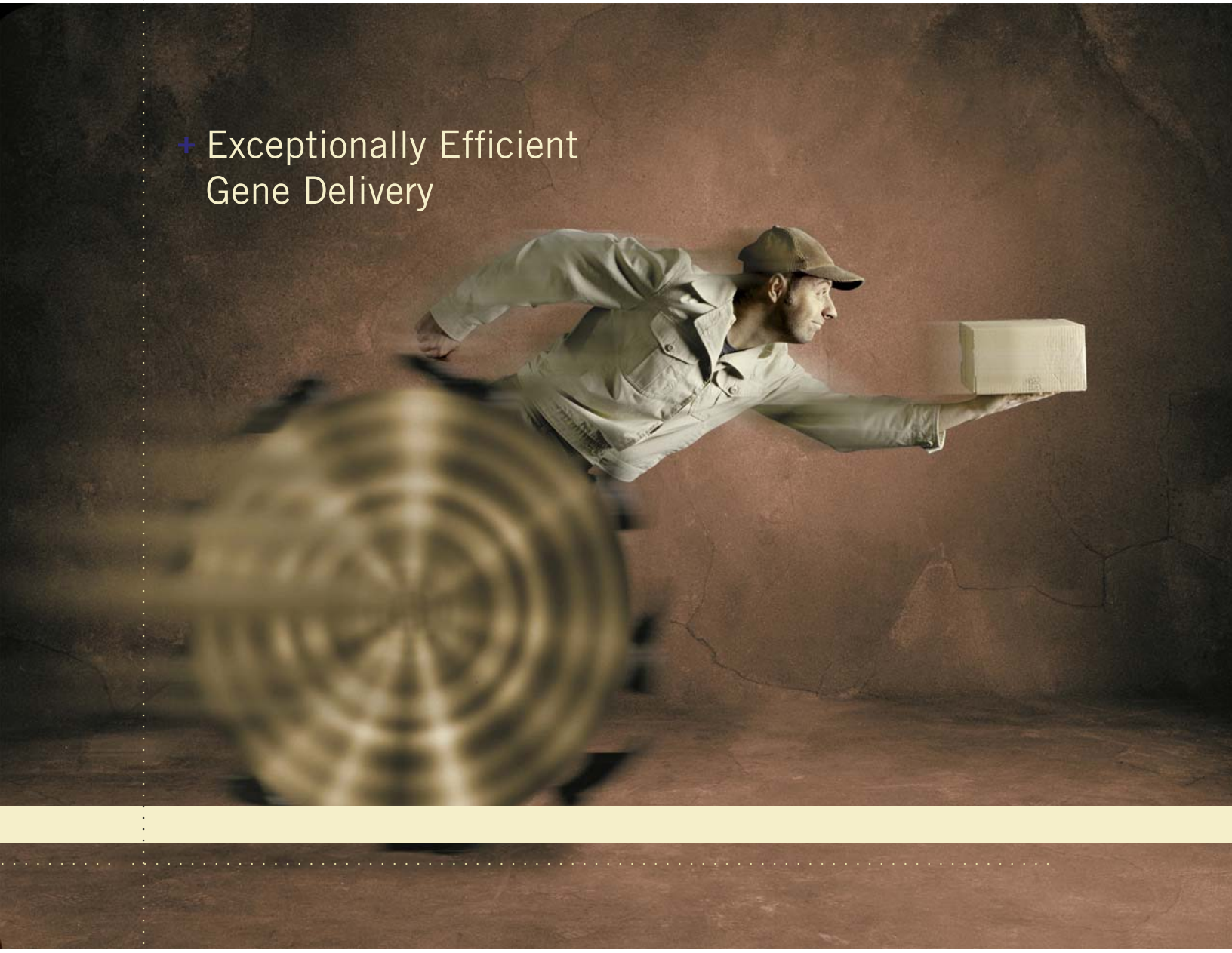
CLONING

Viral Delivery Systems



STRATAGENE

An **Agilent Technologies** Company

Cutting-Edge Gene Delivery

As science moves from gene discovery to understanding gene function, the need to study gene expression in a native host is becoming increasingly important. Many of these hosts are difficult or impossible to transfect, which means functional studies may be limited to hosts that easily accept DNA using traditional transfection methods. To solve this problem, viral-based gene delivery systems, like our AAV Helper-Free System, AdEasy™ adenovirus system, and ViraPort® retroviral cDNA expression system have been developed for exceptionally high-efficiency gene delivery to a broader range of hosts.

