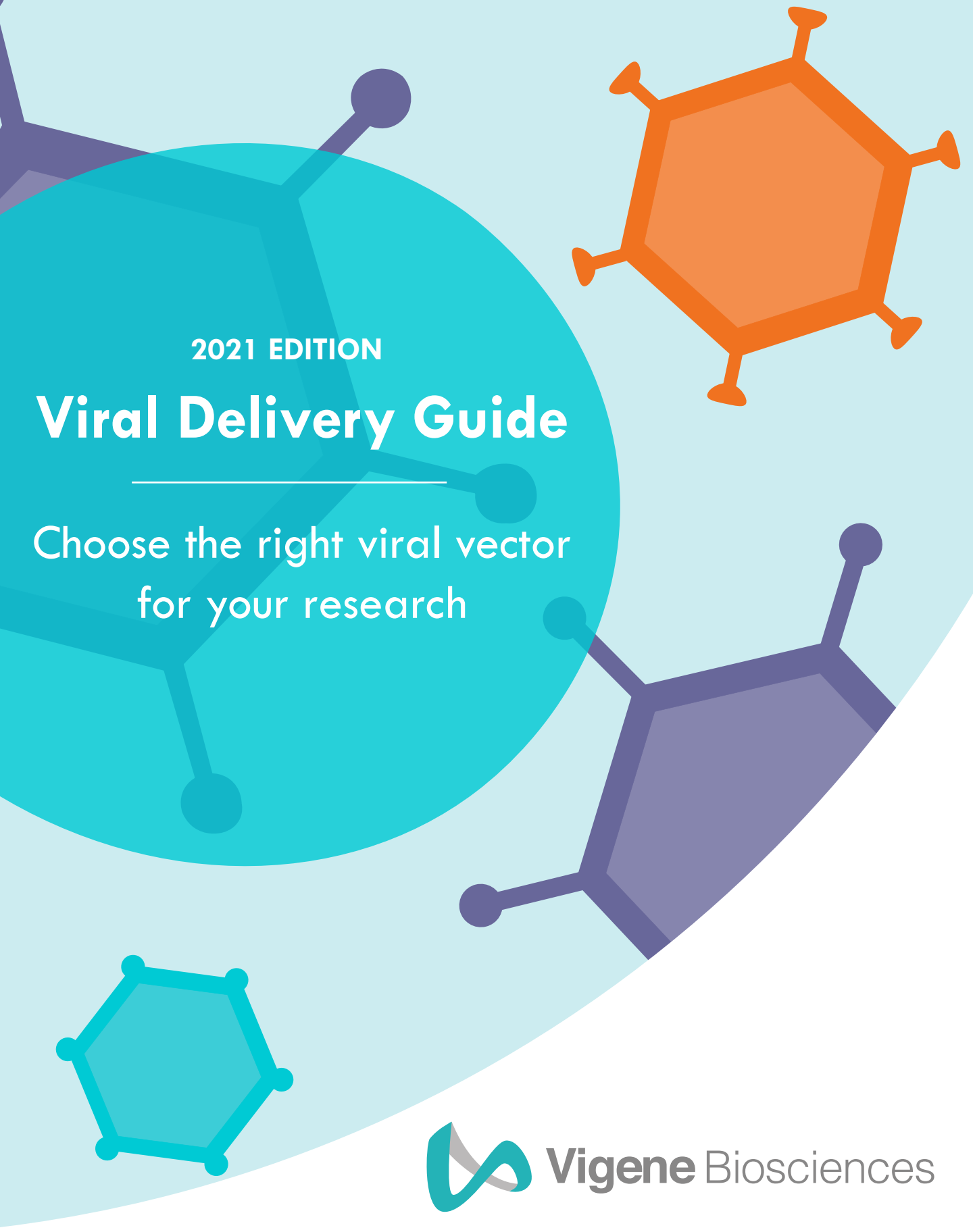
2021 EDITION

Viral Delivery Guide

Choose the right viral vector for your research



Vigene Biosciences

OVERVIEW

Transfection, the introduction of foreign DNA or RNA into eukaryotic cells, is an indispensable tool for studying gene expression and function. Classical transfection strategies rely on chemical or physical methods to transiently destabilize the cell membrane to allow for uptake of exogenous genetic material. While these methods are easy to use, they are not always desirable or practical as it can be damaging to cells and some cell types are difficult or impossible to transfect. A more refined method that overcomes this issue utilizes one of the most highly evolved and efficient delivery systems found in nature: viruses.

