHybLex/Zeo (bait fused to LexA DBD)	leucine-deficient	β-galactosidase assay
pHybcl/HK (bait fused to cI DBD)	lysine-deficient	β-glucuronidase assay

Just What You Need. The Dual Bait Hybrid Hunter™ Yeast Two-Hybrid System allows you to assay two bait proteins in a single screen, offering significant savings in time and resources. The Dual Bait Hybrid Hunter™ Yeast Two-Hybrid System is supplied as a complete kit to give you all the tools you need. If you already have the Hybrid Hunter™ Yeast Two-Hybrid System, the versatile Dual Bait vectors and convenient host strain are available separately. Call today for more information on this revolutionary technology.

Product	Quantity	Cat. no.	Price
Dual Bait Hybrid Hunter™ Yeast Two-Hybrid System	1 kit	K5200-01	\$595
pHybLex/Zeo Vector	20 µg	V610-20	\$245
pHybcl/HK Vector	20 µg	V614-20	\$245
SKY48/pLacGUS <i>S. cerevisiae</i> Strain	500 µl	C832-00	\$65
cI Antibody	50 µl*	R991-25	\$135
Anti-LexA Antibody	50 µl*	R990-25	\$135

* Sufficient antibody is supplied for 25 western blots.

References:

1. Fields, S. and Song O. (1989) *Nature* **340**: 245-246.
2. Gyuris, J. et al. (1993) *Cell* **75**: 791-803.
3. Serebriiskii, I. et al. *J. Biol. Chem. in press.*

Maximizing productivity in your laboratory, whether it is academic or commercial, is always a top priority. This becomes challenging when resources such as time, facilities, staff, and expertise are limited. The Custom Laboratory 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 Laboratory Services provide complete, fully-integrated molecular biology and protein expression services for a variety of projects. Integrated capabilities allow the smooth transfer from one step to the next to provide quick turn-around of your project. Table 1 summarizes some of the laboratory services available. From project initiation to completion, or anywhere in between, our staff of experts will work with you to meet your goals.

“From project initiation to completion, or anywhere in between, our staff of experts will work with you to meet your goals.”

Table 1 - Summary of Custom Laboratory Services

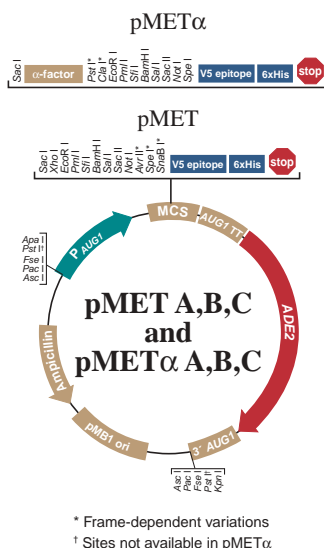
Service	Capabilities
Molecular Biology	Cloning (includes PCR subcloning). DNA sequencing (includes single-pass and publication quality). PCR amplification and optimization. Site-directed mutagenesis.
Baculovirus Expression	Transfection and purification of recombinant baculovirus. Generation of high-titer stocks. Amplification of viral stocks. Pilot protein production.
<i>Pichia pastoris</i> Expression <i>Exclusively from Invitrogen</i>	Transformation and selection of <i>Pichia pastoris</i> strains. Expression verification via western analysis (antibody required). Optimization of expression for fermentation. Scale-up protein production by fermentation.

Services on Demand. The principal benefit of outsourcing research projects to Invitrogen's Custom Laboratory 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 Laboratory Services Representative at 800-955-6288, ext. 265 today.

A New High-Level, Regulated Yeast Expression System

Pichia methanolica Expression System

Figure 1 - pMET and pMET α *P. methanolica* Expression Vectors



Yeast expression systems have proven to be both practical and economical for producing industrial-scale quantities of eukaryotic proteins. A new system is now available that uses the novel methylotrophic yeast *Pichia methanolica* for high-level, regulated expression of recombinant proteins. *P. methanolica* offers the advantages of easy scale-up for the high-level production of proteins that require eukaryotic posttranslational modifications.

