

Expressions

A newsletter for gene cloning and expression



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Streamline Expression Analysis with Isogenic Cell Lines

Flp-In™ System

“Generating isogenic cell lines with the Flp-In™ System is the only way to perform functional analysis studies of different genes without worrying about expression variation due to chromosomal position effects.”

Many new reagents and procedures have recently been developed that improve the efficiency of transfecting and selecting stable mammalian expression cell lines. Invitrogen's new Flp-In™ System further advances this process improvement by modifying the host cell line to ensure that the expression vector efficiently integrates at a specific site in the host genome. With the Flp-In™ System, all stably selected cells will be isogenic and therefore produce homogeneous levels of the gene of interest. This minimizes the number of clones that need to be screened for expression, streamlining the overall analysis process.

Efficient Homologous Recombination is Key. The Flp recombinase enzyme mediates homologous recombination between specific DNA sequences known as Flp Recombination Target (FRT) sites. When two FRT sites are brought into proximity of each other in the presence of Flp recombinase, the enzyme catalyzes a conservative, directional strand exchange between them (1,2). The Flp-In™ System takes advantage of this activity to direct the integration of a Flp-In™ expression vector into a single genomic locus containing a FRT site.

The Advantage of Directed Integration. Directing the integration of all Flp-In™ expression vectors to the same locus generates an isogenic population of transfected cells. Individual foci that arise following transfection and selection will express equivalent levels of the gene of interest. As a result, only a few clones need to be screened to verify expression

before expansion and analysis. Alternatively, the Flp-In™ System can be used to rapidly generate stable populations for analysis of the protein of interest. The entire population of Flp-In™ transfected clones can be pooled and expanded *en masse*. Both of these Flp-In™ System protocols will save you time and effort compared to methods that integrate the expression vector at random loci. In addition, generating isogenic cell lines with the Flp-In™ System is the only way to perform functional analysis studies of different genes without worrying about expression variation due to chromosomal position effects.

Simple Modification of the Parental Cell Line. The first step in the Flp-In™ System is to create a Flp-In™ host cell line. Your mammalian cell line of choice is transfected with the Flp-In™ target site vector pFRT/*lacZeo* (Figure 1A). This vector uses the SV40 promoter to express a fusion of the *lacZ* and *Zeocin*™ resistance genes (*lacZ-Zeocin*™). A FRT site is located 3' to the start codon of the *lacZ-Zeocin*™ protein coding sequence. *Zeocin*™-resistant transfectants will have a FRT site integrated into their genome (Figure 1B). To identify clones that have randomly integrated pFRT/*lacZeo* into a transcriptionally active genomic locus, assay *Zeocin*™-resistant clones for β-galactosidase expression. Southern blot analysis should be performed to confirm that a single copy of the vector has integrated.

Powerful Flp-In™ Expression Vector. The second step in the Flp-In™ System is to cotransfect the Flp-In™ host cell line with the Flp-In™ expres-

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Figure 1A - The Flp-In™ Target Site Vector pFRT/*lacZeo*

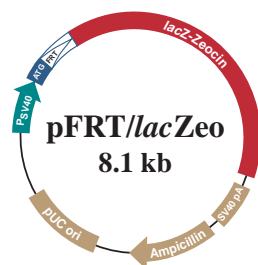
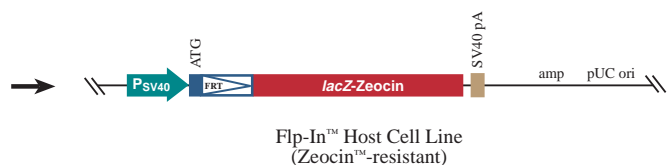


Figure 1B - Genetic Structure of the Flp-In™ Host Cell Line Following Integration of pFRT/*lacZeo*



The Flp-In™ System, continued.

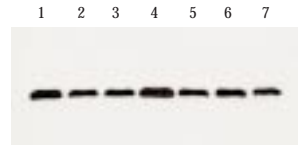
sion vector, pcDNA5/FRT (Figure 2A), and the Flp recombinase expression vector, pOG44. The pcDNA5/FRT vector provides the CMV promoter for high-level expression of cloned genes. This vector also carries a non-expressing hygromycin B resistance gene that lacks a promoter and a translation start site. A FRT site is positioned within the 5' protein coding sequence of this hygromycin B resistance gene. When pcDNA5/FRT and pOG44 are cotransfected into the Flp-In™ host cell line, homologous recombination between the FRT site in pcDNA5/FRT and the genomic FRT site from pFRT/*lacZeo* directs integration of pcDNA5/FRT into that predetermined genomic locus. As shown in Figure 2B, this integration event displaces the *lac-Zeo* gene and places the hygromycin B resistance gene downstream of the SV40 promoter and translation start codon for expression. Consequently, all hygromycin B-resistant cells will have identical genetic structures at the Flp-In™ expression locus and will produce equivalent protein levels.

Homogeneous Expression. To demonstrate the homogeneous expression produced by the Flp-In™ expression cell lines, the green fluorescent protein (GFP) gene was cloned into pcDNA5/FRT and cotransfected with pOG44 into a Flp-In™-293 host cell line. This cell line has a single genomic copy of the Flp-In™ target site vector integrated at a transcriptionally active site. Individual hygromycin B-resistant clones were isolated, expanded, and analyzed for expression of GFP by western blot

(Figure 3). In contrast to traditional selection protocols, every Flp-In™ expression clone analyzed expressed GFP at equivalent levels.

Improve Generation of Your Expression Cell Lines. The Flp-In™ System uses an innovative technology based on Flp recombinase to generate isogenic stable cell lines for homogenous expression of the protein of interest. This saves you time and effort because there's no need to isolate, expand, and screen many individual clones. In addition, the Flp-In™ System makes it possible to accurately compare multiple expression constructs by eliminating position effects. The Flp-In™ Core Kit includes pFRT/*lacZeo*, pcDNA5/FRT, pcDNA5/FRT/CAT as an expression control, pOG44 for expression of Flp recombinase, and sequencing primers. The Flp-In™ Complete System includes the Flp-In™ Core Kit along with Zeocin™ and hygromycin B at a considerable cost savings. Call Invitrogen today to obtain the Flp-In™ System and begin homogeneous stable expression tomorrow.

Figure 3 - Stable Expression of GFP from Individual Flp-In™ Expression Clones



One microgram of pcDNA5/FRT/GFP and nine micrograms of pOG44 were cotransfected into the Flp-In™-293 host cell line. Hygromycin B-resistant foci were expanded in separate cultures and analyzed for GFP expression by western blot using Invitrogen's Anti-GFP Antibody.

Lanes 1-7: GFP expression from individual hygromycin B-resistant clones

Product	Quantity	Cat. no.	Price
Flp-In™ pcDNA5/FRT Complete System	1 kit	K6010-01	\$695
Flp-In™ pcDNA5/FRT Core Kit	1 kit	K6010-02	\$490
pcDNA5/FRT	20 µg	V6010-20	\$235

References:
 1. Sauer, B. (1994) *Cur. Opin. Biotechnol.* 5: 521-527.
 2. O'Gorman, S. *et al.* (1991) *Science* 251: 1351-1355.

