Tools for Expressing and Analyzing Proteins in Bacterial and Mammalian Systems

STRATAGENE

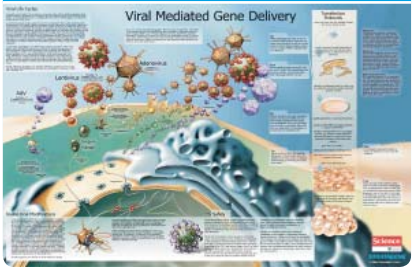
An **Agilent Technologies** Company

Accelerate your research with innovative tools that give you more speed, better accuracy and greater control in gene manipulation, protein expression, novel protein detection and *in vivo* studies of protein interactions. From the fastest, most efficient site-directed mutagenesis kits to unique bacterial and mammalian-based protein expression systems, we offer a comprehensive range of solutions to help you elucidate protein function, protein-protein interactions and decipher complicated cell signaling pathways.

Stratagene: Your Partner in Protein Expression and Analysis

Discover the value of rapid results with our QuikChange Lightning site-directed mutagenesis kits at www.stratagene.com/QCLightning.

For your free copy of the Viral Mediated Gene Delivery Poster, visit **www.stratagene.com/viralposter.**



To help you maximize the power of mutagenesis, we offer the most comprehensive portfolio of reagents and kits today. Integrating advanced technology, enzyme engineering expertise and proven protocols, our products make it easy for you to achieve accurate, reliable results — saving you valuable time. Our ongoing product innovations continue to set new standards. The new QuikChange Lightning site-directed mutagenesis kit[a] dramatically shortens experiment time while maintaining high efficiency and fidelity of results. Our new topoisomerase-based StrataClone expression system provides a quick, easy and reliable solution for protein expression in both prokaryotic and eukaryotic systems.

To address one of the biggest hurdles in expressing non-native genes in bacterial cells, we have designed both vector and cell-based solutions, such as our ArcticExpress competent cells, which improve solubility of the gene product. To overcome codon bias issues in *E. coli,* we engineered special BL21-CodonPlus competent cells that contain extra copies of rare tRNA genes for expression.

The Stratagene BacterioMatch II two-hybrid system offers a simple method for *in-vivo* detection of protein-protein interactions, mapping interaction domains and analyzing interaction strength.

Our advances in eukaryotic gene delivery and detection can be applied to a broad spectrum of cell types, with high efficiency and increased yield. The novel polyamine formulation in GeneJammer transfection reagent provides high-level gene expression in both established lines and primary cells. Alternatively, our AdEasy™ adenoviral and AAV helper-free vector systems offer a complete solution for delivery of genes into cells that will not respond to transfection reagents and enables high-level gene expression in problematic cell types. In addition, our epitope tagging systems enhance detection of the fusion protein in downstream applications.

Now, as an Agilent Technologies company, Stratagene brings even more resources to our partnership with our customers.

Protein Expression Workflows

Protein Production

Bacterial Protein Expression & Analysis

Protein Expression
Challenge: Sufficient expression and solubility of heterologous proteins in *E. coli*.

Solution: **BL21 Gold, CodonPlus** and **ArcticExpress Competent Cells** provide cell-based systems for increased yields and improved solubility of heterologous proteins.

Protein Purification
Challenge: Greater solubility and trouble-free purification of heterologous proteins in *E. coli*.

Solution: **VariFlex Bacterial Protein Expression Systems,** featuring a combination of epitope affinity SET/SBP tags for N- or C- terminals to enhance solubility and provide easy purification.

Protein-Protein Relationships
Challenge: Discovering and analyzing interacting protein partners.

Solution: **BacterioMatch II Two-Hybrid kit** for the rapid determination of protein-protein interactions in *E. coli*.

Functional Analysis

Mammalian Protein Expression & Analysis

Gene Delivery & Protein Expression
Challenge: Variability of gene delivery into mammalian cells.

Solution: Transfection with **GeneJammer** and viral delivery systems **AdEasy™ Adenoviral Vector System** or **AAV Helper-Free Systems** to ensure delivery into most any cell type.

Protein Expression
Challenge: Easy detection of proteins using commercially available antibodies.

Solution: ***New!*** **StrataClone Mammalian Expression Systems** featuring FLAG®- or c-Myc epitope tags for N- or C-terminals simplifies protein detection without interfering with protein function.

Protein-Protein Relationships
Challenge: Understanding the intricacies of protein interactions.

Solution: **InterPlay Mammalian TAP System** or **InterPlay Adenoviral TAP System** provides improved detection of interacting partners in mammalian cells, allowing for analysis of complex protein aggregates.

Mutagenesis
Manipulate your gene prior to expression with **QuikChange® Site-Directed Mutagenesis Kits.**

Protein Quantification and Quality Check
Challenge: Quick and accurate protein quantity and quality analysis.

Solution: The **2100 bioanalyzer** replaces SDS-PAGE analysis, providing a rapid means for protein sizing, purity and quantification of 10 samples in 30 minutes.

Intact Protein Analysis
Challenge: In-depth and accurate analysis of both the intact and digested forms of the protein.

Solution: With exceptional analytical performance and enhanced resolution, the **6520 Accurate-Mass Q-TOF** detects isoforms, variants and impurities. **MassHunter Bioconfirmation Software** enables very high definition between protein components and is especially valuable when looking for minor protein isoform changes.

Increasing the Efficiency of Heterologous Protein Production in *E. coli*

One of the biggest hurdles in expressing non-native genes in bacterial cells is the solubility of the gene product. Forced high-level expression of heterologous proteins in *E. coli* can produce incorrectly folded proteins and inclusion bodies. Although aggregated protein may be easy to purify, obtaining active protein from inclusion bodies typically requires protein-specific *in vitro* refolding steps, with no guarantee of obtaining biologically active product.

