

Expressions

A newsletter for gene cloning and expression



A New Beginning in Stable Insect Cell Expression

2 Rapid, Stable Expression in Insect cells

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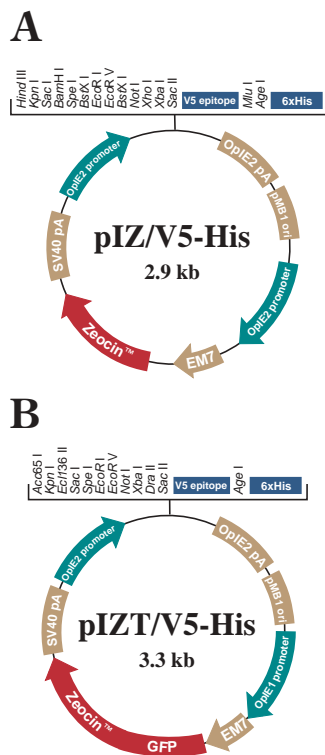
14 New Reporters and Libraries for Two-Hybrid Screens



InsectSelect™ Accelerates Stable Protein Production in Insect Cells

“The InsectSelect™ System simplifies rapid expression of high-quality recombinant proteins in insect cells.”

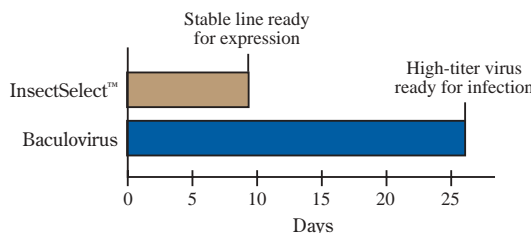
Figure 2 - InsectSelect™ Expression Vectors



Invitrogen’s new InsectSelect™ System allows you to stably produce recombinant proteins in insect cells in less time and with less effort than is required by baculovirus expression systems. That’s because InsectSelect™ is a virus-free system that allows you to create stable cell lines that continuously produce high-quality protein. With InsectSelect™, cell lysis is completely eliminated improving the quality of your recombinant protein.

Virus-Free System. InsectSelect™ is a plasmid-based system that eliminates the use of a baculovirus for expression of recombinant proteins in insect cells. The InsectSelect™ expression vector carries an antibiotic resistance gene for selecting stable insect expression cell lines. Stable cell lines can be generated in as little as 9 days (Figure 1) and can be used for continuous, long-term production of recombinant proteins. InsectSelect™ eliminates cotransfections with linear viral DNA and plaque assays to titer virus stocks so you save significant time and effort.

Figure 1 - Time Requirements for InsectSelect™ and Baculovirus Systems



High-Quality Protein. Not only is InsectSelect™ faster and easier to use than baculovirus expression systems, it can also produce higher quality proteins. Baculovirus-mediated cell lysis releases proteases that reduce the quality and yield of many recombinant proteins. In addition, the secretory pathway is rapidly shut down during later stages of baculovirus infection. This can have a significant effect on yield and modification of secreted proteins. Since InsectSelect™ is a non-lytic system, proteins are produced under optimal conditions for secretion and posttranslational processing. Under non-lytic conditions, the expressed protein is not subjected to the proteases that can decrease yield and quality.

Versatile Expression Vectors. The InsectSelect™ expression vectors use the immediate-early promoter, OpIE2, from the Douglas Fir Tussock moth OpMNPV baculovirus for expression of the gene of interest (Figure 2). OpIE2 is a strong transcriptional promoter in lepidopteran (Sf9, Sf21, High Five™) as well as mosquito and dipteran cell lines (1). Therefore, the InsectSelect™ System has a wider host range than baculovirus or other stable insect expression systems. The expression vector plZ/V5-His (Figure 2A), included in the InsectSelect™ System Kits, offers several features to simplify production and analysis of recombinant proteins in insect cells:

- The OpIE2 promoter for high-level expression of genes cloned into the extensive multiple cloning site
- The Zeocin™-resistance gene for rapid selection of stably transfected cells
- C-terminal tag encoding the V5 epitope for detection with Invitrogen’s Anti-V5 Antibodies
- C-terminal polyhistidine (6xHis) sequence for rapid purification with ProBond™ resin

A second InsectSelect™ vector, plZT/V5-His (Figure 2B), is available that expresses a fusion of the green fluorescent protein and Zeocin™ resistance genes (GFP-Zeo). Co-expression of the GFP-Zeo fusion protein permits non-invasive monitoring of stably transfected cell lines.

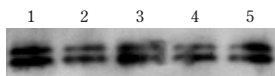
Stable Continuous Protein Production. Stable Zeocin™-resistant insect cell lines generated with the InsectSelect™ System have the capacity to produce large quantities of recombinant proteins. This is because expression is maintained at a constant level over many passages in culture. To demonstrate this, a stable High Five™ cell line expressing a secreted form of recombinant human IL6 was established using plZT/V5-His. This stable cell line was maintained in suspension culture for over 6 weeks without selection. Samples of the expression medium were harvested periodically for western analysis using an Anti-IL6 antibody (Figure 3). The hIL6 protein was expressed as two glycosylated isoforms at relatively constant levels throughout the six-week period.



Rapidly Conjugate HRP to any Protein

InsectSelect™ System, continued.

Figure 3 - Stable, Long-Term Secretion of hIL6 from High Five™ Cells



Time course samples of the growth medium from a High Five™ cell line stably transfected with pIZT/V5-His/hIL6 were analyzed by western blot using rat anti-IL6 antibody and an alkaline phosphatase-conjugated goat-anti-rat secondary antibody.

Lanes 1-5: Samples taken at intervals over a six-week period

Get Started Right Away! The InsectSelect™ System simplifies rapid expression of high-quality recombinant proteins in insect cells. The complete InsectSelect™ System includes the cloning vector pIZ/V5-His, an expression control, sequencing primers, Zeocin™, a choice of insect cell line, culture medium, and InsectinPlus™ transfection reagent. If you already have an established insect cell line, choose the InsectSelect™ Vector Kit. Contact Invitrogen today to accelerate protein production in insect cells with the InsectSelect™ System.

Product	Quantity	Cat. no.	Price
InsectSelect™ System with High Five™ Cells	1 kit	K805-01	\$745
InsectSelect™ System with Sf9 Cells	1 kit	K800-01	\$695
pIZ/V5-His Vector Kit	1 kit	V8000-01	\$325
pIZT/V5-His Vector Kit	1 kit	V8010-01	\$345
Zeocin™	1 gm	R250-01	\$160
InsectinPlus™	1 kit	K2695-01	\$225
High Five™ Cells	3 x 10 ⁶ cells	B855-02	\$380
Sf9 Cells	10 ⁷ cells	B825-01	\$150
Antibodies			
Anti-V5	50 µl*	R960-25	\$135
Anti-V5-HRP	50 µl*	R961-25	\$160
Anti-His(C-term)	50 µl*	R930-25	\$135
Anti-His(C-term)-HRP	50 µl*	R931-25	\$160
ProBond™ Resin	50 ml	R801-01	\$285

*Quantity is sufficient for 25 western blots.

Reference:

1. Pfeifer, T.A. *et al.* (1998) *Gene* 188: 183-190.

The new Linx™ HRP Rapid Protein Conjugation Kit allows you to conjugate horseradish peroxidase (HRP) to your protein of interest in as little as one hour. HRP-conjugated proteins are ideal for sensitive chemiluminescent immunodetection as well as protein-protein interaction analyses.

Novel Chemistry is the Key. The Linx™ HRP Rapid Protein Conjugation Kit takes advantage of the novel Linx™ chemistry, which relies on the specific interaction between two compounds, phenyldi-boronic acid (PDBA) and salicylhydroxamic acid (SHA). In the Linx™ HRP Rapid Protein Conjugation Kit, PDBA is provided conjugated to the protein crosslinker N-hydroxysuccinimide (NHS) to allow you to covalently couple any protein that contains primary amine groups. SHA is provided coupled to HRP. The PDBA-protein and SHA-HRP complexes interact to create an HRP-conjugated protein that is stable under a variety of conditions.