Figure 2A - The Flp-In™ Expression Vector pcDNA5/FRT

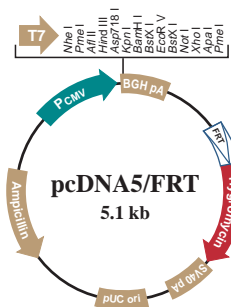
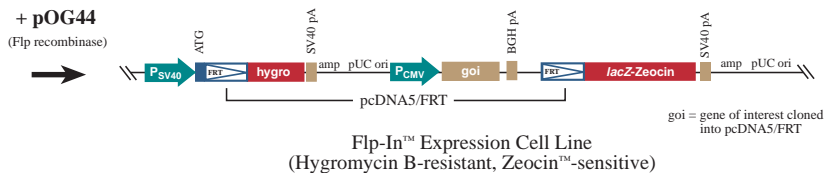


Figure 2A - Genetic Structure of the Flp-In™ Expression Cell Line Following Homologous Recombination and Integration of pcDNA5/FRT at the Genomic FRT Site



Greater Value with the Human Gene Resource

GeneStorm™ Expression-Ready Human Clones

Additional components and new lower prices make the GeneStorm™ Clones a great deal.

The GeneStorm™ resource of expression-ready human genes saves you months of time. Each GeneStorm™ Clone is a human gene PCR cloned into a powerful mammalian expression vector, partially sequenced to confirm insert identity* and proven to express so it's ready for your expression experiments. For added convenience, each GeneStorm™ order now includes competent *E. coli* and a high-quality monoclonal antibody, components you'll find useful in many experiments. When you use GeneStorm™ Clones with the new components, you're getting even greater value from the human gene resource.

pcDNA3.1/GS® negative control vector so you can monitor transformation, transfection efficiencies, and expression relative to background.

Multiple Clones Save you Money. Now you can enjoy big savings when you buy as few as two GeneStorm™ Clones. New pricing for multiple-clone purchases starts with a 25% discount off the regular price when you buy any two GeneStorm™ Clones. Save up to 40% when you buy five GeneStorm™ Clones. With each clone you purchase, you'll receive One Shot® TOP10 Chemically Competent *E. coli* and the Anti-V5-HRP Antibody.

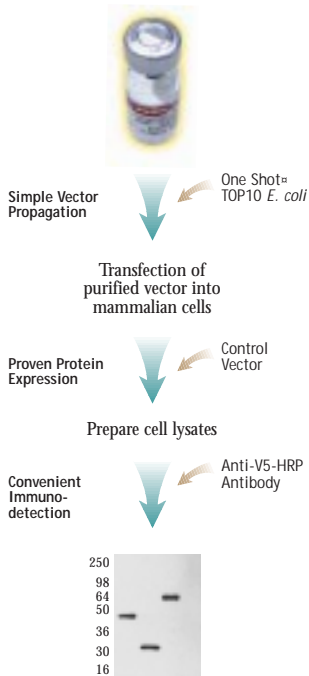
Figure 1 - Added Convenience in the GeneStorm™ Process

Powerful Vector. The GeneStorm™ Clones are provided in the powerful pcDNA3.1/GS® mammalian expression vector. pcDNA3.1/GS® offers the following great features:

- Strong CMV promoter provides high-level expression in mammalian cells
- C-terminal V5 epitope tag for sensitive protein detection using an Anti-V5 Antibody
- Polyhistidine (6xHis) tag for simple protein purification using ProBond™ resin

Trying GeneStorm™ is Easy. A complete listing of the thousands of GeneStorm™ Expression-Ready Human Clones can be found on the easy-to-use GeneStorm™ website database (www.invitrogen.com/genestorm/). The One Shot® TOP10 Chemically Competent *E. coli*, the Anti-V5-HRP Antibody, pcDNA3.1/GS® negative control plasmid, and the multi-clone savings make GeneStorm™ Clones a great value. Order your GeneStorm™ Expression-Ready Human Clones today and begin your gene expression experiments tomorrow.

Great Clones, Greater Value. To give the GeneStorm™ Expression-Ready Human Gene collection even greater value, each clone now comes with TOP10 chemically competent *E. coli* and an Anti-V5-HRP Antibody (Figure 1). TOP10 *E. coli* are included to support efficient vector propagation, which is necessary before transfecting into mammalian cells. TOP10 cells are supplied in the convenient, single-use One Shot® format. With the One Shot® format you only thaw the cells you need so there is no loss in efficiency due to multiple freeze-thaw cycles and no money wasted on unused cells. For even more value, each GeneStorm™ Human Clone also comes with the Anti-V5-HRP Antibody. This antibody is supplied already conjugated to horse-radish peroxidase, so there's no need to use a secondary antibody. With the Anti-V5-HRP Antibody you can easily detect your protein without having to generate or purchase a gene-specific antibody. In addition, each order includes



Western blot of three GeneStorm™ Human Clones detected with the Anti-V5-HRP Antibody.

Product	Cat no.	Price†	New Value Price
GeneStorm™ Expression-Ready Human Clones (pcDNA3.1/GS® vector)			
Any 1 Clone			
	H-(GenBank #)M	\$913	\$650
Any 2 Clones			
	H-K1000-02	\$1,563	\$975
Any 3 Clones			
	H-K1000-03	\$2,213	\$1,350
Any 4 Clones			
	H-K1000-04	\$2,863	\$1,740
Any 5 Clones			
	H-K1000-05	\$3,513	\$2,125

* GeneStorm™ Clones are not guaranteed to match Genbank sequence exactly and may differ by one or more bases.
 † This price represents the cost of compiling individual components from available catalog numbers.

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Save Time with Competent *E. coli* for High-Throughput Cloning

MultiShot™ TOP10 Chemically Competent E. coli in a 96-Well Format

The rapid growth of genomics and proteomics requires high-throughput (HT) methods and tools for cloning. The new MultiShot™ TOP10 Chemically Competent *E. coli* allow rapid transformation in a convenient 96-well format for all of your HT cloning needs. MultiShot™ plates are pre-qualified and supplied ready to use, so they save you days of research time.

96-Well Format Saves Time. MultiShot™ TOP10 *E. coli* save you time because all of the preparation work is done for you. You won't have to spend time preparing, aliquoting, or testing competent *E. coli* again. Each well in the MultiShot™ plate contains 15 µl of high-efficiency, chemically competent TOP10 *E. coli* (Figure 1). Just add your DNA (plasmid or ligation), heat shock, and add SOC medium directly to each well. We've optimized the protocol so that each transformation yields approximately 200-500 colonies when plated on the appropriate selective medium. This means you'll always have plenty of clones for analysis.

Figure 1 - MultiShot™ TOP10 Chemically Competent *E. coli*



Optimized for Guaranteed Success. To ensure quality results, we tested the MultiShot™ TOP10 96-well plates by transforming a variety of products. Both supercoiled DNA and ligation reactions were transformed into TOP10 *E. coli* in the MultiShot™ format. As shown in Table 1, we've optimized the transformation procedure so you're sure to have enough clones for downstream analysis.

Table 1 - Transformation Results Using MultiShot™ TOP10 *E. coli*

Transformation	Average No. of Colonies
pCR®2.1 TA Cloning® Vector + PCR-amplified 750 bp insert	328
pCR®-XL-TOPO® TA Cloning® Vector + PCR-amplified 7 kb insert	149

The pCR®2.1 TA Cloning® ligation was diluted 1:50. Two microliters each of the diluted pCR®2.1 TA Cloning® ligation and the pCR®-XL-TOPO® TA Cloning® ligation were transformed according to the MultiShot™ protocol. Each transformation reaction (105 µl) was plated on the appropriate antibiotic plate and grown for 16 hours at 37°C. Colony counts represent an average of 32 transformations. To pass quality control standards, transformation efficiency using supercoiled pUC18 must be greater than 1 x 10⁸ cfu/µg supercoiled DNA.