Recombinant viruses are exceptionally powerful tools that can be used to overexpress, silence, and even edit genes. However, it is important to know which tool is right for the job when planning experiments as each viral delivery system has a unique set of strengths and weaknesses. We designed this guide to help researchers decide which of the three most popular recombinant viruses, AAV, adenovirus, or lentivirus, will work best for their specific applications.

About Us

Vigene Biosciences is unique in that we are the only company that develops virus production technologies to serve customers from both research and clinical communities. In addition to our extensive product catalog, we provide custom viral packaging services that range from small-scale packaging for *in vitro* work, to large-scale packaging for animal studies, all the way up to cGMP production for use in clinical and commercial applications. No matter what stage of research, we will work with you to design and produce the viral particles to best meet your needs.

Table of Contents

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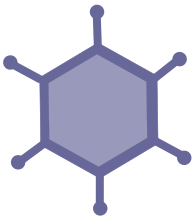
Vigene Biosciences

INTRODUCTION

Adeno-associated Virus (AAV)

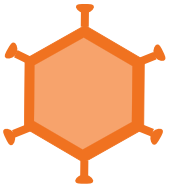
Although AAV has a high prevalence in humans, it is non-pathogenic and elicits a very mild immune response. While wild-type AAV is able to integrate into the host genome at the AAVS1 loci, recombinant AAV lacks two essential genes for viral integration and replication. As a result, recombinant AAV remains primarily episomal and can persist in non-dividing cells for long periods of time. Because of these characteristics, along with the ability to target specific tissue types, **AAV has become one of the main viral vectors used for research and gene therapy applications.**

Adenovirus



Adenoviruses can cause a wide range of illnesses in humans, such as respiratory tract infections. To make adenovirus an effective and safe tool for gene delivery, two genes involved in viral replication and modulating host immune response are deleted. Once inside the cell, recombinant adenovirus remains episomal and does not integrate into the host genome. **Adenovirus is a popular choice for gene delivery applications primarily because of its large packaging capacity, high transgene expression, and ability to infect most cell types.**

Lentivirus



As a subgroup of retroviruses, lentiviruses use viral reverse transcriptase to produce DNA from their RNA genomes before integrating into the host genome. However, unlike other retroviruses, lentiviruses have the ability to infect both dividing and non-dividing cells. To increase safety, the components for virus replication are divided across multiple plasmids during recombinant lentivirus production. **Lentivirus is a particularly useful tool for generating stable cell lines or for gene therapy applications, such as CAR-T.**

Characteristic	AAV	Adenovirus	Lentivirus
Genome	4.8 kb (ssDNA)	36 kb (dsDNA)	9 kb (ssRNA)
Packaging Capacity	4.7 kb	7.5 kb	9 kb
Infection	Most dividing and non-dividing cells	Most dividing and non-dividing cells	Most dividing and non-dividing cells
Transduction Efficiency	Moderate	High	Moderate
Integration	Non-integrating	Non-integrating	Integrating
Expression	Transient or stable	Transient	Stable
Immunogenicity	Very Low	High	Low
Biosafety	BSL-1	BSL-2	BSL-2

ADENO-ASSOCIATED VIRUS (AAV)

Advantages

- Tissue-specific targeting by choice of serotype
- Very low immunogenicity
- Non-integrative
- Potential for long-term gene expression

Limitations

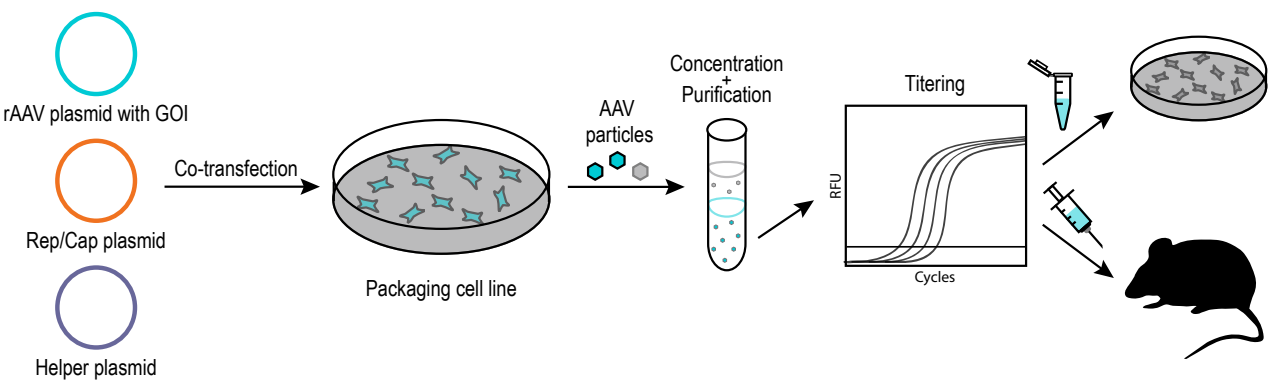
- Moderate expression *in vitro* (except for AAV-DJ)
- Relatively small packaging capacity (~4.7 kb)
- Not useful for making stable cell lines

You can send us your plasmid to package into AAV or work with our team of scientists to design a construct for you. See page 9 for a full list of Vigene's services!