So how do you decide which viral delivery system to choose? The tables below compare our three viral gene delivery systems to make it easy to find the best system for your application.

Application	Long-Term Gene Expression	Transient, High-Level Gene Expression	Functional Cloning Assays
System	**AAV Helper-Free System**	**AdEasy™ XL Adenoviral System**	**ViraPort® Retroviral cDNA Expression System**
Advantages	+ Infects both dividing and non-dividing cells + Long-term, stable gene expression + Unparalleled biosafety profile	+ High-level protein production + Infects both dividing and non-dividing cells + Homologous recombination in *E. coli* saves weeks of work	+ Integrates into host genome for stable expression + Copy number controlled by multiplicity of infection + Functionally screen cDNA libraries in mammalian cells + Premade libraries available

HOW TO CHOOSE THE RIGHT SYSTEM BY APPLICATION

System	AAV	AdEasy XL	ViraPort	Transfection
Gene delivery efficiency	>90%	>90%	>90%	~20%
Host: Dividing cells	+	+	+	+
Host: Non-dividing cells	+	+	–	–
Long-term expression	+	–	+	+
Transient expression	–	+	–	+
High-titer virus	+	+	–	NA
Host immunogenecity	–	+	–	NA
Maximum insert size	3 kb	7.5 kb	<8 kb	Variable
Selection for stable cells	+/-	NA	–	+

COMPARISON OF VIRAL DELIVERY SYSTEMS We compare viral delivery systems to each other and to typical transfection systems.

Long-Term, Stable Expression

AAV Helper-Free System

The AAV Helper-Free System[1] improves upon recombinant adeno-associated virus-2 (AAV-2) technology by eliminating the need for helper virus. It allows safe, high-efficiency gene delivery to a broad range of hosts. This powerful gene delivery system allows stable, long-term gene expression.

Optimal in Non-Dividing Cells

Using the AAV Helper-Free system, you can deliver genes into a wide range of hosts and achieve viral titers of $\geq 10^7$ (Figure 1). Upon transduction, gene expression occurs when the single-stranded recombinant viral genome becomes double-stranded. The AAV genome typically remains epichromosomal, and in slowly dividing or non-dividing cells, expression will be long-term and stable. Rapidly dividing cells will lose the epichromosomal AAV vector. However, integration into the cell genome can occur when you use extremely high multiplicity of infection (MOI) or in the presence of adenoviral replicase.

Safe System

The AAV virus normally requires coinfection with an unrelated helper virus, like adenovirus, to generate AAV virions. Our AAV Helper-Free System uses a vector containing the necessary genes from adenovirus (pHelper vector) to induce the lytic phase of AAV. Additionally, the viral proteins (rep and cap) needed in wild-type AAV to package the virus are located on a separate plasmid (pAAV-RC) that shares no regions of homology with the cloning vector, eliminating the chance of generating wild-type AAV virus (Figure 2). You can use wild-type adenovirus to increase AAV integration into the host genome of dividing cells for long-term expression studies, but its use reduces the biosafety of the AAV system.

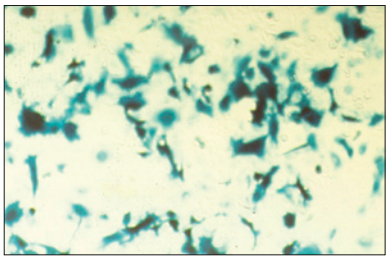


FIGURE 1. EFFICIENT GENE DELIVERY HT1080 cells were infected with pAAV-LacZ and in situ ß-galactosidase staining was performed 48 hours after transduction.