The Best Strain for Cloning. We chose the popular TOP10 *E. coli* strain for the MultiShot™ 96-well plate format because of its optimized genotype. TOP10 *E. coli* are perfect for almost every cloning application. The TOP10 genotype features:

- Efficient cloning of unmethylated DNA from PCR amplifications (*hsdR*)
- Efficient cloning of methylated DNA from genomic preparations (*mcrA*)
- Cleaner preparations of DNA and better results in downstream applications due to the elimination of non-specific digestion by Endonuclease I (*endA1*)
- Reduced occurrence of unwanted recombination in cloned DNA (*recA1*)

Using the TOP10 *E. coli* host ensures you'll get the best results in all of your HT cloning applications.

Pick a Pack of MultiShot™ Plates. MultiShot™ TOP10 Chemically Competent *E. coli* in the 96-well format are available in a pack of 5 plates.* Each kit includes qualified SOC medium for optimum outgrowth of your transformations. Save time in all of your high-throughput transformations with high-quality MultiShot™ TOP10 Chemically Competent *E. coli* in a 96-well format. Call and pick a pack of plates today.

Product	Plates	Cat. no.	Price
MultiShot™ TOP10 Chemically Competent <i>E. coli</i>	5 (480 rxns.)	C400-05	\$2,990

* For larger quantities, call for special pricing.

A T-SHIRT FOR YOUR THOUGHTS

In order to continue to provide you with high-quality, innovative products, we want to learn more about your cloning and expression needs. Just let us know what you're thinking by filling out the short Cloning and Expression Survey on our website (www.invitrogen.com/survey1.html). For telling us what's on your mind, we'll send you a cool Invitrogen T-shirt absolutely FREE! It's that easy.

Effortless, Stable Insect Expression: Your Questions Answered

InsectSelect™ System

InsectSelect™ is a high-level expression system designed for effortless, stable expression in insect cells. By using stably integrating vectors rather than viral infections, InsectSelect™ streamlines expression experiments. Read on to learn more about this revolution in insect expression technology.

Q. What are the benefits of using insect cells for protein expression?

A. Insect cells are a popular protein expression system for many reasons. They can produce high protein yields and allow flexible scale-up to expression culture volumes as high as 25 liters. In addition, insect cells are higher eukaryotic hosts and offer many of the posttranslational modifications that are not possible in bacterial systems. By expressing in insect cells, you can obtain large amounts of protein with the modifications that are critical to your experiment's success.

Q. What are the differences between the InsectSelect™ System and baculovirus insect expression systems?

A. Traditional baculovirus insect systems require you to make viral stocks, perform plaque assays, and infect cells. These time-consuming procedures are eliminated with the stable, plasmid-based InsectSelect™ System. With InsectSelect™ you simply clone your gene of interest into a pIZT/V5-His or pIZT/V5-His vector (Figure 1) and transfect the construct into cells. Transient expression can be quickly assayed or stable cell lines can be easily generated for continuous expression.

Q. What selectable marker is used in the InsectSelect™ system?

A. The pIZ/V5-His and pIZT/V5-His vectors carry the Zeocin™ resistance gene for selection. Using Zeocin™, it takes as little as 10 days to generate stable clones for expression testing. The pIZT/V5-His vector has the Zeocin™ resistance gene fused to the gene encoding green fluorescent protein (GFP) for quick identification of transfected cells by fluorescence microscopy.

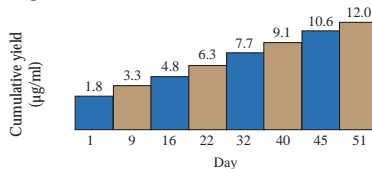
Q. What cell lines can be used with InsectSelect™?

A. The InsectSelect™ System uses the OpIE2 promoter from the *Orgyia pseudotsugata* multicapsid nucleopolyhedrosis virus for high-level expression. This promoter is active in many common insect cell types including Sf9, Sf21, High Five™, and S2 cells.

Q. How high are expression levels from the InsectSelect™ System?

A. To demonstrate typical expression levels, the human IL6 gene was cloned into the pIZT/V5-His vector and stably expressed in High Five™ cells as a secreted protein. Over a period of 51 days, protein was collected eight times from the culture supernatant. At each harvest point, protein expression levels were approximately 1.5 µg/ml culture. Even at day 51, hIL6 is still expressed at 1.4 µg/ml. With InsectSelect™, you'll get high accumulated levels of protein.

Figure 2 - Continuous Expression of Secreted hIL6 in High Five™ Cells



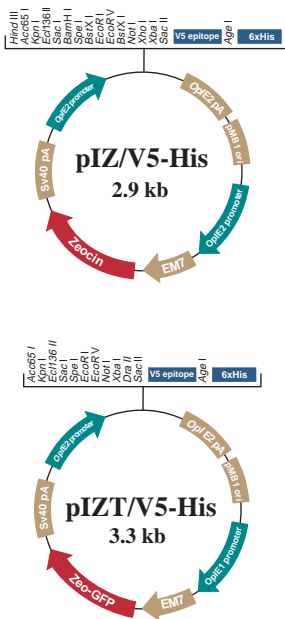
High Five™ cells stably transfected with pIZT/V5-His/IL6 were maintained in High Five™ Serum-Free medium without Zeocin™ at cell densities between 0.8 and 4 x 10⁶ cells/ml. To quantitate hIL6 expression, 1 ml samples of the culture supernatant were assayed at regular intervals by ELISA.

Simple, Non-lytic Insect Expression.

InsectSelect™ is the simplest method for producing your protein in insect cells. There's no plaque assays to perform and no viral stocks to generate and titer so you'll save time and effort. Call Invitrogen to order InsectSelect™ today!

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
pIZT/V5-His Vector Kit	1 kit	V8010-01	\$345

Figure 1 - InsectSelect™ Expression Vectors



Economical Recombinant Protein Production in Milk

pBC1 Milk Expression Vector

Expression in the milk of transgenic animals is a cost-effective way to produce extraordinarily high quantities of recombinant proteins. The pBC1 Milk Expression vector, developed by Genzyme Transgenics Corporation, permits rapid expression in the milk of transgenic mice. Expression levels in mouse milk are typically indicative of levels that can be achieved in larger transgenic animals. Therefore, pBC1 is ideal for high-level protein production in mice as well as economical feasibility studies prior to production scale-up in larger animals.

Tissue-Specific Expression. The pBC1 vector uses tissue-specific regulatory sequences from the goat β -casein gene that limit expression of transgenes to mammary epithelial tissue. These sequences include the enhancer-promoter region as well as the first two exons from the goat β -casein gene. In addition, there is a transcriptional insulator located upstream from the transgene to shield it from positional effects on transcription. As a result, yields of recombinant protein can reach levels above 1 mg/ml (Table 1).

Table 1 - Expression Levels of Various Proteins in Transgenic 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

* Data provided by Genzyme Transgenics Corporation.

Learn More. Visit the Invitrogen website or contact our Technical Service Department for additional information about pBC1 and high-level production of recombinant proteins in the milk of transgenic animals.

Product	Quantity	Cat. no.	Price
pBC1 Milk Expression Kit	1 kit	K270-01	\$695

Isolate High Yields of High-Quality mRNA

FastTrack® 2.0 mRNA Isolation Kit

The FastTrack® 2.0 mRNA Isolation Kit delivers high yields of high-quality mRNA from tissue, cells, or total RNA. Isolated mRNA is ideal for techniques such as microarray analysis, RT-PCR, or cDNA library construction.