A second limitation is the rarity of certain codons in *E. coli* compared with those in the species of origin. Forced high-level expression of heterologous proteins can deplete the pool of rare tRNAs and stall or halt translation altogether.

Enhancing recombinant prokaryotic protein expression

E. coli is often the first choice for recombinant protein expression studies, as it is fast and easy and produces a lot of protein. pET vectors provide the highest level and allow greater control over protein expression. Each protein often has different problems, and one vector system will not give optimal or even acceptable expression levels for all recombinant proteins. With the VariFlex system, you have many choices, allowing you to find vector systems for most of your bacterial expression needs.

Increase solubility and simplify purification with SET and SBP epitope tags

The VariFlex bacterial protein expression system[b] is a series of pET-based vectors that help promote proper protein folding and enhance the utility of *E. coli* as an expression host by adding a Solubility Enhancement Tag (SET) to your gene prior to expression. SET enhances the solubility of fusion proteins by providing a net negative charge, which in turn helps prevent protein aggregation and provides more time for correct protein folding *in vivo* (Table 1). These short tags (40–45 amino acids each) do not affect the activity of recombinant proteins (Figure 1).

Each VariFlex vector carries one or two tags in different combinations, providing flexibility depending on the desired applications. These tags include the solubility enhancement tags 1–3 and the streptavidin binding peptide (SBP) for easy protein purification. The tags are available as fusions to either the N- or C-terminus of the protein of interest, and the N-terminal vectors are provided in all three reading frames. Therefore, we offer our SET-tagged vectors as a complete set. When you order any of our bacterial expression plasmids with the SET tag, you receive all three SET variants.

Lower growth temperatures enhance protein solubility

E. coli is often the first choice for recombinant protein expression because it is fast, simple, and

Table 1. Demonstrated effectiveness of solubility enhanced Tags (SETs)

Protein	Molecular Weight (kD)	SET Effect
CAR D1	13.8	+
MPOex	14.2	+
Imidazoleglycerolphosphate dehydratase (IDPD)	23.8	+
5-formyltetrahydrofolate cyclo-ligase	24.1	–
RAD10	24.3	–
aeGFP	26.9	–
Exosome complex exonuclease RRP41	27.6	+#
UMP pyrophosphorylase	28.8	+#
Prohibitin	31.3	–
RSV Integrase	31.6	–
HIV Integrase	32.2	–
clpX	46.2	+
ilvI	62.8	+
β-galactosidase*	123.5	+

\+ Improves protein solubility
– Does not improve protein solubility
\# Soluble at room temperature
* Insoluble when over-exposed at high temperature

typically produces a lot of protein. Our ArcticExpress competent cells[c] provide an *in vivo* approach to increasing the yield and biological relevance of protein produced in *E. coli*; and have been engineered to include cold-adapted chaperonins for improved protein processing at low temperatures (Figure 2).

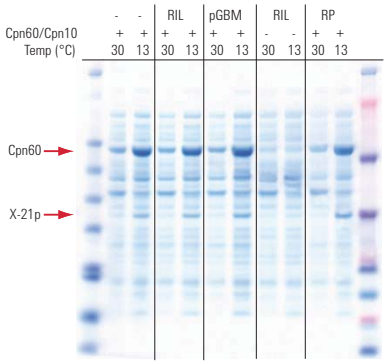


Figure 2. Enhanced protein solubility at lower growth temperatures
Solubility of protein X-21p was enhanced in all cells expressing Cpn60 and Cpn10, especially at 13°C. The CodonPlus vector (pGBM) without tRNA genes did not hinder this effect.

Overcoming codon bias in *E. coli*

To overcome codon bias issues in *E. coli*, the Stratagene BL21-CodonPlus competent cells[d] contain extra copies of rare tRNA genes for expression, which allows for high-level expression of many heterologous proteins that are difficult or impossible to express in conventional *E. coli* hosts (Table 2). The BL21-CodonPlus cells enable protein expression without having to change codons.

Six derivatives of the BL21-CodonPlus competent cells are now available for different levels of expression (Table 3). The BL21-CodonPlus (DE3)-RIL[d] and BL21-CodonPlus (DE3)-RP[d] competent cells, for AT and GC rich genomes, respectively, are all-purpose strains for high-level protein expression and easy induction with IPTG. Copies of each rare tRNA are contained in our BL21-CodonPlus (DE3)-RIPL[d] competent cells.

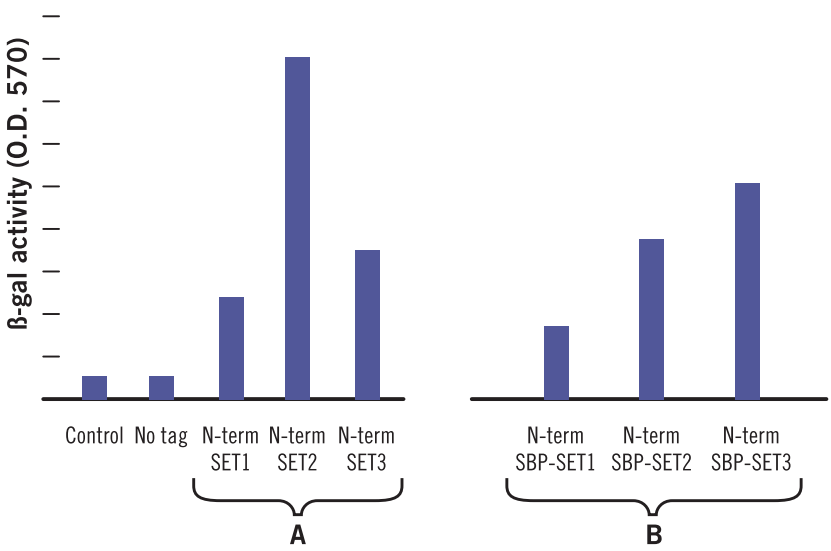


Figure 1. Solubility enhancement tags (SETs) increase protein solubility
We expressed β-galactosidase, a known insoluble protein, in BL21-Gold (DE3) cells. We used each of the three Variflex SET tags alone (A) and in tandem with SBP purification tag (B). We then measured β-galactosidase activity to assay for functional and, therefore, soluble protein. The SET tags dramatically improve the solubility of this problem protein.