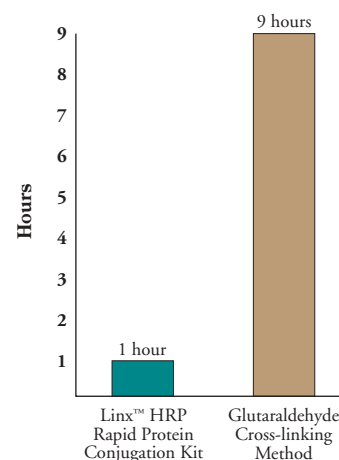
One Hour Conjugation. With the Linx™ technology you can easily HRP-conjugate virtually any protein in as little as one hour. This saves you hours of time compared to the popular glutaraldehyde HRP-conjugation cross-linking protocol (Figure 1).

Fast and Easy. The Linx™ HRP Rapid Protein Conjugation Kit is ideal for the preparation of HRP-conjugated antibodies. A PDBA-antibody conjugate can be prepared in 45 minutes and will be stable for at least one year. Whenever you need HRP-conjugated antibody, simply mix the PDBA-antibody conjugate with the supplied SHA-HRP. In just 5 minutes your HRP-conjugated antibody is ready for detection using your method of choice.

Why Wait? Link up with the Linx™ HRP Rapid Protein Conjugation Kit to quickly and easily HRP-conjugate your protein of interest for all of your detection needs. Order today.

Product	Conjugations	Cat. no.	Price
Linx™ HRP Rapid Protein Conjugation Kit	5	K8050-01	\$275

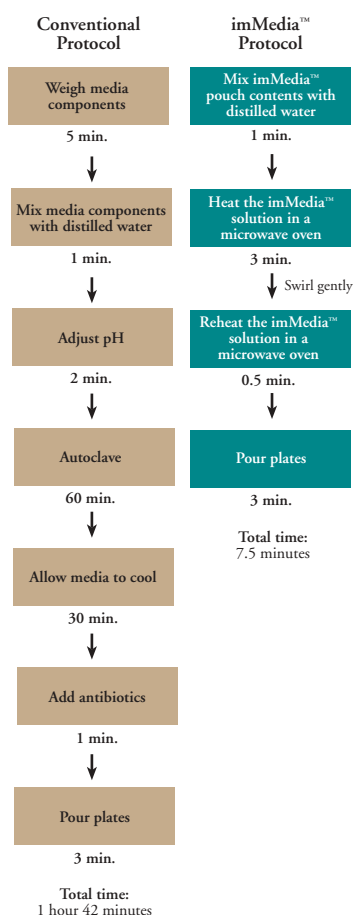
Figure 1 - Comparison of HRP Protein Conjugation Time





imMedia™ –Fast Food for *E. coli*

Figure 1 - Comparison of Protocols for Preparing Agar Plates



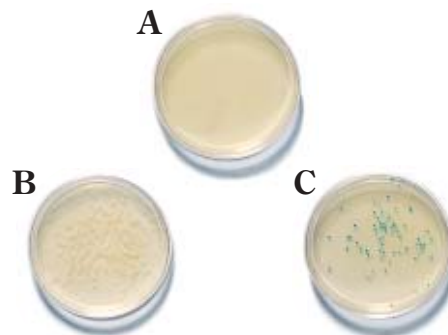
imMedia™ is specially-formulated *E. coli* growth medium that eliminates the need for the time-consuming autoclaving step in media preparation. It is ready to use after heating for only 3.5 minutes in a microwave oven. imMedia™ is fast food for *E. coli* because it saves you hours of media preparation time.

The Ultimate Convenience. imMedia™—provided in individual, easy-to-open pouches—is the ultimate in medium preparation convenience. With imMedia™, you no longer need to measure and mix medium components, adjust pH, autoclave, or wait for the medium to cool in order to add antibiotics. All of these steps are replaced by 3.5 minutes of heating in a microwave oven. imMedia™ contains everything you need in a single pouch, including low-salt medium components and your choice of antibiotic. For additional convenience, imMedia™ Blue products contain IPTG and X-gal to allow blue/white colony screening. With imMedia™, preparing *E. coli* growth medium is more convenient than ever before.

Prepare Plates in Ten Minutes. imMedia™ offers the fastest way to prepare bacterial growth medium. To demonstrate this, a conventional protocol for preparing ten LB agar plates is compared to the imMedia™ protocol (Figure 1). With imMedia™, you simply empty the contents of the pouch into a clean flask, add distilled water, microwave, and pour plates. Unlike conventional protocols, which take more than 1.5 hours, with imMedia™ you can pour plates in 4.5 minutes.

Quality Results. With imMedia™ you get the same results you would expect from conventional low-salt LB medium. To demonstrate, TOPO10 strains were streaked on imMedia™ plates. Figure 2 shows that the imMedia™ plate is sterile (plate A) and that the *E. coli* colonies exhibit excellent growth and optimal density (plate B). In addition, the blue colonies on the imMedia™ Blue plates are clearly distinguishable after overnight incubation at 37°C (plate C). To ensure the highest quality and consistent results, each lot of imMedia™ is stringently tested for colony growth, sterility, antibiotic selection, and blue color development.

Figure 2 - Typical Growth on imMedia™ Plates



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

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

Get What You Need. To satisfy your research needs, imMedia™ is available for the preparation of liquid medium or agar plates with or without IPTG and X-gal. In addition, imMedia™ is provided pre-mixed with your choice of three antibiotics—ampicillin, kanamycin, or Zeocin™. Each imMedia™ pouch contains enough reagents to prepare 200 ml of liquid media or 8-10 agar plates—perfect for a typical cloning experiment. imMedia™ is supplied in a 20-pouch box and includes an easy-to-follow, pocket-size instruction card. Start preparing your *E. coli* growth medium the imMedia™ way and enjoy great time savings. 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

* imMedia™ pouches contain reagents to prepare 200 ml of liquid media or 8-10 standard 100 mm agar plates.

imMedia™ is manufactured by CAYLA.



Rapidly Clone PCR Products and Get the Highest Percentage of Recombinants

There are several variables in conventional PCR cloning procedures that make cloning PCR products difficult and inefficient. These variables include vectors with ends that are incompatible with the PCR products, inefficient ligations, and low-efficiency competent cells. The TOPO® TA Cloning® Kit solves these problems. The kit uses a novel vector and cloning method, as well as highly-efficient competent cells, to save hours in cloning time and yield $\geq 95\%$ recombinants.

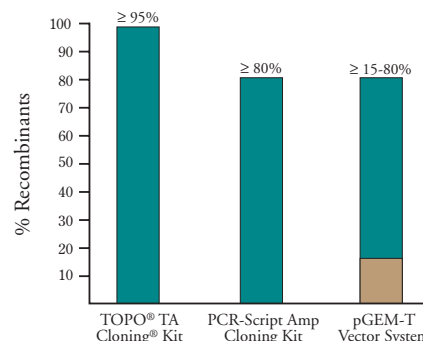
3'-T Overhangs Save You Time. To save you time when cloning *Taq*-amplified PCR products, the TOPO® TA Cloning® method takes advantage of the terminal transferase activity of *Taq* DNA polymerase. During PCR, *Taq* adds a single 3'-A overhang to each end of the PCR product. To take advantage of this activity, each pCR®-TOPO vector is supplied linearized with 3'-T overhangs. This enables direct ligation of PCR products without the need for vector preparation, synthesis of special PCR primers containing restriction sites, or modification of PCR products (1,2). By eliminating these additional steps, you save hours of cloning time.

Topoisomerase Instead of Ligase. The novel pCR®-TOPO vectors are provided linearized with topoisomerase I covalently bound to the 3' phosphates (Figure 1). When a *Taq*-amplified PCR product is added to the vector, topoisomerase I efficiently ligates the insert into the vector in just 5 minutes (3). This eliminates the need for ligase—a common source of nuclease contamination that reduces cloning efficiencies. Because it is covalently bound to the 3' ends, topoisomerase I can protect the vector from degradation by exonucleases. The overall result is a highly-efficient ligation reaction that yields $\geq 95\%$ recombinants.