Quality Means Full-Length, Intact mRNA. The FastTrack® 2.0 mRNA isolation protocol minimizes the number of sample-handling steps in order to preserve the quality of the mRNA. The FastTrack® 2.0 Kit offers:

- Gentle sample lysis with the unique RNase/Protein Degradator to keep mRNA intact
- Reduced sample manipulation for recovery of full-length mRNA
- Premeasured oligo dT cellulose powder to efficiently bind mRNA for the highest yields
- RNase-free reagents for purifying full-length, intact mRNA

These features ensure that mRNA isolated with the FastTrack® 2.0 Kit is of the highest quality for all of your downstream applications.

High Yields. Most methods for isolating mRNA require multiple rounds of mRNA isolation and result in low yields. Using the FastTrack® 2.0 Kit you'll get the high mRNA yields you need from a single isolation. We've isolated mRNA from several sources with the FastTrack® 2.0 Kit to show that you get great results every time (Table 1).

Get on the FastTrack® Isolate the highest yields of high-quality, full-length mRNA from all of your tissue, cell, and total RNA samples. Find out more about the FastTrack® 2.0 Kit by simply mailing back the enclosed business reply card located between pages 3 and 4. Get on the FastTrack® and order your kit today.

Product	Reactions	Cat. no.	Price
FastTrack® 2.0 Kit	6	K1593-02	\$312
	18 (3 x 6)	K1593-03	\$835

Table 1 - mRNA Yields Obtained with FastTrack® 2.0

Sample	Starting Material	mRNA Yield
<i>Tissue</i>		
Rat spleen	1 g	87 μ g
Rat liver	1 g	71 μ g
Rat kidney	1 g	50 μ g
Dog heart	1 g	31 μ g
Calf intestine	1 g	54 μ g
<i>Cells</i>		
NIH3T3	6.4 x 10 ⁷ cells	54 μ g
Sf9 (<i>Spodoptera frugiperda</i>)	1 x 10 ⁸ cells	71 μ g
<i>Fungus</i>		
<i>Trametes versicolor</i>	1 g	50 μ g

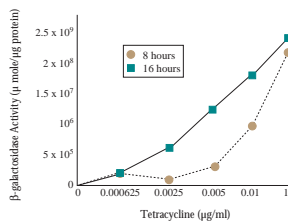
FREE T-SHIRT!

Get on the FastTrack® and get cool stuff. Each time you purchase a FastTrack® 2.0 mRNA Isolation Kit you'll receive a proof-of-purchase card that can be redeemed for free prizes. Check out the postcard enclosed in this issue of *Expressions* for more information.

Powerful Tool for Controlled Expression in Mammalian Cells

T-REx™ System

Figure 1 - Modulation of Gene Expression



T-REx™-293 cells were stably transfected with pcDNA4/TO/*lacZ* and induced with tetracycline at the indicated concentrations for 8 or 16 hours. Samples were normalized for protein content and then assayed for β-galactosidase activity.

The human cytomegalovirus (CMV) immediate-early enhancer-promoter is one of the strongest known promoters for expression of recombinant proteins in mammalian cells. However, overexpression of some proteins can be toxic or produce undesired phenotypic changes in the host cell. The Tetracycline-Regulated Expression (T-REx™) System allows you to overcome these limitations and effectively control expression levels from the complete CMV enhancer-promoter.

Controlling the CMV Promoter. The CMV enhancer-promoter sequence in the T-REx™ expression vectors, pcDNA4/TO and pcDNA4/TO/*myc*-His, has been modified to include two copies of the bacterial Tet operator (TetO₂) sequence (1,2). The Tet repressor (TetR) protein, expressed from the pcDNA6/TR vector, binds the TetO₂ sequences and effectively blocks transcription of genes inserted into the multiple cloning site of the expression vector. When tetracycline is added, it binds the TetR protein and causes TetR to release the TetO₂ sequences. This derepresses the CMV promoter and allows high-level transcription of the cloned gene.

Easily Modulate Gene Expression. Modulating expression allows you to study dose-dependent effects of your protein on cellular processes or pathways. You can easily modulate gene expression levels in the T-REx™ System by varying the amount of tetracycline added to cells transfected with a T-REx™ expression vector. To demonstrate modulated expression, T-REx™-293 cells stably expressing the TetR protein were transiently transfected with the β-galactosidase expression control vector, pcDNA4/TO/*lacZ*. The transfected cells were induced with varying amounts of tetracycline, harvested after 8 to 16 hours, and then assayed for β-galactosidase activity (Figure 1). This ability to modulate expression makes the T-REx™ System ideal for functional analysis of recombinant proteins.

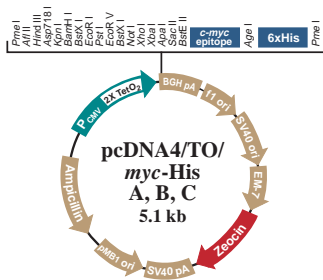
Simplified Detection and Purification. The pcDNA4/TO/*myc*-His T-REx™ expression vector (Figure 2) offers the same regulated expression as its parent vector, pcDNA4/TO. In addition,

pcDNA4/TO/*myc*-His is ideal for the analysis and production of novel proteins that do not have specific antibodies or purification protocols. pcDNA4/TO/*myc*-His offers the following features:

- A C-terminal *c-myc* epitope for simple detection with Invitrogen's Anti-*myc* Antibodies
- A C-terminal polyhistidine (6xHis) sequence for rapid purification with ProBond™ nickel-chelating resin and detection with Invitrogen's Anti-His(C-term) Antibodies
- Supplied in three reading frames to simplify cloning in frame with the C-terminal tag

Effective Control of Expression. The T-REx™ System offers the power of controlled expression from the complete CMV enhancer-promoter. The T-REx™ Core Kit includes pcDNA6/TR, pcDNA4/TO or pcDNA4/TO/*myc*-His, and sequencing primers. The T-REx™ Complete Kit includes the Core Kit, selection agents, and tetracycline. T-REx™ cell lines that stably express the TetR protein are also available to expedite regulated expression of your proteins. Call Invitrogen today to order this versatile tool.

Figure 2 - T-REx™ pcDNA4/TO/*myc*-His Expression Vector



Description	Quantity	Cat. no.	Price
T-REx™ Complete Kit	1 kit		
with pcDNA4/TO		K1020-01	\$695
with pcDNA4/TO/ <i>myc</i> -His		K1030-01	\$750
T-REx™ Core Kit	1 kit		
with pcDNA4/TO		K1020-02	\$490
with pcDNA4/TO/ <i>myc</i> -His		K1030-02	\$545
T-REx™-293 Cells	3 x 10 ⁶	R710-07	\$395
T-REx™-U2OS Cells	3 x 10 ⁶	R712-07	\$395
T-REx™-HeLa Cells	3 x 10 ⁶	R714-07	\$395

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.

As an added bonus you'll receive a 50% discount on the T-REx™ cell line of your choice. Just mention PROM157 when you purchase a T-REx™ Complete Kit before December 31, 1999.

Toolbox for Optimized Expression in *Saccharomyces cerevisiae*

YES™ Expression Vector Collection

Saccharomyces cerevisiae is a popular host for gene expression because it offers the ease of use and economy of a microbial host with many of the posttranslational modifications found in higher eukaryotes. Now there's a new tool—the YES™ (Yeast Expression System) Vector Collection—designed to optimize your gene expression results in *S. cerevisiae*.

Regulated Expression. Each YES™ vector carries the promoter and enhancer sequences from the *GALI* gene for tightly-regulated, high-level expression. The *GALI* promoter is one of the most widely used yeast promoters because of its strong transcriptional activity upon induction with galactose.