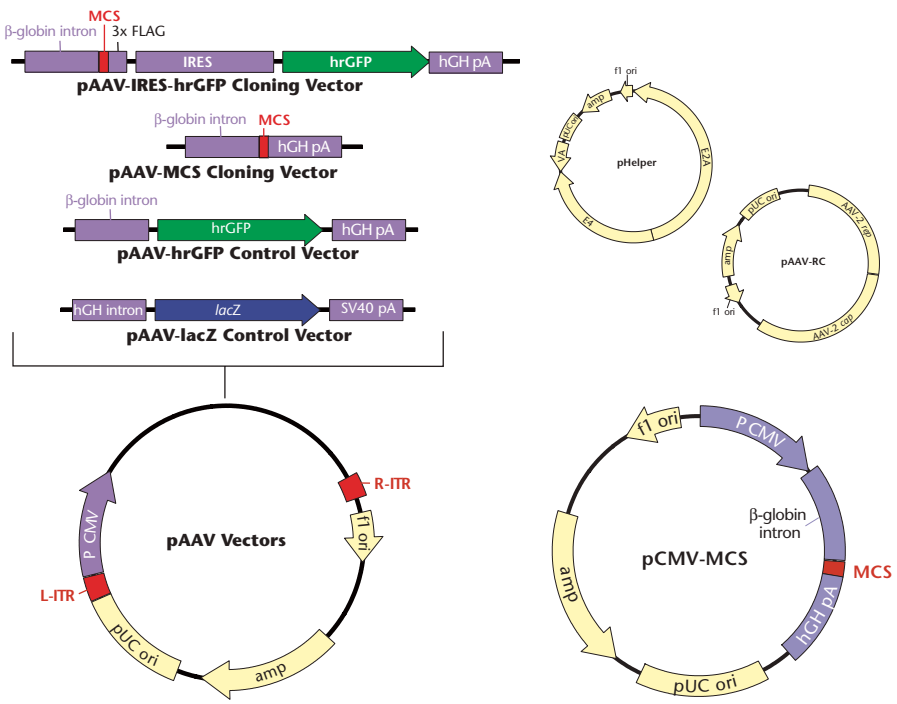


FIGURE 2. INNOVATIVE HELPER-FREE VECTORS[2,3] pAAV is the basic cloning vector for the AAV system. If, before virus production, you plan to modify your gene of interest (using mutagenesis, etc), we recommend first cloning into the pCMV-MCS shuttle. Other system vectors are the pHelper, which supplies 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.

High-Level Transient Expression

AdEasy™ XL and AdEasy™ Systems

The AdEasy™ XL and AdEasy™ Adenoviral Vector Systems[4] save you a month of work over traditional methods by producing the recombinant adenoviral plasmid by homologous recombination in *E. coli*. Now you can obtain your recombinant plasmid after a simple transformation!

Homologous Recombination in *E. coli* Saves Time

Traditional methods of adenovirus production rely on one of two steps. The first involves cloning directly into the adenoviral genome. However, the scarcity of unique restriction sites and the prohibitive size of the genome (36 kb) make this method technically challenging. The second, more common, method of adenovirus production involves transfection of a shuttle vector and the adenoviral backbone into a packaging cell line and allowing homologous recombination to occur between the shuttle vector and the adenoviral genome. This laborious method requires weeks of work in tissue culture, performing multiple rounds of plaque purifications.

The AdEasy XL and AdEasy systems dramatically improve on traditional methods by taking the homologous recombination out of mammalian cells and transferring it into *E. coli*. Instead of waiting weeks to obtain your recombinant adenovirus genome, you can have recombinants after a single transformation and overnight incubation (Figure 1).

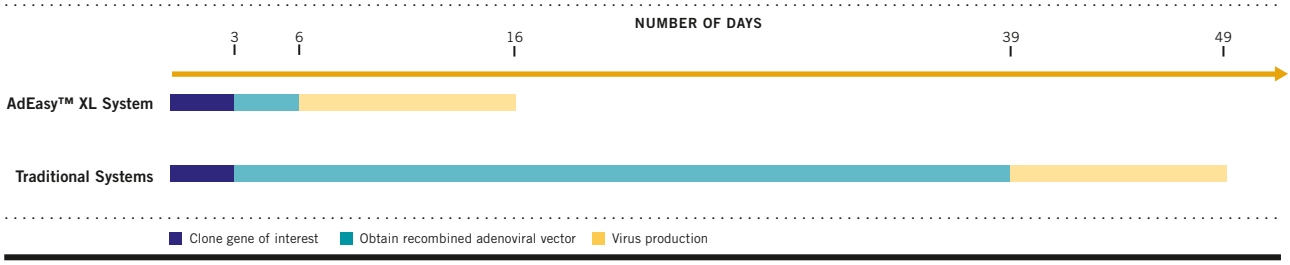


FIGURE 1. SAVE WEEKS WITH THE ADEASY XL SYSTEM With the AdEasy™ and AdEasy™ XL adenoviral vector systems, generating adenoviral vectors in *E. coli* is easy and takes only three days. Compare that with traditional plaque purification, which can require 24 to 44 days. Your experiments will be complete while others are still purifying plaques!