Recombinant AAV Production

AAV belongs to the *Dependovirus* genus, meaning it depends on a co-infecting helper virus (usually adenovirus) for productive infection to occur. In addition, the recombinant AAV genome has two essential genes removed to prevent integration and replication to make it a safe and efficient research tool for gene delivery. Therefore, in order to generate more AAV particles, essential genes must be provided *in trans*.

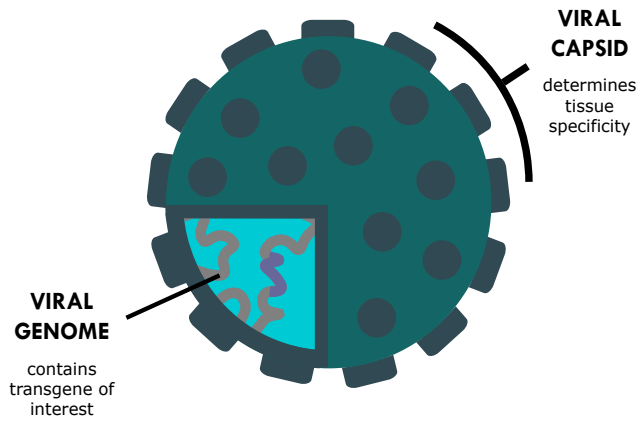
Vigene uses the triple transfection strategy for AAV production, which involves co-transfecting the packaging cell line with the recombinant AAV plasmid containing the gene of interest (GOI), a plasmid containing the essential *rep* and *cap* genes, and a third adenovirus-derived helper plasmid supplying genes needed for replication. The AAV particles are collected from the cell lysates. For our large-scale packaging service, AAV particles are purified using iodixanol (IDX) gradient ultracentrifugation to remove impurities and empty viral capsids for safe use *in vivo*. Viral titer is then determined using quantitative PCR with primers to the inverted terminal repeats present in the viral genome. The viral particles are then ready to use for *in vitro* or *in vivo* research.



Choosing Your Serotype

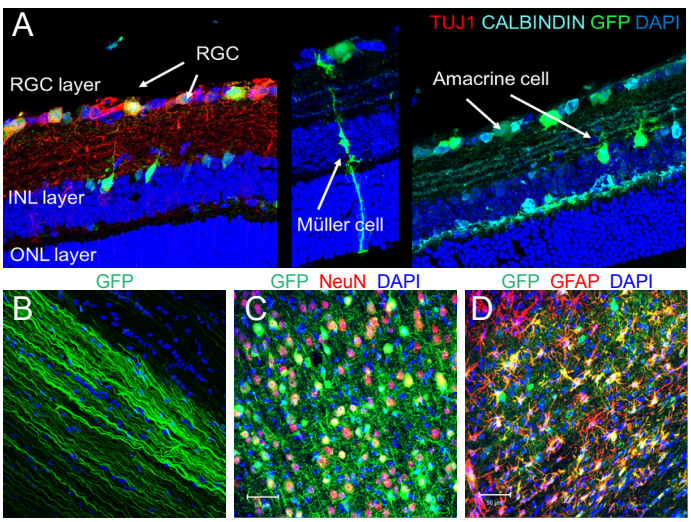
The serotype of AAV is determined by its viral capsid, which defines its tissue specificity. For research and gene therapy, this specificity allows you to target your gene of interest to certain tissues and cells, so it is important to select an appropriate AAV serotype to ensure optimal gene delivery. Below is a list of our serotype recommendations for different tissue types.

Tissue	Recommended Serotypes
Muscle	AAV1, AAV6, AAV8, AAV9
Liver	AAV8, AAV9, AAV-DJ
Lung	AAV6, AAV9
CNS	AAV1, AAV5, AAV8, AAV9, AAV-DJ
Retina	AAV1, AAV2, AAV5, AAV8
Pancreas	AAV8
Kidney	AAV2, AAV9
Heart	AAV1, AAV8, AAV9



If you are unsure which serotype is right for your experiments, Vigene offers the **AAV Testing Kit** as a quick and cost-effective way to determine which serotype to use. Contact us for more details.