High-Level, Controlled Expression. Scientists at Zymogenetics Corporation (1) created the powerful *P. methanolica* expression system. The system takes advantage of the *P. methanolica* methanol-inducible alcohol oxidase (*AUG1*) promoter and the yeast's ability to grow to extremely high cell density. In growth medium without methanol, transcription from the *AUG1* promoter is tightly repressed. When methanol is the sole carbon source, transcription from the *AUG1* gene is rapidly induced to extremely high levels. *P. methanolica* can be grown to cell densities greater than 50% cells per volume. When the *AUG1* promoter is harnessed to express recombinant genes in cells grown to these high densities, extraordinary amounts of protein can be produced (1).

Versatile Expression Vectors. The *P. methanolica* System provides two expression vectors, pMET and pMET α (Figure 1), to allow intracellular or secreted expression of recombinant proteins, respectively. The vectors offer many features to facilitate cloning, expression, and analysis of heterologous protein including:

- The *AUG1* promoter for high-level, regulated expression of recombinant proteins
- A C-terminal V5 epitope for convenient detection of recombinant proteins with an Anti-V5 Antibody
- A C-terminal 6xHis sequence for rapid purification on ProBond™ resin
- The *ADE2* gene for auxotrophic selection of cells transformed with *P. methanolica* expression vectors

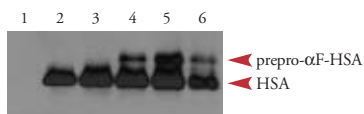
In addition, pMET α encodes the prepro- α factor signal peptide for secretion of the protein product

into the culture medium. This simplifies downstream purification of many recombinant proteins.

High-Level Expression. High-level *P. methanolica* expression strains can be generated in just a few days. The recombinant expression vector containing the gene of interest is first linearized by restriction digest. This linear DNA is used to transform a *P. methanolica* host using either chemical or electroporation methods. Transformed cells are selected on minimal medium lacking adenine and then screened for inducible expression. Regulated expression of human serum albumin (HSA) is shown in Figure 2. When grown in medium containing glucose, expression is undetectable, even by western blot with the Anti-V5-HRP Antibody. However, when the cells are grown in medium with methanol as the sole carbon source, HSA is induced to very high levels.

Powerful Expression. The *P. methanolica* Expression System can address your high-level expression needs. The kit comes complete with host strains, expression vectors, sequencing primers, and specialized expression medium. Give Invitrogen a call today to order this powerful gene expression system for your lab.

Figure 2 - Regulated Expression of HSA in *P. methanolica*



The *P. methanolica* strain PMET16 was transformed with the recombinant vector pMET α /HSA which expresses human serum albumin (HSA) with the N-terminal prepro-alpha factor signal sequence and the V5-His C-terminal polypeptide. A single transformant was used to inoculate 10 ml of BMGY (buffered minimal glycerol yeast extract) in a 50 ml conical tube. Expression of HSA was induced by replacing the medium with BMMY (buffered minimal methanol yeast extract). Samples of the growth medium were harvested immediately before induction and every 24 hours after induction. Expression of HSA was analyzed by western blot using the Anti-V5-HRP Antibody.

Lane 1: Before induction
Lane 2: 24 hours postinduction
Lane 3: 48 hours postinduction
Lane 4: 72 hours postinduction
Lane 5: 96 hours postinduction
Lane 6: 120 hours postinduction

Product	Quantity	Cat. no.	Price
<i>P. methanolica</i> Expression System	1 kit	K1780-01	\$725
Anti-V5 Antibody	50 μ l*	R960-25	\$135
Anti-V5-HRP Antibody	50 μ l*	R961-25	\$160
ProBond™ Resin	50 ml	R801-01	\$285

* Sufficient antibody is supplied for 25 western blots.

Reference:

1. Raymond, C.K. *et al.* (1997) *Yeast* 14: 1-13.



Expression-Ready Human Gene Resource Saves Time and Money

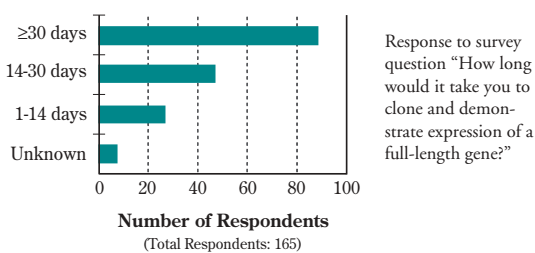
GeneStorm™ Expression-Ready Human Clones

GeneStorm™ Expression-Ready Human Clones, the first and only cloned, expression-ready genes, now number in the thousands. Each GeneStorm™ Clone contains a human gene that has been cloned into a versatile mammalian or bacterial expression vector, partially sequenced to verify insert identity, and expression tested via western blot. All the cloning work is done for you so you'll save weeks of time and effort.