300% More Recombinants

The AdEasy XL system uses specialty competent cells, BJ5183-AD-1 cells, that are pretransformed with the adenoviral backbone (pAdEasy-1 plasmid). This reduces the cotransformation of both the shuttle vector and large adenoviral backbone in the original system to a single transformation of the shuttle vector containing your gene of interest. These specialty cells dramatically reduce background and increase the number of colonies containing your positive recombinant by 300% (Figure 2).

Flexible Vector Choice

The AdEasy system offers flexibility in the pShuttle cloning vector. The basic pShuttle vector contains all of the regions of homology required to recombine with the adenoviral backbone. The pShuttle-CMV vector has the same regions of homology but also has the high-level constitutive CMV promoter and the SV40 poly(A) tail. We also offer pShuttle-CMV vectors with the Vitality® hrGFP-reporter (Figure 3).

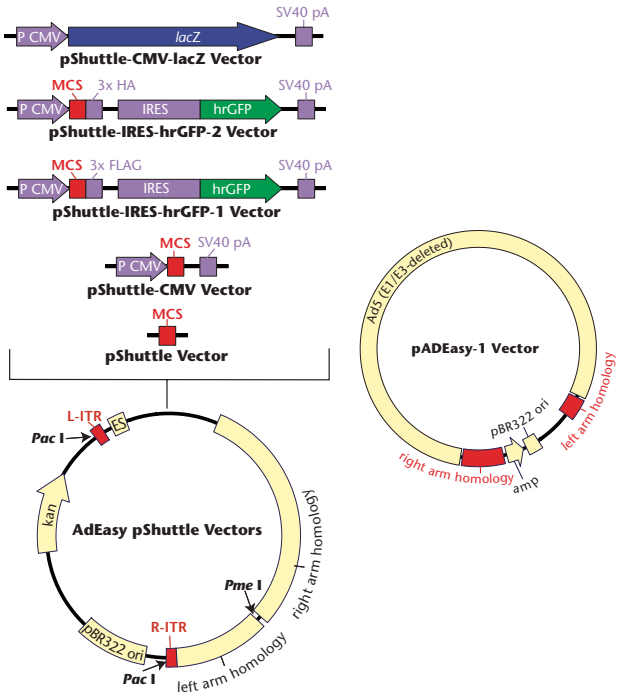


FIGURE 3. VERSATILE EXPRESSION CASSETTES

E. coli	Transformed DNA		Results		
	pAdEasy-1	*pShuttle-CMV-LacZ*[a]	Recombinants[b]	Total # of Colonies	% Recombinants
BJ5183-pAD-1[c]	—	Prep 1 (0.03 µg)	>1500	1600	**94%**
	—	Prep 2 (0.03 µg)	920	980	**94%**
	—	Prep 3 (0.03 µg)	1500	1540	**94%**
BJ5183	0.1 µg	Prep 1 (0.03 µg)	120	800	18%
	0.1 µg	Prep 2 (0.03 µg)	72	214	34%
	0.1 µg	Prep 3 (0.03 µg)	108	546	21%

a. Each preparation of shuttle vector is an independent preparation of linearized gel-purified *pShuttle CMV-LacZ*
b. Recombinants are typically small colonies. In 17 different transformation reactions, over 98% of 140 randomly picked small colonies were confirmed to be recombinants by restriction digestion.
c. Colony counts were performed on plates on which 25 to 50 µl of transformation reaction was plated, and values multiplied by the appropriate factor.

FIGURE 2. IMPROVE RECOMBINANT ADENOVIRUS PRODUCTION WITH BJ5183-AD-1 CELLS

Functional Cloning Assays

ViraPort® Retroviral cDNA Expression System

Our ViraPort® retroviral gene expression system[5] is superior to standard transfection technology. High transduction efficiency and large cloning capacity (8 kb for the pFB vector) make the system ideal for building and screening complex libraries. Because the virus naturally integrates into the cellular genome, every transduced cell is a stable cell.

Powerful Functional Cloning

The ViraPort Retroviral cDNA Expression System is an extremely powerful method to perform functional screening assays, as it allows you to control the copy number of recombinant plasmid that is transduced into each cell. Once a specific assay has been developed for a desired function, use our extensive collection of retroviral libraries to screen for that function of interest (Table 1). Genes delivered by retrovirus naturally integrate into the host's genome, so every transduced cell is a stable cell.