High-Efficiency Competent Cells. To ensure your results, the TOPO® TA Cloning® Kit includes One Shot™ competent *E. coli*. These cells offer high transformation efficiencies—up to 1×10^9 cfu/ μ g DNA—so your transformation yields hundreds of colonies. In addition, One Shot™ cells are supplied in single-use, 50 μ l aliquots to eliminate losses in efficiency caused by multiple freeze-thaw cycles.

The Highest Percentage of Recombinants. The combination of 3'-T overhangs, topoisomerase I activation, and highly-efficient One Shot™ competent *E. coli* cells gives you the best results when cloning PCR products. The percent recombinants obtained using the TOPO® TA Cloning® Kit are on average 15% higher than other manufacturers' kits (Figure 2).

Figure 2 - Cloning Efficiencies Using the TOPO® TA Cloning® Kit and Other Manufacturers' Kits



PCR cloning efficiencies using a control insert (manufacturers' claims).

Everything You Need. To ensure the highest percentage of recombinants possible, each TOPO® TA Cloning® Kit comes with everything you need for cloning PCR products. The kit includes cloning-ready, linearized, topoisomerase I-activated vector; PCR reagents (except a thermostable polymerase); One Shot™ Competent Cells; and controls. For efficient cloning of PCR products yielding $\geq 95\%$ recombinants, contact the PCR Cloning Experts at Invitrogen and order a TOPO® TA Cloning® Kit today.

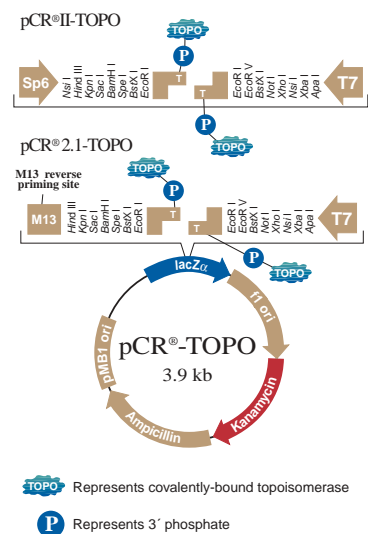
Product	Reactions	Cat. no.	Price
TOPO® TA Cloning® Kit with pCR® 2.1-TOPO vector	20	K4500-01	\$305
	40	K4500-40	\$540
TOPO® TA Cloning® Kit Dual Promoter with pCR® II-TOPO vector	20	K4600-01	\$305
	40	K4600-40	\$540

References:

- Clark, J.M. (1988) *Nuc. Acido Res.* 16: 9677-9686.
- Mead, D. et al. (1991) *Bio/Technology* 9: 657-663.
- Shuman, S. (1994) *J. Biol. Chem.* 269: 32678-32684.

“The combination of 3'-T overhangs, topoisomerase I activation, and highly-efficient One Shot™ competent *E. coli* cells gives you the best results when cloning PCR products.”

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





Expression and Immunofluorescent Detection of Proteinase 3 in COS Cells

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Figure 1 - pcDNA3.1(-)/myc-His Vector

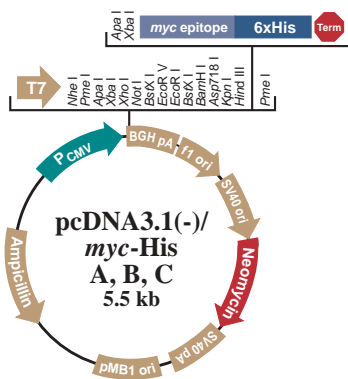
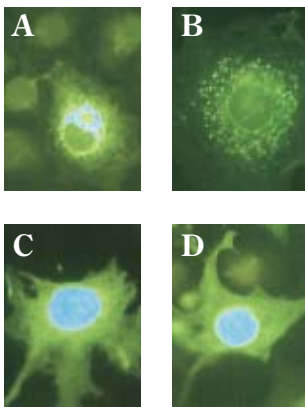


Figure 2 - Immunofluorescence Detection of PR3 Peptides



Different PR3 peptides were cloned into pcDNA3.1(-)/myc-His. Resulting constructs were electroporated into COS-7 cells. Expression was detected by immunofluorescence with either the Anti-myc Antibody (A, B) or the Anti-His(C-term) Antibody (C, D).

Antineutrophil cytoplasmic antibodies (ANCA) are associated with vasculitic diseases involving the kidney. These autoantibodies are specific for neutrophil granule proteins and monocyte lysosome proteins. ANCA can be detected by immunofluorescence microscopy of ethanol-fixed neutrophils incubated with patient serum (1). There are two subtypes of ANCA, cytoplasmic ANCA (C-ANCA) and perinuclear ANCA (P-ANCA), which are differentially localized in the neutrophil. C-ANCA and P-ANCA recognize the neutrophil granule proteins Proteinase 3 (PR3) and Myeloperoxidase, respectively (2). To learn more about the pathogenicity of ANCA, the role of PR3 is being investigated. Isolating native protein for functional studies is time consuming and costly, therefore we are using the pcDNA3.1(-)/myc-His vector to transiently express recombinant human PR3. Furthermore, we are expressing small peptides of PR3 to determine epitope specificity. Expression of these constructs is initially determined via immunofluorescence detection.

Methods.

Cloning and Transfection. The PR3 protein is approximately 270 amino acids. DNA encoding the full-length protein and three small peptides of PR3, ranging in size from 50-110 amino acids, were amplified by PCR with Vent® polymerase (New England BioLabs). PCR primers were designed with restriction sites to allow the PCR products to be directionally cloned into the *EcoR* V and *EcoR* I sites of pcDNA3.1(-)/myc-His. In pcDNA3.1(-)/myc-His, the gene of interest is expressed from the CMV promoter and is fused to a C-terminal *c-myc* epitope and polyhistidine (6xHis) tag (Figure 1). All constructs were confirmed by sequencing. For transfection, 8×10^6 COS-7 cells were electroporated with 30 µg of plasmid DNA in 0.6 ml RPMI (serum-free) at 300V and 960 µF. Transfection efficiencies ranged from 50% to 75%. Electroporated cells were harvested 16-48 hours posttransfection. Expression of the fusion peptide was analyzed by western blot and immunofluorescence with either the Anti-myc or Anti-His(C-term) Antibody (Invitrogen).

Immunofluorescence. COS-7 cells transfected with pcDNA3.1(-)/myc-His constructs were seeded onto cover slips, allowed to attach, and then fixed with 2% paraformaldehyde in PBS for 10 minutes at room temperature. Fixed cells were washed in PBS, permeabilized with acetone and washed in PBS again to remove residual acetone. Cells were then blocked with 5% goat serum in PBS for 30 minutes at room temperature. The fixed cells were incubated with primary antibody, either Anti-myc or Anti-His(C-term) diluted 1:300 in 0.2% goat serum in PBS for 1 hour. Cells were then washed and incubated with a 1:200 dilution of a FITC-labeled donkey anti-mouse secondary antibody (Jackson Laboratories) for 1 hour. Cells were washed again and mounted on slides with Fluoromount (Southern Biotech). Cells were then viewed under a microscope with a standard FITC filter to determine where the expressed PR3 peptides localized (Figure 2).

Discussion. We were able to use the pcDNA3.1(-)/myc-His vector to successfully express recombinant PR3 protein and several small PR3 peptide regions in COS-7 cells. Because this vector contains a C-terminal *myc*-His tag, we did not have to spend the time, effort, and money required to generate antibodies specific to our peptides. The fusion peptides were easily detected in different cellular locations using the Anti-myc and Anti-His(C-term) Antibodies in immunofluorescence experiments. These antibodies proved to be very sensitive and highly specific.

Product	Quantity	Cat. no.	Price
pcDNA3.1(-)/myc-His A, B, & C	20 µg ea.	V855-20	\$320
Anti-myc Antibody	50 µl*	R950-25	\$135
Anti-His(C-term) Antibody	50 µl*	R930-25	\$135

* Sufficient antibody is supplied for 25 western blots.