Expression-Ready Clones Save Time and Money.

A recent survey of industrial and academic researchers suggests that the process of cloning a gene and testing for expression can take weeks or often months of work (Figure 1). The GeneStorm™ clones have already undergone an extensive multi-step cloning and testing process. This eliminates the time you would have spent preparing a clone for your experiments (Figure 2). In addition to the time saved, the GeneStorm™ Clones are priced at a fraction of what it would cost you to clone and demonstrate expression of these genes yourself (Figure 2).

Figure 1 - Estimated Time to Clone and Express a Gene



Convenient Vectors. The GeneStorm™ Expression-Ready Human Clones are available cloned into one or more expression vectors (Table 1). Three expression vector formats are available: constitutive mammalian, inducible mammalian, and inducible bacterial. To save you time during protein analysis, each vector carries convenient features for recombinant protein detection and purification.

Table 1 - GeneStorm™ Vector Formats

GeneStorm™ Vector	Expression	Features
pcDNA3.1/GS*	Constitutive expression in mammalian cells	Strong expression from CMV promoter. C-terminal V5 epitope for immunodetection and polyhistidine (6xHis) sequence for rapid purification using ProBond™ resin.
pIND/GS	Inducible expression in mammalian cells	Low basal expression. Used with the Ecdysone-Inducible Mammalian Expression System. C-terminal V5 epitope for immunodetection and polyhistidine (6xHis) sequence for rapid purification using ProBond™ resin.
pCR®T7/GS	Inducible expression in <i>E. coli</i>	T7 promoter inducible with IPTG. N-terminal enterokinase cleavage site for removal of fusion tags, polyhistidine (6xHis) sequence for rapid purification using ProBond™ resin.
pBAD/ThioGS	Tightly-regulated expression in <i>E. coli</i>	Regulatable <i>araBAD</i> promoter for high-level expression. C-terminal V5 epitope for immunodetection and polyhistidine (6xHis) sequence for rapid purification using ProBond™ resin.

GeneStorm™ is the Gene Resource. With more than 2,300 human clones to choose from, the growing GeneStorm™ collection is a valuable resource for life science researchers. Gene categories available focus on signal transduction, cell signaling, apoptosis, and tumor suppressor proteins.

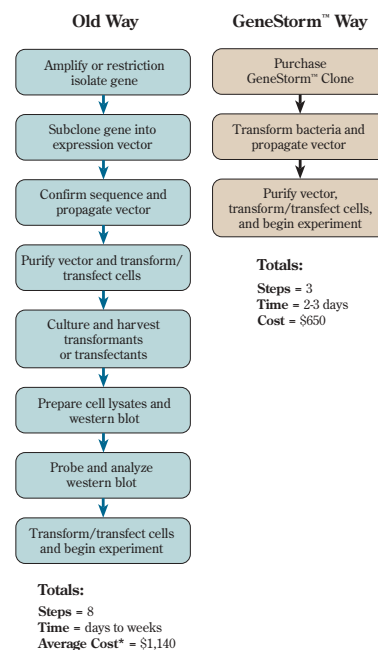
Use GeneStorm™ and Save. The large collection of readily-available and expression-tested GeneStorm™ Expression-Ready Human Clones eliminates gene expression guess work and saves you time and resources. To place an order or obtain the complete list of the GeneStorm™ Expression-Ready Human Clones now available, contact an Invitrogen Technical Service Representative (800-955-6288, ext. 2) or visit our GeneStorm™ web site (www.invitrogen.com/genestorm) today.

Product	Quantity	Price
GeneStorm™ Expression-Ready Human Clones	50 ng	\$650

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Figure 2 - Comparison of Cloning and Gene Expression



* Average cost includes labor, materials, and overhead (in U.S. dollars).

Simplified Construction of Blasticidin Selection Vectors

BsdCassette™ Vectors

Blasticidin is a potent selection agent that allows you to generate stable mammalian cell lines in one week. With BsdCassette™ vectors, you can easily construct customized vectors that confer resistance to blasticidin and save time.