Table 2. Codon prevalence in humans and *E. coli* is quite different, often resulting in depletion of available tRNAs

Codon	Amino Acid	Frequency* in *H. sapiens*	Frequency* in *E. coli*
AGG/AGA	Arginine	21.3/9.3	1.4/2.1
CGA	Arginine	6.1	3.1
CUA	Leucine	6.5	3.2
AUA	Isoleucine	6.9	4.1
CCC	Proline	20.3	4.3

* Frequency per 1,000 codons

Table 3. BL21-CodonPlus competent cells encode tRNAs rare in Wild Type *E. coli*

Codon Plus Solution	Extra tRNAs Included	Codons Recognized	Corresponding Amino Acids
RIL	*argU, ileY, leuW*	AGG/AGA, AUA, CUA	Arginine, Isoleucine, Leucine
RP	*argU, proL*	AGG/AGA, CCC	Arginine, Proline
RIPL	*argU, ileY, leuW, proL*	AGG/AGA, AUA, CUA, CCC	Arginine, Isoleucine, Leucine, Proline

An Effective Bacterial System for Detection and Analysis of Protein-Protein Interactions

Elucidating protein-protein interactions and understanding the underlying mechanisms—for example, interaction domains and intra- and extracellular conditions affecting interactions—are essential in dissecting larger cell-signaling cascades.

A bacterial model for *in-vivo* detection

The Stratagene BacterioMatch II two-hybrid system is a simple alternative or complement to yeast two-hybrid systems for *in-vivo* detection of protein-protein interactions, mapping interaction domains and analyzing interaction strength. The system is based on transcriptional activation of a primary Ampicillin-resistant reporter and a secondary β-galactosidase reporter for validation (Figure 3).

This two-hybrid assay performed in bacteria provides easier and quicker screens than assays performed in yeast. Transformation efficiencies are often 1000-fold higher than in yeast. Plating is easy and colonies grow by the next day. The increased efficiency and speed open up new possibilities for your experiments:

- Screen larger libraries to identify binding partners.
- Identify novel target proteins from a cDNA library.
- Verify protein-protein interactions.
- Characterize interactions between a known protein pair.

Stratagene offers many premade human, mouse and rat cDNA libraries for the BacterioMatch two-hybrid system. For currently available premade libraries and a complete list of our BacterioMatch II products, please visit www.stratagene.com/bacteriomatch.

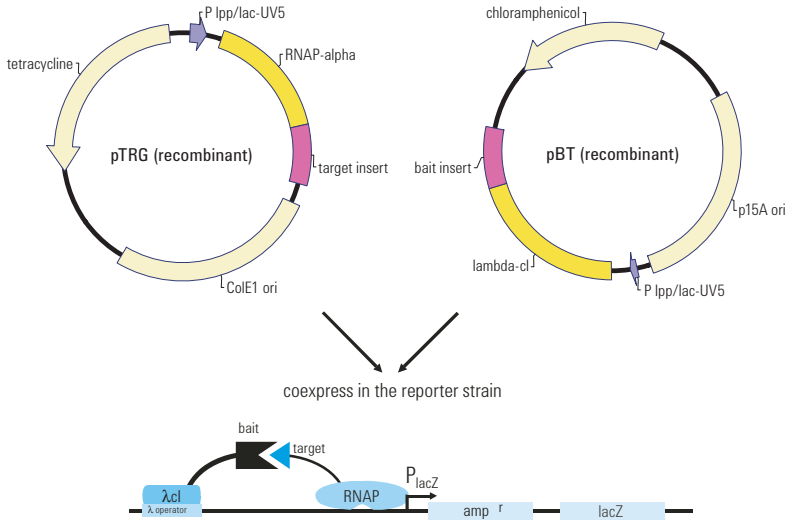


Figure 3. The BacterioMatch II two-hybrid system is a simple alternative or complement to yeast two-hybrid systems.
When the bait and target interact, they recruit and stabilize the binding of RNA polymerase close to the promoter and activate transcription.

Product citations:

A. J. Shaywitz, S. L. Dove, M. E. Greenburg, A. Hothschild (2002) *Science's STKE (Signal Transduction Knowledge Environment).*

Sun AQ, Balasubramaniyan N, Liu CJ, Shahid M, Suchy FJ (2004) *J Biol Chem*, Vol 279(16):16295—300.

Streamline Mammalian Protein Studies

Simplified eukaryotic gene delivery

Stratagene's new StrataClone mammalian expression[b] offers versatile mammalian expression vectors that provide a variety of options for functional studies of gene products. These options include the insertion of regulatory sequences for high expression levels and the presence of epitope tags in variable positions for *in-vivo* studies of gene products. Our new system provides a simple, effective and rapid way to clone a gene into a mammalian expression vector.

- More accurate method compared to other commercially available kits
- Method completed in less than one day versus two days with restriction enzymes
- Allows for rapid protein detection using either FLAG® or c-Myc epitope tags

Overview of the StrataClone mammalian expression technology

Our new StrataClone Mammalian Expression system consists of five different vectors for maximum flexibility. Each vector is supplied as two linear topoisomerase-activated arms (Figure 4). Our system uses epitope tagging technologies that enable easier detection and analysis of your protein using commercially available antibodies. There is no need to produce specific antibodies to identify your protein.