References:

- Jennette, J.C. and Falk, R.J. (1997) *N. Engl. J. Med.* **337**: 1512-1523.
- Yang, J.J. et al. (1996) *Amer. J. Pathol.* **149**: 1617-1626.



New Mammalian Vector Provides Increased Expression Levels

The human cytomegalovirus (CMV) promoter drives high-level expression of recombinant proteins in a wide variety of cell types (1). To enhance the expression abilities of the CMV promoter, the new pcDNA4/HisMax vector carries a novel translational enhancer. This powerful vector can produce expression levels that are up to five fold higher than those obtained with the CMV promoter alone.

Maximized Expression. pcDNA4/HisMax can express high levels of recombinant protein because it takes advantage of the ability of a unique sequence, QBI SP163, to act as a translational enhancer. QBI SP163 is a 163 base pair DNA sequence isolated from the 5' untranslated region of the mouse vascular endothelial growth factor (VEGF) gene. It functions in a manner similar to an internal ribosome entry site (IRES) to ensure VEGF expression under stressful conditions (2). When placed adjacent to the open reading frame of the gene of interest, QBI SP163 is able to maintain cap-independent translation initiation (2). This increases the overall rate of translation and leads to increased yields of protein. The result is expression levels that are up to five times higher than those achieved using the CMV promoter alone.

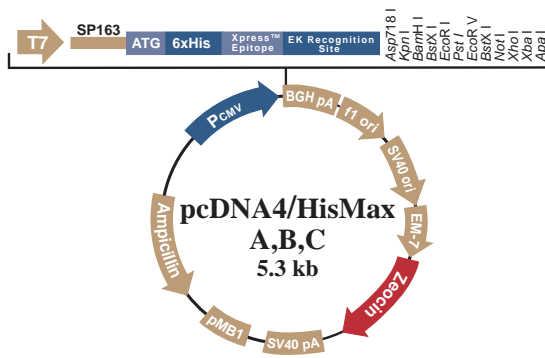
The Data Proves It. To demonstrate the ability of the QBI SP163 element to increase expression levels, three different reporter proteins were expressed in both pcDNA4/HisMax (with the QBI SP163 element) and an identical vector that lacks the QBI SP163 sequence. The data clearly show that protein yields of each of the three reporter proteins are higher when expressed from pcDNA4/HisMax (Figure 1). This increase in expression is due to the presence of the QBI SP163 translational enhancer.

Versatile Design. In addition to offering enhanced expression, pcDNA4/HisMax contains features that facilitate cloning, expression, detection, and purification of recombinant proteins (Figure 2).

- The ATG upstream of the multiple cloning site ensures proper translation from the QBI SP163 sequence.
- The N-terminal polyhistidine (6xHis) tag and Xpress™ epitope allow simple purification and rapid detection of recombinant proteins.

- The enterokinase cleavage site permits efficient removal of the N-terminal Xpress™ tag, returning the protein to its native sequence.
- A large multiple cloning site in three reading frames simplifies cloning in frame with the N-terminal tag.
- The Zeocin™ resistance gene allows effective generation of stable mammalian cell lines.

Figure 2 - pcDNA4/HisMax



Increase Protein Expression Today. By combining the power of the CMV promoter with the translational enhancing ability of the QBI SP163 element, the pcDNA4/HisMax vector allows you to achieve even higher levels of expression. Order pcDNA4/HisMax today and take advantage of this powerful vector.

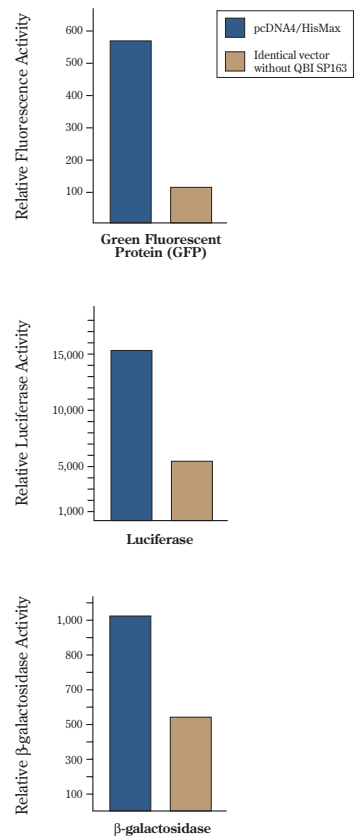
Product	Quantity	Cat. no.	Price
pcDNA4/HisMax A, B, & C	20 µg ea.	V864-20	\$310
Zeocin™	1 g	R250-01	\$160
	5 g	R250-05	\$660
Anti-Xpress™ Antibody	50 µl*	R910-25	\$135
Xpress™ Purification System	6 purifications	K850-01	\$275
EKMax™	250 units	E180-01	\$280
	1000 units	E180-02	\$935

* Sufficient antibody is supplied for 25 western blots.

References:

1. Foecking, M.K. and Hofstetter, H. (1986) *Gene* 45:101-105.
2. Stein, I. et al. (1996) *Mol. Cell. Biol.* 18: 3112-3119.

Figure 1 - Comparison of Expression with and without QBI SP163



All expression experiments were performed in COS-1 cells. The genes encoding GFP, luciferase, and β-galactosidase were cloned into both pcDNA4/HisMax and an identical vector that does not contain the QBI SP163 element. 2 x 10⁵ COS-1 cells were transfected by calcium phosphate or lipid-mediated transfection procedures. Forty-eight hours posttransfection cells were assayed for activity. Samples were normalized for transfection efficiency. GFP fluorescence was assayed by fluorimeter. The activities of luciferase and β-galactosidase were measured with a luminometer. Experiments were performed in duplicate and the average of the results is shown.

Western blots comparing expression levels of GFP in COS-1, CHO, HEK 293, NIH3T3, and HeLa cells can be viewed on the Invitrogen web site at www.invitrogen.com/tech/pcdna4hismax.html.

Expression-Ready Human Gene Resource: Your Questions Answered

GeneStorm™ Expression-Ready Human Clones provide the most convenient, time-saving, and cost-effective human gene resource available.

Every gene in the collection has been cloned into a versatile expression vector, sequenced for identity, and tested for expression. Some of the most frequently-asked questions about GeneStorm™ Clones are addressed here to give you an overview of this unique gene resource.

Q. How are the GeneStorm™ Expression-Ready Human Clones produced?

A. GeneStorm™ Expression-Ready Human Clones are made using the highly-efficient ACE™* (Accelerated Cloning and Expression) process. This process applies PCR cloning, DNA sequencing, and protein expression testing in a high-throughput format. Human genes are amplified using gene-specific primer sets designed to amplify only full-length open reading frames. The PCR products are then efficiently TOPO® Cloned into several versatile GeneStorm™ (GS) bacterial and mammalian expression vectors (Table 1). The GeneStorm™ plasmid DNA is lyophilized in vacuum-sealed glass vials to maintain stability.

Q. How are the GeneStorm™ Human Clones quality tested?

A. After verifying the gene orientation by PCR, each clone is tested in the appropriate cell-based expression system. Cell lysates from transfections (or transformations) are analyzed by western blot. A positive result in this expression test ensures that every clone is expression ready. Finally, each clone is partially sequenced and compared to known sequences from public databases such as GenBank. This test unequivocally confirms the identity of the gene insert.

Q. How are the genes for the GeneStorm™ Human Gene Resource selected?

A. The genes used to create the GeneStorm™ Human Clones are selected based on the most widely-studied research areas. Some of the gene categories included in the GeneStorm™ Gene Resource are shown in Table 2.

Q. What can I do if I need a different gene/vector combination than those available?

A. GeneStorm™ Expression-Ready Human Clones are available in several versatile bacterial and mammalian expression vectors, each of which carries elements for protein detection and purification (Table 1). However, if you are interested in a specific gene/vector combination that is not available, the Biological Operating System (BOS™) allows you to easily shuttle a gene from any GeneStorm™ clone into a variety of expression vectors. BOS™ combines the GeneStorm™ Expression-Ready Human Clones, Invitrogen's collection of TOPO® Cloning Kits, and the gene-specific human GeneStorm™ Primer Sets. A gene can be amplified from a GeneStorm™ clone using the appropriate gene primer set and efficiently transferred to any of the topoisomerase I-activated vectors available. The TOPO® Cloning technology allows you to clone your amplified gene with high efficiency in five minutes at room temperature.