Copy Number Matters. To accommodate research that requires high- or low-plasmid copy number, two types of YES™ vectors are available: pYES and pYC. The pYES vectors carry the 2μ origin and are episomally maintained in high-copy numbers (10-40 copies per cell). They are ideal for high-level overexpression of recombinant proteins in *S. cerevisiae*. When you need a low-copy expression vector to obtain expression levels that are similar to those of wild-type yeast, use a pYC vector. The CEN6/ARSH4 sequence in the pYC vectors allows the plasmid to be maintained at 1-2 copies per cell (1). The pYC vectors provide more suitable expression levels for functional studies of the protein of interest.

Table 1 - YES™ Vector Collection

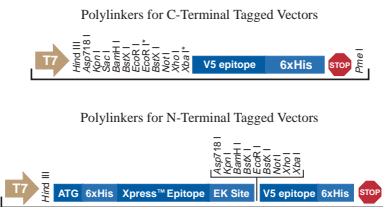
Vector	Origin	Selection Marker	Tag
pYES2	2μ	URA3	-
pYES2/NT	2μ	URA3	NT Xpress™
pYES2/CT	2μ	URA3	CT V5-6xHis
pYES2.1/V5-His-TOPO®	2μ	URA3	CT V5-6xHis
pYES3/CT	2μ	TRP1	CT V5-6xHis
pYES6/CT	2μ	Blasticidin	CT V5-6xHis
pYC2/NT	CEN6 ARSH4	URA3	NT Xpress™
pYC2/CT	CEN6 ARSH4	URA3	CT V5-6xHis
pYC6/CT	CEN6 ARSH4	Blasticidin	CT V5-6xHis

CT = C-Terminal

NT = N-Terminal

Excellent Expression Tools. The YES™ expression vector collection offers a variety of features that make them excellent gene expression tools (Table 1). The pYES6/CT and pYC6/CT vectors carry the *bsd* gene that confers resistance to blasticidin, a potent selection agent in yeast. This dominant selection marker allows you to use these expression vectors in your favorite *S. cerevisiae* strain without worrying about compatibility of auxotrophic selection. For fast, direct cloning of PCR products, use the pYES2.1/V5-His-TOPO® vector. This vector is topoisomerase I-activated and allows 5-minute TOPO® Cloning of *Taq*-amplified PCR products with >85% efficiency. In addition, for simplified protein detection and purification, we have incorporated N- or C-terminal fusion tags into all of our *S. cerevisiae* expression vectors (Figure 1).

Figure 1 - Polylinkers for the YES™ Vector Collection



* Not unique in all vectors.

Choose Your Tool Today. Invitrogen's collection of YES™ expression vectors enables you to optimize recombinant protein expression in *Saccharomyces cerevisiae*. Call us today to order the YES™ expression vector that's right for you.

Description	Quantity	Cat. no.	Price
pYES2	20 μg	V825-20	\$235
pYES2/NT A, B, & C	20 μg ea.	V8252-20	\$320
pYES2/CT	20 μg	V8251-20	\$255
pYES2.1/V5-His TOPO TA Cloning® Kit	20 rxn.	K4150-01	\$345
pYES3/CT	20 μg	V8253-20	\$255
pYES6/CT	20 μg	V8254-20	\$255
pYC2/NT A, B, & C	20 μg ea.	V8256-20	\$320
pYC2/CT	20 μg	V8255-20	\$255
pYC6/CT	20 μg	V8257-20	\$255

Reference:

1. Sikorski, R.S. and Hieter, P. (1989) *Genetics* 122: 19-27.

A T-SHIRT FOR YOUR THOUGHTS

In order to continue to provide you with high-quality, innovative products, we want to learn more about your cloning and expression needs. Just let us know what you're thinking by filling out the short Cloning and Expression Survey on our website (www.invitrogen.com/survey1.html). For telling us what's on your mind, we'll send you a cool Invitrogen T-shirt absolutely *FREE!* It's that easy.

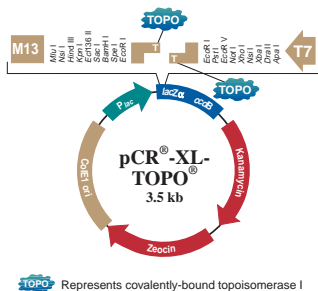
TOPO® Cloning Yields Great Results for Long PCR Cloning

TOPO® XL PCR Cloning Kit

"With the TOPO® XL PCR Cloning Kit I cloned a 9 kb PCR product and got great results. I think that this kit is much better than other methods, like restriction site cutback, because it is much easier, saved 3 to 5 days in the cloning process, and yielded better results."

- Baoxue Yang M.D., Ph.D., University of California San Francisco, Department of Physiology

Figure 1 - pCR®-XL-TOPO®



At Invitrogen, we often hear statements like this one from Dr. Yang. Many researchers who experienced difficulty cloning long PCR products* in the past are finding success with the TOPO® XL PCR Cloning Kit. That's because the TOPO® XL PCR Cloning Kit is specifically designed to eliminate problems associated with cloning long PCR products such as high vector background, low transformation efficiency, and few recombinants. The TOPO® XL PCR Cloning Kit includes a novel vector and optimized protocol that work together to improve your results when cloning long PCR products.

Table 1 - Results from TOPO® Cloning Long PCR Products Stained with Crystal Violet or Ethidium Bromide

	Crystal Violet	Ethidium Bromide
Total Colonies (Kan ^R)	275	15
Colonies w/insert (Kan ^R -Amp ^R)	258	9
Percent Recombinants	94%	60%

A 7 kb ampicillin resistance gene sequence was PCR amplified with Expand™ polymerase. PCR products were loaded onto one gel with crystal violet and another gel stained with ethidium bromide. The 7 kb products were gel purified and TOPO® Cloned into pCR®-XL-TOPO®. The number of recombinants was determined by plating 125 µl of each transformation on LB plates containing either kanamycin or kanamycin and ampicillin.

Eliminate Vector Background. To eliminate vector background, the TOPO® XL PCR Cloning Kit includes the novel pCR®-XL-TOPO® vector (Figure 1). pCR®-XL-TOPO® carries the lethal *ccdB* gene for positive selection of recombinants. Without an insert, pCR®-XL-TOPO® expresses the toxic CcdB protein that kills cells transformed with vector alone. When a long PCR product is ligated into the vector, expression of the CcdB protein is disrupted and cells are able to grow. The vector is provided linearized and cloning-ready with topoisomerase I covalently bound to each end for rapid, high-efficiency TOPO® Cloning (see article on page 13 for more information on how TOPO® Cloning works). The overall result is an efficient ligation that yields virtually no background and ≥80% recombinant colonies.

Improved Results with Gel Purification. To enhance your results, the TOPO® XL PCR Cloning Kit utilizes a unique crystal violet gel purification step. Traditional gel purification of PCR products requires exposure to ethidium bromide and UV light, which can nick and damage long PCR prod-

ucts, and reduce cloning efficiency. In contrast, the TOPO® XL PCR Cloning Kit uses crystal violet in the agarose gel to allow visualization of DNA under ambient light. This eliminates the need for ethidium bromide and exposure to UV light for safe gel purification of long PCR products. Using crystal violet results in higher efficiency cloning of long PCR products. Our experiments demonstrate at least a 10-fold increase in the number of colonies and 1/3 more recombinants than ethidium bromide gel purification procedures (Table 1).

Order a Kit Today. The TOPO® XL PCR Cloning Kit contains all of the reagents you need to get great results in long PCR cloning. The kit includes the pCR®-XL-TOPO® vector, PCR and cloning reagents (except a thermostable enzyme), gel purification reagents, primers for sequencing your insert, and a One Shot® TOP10 Competent Cell Kit. Like Dr. Yang and hundreds of others, you can get great results in long PCR cloning. Order a TOPO® XL PCR Cloning Kit today.