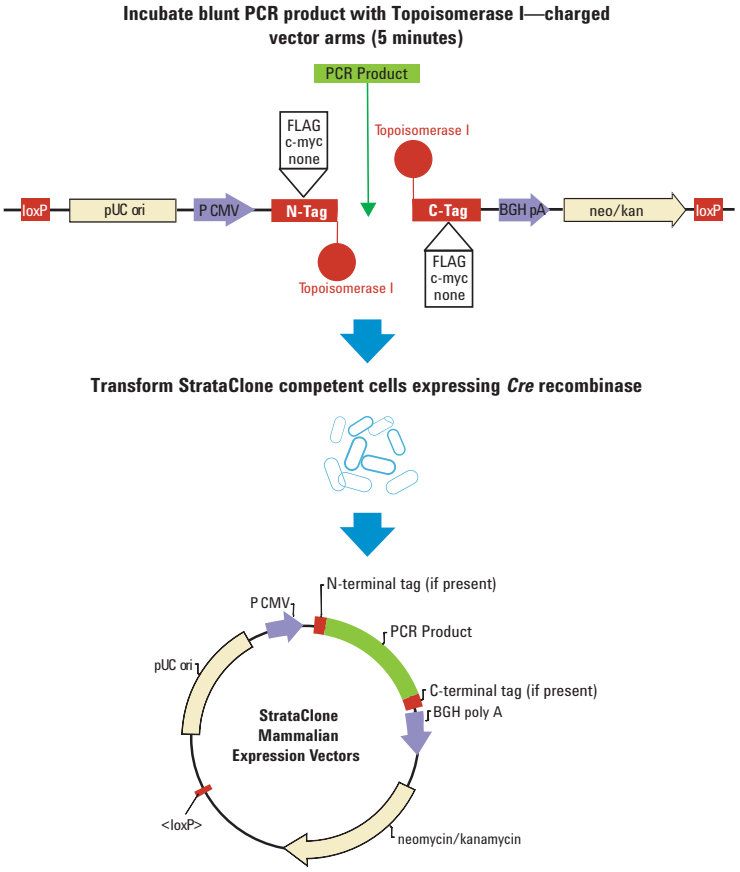


Figure 4. The StrataClone mammalian expression system
Each vector is topoisomerase-activated and available with or without epitope tags.

The demand for versatile mammalian expression vectors is growing, since many researchers study eukaryotic gene transcription and translation. In many instances, achieving post-translational modifications of a gene product of interest is necessary to obtain an active eukaryotic protein. Vectors with convenient options, such as capacity for high-fidelity PCR cloning and epitope tagging for characterization studies, have become integral to sophisticated research inquiries.

Adding an epitope tag to a protein makes detection simple. The epitope tagging technique involves fusing the protein to a small peptide epitope that is recognized by a readily available antibody. With this technique, expression of the fusion protein is monitored using a tag-specific antibody, allowing a new protein to be studied without the need for an antibody specific to the protein of interest. Epitope tagging can be used to localize gene products in living cells, identify associated proteins, detect the recombinant protein within the cell, or characterize new proteins by immunoprecipitation.

Learn about comprehensive mammalian protein expression workflow solutions at www.stratagene.com/mpe

Stronger signal simplifies functional analysis

Epitope-tagging technology provides a powerful means to functionally analyze your protein of interest without creating an antibody specific to each new protein under study. Our new StrataClone mammalian expression system utilizes c-Myc- and FLAG® tags for simplified detection of your protein. This advanced epitope tagging system provides three copies of either the FLAG or c-Myc tags that are added to your protein for easy detection. Three copies of each tag consistently enhances signal strength in all of your protein characterization studies (Figure 5). The FLAG and c-Myc tags are small — 8 (DYKDDDDK) and 10 amino acid residues (EQKLISEEDL), respectively:

- Highly immunoreactive
- Greater signal strength
- Do not interfere with the function of your protein
- Easily detected with commercially available antibodies

These vector arms are available in a variety of combinations or alone for maximum flexibility and are available as fusions to either the N- or C-terminus of the protein of interest:

- No epitope tag
- N-terminal FLAG tag
- N-terminal c-Myc tag
- C-terminal FLAG tag
- C-terminal c-Myc

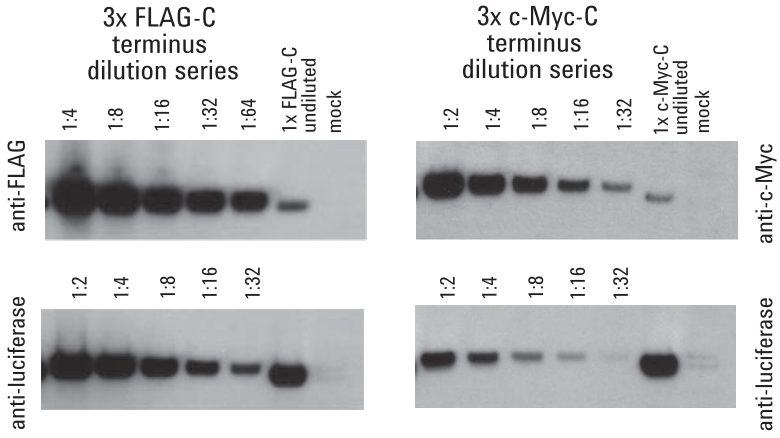


Figure 5. Triple epitope tagging results in 60-fold increase in detection sensitivity compared to single epitope tagging
This figure shows serial dilutions of a luciferase protein expressed with one or three copies of either the FLAG or c-Myc epitope tags detected in a Western blot. Note triple tagged luciferase has a higher molecular weight than single tagged.

High-efficiency, low-cytotoxicity eukaryotic transfection

Achieve high-level gene expression while maintaining cell viability with our GeneJammer transfection reagent. Our novel polyamine formulation dramatically reduces cell damage and death compared to liposomal reagents; this is especially significant when the post-transfection state of the cell is important. Our GeneJammer transfection reagent provides a unique cellular entry mechanism for adenoviruses in receptor-deficient cells, enabling high-level transduction efficiency and transgene expression in cells without adenovirus-specific receptors such as the coxsackie-Ad receptor (CAR) (Figure 6):

- Works with dividing or non-dividing cells
- Achieves high efficiencies in problematic conditions
- Effective in presence of serum (no media changes or washing required)

The Elizabeth and Alan Davis lab at Baylor College of Medicine in collaboration with Dr. Steven Stice at Emory University recently discovered that the GeneJammer transfection reagent provides a novel cellular entry mechanism for adenoviruses in receptor-deficient cells, resulting in increased transduction efficiency and transgene expression[1,2].