Q. Where can I find a list of GeneStorm™ Expression-Ready Human Clones?

A. The most up-to-date list of all GeneStorm™ Expression-Ready Human Clones and GeneStorm™ Primer Sets can be found in a searchable database at the GeneStorm™ web site (www.invitrogen.com/GS3.html). In addition to GeneStorm™ products, you will find valuable information about TOPO® Cloning kits and the GeneStorm™ vectors.

To place an order or learn more about the most convenient human gene resource available, visit the GeneStorm™ website or contact an Invitrogen Technical Service Representative at 1-800-955-6288, ext. 2 today.

Product	Quantity	Price
GeneStorm™ Expression-Ready Human Clones	50 ng	\$650
Human GeneStorm™ Primer Sets		
<i>Forward and Reverse (with or without stop codon)</i>	2 µg ea.	\$90

* For more information about the ACE™ process, contact Invitrogen at 1-800-955-6288, ext. 2.

Table 1 - GeneStorm™ Expression Vectors Available

Vector	Application
pcDNA3.1/GS ⁺	Constitutive mammalian expression
pIND/GS	Inducible mammalian expression
pCR ⁺ T7/GS	Inducible high-level expression in <i>E. coli</i>
pBAD/ThioGS	Tightly-regulated expression in <i>E. coli</i>

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Table 2 - GeneStorm™ Expression-Ready Human Clone Categories

Category	Genes Represented
Protein modification genes	178
Oncogenes and tumor suppressor genes	82
Ion channel and transport genes	46
Hormones and growth factor genes	20
DNA binding and transcription-related genes	29
DNA repair and recombination genes	15
Cell surface antigens and adhesion molecule genes	43
Extracellular cell signaling and communication protein genes	30
Cell receptor genes	128
Cell cycle control protein genes	49
Apoptosis-related protein genes	55
Immunology and hematology genes	58
Neuro-related Genes	75

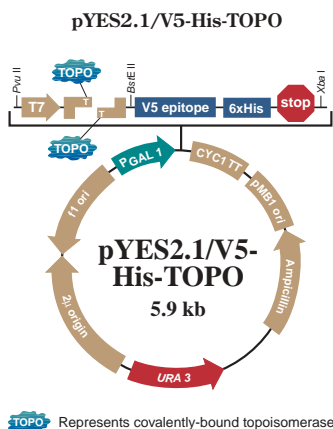


Simplified Cloning of PCR Products for Expression in *S. cerevisiae*

Researchers often choose *Saccharomyces cerevisiae* for gene expression because it grows quickly in defined medium, is less expensive to work with than mammalian cells, and provides many of the posttranslational modifications found in higher eukaryotes. The new pYES2.1 TOPO® TA Cloning® Kit offers five-minute TOPO® Cloning of *Taq*-amplified PCR products into a galactose-inducible *S. cerevisiae* expression vector. This saves you hours of time and effort by allowing you to go directly to inducible expression without subcloning.

High-Level, Inducible Expression. The pYES2.1/V5-His-TOPO vector is a high-copy episomal *S. cerevisiae* expression vector. The vector carries the promoter and enhancer sequence from the *S. cerevisiae* *GAL1* gene for high-level, tightly-regulated, inducible transcription. In addition, the vector contains the following features:

- The 2 μ origin of replication and *URA3* gene for high-copy maintenance and selection in yeast
- C-terminal V5 epitope to allow simplified fusion protein detection with an Anti-V5 Antibody
- Polyhistidine (6xHis) sequence for rapid purification of fusion protein



TOPO® TA Cloning® is Fast and Efficient. The pYES2.1/V5-His-TOPO vector is provided cloning ready with 3'-T overhangs that are activated with topoisomerase I. This enables 5-minute cloning of *Taq*-amplified PCR products right on your bench top. TOPO® TA Cloning® is the most efficient way to clone *Taq*-amplified PCR products. Yields achieved

with the pYES2.1/V5-His-TOPO vector are ≥85% recombinants. In addition, TOPO® TA Cloning® saves time by eliminating vector preparation, synthesis of special primers containing restriction sites, modification or purification of PCR products, ligase, and the lengthy overnight ligation step.

The Perfect Yeast Expression Tool. To demonstrate the utility of the pYES2.1/V5-His-TOPO vector for expression in *S. cerevisiae*, PCR fragments encoding three different proteins were TOPO® Cloned into the vector and expressed in the yeast strain INVSc1. All three proteins were successfully expressed using the pYES2.1/V5-His-TOPO vector (Figure 1).

Complete Kit Ensures Results. For optimal results, the pYES2.1 TOPO® TA Cloning® Kit includes linearized, topoisomerase I-activated pYES2.1/V5-His-TOPO vector, reagents for PCR (except a thermostable polymerase), sequencing primers, One Shot™ TOP10F' competent cells, and controls. Put the power of the pYES2.1/V5-His-TOPO vector to work in your lab with the pYES2.1 TOPO® TA Cloning® Kit. For more information or to order your kit, contact Invitrogen's Technical Services Representatives today.

Product	Quantity	Cat. no.	Price
pYES2.1 TOPO® TA Cloning® Kit	20 rxn	K4150-01	\$345
Anti-V5 Antibody	50 μl	R960-25	\$135

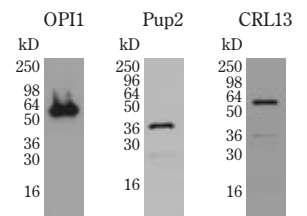
Cloned Yeast Genes Save You Time

Invitrogen offers more than 1,500 *S. cerevisiae* genes already cloned into pYES2.1/V5-His-TOPO and guaranteed to express. The pYES2/GS† GeneStorm™ Expression-Ready Yeast Clones are ideal for many functional genomics applications including functional studies, pathway elucidation, complementation assays, and more. To obtain a complete list of clones available, visit the GeneStorm™ website at www.invitrogen.com/GS3.html.

Quantity	Cat. no.	Price
pYES2/GS GeneStorm™ Expression-Ready Yeast Clones	50 ng (ORF locus convention)Y	\$330

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Figure 1 - Expression of OPI1, Pup2, and CRL13 from pYES2.1/V5-His-TOPO



PCR-amplified inserts were TOPO® Cloned into the pYES2.1/V5-His-TOPO® vector, transformed into INVSc1, and induced with galactosidase. Cell lysates were separated on 12% Tris-glycine polyacrylamide gels and transferred to nitrocellulose membranes. Recombinant fusion protein expression and size were analyzed via western blot using HRP-conjugated Anti-V5 Antibody.

OPI1: Negative regulator of phospholipid biosynthesis

Pup2: Proteasome subunit

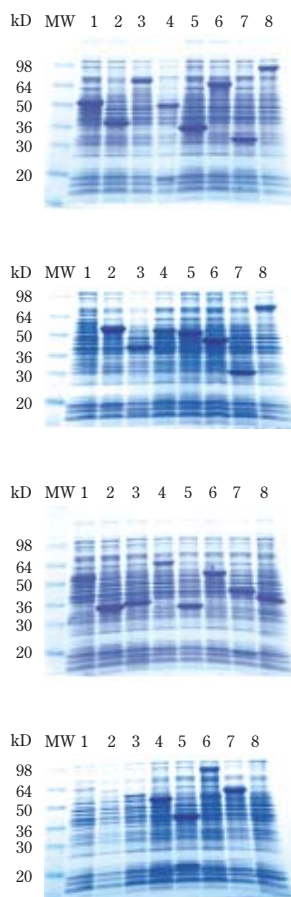
CRL13: ATPase/component of the 26S proteasome CAP subunit



High-Level Expression of Human Genes in *E. coli*

Marc Nasoff, Research and Development, Invitrogen Corporation

Figure 2 - Expression of Human Genes from pBAD/Thio-TOPO

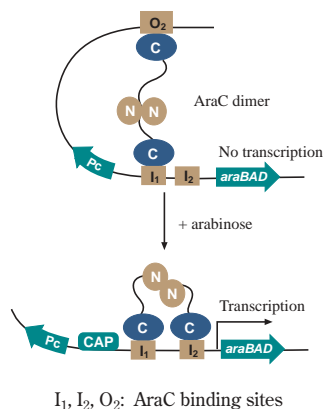


Two hundred and eighty human genes were amplified and cloned in pBAD/Thio-TOPO. Recombinant fusion proteins were expressed in TOP10 *E. coli*. Fifty microliters of each cell lysate was analyzed on a Coomassie-stained 10-20% glycine-SDS gel. Each lane represents a different human gene. The prominent band in most lanes indicates the expressed human gene product.