Product	Reactions	Cat. no.	Price
TOPO® XL PCR Cloning Kit†			
<i>with One Shot® TOP10 Electrocomp™ Cells</i>			
	10	K4700-10	\$215
	20	K4700-20	\$360
<i>with One Shot® TOP10 Chemically Competent Cells</i>			
	10	K4750-10	\$215
	20	K4750-20	\$360

* 3-10 kb in length.

† With chemically competent cells, you can expect ≥70% recombinants. For the best results, we recommend using electrocompetent cells. Electroporation yields higher transformation efficiencies than chemical methods and is not biased against large constructs.

References:

- Shuman, S. (1994) *J. Biol. Chem.* 269: 32678-32684.
- Bernard P. et al. (1993) *J. Mol. Biol.* 234: 534-541.
- Rand K. N. (1996) *Elsevier Trends Technical Tips Online.*

Expand™ is a trademark of Roche.

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

Convenient Dual Protein Expression from a Single Vector

pBudCE4

To efficiently study a functional heterodimeric protein, both subunits must be co-expressed within the same cell. Often this task requires the use of two vectors, expensive dual selection procedures, and tiresome testing to ensure the presence of both genes. The pBudCE4 mammalian expression vector offers cloning and expression elements for the simultaneous expression of two genes. Using pBudCE4 you can overcome these inconveniences and make expressing two distinct proteins in a single cell more convenient than ever before.

The Single Vector Advantage. Expressing two proteins within a single mammalian cell doesn't have to mean using multiple vectors. The pBudCE4 vector is all you need. By performing dual protein expression experiments with a single vector you will:

- Save time, effort, and reagents because there's only one vector to prepare for transfection
- Ensure that transfected cells carry both genes of interest
- Reduce expression differences of stably integrated plasmids due to position effects

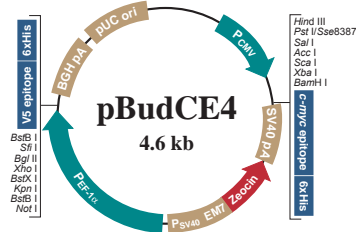
In addition, there's only one selection marker so you'll save money on selection agents when establishing stable cell lines.

The Only Vector You Need. pBudCE4 (Figure 1) carries all the features you need to independently express, differentially identify, and quickly purify two proteins in a mammalian system. These include:

- Two promoters: The human cytomegalovirus (CMV) promoter and the human elongation factor-1 α (EF-1 α) subunit promoter for high-level expression of two genes of interest.
- Two C-terminal tags: One downstream of each multiple cloning site, encoding either the *c-myc* or V5 epitope and a polyhistidine (6xHis) sequence for specific detection and simple purification of the fusion protein.
- One selectable marker: The Zeocin[™] resistance gene for efficient generation of stable cells.

For dual protein expression studies, pBudCE4 is the only vector you'll ever need.

Figure 1 - pBudCE4 Vector



Tested for Expression. The pBudCE4 vector has been tested to ensure that high-level expression is achieved from both the CMV and EF-1 α promoters. To demonstrate, the β -galactosidase gene was cloned downstream of the CMV promoter and the gene encoding chloramphenicol acetyl transferase (CAT) was cloned downstream of the EF-1 α promoter. The vector was transfected into COS-1 cells and then assayed for protein expression by western blot (Figure 2). The data clearly show that both proteins are present in the cell lysate sample.

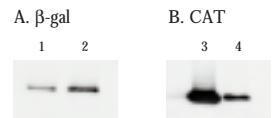
Convenient Expression. pBudCE4 provides the most convenient way to independently express two proteins within a single mammalian cell. To save time and effort in your dual protein expression experiments, place your pBudCE4 order today.

Product	Quantity	Cat. no.	Price
pBudCE4	20 μ g	V532-20	\$265
Zeocin [™]	1 gram	R250-01	\$160
	5 grams	R250-05	\$660
Anti- <i>myc</i> Antibody	50 μ l*	R950-25	\$135
Anti- <i>myc</i> -HRP Antibody	50 μ l*	R951-25	\$160
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.

"pBudCE4 carries all the features you need to independently express, differentially identify, and quickly purify two proteins in a mammalian system."

Figure 2 - Dual Protein Expression



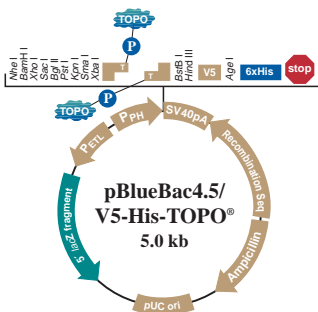
The pBudCE4/*lacZ*/CAT vector was transfected into 2×10^5 COS-1 cells using the Calcium Phosphate Transfection Kit (Invitrogen). Forty-eight hours posttransfection, 10 μ g of cell lysate was separated by SDS-PAGE (lanes 1 and 3) and transferred to nitrocellulose. Purified β -galactosidase-*myc* (lane 2) or CAT-V5 (lane 4) proteins were run as positive controls. The blots were probed with either the Anti-*myc*-HRP Antibody (Panel A) or the Anti-V5-HRP Antibody (Panel B).

Lane 1: Expression of β -galactosidase from pBudCE4/*lacZ*/CAT
 Lane 2: β -galactosidase-*myc* Control Protein
 Lane 3: Expression of CAT from pBudCE4/*lacZ*/CAT
 Lane 4: CAT-V5 Control Protein

Fast and Efficient Cloning into a Baculovirus Transfer Vector

pBlueBac4.5/V5-His TOPO TA Cloning® Kit

Figure 1 - pBlueBac4.5/V5-His-TOPO® Vector Map



 Represents covalently-bound topoisomerase I

The baculovirus expression system is a powerful tool for expressing very high levels of recombinant protein in a eukaryotic system. The baculovirus experts at Invitrogen have added the revolutionary TOPO® Cloning technology to a baculovirus transfer vector to make cloning your gene of interest faster than ever before.

Five Minute, Efficient Cloning. TOPO® Cloning takes advantage of the natural property of topoisomerase I to ligate DNA and uses it in a simple and fast reaction to insert a PCR product into the pBlueBac4.5/V5-His-TOPO® vector (Figure 1). pBlueBac4.5/V5-His-TOPO® is provided linearized and topoisomerase I-activated. All you need to do is mix your *Taq*-amplified PCR product with 1 µl of the prepared TOPO® vector, and incubate the reaction briefly on your benchtop. In just 5 minutes the ligation is complete and ready to be transformed into *E. coli*. You'll save time and reagents because there's no need for overnight incubations, extra insert preparation, or additional subcloning steps.

Powerful Vector Design. pBlueBac4.5/V5-His-TOPO® is a great cloning and expression vector. The following features make it ideal for your expression experiments:

- Strong promoter for high-level expression: The polyhedrin promoter from *Autographa californica* expresses levels as high as 500 mg per liter of functionally active protein (1).
- TOPO® activation speeds up cloning: Cloning takes just 5 minutes and results in >80% recombinants.
- Convenient tags for purification and detection: A C-terminal V5 epitope and polyhistidine (6xHis) tag allows detection of the fusion protein with either our Anti-V5 Antibody or Anti-His (C-term) Antibody. Protein purification is simplified by using the polyhistidine (6xHis) tag for purification on Invitrogen's ProBond™ resin.