1. Stice, S.L., et al. (2005) *Human Gene Therapy* 16:1287—1297.
2. Stice, S.L. et al. (2006) *Molecular Reproductive Development*, Vol 73(11):1393—1394.

Transfection reagents provide an effective vehicle for gene delivery into eukaryotic cells, with easy-to-follow protocols and short times to end-point analysis compared to alternative delivery vectors such as viral delivery. It is important to select a reagent that exhibits low cytotoxicity as well as compatibility with a broad spectrum of cell types, including both established immortalized lines and primary cells.

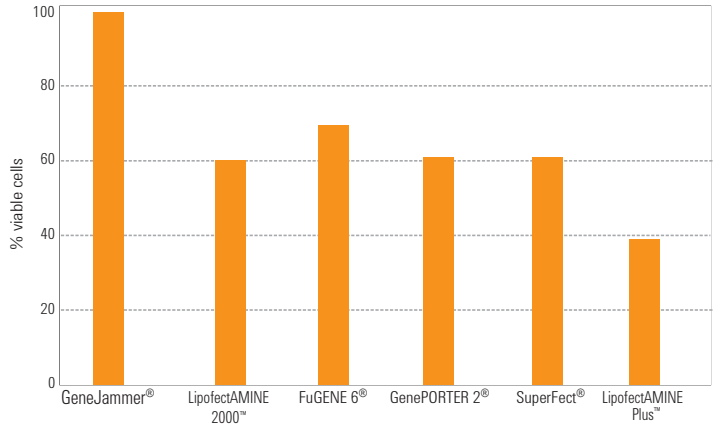


Figure 6. The GeneJammer reagent demonstrates very low toxicity and provides a high number of viable cells
To compare viability, HeLa cells were transfected with pCMV-ß-gal and either the GeneJammer reagent or a competitive reagent. Twenty-four hours after transfection, cell viability was evaluated by counting cells stained with trypan blue. The proportion of viable transfected cells was defined as the percent of transfected cells multiplied by the percent of viable cells.

Cutting-Edge Viral-Based Gene Delivery

For delivering genes into cells that will not respond to transfection reagents, viral-based delivery provides an alternative method. Recombinant adenoviruses are a versatile tool for gene delivery and expression. Several features of adenovirus biology have made such viruses the vectors of choice for certain applications. For example, adenoviruses can infect a broad spectrum of cell types, and infection is not dependent on active cell division. Additionally, high titers and high-level gene expression can be obtained, which lend themselves well to protein production in mammalian cells. Since many hosts are difficult to transfect, functional studies are often limited by the cell line and transfection method used. To solve this problem, viral-based gene delivery systems, like our AdEasy™ gene expression systems and AAV helper-free systems[e] have been developed for exceptionally high-efficiency gene delivery to a broader range of hosts (Table 4).

Viral-based gene delivery for high-level transient expression

For simple high-level delivery of genes into cells that will not respond to transfection agents, Stratagene offers a complete adenoviral solution, including reagents and kits that enable you to rapidly package, concentrate, purify, and deliver your packaged virus. Our AdEasy™ XL and AdEasy adenoviral vector systems utilize recombinant, replication-deficient adenoviral particles to introduce a gene into a host cell. This approach saves a month of work compared to traditional adenoviral vector construction methods. Our AdEasy system uses homologous recombination of *E. coli* to produce viral vectors containing your gene (Figure 7):

- Generate recombinant vectors in three days
- High-level protein expression with proper post-translational modification
- Infects dividing and non-dividing cells

Rapid, affordable adenovirus purification and titer determination

Achieve unparalleled cell viability and infection rates using our new AdEasy virus purification kits, which offer a rapid and affordable alternative to cesium chloride density gradients. With these kits, you can concentrate and purify your viral stocks in 2—3 hours and achieve viral titers of up to 3 x 10^{13} viral particles per milliliter. Easily identify and count positively infected cells using a standard microscope. For viral titer determination, our AdEasy Viral Titer kit is an enzyme-linked immunoassay that quantitates adenoviral stocks in three simple steps.

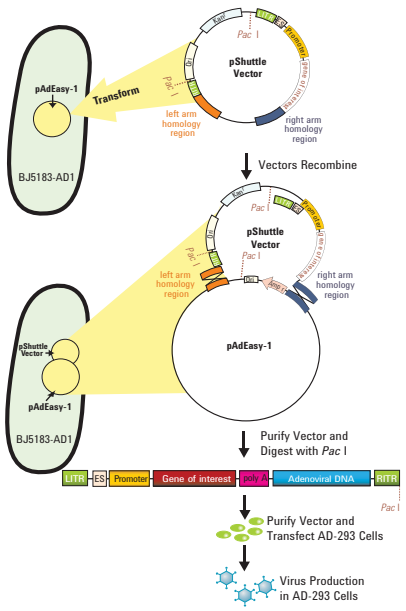


Figure 7. AdEasy reagents provide a complete solution for highly effective adenoviral-mediated gene delivery **With Stratagene's AdEasy family of reagents, it is easier than ever to construct, purify, concentrate, titer and deliver an adenovirus containing your gene.**

Table 4. Features of the different Stratagene viral delivery systems

	Adenovirus	Adeno-Associated Virus	Retrovirus
Host: Dividing Cells	+	+	+
Host: Non-Dividing Cells	+	+	–
Viral Integration into Host Genome	–	5—10% of the time	+
Long-Term Expression in Dividing Cells	–	5—10% of the time	+
Long-Term Expression in Non-Dividing Cells	–	+	–
Host Immunogenicity	+	–	–
Maximum Insert Size	7.5 kb	3 kb	8.4 kb

+ **Recommended**
– **Not Recommended**

Safe, long-term stable expression

Our AAV helper-free system uses the pHelper vector containing adenoviral genes to produce recombinant, replication-defective AAV virions (Figure 8). The AAV genome with your gene of interest typically remains epichromosomal, resulting in stable, long-term expression in slowly dividing or non-dividing cells. A high multiplicity of infection (MOI) results in integration into the host cell genome and stable expression in rapidly dividing cells.