High Efficiency Gene Delivery

The strategy is based on cotransfection of three plasmids into a packaging cell line such as HEK293 cells. One plasmid contains your gene of interest flanked by the LTRs and packaging signal required to package the virus particles and insert the gene of interest into the host's genome. The other two plasmids contain viral structural genes that are required to make infection-competent viruses. However, the three plasmids do not contain any regions of homology to each other, preventing the generation of wild-type virus (Figure 1). Once viruses are packaged, the target cell can be transduced, and functional screening can occur.

Gene Class	Selection /Screen
Cell-surface proteins	FACS sorting
Extracellular receptors	FACS sorting Proliferation or survival
Growth factors	Factor-dependent growth
Oncogenes	Loss of contact-inhibition
Cell-cycle proteins	Loss of contact-inhibition Phenotypic complementation
Signaling proteins	Loss of contact-inhibition Phenotypic complementation Reporter activation
Transcription factors	Reporter activation
Apoptosis inhibitors	Survival: resistance to apoptosis inducers
Metastasis-inducing genes	In vivo metastasis In vitro invasion
Differentiation-inducing genes	Phenotype: differentiation
Ion channels	Phenotype: ion-specific indicator or tracer

TABLE 1. GENES CLONED BY EXPRESSION SCREENING You can clone mammalian genes by their function in mammalian cells transduced with the ViraPort retrovirus. Selection or screening depends on the class of gene in which you are interested.

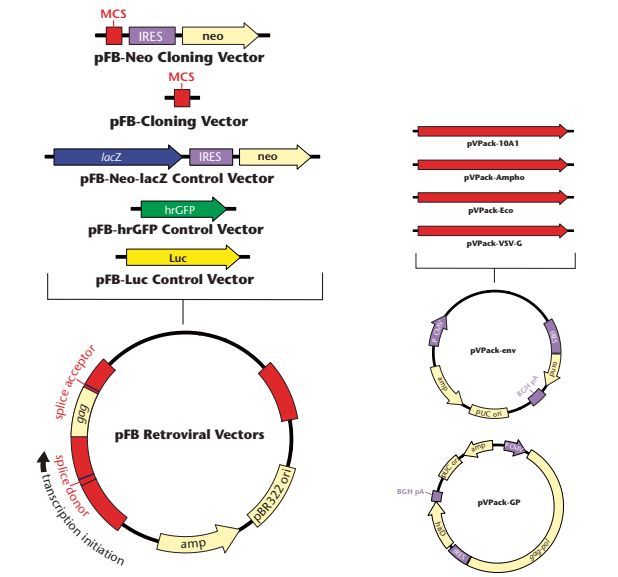


FIGURE 1. VIRAPORT® SYSTEM VECTOR MAPS The pFB vector is the basic cloning vector for the ViraPort® system. The pVPack-GP[6,7] vector supplies the gag and pol genes, and the pVPack-env vectors supply the env gene that determines the ability to infect target cells.

Transfecting Viral Plasmids

ViraPack™ Transfection Kit for Viral Delivery Systems

A critical success factor in any viral gene delivery experiment is transfecting your viral plasmid into the packaging cell line. Inefficient transfection can lead to low titers, potentially decreasing the chances of success in your studies.

Transfection Just for Packaging

One of the critical steps in viral gene delivery systems is transfecting your viral plasmid into the packaging cell line. Inefficient transfection into HEK293 cells often leads to low viral titers. We developed the ViraPack transfection kit[8] specifically for transfecting your vectors into the HEK293 packaging cell line at a very high transfection efficiency (Table 1).

The protocol is based on a modified calcium phosphate method of transfection that has been optimized to be highly efficient in HEK293 cells. The kit contains an enhancing factor that increases its transfection efficiency over traditional calcium phosphate methods of transfection.

	RLU/cell
ViraPack™ Transfection Kit	194.96
Lipofectamine 2000	11.78
Lipofectamine Plus	12.55

TABLE 1. COMPARING TRANSFECTION EFFICIENCIES Triple transfections of pFB-Luc, pVPack-Eco, and pVPack-GP were carried out in HEK293 cells following the recommended protocols for each transfection reagent. Transfection efficiency was assessed by measuring the expression of the luciferase reporter gene and normalizing to the total number of cells 48 hours following transfection.