E. coli is an easy-to-use, inexpensive host for expressing heterologous proteins. The most common problem associated with *E. coli* expression is low protein yield which is often caused by the growth-inhibitive effects of foreign proteins. An effective method to overcome this problem is to use a promoter that provides low basal expression levels and high inducibility. Here we describe the high-level expression of 280 human genes in *E. coli* using the pBAD/TOPO ThioFusion™ Expression System.

Tightly-Regulated *araBAD* Promoter. The pBAD/Thio-TOPO vector was chosen to express the human genes because it uses the *araBAD* promoter. The *araBAD* promoter is regulated by the AraC protein which effectively represses basal level expression in the absence of the inducer arabinose. Upon induction, AraC changes its conformation and activates transcription (Figure 1). In addition, the pBAD/Thio-TOPO vector encodes an N-terminal cleavable thioredoxin fusion to improve translation initiation and solubility of the expressed protein (1).

Figure 1 - Regulation of the *araBAD* Promoter



Methods.

Cloning. Each of the 280 full-length human genes was amplified by PCR. Two and one half microliters of each PCR product was TOPO® Cloned into 2.5 µl of pBAD/Thio-TOPO vector using a 96-well microtiter plate format. Each cloning reaction was transformed into 50 µl of TOP10 competent *E. coli*. Eight ampicillin-resistant colonies were isolated from each transformation. Diagnostic PCR (using the pBAD Forward Primer and a 3' gene-specific

primer) was performed directly on the colonies to identify clones that contain the human gene insert in the correct orientation.

Expression. Positive clones identified by diagnostic PCR were inoculated into deep well blocks (96 x 2 ml) and grown overnight at 37°C in LB medium supplemented with ampicillin (50 µg/ml). The following day, 20 µl of each culture was diluted 1:50 in deep well blocks, grown to mid-log phase, and induced with 0.02% arabinose for 3 hours at 37°C. Cell pellets from 1 ml of each culture were resuspended in Laemmli loading buffer and placed in a boiling water bath for 10 minutes to lyse the cells. Cell lysates were analyzed by SDS-PAGE.

Results. Our results demonstrate that the pBAD/TOPO ThioFusion™ Expression System is able to consistently deliver high-level expression of a variety of human genes. Following induction, 210 of the 280 (75%) clones generated easily recognizable protein bands of the correct molecular weight by Coomassie-stained SDS-PAGE (Figure 2). An estimate of the recombinant protein yields from 1 ml cultures ranged from 1-50 µg/ml. With the pBAD/TOPO ThioFusion™ Expression System, both the overall expression success rates and protein yields were better than those achieved from the *trc*- or T7-based expression systems that were also tested (data not shown).

Discussion. The pBAD/TOPO ThioFusion™ Expression System generates high-level expression of human genes in *E. coli*. This is made possible by the tight regulation of the *araBAD* promoter which minimizes the detrimental growth effects of heterologous proteins. In addition, because thioredoxin can be expressed in its native form at high levels in *E. coli*, the N-terminal thioredoxin fusion may improve the efficiency of translation initiation of the fusion protein leading to increased protein yields.

Product	Quantity	Cat. no.	Price
pBAD/TOPO ThioFusion™ Expression System	20 rxns	K370-01	\$345

Reference:

1. LaVallie, E.R. et al. (1995) *Bio/Technology* 11: 187-193.



Human Genes Cloned into pBAD/Thio-TOPO

The pBAD/TOPO ThioFusion™ Expression System provides a highly effective means of expressing a wide variety of human proteins in *E. coli* (see Application Note on page 10 of this issue). Hundreds of full-length human genes have been cloned into the pBAD/Thio-TOPO expression vector for use in your research applications. The resulting GeneStorm™ pBAD/ThioGS Human Clones save you the time and effort of isolating genes, cloning, and expression testing, and offer high-level inducible expression in *E. coli*.

Powerful Tools. The pBAD/ThioGS GeneStorm™ Expression-Ready Human Clones are powerful tools for regulated, high-level expression of recombinant proteins in *E. coli*. Each of the pBAD/ThioGS Human Clones has been confirmed by partial sequencing and expression tested to save you time and ensure the best expression performance (see GeneStorm™ Q&A on page 8 in this issue). Suitable applications for the GeneStorm™ pBAD/ThioGS human clones include antigen generation and regulated expression of recombinant proteins. There are hundreds of pBAD/ThioGS human clones to choose from, including many kinases.

Figure 1 - Promoter Through Stop Region of pBAD/ThioGS Vector



Try the Gene Resource and Save. The Expression-Ready GeneStorm™ pBAD/ThioGS Human Clones are designed to save you time and effort. For more information about the GeneStorm™ pBAD/ThioGS clones and other GeneStorm™ products, please visit the GeneStorm™ web site (www.invitrogen.com/GS3.html) today.

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

Cost-Effective and Convenient Sequencing

When DNA sequencing is a necessary step in completing your research project, you have two options: sequence the DNA yourself or have someone else do it for you. Invitrogen's Contract Sequencing Service makes that decision easier. Manual DNA sequencing and analysis is cumbersome when you have multiple samples, and automated DNA sequencing is expensive when you have only a few. The sequencing specialists at Invitrogen provide convenient, cost-effective, single-pass and publication-quality DNA sequencing that is tailored to your specific research needs.

Choose from Several Options. Invitrogen's Contract Sequencing Service offers a range of options to fit individual circumstances. Economical single-pass sequencing services are ideal for insert confirmation. Simply send us your DNA template and primer and we will return printed and/or electronic sequencing results (typically 400-600 base pairs per primer) within five business days. For your publication needs, our sequencing specialists will design and synthesize specific primers and sequence both strands of DNA to completion. Your results will be provided in a comprehensive sequence analysis report, including information on primer design, consensus alignment, and electronic and/or printed DNA sequence data. You can rest assured that your sequencing will be done correctly and in a timely manner.

The Time is Right. If you have special DNA sequencing needs, the time is right for you to try Invitrogen's Contract Sequencing Service. Our sequencing specialists are ready to help you meet your goals on time and within budget. To learn more about this valuable service, contact Invitrogen's Contract Services Representative today (1-800-955-6288, ext. 225).

Service	Cat. no.	Price
Single-Pass Sequencing		
1 to 4 reactions	M1002-01	\$40/reaction
5 to 20 reactions	M1002-02	\$30/reaction
21 to 95 reactions	M1002-03	\$25/reaction
96 or more reactions	<i>Please Inquire</i>	
Publication-Quality Sequencing		
	M1001-02	\$1/base



Micro-FastTrack™ 2.0: New and Improved for Twice the Yield of mRNA

FastTrack® Frequent Buyer Club

When you use the Micro-FastTrack™ 2.0 Kit you automatically become a member of the FastTrack® Frequent Buyer Club. Frequent Buyer Club members win cool prizes by redeeming the proof-of-purchase cards supplied in each kit. You only need three cards to get a T-shirt or save them up for other prizes like a baseball cap (5 cards) or a denim shirt (10 cards).

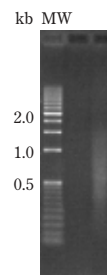
If you isolate mRNA from small samples for RT-PCR, northern blots, library construction, or differential display, then you need the highest possible yields and the highest quality mRNA you can get. Now, the Micro-FastTrack™ Kit has been improved to give you higher yields of high-quality mRNA.