Super Potent Selection Agent. Blasticidin is a nucleoside antibiotic isolated from *Streptomyces griseochromogenes*. It causes cell death in both prokaryotic and eukaryotic cells by inhibiting protein translation (1,2). Blasticidin is especially potent in mammalian cells. To demonstrate, blasticidin kill curves were generated using COS-1, CHO, 293, NIH3T3, and HeLa cells. In all cell types, blasticidin caused complete cell death at a concentration of 10 µg/ml or less in fewer than 7 days (Table 1). This means that you can select stable cell lines two to four times faster with blasticidin than with other selection agents (e.g. G418).

Table 1 - Blasticidin Concentration Required to Establish Stable Cell Lines

Cell Line	Blasticidin Concentration	Days to Complete Selection
COS-1	5 µg/ml	7
CHO	10 µg/ml	6
293	10 µg/ml	4
NIH3T3	5 µg/ml	7
HeLa	3 µg/ml	4

Designed for Simplicity. Each BsdCassette™ vector contains the BsdCassette™. The BsdCassette™ is composed of the blasticidin resistance gene (*bsd*) and a powerful promoter and is flanked by two extensive polylinkers (Figure 1). This design allows you to easily remove the *bsd* gene and its promoter and insert the cassette into your vector of choice. Alternatively, the BsdCassette™ vectors can be used as the backbone for construction of your own unique vector. Simply clone your promoter, gene of interest, and any other important elements into the polylinkers and you can create a blasticidin selection vector that has all of the features you need.

Choice of Promoters. Five BsdCassette™ vectors are available, all of which carry the small prokaryotic EM7 promoter for blasticidin selection in *E. coli*

(Figure 2). Three of the BsdCassette™ vectors also carry a promoter for expression in mammalian cells (pCMV/Bsd, pEF/Bsd, and pUB/Bsd) and one for expression in yeast (pTEF1/Bsd). The fifth vector, pEM7/Bsd, contains only the EM7 promoter and is ideal for constructing vectors that carry a species- or tissue-specific promoter to drive *bsd* expression. BsdCassette™ vectors allow you to take advantage of the potency of blasticidin selection in virtually any cell type.

One Cassette, Dual Selection. Blasticidin selection is effective in both *E. coli* and eukaryotes. Therefore, there's no need to have more than one resistance gene in blasticidin-resistant vectors. Vectors can be smaller than standard eukaryotic expression vectors making them easier to manipulate and maintain.

Build Your Ideal Vector. With the BsdCassette™ vectors you can easily construct your own blasticidin-resistant vector and save time by taking advantage of the high potency of blasticidin. Call and order today!

Product	Quantity	Cat. no.	Price
pCMV/Bsd Starter Kit	1 kit*	K510-01	\$310
pEF/Bsd Starter Kit	1 kit*	K511-01	\$310
pUB/Bsd Starter Kit	1 kit*	K512-01	\$310
pTEF1/Bsd Starter Kit	1 kit*	K513-01	\$310
pEM7/Bsd Starter Kit	1 kit*	K514-01	\$310
pCMV/Bsd	20 µg	V510-20	\$200
pEF/Bsd	20 µg	V511-20	\$200
pUB/Bsd	20 µg	V512-20	\$200
pTEF1/Bsd	20 µg	V513-20	\$200
pEM7/Bsd	20 µg	V514-20	\$200
Blasticidin	50 mg	R210-01	\$130

* Each starter kit includes 20 µg of one BsdCassette™ vector and 50 mg of blasticidin.

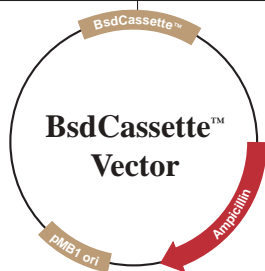
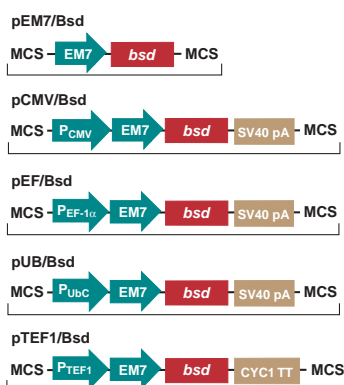
References:

1. Yamaguchi, H. et al. (1965) *J. Biochem.* 57: 667-677.
2. Izumi, M. et al. (1991) *Exp. Cell Res.* 197: 229-233.

Figure 1 - Structure of a BsdCassette™



Figure 2 - BsdCassette™ Vectors*



* The complete multiple cloning site is available on the web.