Use this unique system to deliver your gene of interest to a wide range of cells and achieve viral titers of more than 10^{7}:

- Broad host range
- Unparalleled biosafety profile—eliminates need to use wild-type virus
- Provides for long-term, stable gene expression

Improved characterization of protein-protein interactions in mammalian cells

The Stratagene InterPlay mammalian TAP system[f] allows you to purify interacting proteins from mammalian cells. The Tandem Affinity Purification (TAP) method is based on expression of a protein of interest fused to two affinity tags: a streptavidin-binding peptide (SBP) and a calmodulin-binding peptide (CBP). Gentle washing and elution conditions leave interacting proteins intact (Figure 9). There is no need to perform protease digestions to recover interacting protein partners, since SBP and CBP elute easily from their affinity resins.

Our InterPlay adenoviral TAP system[g] combines the unique tandem affinity purification (TAP) tags with our AdEasy XL adenoviral vector system for adenoviral-mediated gene delivery. Recombinant adenoviruses can infect both dividing and non-dividing cells, allowing you to study interactions in difficult-to-transfect and impossible-to-transfect mammalian cells. High viral titers coupled with the infectivity of adenoviruses can generate the yields necessary to study protein-protein relationships in most mammalian cells.

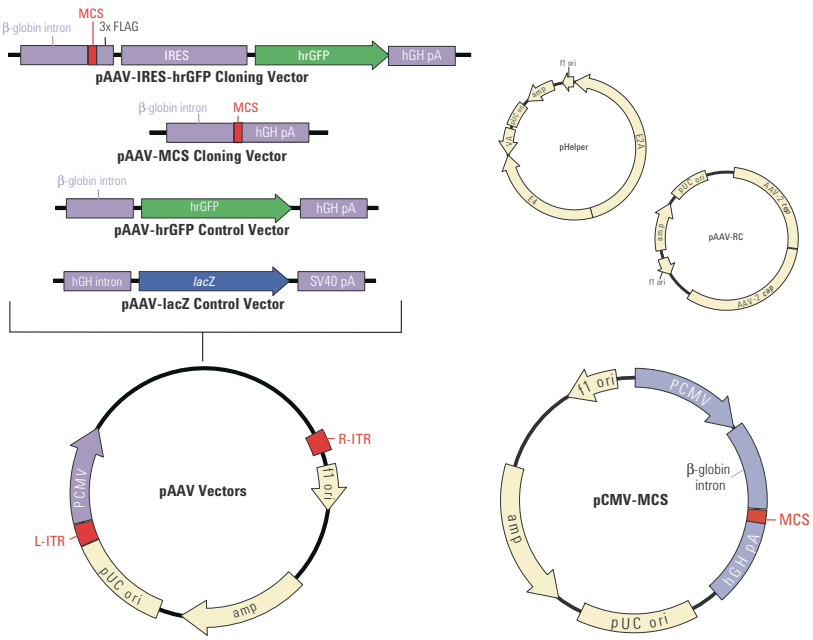


Figure 8. Innovative helper-free vectors

pAAV is the basic cloning vector for the AAV system. If, before virus production, you plan to modify your gene of interest (using mutagenesis, etc), we recommend first cloning into the pCMV-MCS shuttle. Other system vectors are the pHelper, which supplies the necessary adenoviral genes to make infectious virions, and the pAAV-RC vector, which supplies the genes that encode for DNA replication proteins and capsid proteins.

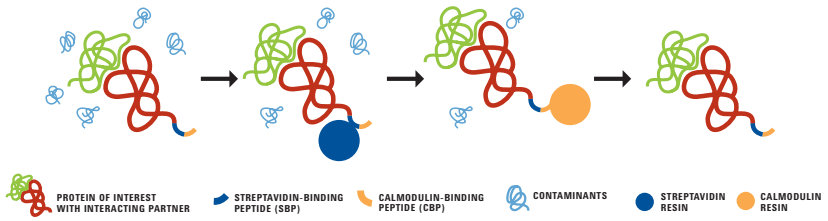


Figure 9. Gentle washing and eluting conditions leave your interacting proteins intact in two easy purification steps

To purify protein with the TAP protocol, apply the mammalian cell lysate to the strepavidin resin, elute with biotin, apply this first eluate to calmodium resin, and elute with EGTA. Two elution steps combined with gentle washing result in ultra-pure protein and intact protein complexes.

Protein Analysis Selection Guide

Functional Cloning	Kit	Advantages
Topoisomerase-based expression	***New!*** StrataClone mammalian expression system	Offers five unique vectors with epitope tagging technologies (c-Myc or FLAG®) that enable easier detection and analysis of your protein. Method completed in less than one day.
Easy detection with epitope tagging	pCMV-3Tag vectors	Three reading frames allow the gene of interest to be fused correctly to the epitope tags (c-Myc or FLAG), providing a stronger signal in immunological assays.
High-level expression	pCMV-Script vectors	Powerful CMV promoter and SV40 polyadenylation sequence for high-level expression.
Multiple constructs	pExchange vectors	Creates multiple expression constructs with different drug-resistance genes.
Protein visualization	Vitality hrGFP II mammalian expression vectors	Available with either a FLAG or HA tag for stable expression. Non-invasive *in-vivo* detection using FACS or microscopy.
Inducible expression	Complete Control System	Dose-responsive inducible mammalian expression with induction as high as 1,700-fold.