The Secret is in the Powder. The Micro-FastTrack™ 2.0 Kit is specifically designed for isolation of mRNA directly from small samples ranging in size from 1×10^2 to 5×10^6 cells, 10 to 200 mg of tissue, or 100 to 500 µg of total RNA. The new Micro-FastTrack™ 2.0 Kit incorporates premeasured aliquots of high-quality oligo(dT) cellulose powder to make yields better than ever. Premeasured oligo(dT) cellulose readily dissolves for more efficient mRNA binding than with oligo(dT) cellulose tablets. Using premeasured oligo(dT) cellulose powder results in consistently higher yields of mRNA—more than twice those obtained from the original Micro-FastTrack™ Kit.

Double the Yield. Extensive studies at Invitrogen have demonstrated that the yield of mRNA can be significantly increased by using premeasured oligo(dT) cellulose powder. mRNA was isolated from human fetal heart tissue, *Spodoptera frugiperda* (Sf9) cells, baby hamster kidney (BHK) cells, and HeLa cells with either the original Micro-FastTrack™ Kit (oligo(dT) cellulose tablet) or the new Micro-FastTrack™ 2.0 Kit (25 mg of oligo(dT) cellulose powder). Results show at least a two-fold increase in mRNA yield using the new Micro-FastTrack™ 2.0 Kit (Table 1).

Great Results. mRNA isolated with the Micro-FastTrack™ 2.0 Kit was analyzed by gel electrophoresis to show that it is full-length and intact (Figure 1). High-quality mRNA is critical when you need the best outcome for your downstream experiments. The Micro-FastTrack™ 2.0 mRNA Isolation Kit provides increased yields while maintaining high quality and reliability.

Figure 1 – Integrity of mRNA Isolated with the Micro-FastTrack™ 2.0 Kit



mRNA was isolated from 5×10^6 BHK cells using the Micro-FastTrack™ 2.0 Kit. The mRNA pellet was resuspended in 30 µl of RNase-free water and 1 µg was loaded onto a 1% non-denaturing agarose gel.

Quality You Can Count On. The Micro-FastTrack™ 2.0 Kit comes complete with buffers and solutions, premeasured oligo(dT) cellulose powder, microcentrifuge tubes, spin columns, and even RNase-free water to ensure consistent mRNA yields and quality. All components are tested to guarantee RNase-free conditions and to ensure your results.

Get On the FastTrack®. Obtain high-quality, full-length, intact mRNA from your small samples and double your yields with the improved Micro-FastTrack™ 2.0 mRNA Isolation Kit. Order your kit today.

Product	Reactions	Cat. no.	Price
Micro-FastTrack™ 2.0 Kit	20	K1520-02	\$372
	60 (3 x 20)	K1520-03	\$980

Table 1 – Comparison of mRNA Yields from the Original and New Micro-FastTrack™ Kits

Sample	Starting Material	Yield with Original Micro-FastTrack™ Kit	Yield with Improved Micro-FastTrack™ 2.0 Kit
Human fetal heart tissue	200 mg	6.2 µg	14.0 µg
<i>Spodoptera frugiperda</i> (Sf9) cells	5×10^6	1.9 µg	4.0 µg
Baby hamster kidney (BHK) cells	5×10^6	1.85 µg	3.8 µg
HeLa cells	5×10^6	0.85 µg	1.8 µg



Translocation of Functional Flp Recombinase by Fusion to VP22

Brian Dalby and Robert Bennett, Research and Development, Invitrogen Corporation

In the past, the most common method of delivering functional protein to cells was microinjection. Unfortunately, microinjection is tedious and limits the number of cells you can work with. The Voyager™ Technology, based on the VP22 protein, eliminates these limitations and allows the efficient delivery of functional recombinant protein to virtually all of the cells in a culture. In this experiment we show that fusing Flp recombinase to VP22 results in translocation of Flp into untransfected cells and the activation of reporter gene expression.

Introduction. VP22 is a 38 kDa protein isolated from the Herpes Simplex Virus I tegument. It has been demonstrated that VP22 as well as VP22-fusion proteins have the ability to translocate between cells in a culture (1-3). When a cell culture transfected with a plasmid encoding a VP22 fusion protein is added to untransfected cells, the expressed VP22 fusion protein translocates out of the transfected cells and into the untransfected cells. In the untransfected cells, the VP22 fusion is localized to the nucleus.

Materials and Methods:

Transfection of CHO cells with a β -galactosidase reporter.

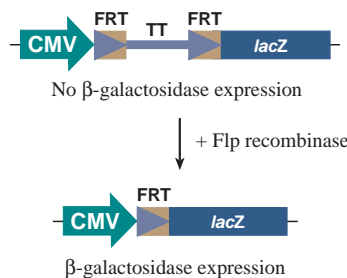
Flp recombinase recognizes and recombines DNA at specific Flp recognition target sites (FRT sites). A reporter plasmid (pFIN4/*lacZ*), containing a transcriptional termination sequence (TT) flanked by FRT sites separating the CMV promoter and the β -galactosidase (*lacZ*) gene (Figure 1), was transiently transfected into CHO cells. Cells transfected with pFIN4/*lacZ* do not express β -galactosidase because the mRNA initiated from the CMV promoter is terminated before the reporter sequence. However, in the presence of Flp recombinase, the transcriptional termination sequence is removed by recombination and the β -galactosidase gene is expressed (Figure 1).

VP22-mediated delivery of Flp recombinase.

COS-1 cells were transfected with a plasmid expressing Flp recombinase, Flp recombinase fused to VP22 (VP22-Flp), or green fluorescent protein (GFP) fused to VP22 (VP22-GFP). Twenty-four hours posttransfection, the CHO cells tran-

siently transfected with the pFIN4/*lacZ* reporter plasmid were added to each of the COS-1 cell transfections at a 1:1 ratio. After 30 hours of incubation, each of the mixtures of cells was fixed and assayed for β -galactosidase activity.

Figure 1 - pFIN4/*lacZ* Reporter Plasmid



Results. Only the CHO cell cultures that were mixed with the VP22-Flp transfected COS-1 cells exhibited β -galactosidase activity (Figure 2). The CHO cell cultures that were mixed with COS-1 cells expressing Flp recombinase alone or the VP22-GFP fusion, did not exhibit β -galactosidase activity (Figure 2).

Discussion. Using the unique ability of VP22 fusions to translocate between cells in culture, the VP22-Flp fusion was able to translocate from COS-1 cells into CHO cells, mediate excision of the transcriptional termination sequence in the pFIN4/*lacZ* reporter, and allow expression of the β -galactosidase reporter gene. This experiment demonstrates the ability of VP22 to translocate a functional protein into eukaryotic cells. VP22 is ideal for the delivery of functional proteins for a variety of studies including oncogene regulation, transcriptional activation, or other nuclear events.

Product	Quantity	Cat. no.	Price
pVP22/ <i>myc</i> -His Vector Kit	20 μ g	V484-01	\$325
pVP22 TOPO® TA Cloning® Kit	20 rxns	K4840-01	\$365

References:

- O'Hare, P. and Elliot, G. (1997) *Cell* **88**: 223-233.
- Phelan, A. et al. (1998) *Nature Biotech.* **16**: 440-443.
- Dilber, M.S. et al. (1999) *Gene Therapy* **6**: 12-21.

Figure 2 - Flp Recombinase-Activated β -galactosidase Expression



pFIN4/*lacZ*-transfected CHO cells were mixed 1:1 with COS-1 cells expressing Flp, VP22-GFP, or VP22-Flp. After 30 hours of incubation, cells were fixed and assayed for β -galactosidase expression. Cells were photographed using light micrograph.



New Reporters and Libraries Enhance the Versatility of the Two-Hybrid System

The Hybrid Hunter™ Two-Hybrid System is the most versatile two-hybrid system available. Now, several new reporter strains, reporter vectors, and premade ‘prey’ libraries are available to allow you to customize your two-hybrid hunt.