True Blue. pBlueBac4.5/V5-His-TOPO® doesn't just make cloning faster, it also simplifies the identification of recombinant plaques. pBlueBac4.5/V5-His-TOPO® is designed to be used in conjunc-

tion with our Bac-N-Blue™ linear viral DNA to generate blue recombinant plaques. Blue plaques can readily be seen with the naked eye and are easier to screen than the clear plaques produced by other systems (Figure 2).

Figure 2 - Blue Plaques from pBlueBac4.5/V5-His/CAT



Blue plaques produced with pBlueBac4.5/V5-His/CAT are easily seen so that you can identify recombinant plaques.

Fast, Efficient Cloning and High-level Expression. For fast, efficient cloning into a baculovirus transfer vector, choose pBlueBac4.5/V5-His-TOPO®. It's the only baculovirus transfer vector that combines the speed of TOPO® Cloning with the power of baculovirus expression. Each pBlueBac4.5/V5-His TOPO TA Cloning® Kit comes complete with linearized, topoisomerase I-activated pBlueBac4.5/V5-His-TOPO® vector, reagents for PCR (except a thermostable polymerase), a CAT expression control, sequencing primers, and One Shot® TOP10 Chemically Competent Cells. You'll have everything you need for fast and efficient TOPO® Cloning. Call Invitrogen and order today!

Product	Quantity	Cat. no.	Price
pBlueBac4.5/V5-His TOPO TA Cloning® Kit	20 rxns.	K2100-20	\$385
Bac-N-Blue™ Transfection Kit	5 transfections	K855-01	\$300
Anti-V5 Antibody	50 µl*	R960-25	\$135
Anti-His(C-term) Antibody	50 µl*	R930-25	\$135
ProBond™ Resin	50 ml	R801-01	\$285

* Sufficient antibody is supplied for 25 western blots.

Reference:

1. Luckow, V. and Summers, M. (1988) *Biotech.* 6: 47-53.

A T-SHIRT FOR YOUR THOUGHTS

In order to continue to provide you with high-quality, innovative products, we want to learn more about your cloning and expression needs. Just let us know what you're thinking by filling out the short Cloning and Expression Survey on our website (www.invitrogen.com/survey1.html). For telling us what's on your mind, we'll send you a cool Invitrogen T-shirt absolutely **FREE!** It's that easy.

Think TOPO® and Improve PCR Cloning Results

TOPO TA Cloning® Kits

Various kits exist for cloning *Taq*-amplified PCR products. Only one—the TOPO TA Cloning® Kit—guarantees ≥95% recombinants in just 5 minutes. That means TOPO TA Cloning® will improve your efficiency in cloning PCR products every time.

Efficient TOPO® Cloning Method. TOPO TA Cloning® improves PCR cloning results. That's because it replaces ligase and overnight ligations with topoisomerase I-activated vectors and a 5-minute, bench-top ligation. The typical role of topoisomerase I in the cell is to relax supercoils by cleaving and religating DNA during replication. Topoisomerase I recognizes the pentameric sequence 5'-(C/T)CCTT-3'; binds to the 3' thymine, and cleaves one strand to allow the DNA to unwind. The enzyme then quickly and efficiently religates the DNA ends and is released. TOPO TA Cloning® takes advantage of the rapid religation activity of topoisomerase I. The TOPO TA Cloning® vectors, pCR®2.1-TOPO® and pCR®II-TOPO® are provided linearized and covalently bound to topoisomerase I. The result is a topoisomerase I-activated vector that you simply mix with 1 µl of your PCR reaction and ligate for 5 minutes to obtain ≥95% recombinants.

Incredible Results. TOPO TA Cloning® yields incredible results because of the novel ligation method. First, ligase is not needed. Ligase can be a problem in cloning because it often contains nucleases that can nick DNA and reduce the number of recombinants. Second, the linearized TOPO® Cloning vectors have topoisomerase I covalently bound to the ends (Figure 1). This maintains the integrity of the vector by protecting the vector ends from exonuclease digestion. Since topoisomerase I is covalently bound, only two molecules—the vector and the PCR product—need to interact for ligation. This is faster and more efficient than a typical ligation reaction in which three molecules—the vector, ligase, and the PCR product must interact in order to produce a ligation product. TOPO TA Cloning® ensures ≥95% recombinant colonies (Figure 2). That means you can screen fewer colonies to obtain your clone, eliminate extra steps required by other methods, and save time and money.

Figure 2 - TOPO® Cloning Results in ≥95% Recombinants



A 750 bp PCR product was TOPO® Cloned into the pCR®II-TOPO® vector, transformed into TOP10 One Shot® Competent Cells, and plated onto LB-Kan plates with X-gal. White colonies represent recombinants.

Think TOPO®. Each TOPO TA Cloning® Kit comes complete to ensure great results every time. The kit includes linearized and topoisomerase I-activated pCR®2.1-TOPO® or pCR®II-TOPO® vector*, dNTPs and 10X PCR buffer for PCR, sterile water, 6X TOPO® Cloning Stop Solution, a control template and primers, M13 forward and reverse primers for sequencing your insert, and a One Shot® Competent Cell Kit for transformation. If you're thinking about getting better results in PCR cloning, think TOPO®.

Product	Reactions	Cat. no.	Price
TOPO TA Cloning® Kit			
<i>with TOP10 One Shot® Cells</i>			
	20	K4500-01	\$305
	40	K4500-40	\$540
<i>with TOP10F' One Shot® Cells</i>			
	20	K4550-01	\$305
	40	K4550-40	\$540
<i>with TOP10 One Shot® Electrocomp™ Cells</i>			
	20	K4560-01	\$305
	40	K4560-40	\$540
TOPO TA Cloning® Kit Dual Promoter			
<i>with TOP10 One Shot® Cells</i>			
	20	K4600-01	\$320
	40	K4600-40	\$566
<i>with TOP10F' One Shot® Cells</i>			
	20	K4650-01	\$320
	40	K4650-40	\$566
<i>with TOP10 One Shot® Electrocomp™ Cells</i>			
	20	K4660-01	\$320
	40	K4660-40	\$566

* The pCR®2.1-TOPO® vector contains the single T7 promoter/primer site. The pCR®II-TOPO® vector contains Sp6 and T7 promoter/primer sites for *in vitro* transcription.

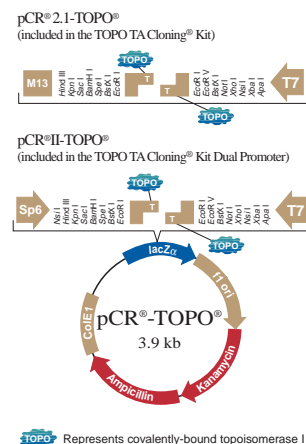
Reference:
Shuman, S. (1994) *J. Biol. Chem.* 269: 32678-32684.

SPECIAL OFFER!



Order one of the TOPO TA Cloning® Kits in this article and we'll send you a cool *Think TOPO®* baseball cap. *Think TOPO®* and call today to order your TOPO TA Cloning® Kit. Use Catalog no. CAP99 for your free baseball cap. This offer lasts only as long as our supply of caps.

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

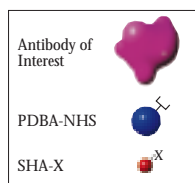


Rapidly HRP- or AP-Conjugate Any Antibody

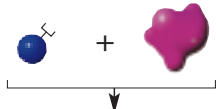
Linx™ Rapid Protein Conjugation Kits

Horseshradish peroxidase (HRP) and alkaline phosphatase (AP) are the two most commonly used enzymes for non-radioactive immunoassays. The new Linx™ HRP and AP Rapid Protein Conjugation Kits allow you to rapidly and easily generate HRP- or AP-conjugated antibodies.