Transfection	Kit	Advantages
Polyamine-based	GeneJammer transfection reagent	Polyamine-based transfection reagent providing significant reduction in cell damage.
Liposome-based	LipoTAXI transfection reagent	Liposome-based transfection reagent for many cell types.
$CaPO_4$-DNA coprecipitate	MBS mammalian transfection kit	Increases transient transfection efficiencies >100-fold.
Modified $CaPO_4$	ViraPack transfection kit	Unprecedented efficiency with retroviral, adenoviral and AAV systems. Exceptional transfection efficiencies for HEK293 cells.

Viral Mediated Delivery	Kit	Advantages
Adenoviral	AdEasy™ adenoviral vector system	Easy, rapid two-step process for homologous recombination in *E. coli* saves weeks of time. Gene delivery across the broadest spectrum of cell types independent of host cell division.
Adeno-associated viral	AAV helper-free system	Facilitates high-efficiency gene delivery to a broad range of hosts. Uses recombinant replication-defective AAV virions.
Retroviral	ViraPort retroviral gene expression system	Transduction efficiencies approaching 100% in a wide range of mitotic cells.

Purification & Protein-Protein Interactions	Kit	Advantages
Affinity purification	InterPlay mammalian TAP system	Easy two-step protocol yields exceptionally clean proteins. Suitable for mass spectrometry.
Affinity purification	InterPlay adenoviral TAP system	Enhanced gene delivery to a broader range of mammalian cells, higher protein yields, and improved purification and analysis. Suitable for mass spectrometry.
Mammalian two-hybrid	Mammalian two-hybrid system	Confirms suspected interactions between two proteins or interactions identified by yeast two-hybrid screenings. Works well in a variety of mammalian cell lines.

Visit **www.stratagene.com/proteinexpression** to discover additional solutions for protein expression and analysis. Now, as an Agilent Technologies company, we bring even more resources to our partnerships with our customers.

Agilent's 2100 bioanalyzer protein chips provide rapid analysis of protein size, purity and quantification. For in-depth and accurate analysis of both the intact and digested forms of the protein, Agilent's 6520 Accurate-Mass Q-TOF detects isoforms, variants and impurities. Agilent's MassHunter Bioconfirmation Software enables very high definition between protein components and is especially valuable when looking for minor protein isoform changes. Visit **www.agilent.com** for additional information.

Ordering Information

Product	Description	Contents	Cat. No.
NEW! QuikChange Lightning site-directed mutagenesis	Fastest high-fidelity site-directed mutagenesis available; suitable for large plasmids >8 kb	10 rxns 30 rxns	210518 210519
QuikChange site-directed mutagenesis kits	QuikChange II kits, QuikChange Multi kits, and other mutagenesis kits	To view our collection of QuikChange kits, visit www.stratagene.com/QuikChange	
VariFlex N-terminal SBP-SET expression system	Double-tagged pET-based bacterial protein expression vectors and related reagents	1 kit	240165
VariFlex C-terminal SBP-SET expression system	Double-tagged pET-based bacterial protein expression vectors and related reagents	1 kit	240177
VariFlex N-terminal SBP-SET vector set	Double-tagged pET-based bacterial protein expression vectors and related reagents	1 kit	240164
VariFlex C-terminal SBP-SET vector set	Double-tagged pET-based bacterial protein expression vectors and related reagents	1 kit	240176
Streptavidin resin	1.25 ml of streptavidin resin	1.25 ml	240105
ArcticExpress competent cells	Enhanced protein folding and solubility of expressed proteins	10 x 0.1 ml	230191
ArcticExpress (DE3) competent cells	T7 RNA polymerase under control of the lacUV5 promoter	10 x 0.1 ml	230192
ArcticExpress (DE3)RIL competent cells	Improves expression for AT-rich genomes. T7 RNA polymerase under control of the lacUV5 promoter	10 x 0.1 ml	230193
ArcticExpress (DE3)RP competent cells	Improves expression for GC-rich genomes. T7 RNA polymerase under control of the lacUV5 promoter	10 x 0.1 ml	230194
ArcticExpress RIL competent cells	Improves expression for AT-rich genomes	10 x 0.1 ml	230195
ArcticExpress RP competent cells	Improves expression for GC-rich genomes	10 x 0.1 ml	230196
BL21-CodonPlus (DE3)-RIPL competent cells	Extra copies of argU, ileY, leuW, proL. T7 RNA polymerase under control of the lacUV5 promoter	10 x 0.1 ml	230280
BL21-CodonPlus (DE3)-RIL competent cells	Extra copies of argU, ileY, leuW. T7 RNA polymerase under control of the lacUV5 promoter	10 x 0.1 ml	230245
BL21-CodonPlus (DE3)-RP competent cells	Extra copies of argU and proL. T7 RNA polymerase under control of the lacUV5 promoter	10 x 0.1 ml	230255
BL21-CodonPlus (DE3)-RIL-X competent cells	Extra copies of argU, ileY, leuW. T7 RNA polymerase under control of the lacUV5 promoter. Methionine label for metabolic labeling	10 x 0.1 ml	230265
BL21-CodonPlus (DE3)-RP-X competent cells	Extra copies of argU and proL. T7 RNA polymerase under control of the lacUV5 promoter. Methionine label for metabolic labeling	10 x 0.1 ml	230275
BL21-CodonPlus-RIL competent cells	Extra copies of argU, ileY, leuW. For extremely toxic proteins, use with lambda CE6 for introduction of the T7 RNA polymerase	10 x 0.1 ml	230240
BL21-CodonPlus-RP competent cells	Extra copies of argU and proL. For extremely toxic proteins, use with lambda CE6 for introduction of the T7 RNA polymerase	10 x 0.1 ml	230250
Lambda CE6 induction kit	Expression of toxic proteins in BL21 and BL21-Gold cells; contains Bacteriophage CE6, BL21 and LE392 cells	1 kit	235200
BacterioMatch II two-hybrid system vector kit	Vectors and cells needed to construct target, bait and control plasmids	Two-hybrid system plasmids, control plasmids, XL1-Blue MRF' Kan glycerol stock for propagating recombinants	240065
BacterioMatch II reporter screening competent cells	Chemically competent reporter strain for screening libraries in the BacterioMatch II Two-Hybrid System	6 x 0.5 ml	200190
BacterioMatch II validation reporter competent cells	Chemically competent reporter strain for validation and testing in the BacterioMatch II Two-Hybrid System	5 x 0.2 ml	200192
BacterioMatch II electrocompetent reporter cells	Electroporation-competent reporter strain for screening libraries in the BacterioMatch II Two-Hybrid System	5 x 0.1 ml	200195
BacterioMatch II two-hybrid system cDNA library construction kit	Complete library construction and interaction screening system	Reagents for construction of 5 directional cDNA libraries in the pTRG target vector, provided pre-digested. Also includes two-hybrid screening components	200414
BacterioMatch premade cDNA libraries	Human, mouse and rat premade cDNA libraries for the identification of protein-protein interactions	To view our collection of premade BacterioMatch II libraries, visit www.stratagene.com/bacteriomatch	