Versatile Reporter Strains and Vectors. Several *Saccharomyces cerevisiae* host strains and reporter vectors are available to allow you to optimize the Hybrid Hunter™ System. The Hybrid Hunter™ Yeast Two-Hybrid System uses the activation of reporter genes to indicate positive protein-protein interactions. Typically two reporter genes are used in a two-hybrid screen; an auxotrophic reporter and *lacZ*. The auxotrophic reporter is usually provided integrated into the *S. cerevisiae* host genome whereas the *lacZ* reporter is transformed into the host on a separate plasmid.

Customize the Stringency of Detection. The Hybrid Hunter™ System uses LexA operator sites upstream of the reporter genes to determine the stringency of the protein-protein interactions that will be detected. More LexA operator sites upstream of the reporter allow the detection of weaker interactions. However, increasing the number of LexA operator sites will increase the number of false positives. Conversely, fewer LexA operator sites upstream of the reporter genes will increase the stringency of the protein-protein interactions that will be detected and reduce the number of false positives. Invitrogen offers three *S. cerevisiae* strains containing two to six pairs of LexA operator

sites upstream of the auxotrophic reporter as well as three vectors containing one to eight pairs of LexA operator sites upstream of *lacZ* (Table 1). By varying your reporter strains and vectors you can customize the stringency of detection for your protein-protein interaction of interest.

High-Quality Hybrid Hunter™ Libraries. To expedite your two-hybrid screen, we offer a continually expanding selection of premade Hybrid Hunter™ Libraries prepared from normal and diseased human tissues. Libraries are constructed in pYESTrp, pYESTrp2, or the original LexA-based two-hybrid prey vector, pJG4-5. Hybrid Hunter™ Premade Libraries are pre-qualified and ready to use to save you time and give you the best possible results. And because most interactions occur between domains of proteins, each Hybrid Hunter™ Library is randomly primed and size-selected between 0.4 and 1.2 kb. This ensures that the library contains a full representation of domains. In addition, all libraries have greater than 1×10^6 primary recombinants and are tested for representation by PCR with primers for actin and clathrin. The presence of the actin gene and the 6 kb region of the clathrin gene demonstrates that a full range of cDNA is present, ensuring the representation of the library.

Customize Your Hunt. Now you can customize your two-hybrid screen with your choice of reporter strains and vectors and a selection of premade libraries specifically designed to complement the Hybrid Hunter™ Two-Hybrid System. Get a hold of Hybrid Hunter™ Two-Hybrid products and start your hybrid hunt today.

Table 1 – Strains and Reporter Vectors Available for Use with the Hybrid Hunter™ Yeast Two-Hybrid System

Product	Marker	Auxotrophic Reporter # of LexA Sites	<i>lacZ</i> Reporter # of LexA Sites	Cat. no.
<i>Strains</i>				
LA0*	<i>HIS3</i>	4	8	C830-00
EGY48*	<i>LEU2</i>	6	–	C835-00
EGY48/pSH18-34*	<i>LEU2</i>	6	8	C836-00
EGY191	<i>LEU2</i>	2	–	C837-00
EGY191/pSH18-34	<i>LEU2</i>	2	8	C838-00
<i>Reporter Vectors</i>				
pSH18-34*	–	–	8	V611-20
pRB1840	–	–	1	V612-20
pJK103	–	–	2	V613-20

* Supplied in the Hybrid Hunter™ System

Product	Quantity	Cat. no.	Price
Hybrid Hunter™ System	1 kit	K5000-01	\$475
Reporter Strains	0.5 ml	see Table 1	\$65
Reporter Vectors	20 µg	see Table 1	\$150

Hybrid Hunter™ Libraries

Tissue	Primary		Price
	Library Size†	Cat. no.	
Colon Tumor	7.98×10^6	A222-01	\$675
Normal Bladder	1.76×10^7	A225-01	\$675
Normal Kidney	6.96×10^6	A223-01	\$675

† cfu/ml

“Now you can customize your two-hybrid screen with your choice of reporter strains and vectors and a selection of premade libraries.”



High-Efficiency Electrocompetent Cells

Electroporation is a simple, highly-efficient method for introducing nucleic acids into a broad range of cell types. To facilitate electroporation in your laboratory, Invitrogen offers high-efficiency Electrocomp™ *E. coli* and high-quality electroporation cuvettes.

High-Efficiency Electrocompetent Cells. For convenient, high-efficiency transformation of *E. coli*, Invitrogen offers Electrocomp™ Kits. For consistent results, Electrocomp™ cells are functionally tested to guarantee transformation efficiencies of $>1 \times 10^9$ cfu/ μ g of supercoiled plasmid DNA.

For Multiple Transformations. If you are performing multiple transformations, the Electrocomp™ Transformation Kits are the ideal choice. Electrocomp™ Transformation Kits are supplied with electrocompetent *E. coli* in 80 μ l aliquots (enough for up to four transformations per tube). Four different *E. coli* strains are available—TOP10, TOP10F', HB101, and MC1061/P3—to fit your specific electroporation needs (Table 1).

One Shot™ Convenience. For maximum convenience and economy, the widely-used TOP10 *E. coli* are available in the One Shot™ Electrocomp™ Kit. One Shot™ Electrocomp™ Kits are supplied with single-reaction aliquots (40 μ l) of cells 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.

High-Quality Electroporation Cuvettes. Invitrogen offers high-quality electroporation cuvettes that are compatible with most common electroporators. All cuvettes feature:

- Gamma-irradiation treatment after manufacture to ensure sterility
- Snap-tight caps and individual packaging to maintain sterility until use
- Your choice of 0.1, 0.2, or 0.4 cm gap width to electroporate bacterial, yeast, or mammalian cells
- Color-coded caps (white, blue, or red) for easy identification of gap width

Call Today. Electroporation is made easy with Invitrogen's high-efficiency electrocompetent *E. coli* and high-quality electroporation cuvettes. Electrocomp™ and One Shot™ Electrocomp™ Kits are supplied with pUC18 control DNA and SOC medium to ensure your results. Call Invitrogen to order your Electrocomp™ cells and electroporation cuvettes today.

Product	Quantity	Cat. no.	Price
<i>Electrocomp™ Transformation Kits</i>			
TOP10F'	400 μ l (5 x 80 μ l)	C665-55	\$121
	800 μ l (10 x 80 μ l)	C665-11	\$218
	2.4 ml (30 x 80 μ l)	C665-24	\$619
TOP10	400 μ l (5 x 80 μ l)	C664-55	\$121
	800 μ l (10 x 80 μ l)	C664-11	\$218
	2.4 ml (30 x 80 μ l)	C664-24	\$619
HB101	400 μ l (5 x 80 μ l)	C661-55	\$121
	800 μ l (10 x 80 μ l)	C661-11	\$218
	2.4 ml (30 x 80 μ l)	C661-24	\$619
MC1061/P3	400 μ l (5 x 80 μ l)	C663-55	\$131
	800 μ l (10 x 80 μ l)	C663-11	\$235
	2.4 ml (30 x 80 μ l)	C663-24	\$668
<i>One Shot™ Electrocomp™ Kit</i>			
TOP10	10 rxns	C4040-50	\$131
<i>Electroporation Cuvettes</i>			
0.1 cm (white)	50/bag	P410-50	\$133
0.2 cm (blue)	50/bag	P450-50	\$133
0.4 cm (red)	50/bag	P460-50	\$133

“Electroporation is made easy with Invitrogen's high-efficiency electrocompetent *E. coli*.”



Table 1 - Electrocomp™ *E. coli* Strains and Applications

Strain	Applications
TOP10	cDNA library construction, blue/white screening, subcloning, and plasmid preparation
TOP10F'	cDNA library construction, blue/white screening, subcloning, plasmid preparation, and single-strand rescue
HB101	subcloning, cloning large or unstable constructs
MC1061/P3	cDNA library construction, subcloning, and <i>supF</i> selection



New Products

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 Rapidly Conjugate HRP to any Protein3

imMedia™
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Application Note

pBAD/TOPO ThioFusion™ Expression System
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Voyager™ Technology
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 by Fusion to VP2213



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