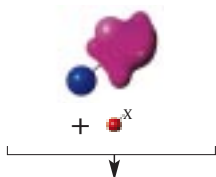
Figure 1 - Linx™ PDBA-SHA Chemistry



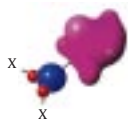
Antibody is incubated with PDBA-NHS reagent. Antibody becomes covalently bound to PDBA.



PDBA-conjugated antibody is incubated with the SHA-HRP or SHA-AP reagent.



HRP or AP-conjugated antibody



PDBA = phenyldiboronic acid
 NHS = N-hydroxysuccinimide
 SHA = salicylhydroxamic acid
 X = horseradish peroxidase or alkaline phosphatase

Three Simple Steps. The Linx™ Rapid Protein Conjugation Kits use the novel Linx™ Chemistry (Figure 1) to rapidly generate HRP- or AP-conjugated antibodies. In just three easy steps, you can conjugate HRP or AP to your antibody of interest:

1. Incubate your antibody with the activated Linx™ PDBA reagent for 45 minutes at room temperature.
2. Use the spin columns provided in the kit to quickly purify the PDBA-conjugated antibody away from any unconjugated PDBA.
3. Add the purified PDBA-conjugated antibody to the Linx™ SHA-HRP or SHA-AP reagent and incubate for 5 minutes at room temperature.

In less than one hour your antibody is HRP- or AP-conjugated and ready to use.

Ready When you Need It. The purified PDBA-antibody conjugate generated from Step 2 (above) is stable for up to one year at +4°C. Whenever you need to HRP- or AP-conjugate this antibody, simply add the SHA-HRP or SHA-AP reagent to your prepared, purified PDBA-conjugated antibody (Step 3). In just 5 minutes your HRP- or AP-conjugated antibody is ready for use in your assay.

Fast and Easy. The Linx™ Rapid Protein Conjugation Kits provide you with the easiest and most rapid way to generate HRP- or AP-conjugated antibodies for all of your immunodetection needs. Order today.

Product	Conjugations	Cat. no.	Price
Linx™ HRP Rapid Protein Conjugation Kit	5*	K8050-01	\$275
Linx™ AP Rapid Protein Conjugation Kit	5*	K8060-01	\$275

* Sufficient reagents are provided for approximately 100 western blots.

Get Maximum Expression with Minimum Effort

Pichia Expression Services

When high levels of heterologous protein with eukaryotic modifications are needed, the *Pichia pastoris* Expression System is the ideal choice. Now you can gain access to this powerful system with minimum investment of your time and effort. You won't need to set up a fermentation lab, learn a new system, train personnel, or be concerned with licensing issues. Just let the experts in Invitrogen's *Pichia* Expression Services do the work for you.

Take Advantage of Our Expertise. The time-tested *Pichia pastoris* Expression System is the system of choice for large-scale expression of recombinant proteins. Over the past six years Invitrogen's expert staff has optimized every aspect of cloning, expression testing, fermentation, and processing used in the *Pichia pastoris* Expression System. We will work with you to design the best strategy and get you the best results from your expression experiment. Table 1 outlines some of the *Pichia pastoris* Expression Services offered by Invitrogen.

Table 1 - *Pichia pastoris* Expression Services

Procedure	Services Provided
Subcloning	Consultation on choice of <i>Pichia</i> expression vector
Transformation	Transform <i>Pichia</i> strain with verified construct Verify transformed strains by PCR Select high-copy number transformants for production
Fermentation	Design optimized protocol for expression Scale-up production (range: 1 to 100 liters)
Processing	Micro-, ultra-, and dia-filtration of supernatants for secreted proteins Microfluidization for isolation of intracellular proteins

Don't Sweat It. Invitrogen's *Pichia* Expression Services can express your recombinant proteins with minimal effort on your part. We will work with you to set up a plan of action for your individual project and keep you updated throughout the process. If you need high levels of recombinant protein expression, take advantage of Invitrogen's *Pichia* Expression Services. Contact our Custom Laboratory Services Representative today at 1-800-955-6288, ext. 265.

Prepare Sterile *E. coli* Growth Medium in Just Five Minutes

imMedia™

Now you can get sterile *E. coli* growth medium immediately. imMedia™ is premixed *E. coli* growth medium specially formulated to eliminate autoclaving. It allows you to prepare high-quality, sterile, low salt LB medium in just 5 minutes using a microwave oven. With imMedia™ you can save hours of media preparation time.

Five-Minute Medium Preparation. Preparing *E. coli* growth medium with imMedia™ is fast. That's because there's:

- No weighing or mixing of media components
- No autoclaving
- No waiting for media to cool before adding antibiotics

Simply mix imMedia™ with water in a clean flask, heat in a microwave oven, and in less than 5 minutes your medium is prepared. With the 5-minute imMedia™ protocol, you can perform your experiment without wasting hours preparing media.

The Ultimate Convenience. imMedia™ means medium preparation without any hassles. All medium components, including the antibiotic, are provided presterilized and premixed in an easy-to-open pouch (Table 1). The presterilized components, microwaving process, and presence of

antibiotic ensure sterility so you don't need to autoclave. For your convenience, imMedia™ is available for preparing liquid medium or agar plates, with or without IPTG and X-gal, and offers a choice of three antibiotics—ampicillin, kanamycin, or Zeocin™.

Ensured Quality. Each type of imMedia™ is stringently tested to ensure *E. coli* growth, antibiotic activity, sterility, and efficient blue/white colony screening. Figure 1 demonstrates the robust results obtained when imMedia™ plates were prepared using non-autoclaved flasks and deionized water. Even when tested under these non-sterile conditions, each lot of imMedia™ must display the same growth support and selection efficiency as LB medium made using a conventional protocol. imMedia™ Blue products are further tested to ensure efficient blue color development. With imMedia™ you not only get your media fast, you can count on quality results as well.

Try imMedia™ Today. By eliminating the need to autoclave, imMedia™ provides the fastest method for preparing *E. coli* growth medium. When you need *E. coli* growth medium in a hurry, you can rely on imMedia™. Order today and you'll have a box of imMedia™ on your shelf tomorrow.

Figure 1 - TOP10 *E. coli* Growth on imMedia™ Plates



TOP10 *E. coli* were plated on imMedia™ plates. The plates were incubated at 37°C for 16 hours.
 A: TOP10 *E. coli* grown on an imMedia™ Amp Agar plate.
 B: Amp^R TOP10 *E. coli* grown on an imMedia™ Amp Agar plate.
 C: Amp^R lacZ⁺ TOP10 *E. coli* grown on an imMedia™ Amp Blue plate.

Table 1 - imMedia™ Components and Ordering Information

imMedia™ Type	Components			Ordering Information				
	Low-Salt LB Media Components	Agar	Antibiotic	IPTG and X-gal	Heat Stabilizers	Quantity (Pouches*)	Cat. no.	Price
imMedia™ Amp Liquid	✓		ampicillin		✓	20	Q600-20	\$127
imMedia™ Kan Liquid	✓		kanamycin		✓	20	Q610-20	\$127
imMedia™ Zeo Liquid	✓		Zeocin™		✓	20	Q620-20	\$200
imMedia™ Amp Agar	✓	✓	ampicillin		✓	20	Q601-20	\$150
imMedia™ Kan Agar	✓	✓	kanamycin		✓	20	Q611-20	\$150
imMedia™ Zeo Agar	✓	✓	Zeocin™		✓	20	Q621-20	\$250
imMedia™ Amp Blue	✓	✓	ampicillin	✓	✓	20	Q602-20	\$200
imMedia™ Kan Blue	✓	✓	kanamycin	✓	✓	20	Q612-20	\$200

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



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