Ordering Information >

Description	Quantity	Catalog #
AAV Helper-Free System		
AAV Helper-Free System + pAAV-MCS vector, 10 µg + pCMV-MCS vector, 10 µg + pAAV-LacZ vector, 10 µg + pAAV-RC vector, 20 µg + pHelper vector, 20 µg + AAV-293 cells,1x10^6 cells + AAV HT1080, 1x10^6 cells	1 kit	240071
pAAV-hrGFP Vector	20 µg	240074
pAAV-IRES-hrGFP Vector	20 µg	240075
AAV-293 Cells	1x10^6 cells	240073
AAV-HT1080 Cells	1x10^6 cells	240109
AdEasy™ and AdEasy™ XL Adenoviral Vector Systems		
AdEasy™ XL System + pShuttle vector, 20 µg + pShuttle-CMV vector, 20 µg + pShuttle-CMV-lacZ control vector, 10 µg + BJ5183-AD1 electroporation-competent cells, 5 x 100 µl + XL10-Gold® ultracompetent cells, 5 x 100 µl + pUC18 DNA control plasmid, 10 µl + AD-293 cells. 1 x 10^6 cells	1 kit	240010
BJ5183-AD1 electroporation-competent cells	5 x 100 µl	200157
AdEasy™ Adenoviral Vector System + pAdEasy-1 vector, 2.5 µg + pShuttle vector, 20 µg + pShuttle-CMV vector, 20 µg + pShuttle-CMV-LacZ vector, 10 µg + BJ5183 electroporation-competent cells, 5 x 100 µl + XL10-Gold® ultracompetent cells, 5 x 100 µl + pUC18 DNA control plasmid, 10 µl	1 kit	240009
BJ5183 electroporation-competent cells	5 x 100 µl	200154
pAdEasy-1 vector	2.5 µg	240005
pShuttle vector	20 µg	240006
pShuttle-CMV Vector	20 µg	240007
pShuttle-CMV-LacZ control vector	10 µg	240008
pShuttle-IRES-hrGFP-1	20 µg	240081
pShuttle-IRES-hrGFP-2	20 µg	240082

Description	Quantity	Catalog #
ViraPort® Retroviral Gene Expression System		
pFB Retroviral Vector	10 µg	217563
pFB-Neo Retroviral Vector	10 µg	217561
pVpack-GP Vector	20 µg	217566
pVpack-Eco Vector	20 µg	217569
pVpack-Ampho Vector	20 µg	217568
pVpack-10A1 Vector	20 µg	217570
pVpack-VSV-G Vector	20 µg	217567
Vitality® pFB-hrGFP plasmid vector	10 µg	240027
pFB-Neo-LacZ plasmid vector	10 µg	240029
pFB-Luc plasmid vector	10 µg	240030
ViraPack Transfection Kit		
ViraPack Transfection Kit	1 kit	200488

License Information

1. Use of AAV vectors is covered by U.S. Patent Nos. 5,622,856, 5,945,335, 6,001,650, 6,004,797 and 6,027,931 and is limited to use solely for research purposes. Any other use of the AAV Helper-Free System requires a license from Avigen, Inc.

2. Certain hrGFP products, and other products containing hrGFP variants, are covered by the following license terms.

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3. Use of the CMV promoter is covered under U.S. Patent Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation and licensed FOR RESEARCH USE ONLY.

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6. Use of the translational enhancer of the pVPack vectors is covered by U.S. Patent No. 4,937,190 and is limited to use solely for research purposes. Any other use of the translation enhancer of the pVPack vectors requires a license from WARF. WARF can be reached at P.O. Box 7365 Madison, WI 53707-7365.

7. The bovine growth hormone (BGH) polyadenylation sequence is licensed under U. S. Patent No. 5,122,458 for research purposes only. "Research purposes" means uses directed to the identification of useful recombinant proteins and the investigation of the recombinant expression of proteins, which uses shall in no event include any of the following:

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(b) Any use in human of protein or other substance expressed or made at any stage of its production with the use of a CLAIMED DNA or a CLAIMED CELL;

(c) Any use in which a CLAIMED DNA or CLAIMED CELL would be sold or transferred to another party other than Stratagene, its affiliate, or its sublicensee;

(d) Any use in connection with the expression or production of a product intended for sale or commercial use; or

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8. U.S. Patent No. 5,330,904.

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