Convenient E-Gels™ Make Electrophoresis Quick and Easy

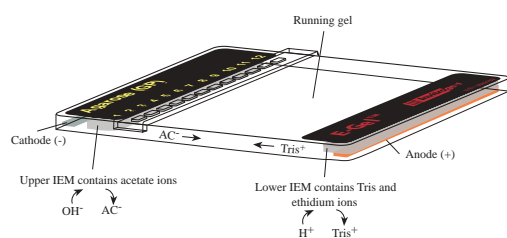
E-Gel™ Electrophoresis System

E-Gels™ are bufferless, precast agarose gels designed for fast and convenient electrophoresis. Each E-Gel™ is a self-contained electrophoresis system that includes agarose, electrodes, ethidium bromide, and ion exchange matrices all packaged inside a UV-transparent cassette. The novel E-Gel™ electrophoresis concept has generated lots of interest from our readers. Some of the most commonly-asked questions are answered below.

Q. What makes E-Gels™ bufferless?

A. To create a bufferless system, each E-Gel™ cassette contains two unique ion exchange matrices (IEMs) that lie between the running gel and the electrodes (Figure 1). The IEMs provide a buffering reservoir that supplies a continuous flow of acetate, Tris, and ethidium ions throughout the gel. This patented technology results in a sustained electric field with enhanced buffering capacity. E-Gels™ eliminate exposure to ethidium bromide and the need to prepare liquid buffer, saving you time.

Figure 1 - The E-Gel™ Cassette



Q. Is a specific power supply recommended for E-Gels™?

A. No. E-Gels™ run in the E-Gel™ Base, which is designed to connect directly to your power supply. Simply set your power supply to 60-70V or 35-50 mA, and electrophorese for 30-minutes. If you do not have access to a power supply, there's the E-Gel™ PowerBase™, a self-contained unit with a built-in power supply (Figure 2) that plugs directly into an outlet. The PowerBase™ maintains a constant current of 38 mA and has a timed program so that it will shut off automatically when electrophoresis is complete.

Q. What is the shelf-life of an E-Gel™?

A. E-Gels™ are stable for 6 months when stored at room temperature.

Q. How can I isolate DNA fragments or transfer DNA from the E-Gel™?

A. The E-Gel™ cassette can be easily opened with the E-Gel™ Opener (Figure 3). Once the cassette is open you can gel purify DNA or transfer samples to a membrane for Southern blot analysis. The thin nature of the E-Gel™ allows efficient isolation of DNA bands from agarose and a quick 1 hour transfer of DNA samples to a membrane.

Q. What do I need to get started using E-Gels™?

A. The E-Gel™ Starter Pak provides everything you need to use E-Gels™. The Starter Pak includes your choice of nine 1.2%, 2%, or 4% agarose gels and an E-Gel™ Base. All you need is your sample.

Why Do it the Hard Way? E-Gels™ provide the easiest, most convenient method for agarose gel electrophoresis. Everything you need is included inside the UV-transparent cassette. All you need to provide is the sample. Several gel percentages and pack sizes are available so you're sure to find one that meets your electrophoresis needs. Call Invitrogen today to place your order or to learn more about the novel E-Gel™ electrophoresis system.

Product	Cat. no.	Price
<i>Starter Paks*</i>		
1.2% General-Purpose Agarose	G5000-01	\$75
2% General-Purpose Agarose	G5000-02	\$75
4% High-Resolution Agarose	G5000-04	\$95
<i>E-Gel™ Equipment</i>		
E-Gel™ Base (Qty 4)	G5100-01	\$75
E-Gel™ PowerBase™	G5200-01	\$125
E-Gel™ Opener	G5300-01	\$210
<i>E-Gel™ Accessories</i>		
Mixed Ladder and Dye Pack	R500-01	\$49

* Each Starter Pak includes nine E-Gels™ and an E-Gel™ Base.

E-Gels™ are covered by U.S. Patent Nos. 5,582,702 and 5,868,974.

“Everything you need is included inside the UV-transparent cassette. All you need to provide is the sample.”

Figure 2 - The E-Gel™ Cassette in the E-Gel™ PowerBase™



Figure 3 - The E-Gel™ Opener





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