Ordering Information

Product	Description	Contents	Cat. No.
Strataclone mammalian expression untagged vector system	Allows for rapid 5-minute PCR ligation; allows you to insert the exact DNA sequence without non-coding regions	20 reactions	240228
StrataClone mammalian expression N-terminal FLAG vector system	Utilizes three copies of FLAG tags for simplified detection of your protein for N-terminus	20 reactions	240229
StrataClone mammalian expression C-terminal FLAG vector system	Employs three copies of FLAG tags for simplified detection of your protein for C-terminus	20 reactions	240230
StrataClone mammalian expression N-terminal c-Myc vector system	Utilizes three copies of c-Myc tags for simplified detection of your protein for N-terminus	20 reactions	240231
StrataClone mammalian expression C-terminal c-Myc vector system	Employs three copies of c-Myc tags for simplified detection of your protein for C-terminus	20 reactions	240232
Anti-FLAG M2 antibody	Monoclonal IgG1. Murine origin	200 µg	200471
Anti-FLAG M2 antibody	Monoclonal IgG1. Murine origin	1 mg	200472
Anti-c-Myc antibody, clone 9E10	Monoclonal IgG1. Murine origin	25 µg	257260
Anti-c-Myc antibody, clone 9E10	Monoclonal IgG1. Murine origin	100 µg	257261
GeneJammer rransfection reagent	1.33 mg/ml reagent in 80% ethanol	0.3 ml trial size	204132
GeneJammer transfection reagent	1.33 mg/ml reagent in 80% ethanol	1.0 ml	204130
GeneJammer transfection reagent	1.33 mg/ml reagent in 80% ethanol	4 x 1.0 ml	204131
AdEasy XL adenoviral vector system	Vectors and cells required to clone and package recombinant adenovirus	1 kit	240010
AdEasy virus purification kit 2 x 100	Purify 2 × 20–100 ml cultures or 1 × 100–200 ml supernatant	1 kit	240243
AdEasy virus purification kit 5 x 100	Purify 5 × 20–100 ml supernatant	1 kit	240244
AdEasy virus purification kit 500	Purify 1 x 500 ml supernatant	1 kit	240245
AdEasy viral titer kit	Enzyme-linked immunoassay for rapid titer determination	1 kit	972500
AAV helper-free system	All vectors and cells needed to package recombinant, helper-free AAV	1 kit	240071
InterPlay C-terminal mammalian TAP system	Vectors, resins and buffers for mammalian tandem affinity purification	1 kit	240104
InterPlay C-terminal mammalian TAP vectors	Vectors for performing tandem affinity purification	1 kit	240102
InterPlay N-terminal mammalian TAP system	Vectors, resins and buffers for mammalian tandem affinity purification	1 kit	240103
InterPlay N-terminal mammalian TAP vectors	Vectors for performing tandem affinity purification	1 kit	240101
InterPlay adenoviral C-terminal TAP system	Vectors, cells, resins and buffers for adenoviral gene delivery and tandem affinity purification	1 kit	240215
InterPlay adenoviral C-terminal TAP vectors	Vectors for adenoviral gene delivery and tandem affinity purification	1 kit	240216
InterPlay adenoviral N-terminal TAP system	Vectors, cells, resins and buffers for adenoviral gene delivery and tandem affinity purification	1 kit	240213
InterPlay adenoviral N-terminal TAP vectors	Vectors for adenoviral gene delivery and tandem affinity purification	1 kit	240214
InterPlay TAP purification kit	Resins and buffers required to perform tandem affinity purification	1 kit	240107
InterPlay TAP purification buffers kit	Buffers required to perform tandem affinity purification	1 kit	240099
MS-grade calmodulin resin	Calmodulin resin qualified for mass spectrometry	0.625 ml	240106
Streptavidin resin	Resin	1.25 ml	240105

Notes:

a. U.S. Patent Nos. 7,176,004; 7,132,265; 7,045,328; 6,734,293; 6,489,150; 6,444,428; 6,391,548; 6,183,997; 5,948,663; 5,932,419; 5,866,395; 5,789,166; 5,545,552, 6,713,285; 6,706,525; 6,489,150; 5,707,841; 5,512,468 and patents pending

b. T7 promoter: U.S. Patent No. 4,952,496. For academic or non-profit laboratories, an assurance letter accompanies the sale of the products. For commercial laboratories, a research use license agreement must be entered into prior to purchase of the products.

c. The T7 expression system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is protected by U.S. patents assigned to Brookhaven Science Associates (BSA). BSA will grant a nonexclusive license for use of this technology, including the enclosed materials, based on the following assurances:

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d. U.S. Patent No. 6,706,525 and patents pending
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