Application Spotlight



AAV-GFP expression in a variety of tissue types. AAV-GFP expression in the mouse (A) retina and (B) optic nerve. AAV-GFP expression in rat (C) neurons and (D) astrocytes. Figures adapted from: (A) Ratican, S. E., Osborne, A. & Martin, K. R. *Neural Plast*. **2018**, 7108948 (2018); (B) Osborne, A. et al. *Cell Death Dis* **9**, 1007 (2018); (C, D) Schober, A. L. et al. *Front Cell Neurosci* **10**, 262 (2016).

ADENOVIRUS

Advantages

- Large packaging capacity (~8 kb)
- Infects most cell types with nearly 100% efficiency
- High levels of expression that can often be observed within 24 hours
- Does not integrate into host genome

Limitations

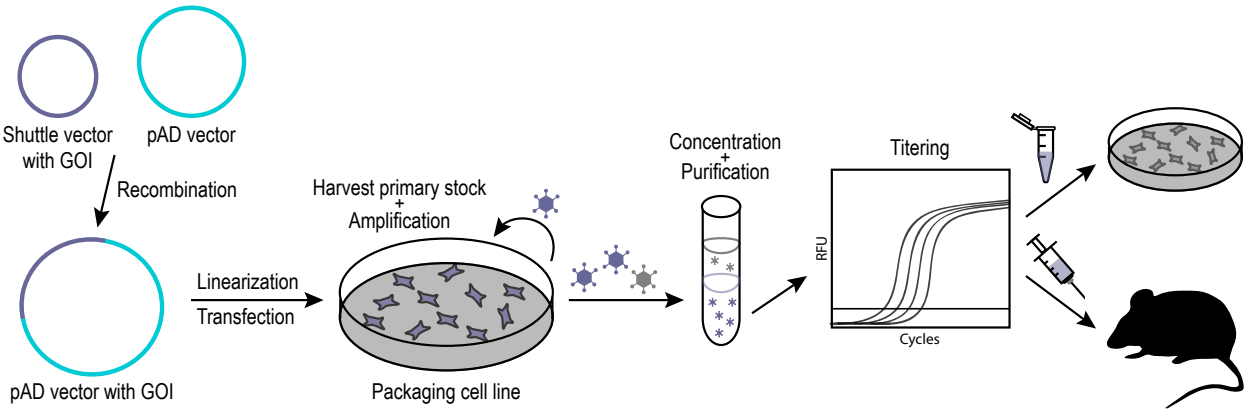
- May trigger a substantial immune response
- Cloning can be challenging due to large genome size
- Transient expression
- Not useful for making stable cell lines

You can send us your plasmid to package into adenovirus or work with our team of scientists to design a construct for you. See page 9 for a full list of Vigene's services!

Recombinant Adenovirus Production

Recombinant adenovirus production requires two vectors: the shuttle vector containing the gene of interest (GOI) and the recombinant adenovirus vector (pAD). To ensure safe use, Vigene uses replication-incompetent (E1/E3 genes deleted) human adenovirus vector type 5 (Ad5). The first step of adenovirus production involves transferring the GOI from the shuttle vector to the recombinant adenovirus vector through recombination.

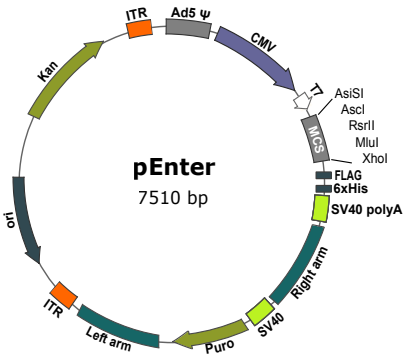
The recombinant adenovirus vector containing the GOI is then linearized and transfected into the packaging cell line to generate the primary stock of recombinant adenovirus. This primary stock is then harvested and amplified through multiple rounds of infection. For our large-scale packaging service, adenovirus particles are purified using iodixanol (IDX) gradient ultracentrifugation to remove impurities for safe use *in vivo*. Viral titer is determined using quantitative PCR. The adenovirus particles are then ready to use for *in vitro* or *in vivo* research.



ORF Shuttling Systems

Adenovirus is one of the most efficient gene delivery vehicles for both *in vitro* and *in vivo* applications. One of the biggest advantages of adenovirus is its large packaging capacity. The ability to accommodate up to ~7.5 kb of cargo sequence makes it an ideal choice for researchers wanting to express larger open reading frames (ORFs), promoters, and/or reporters.

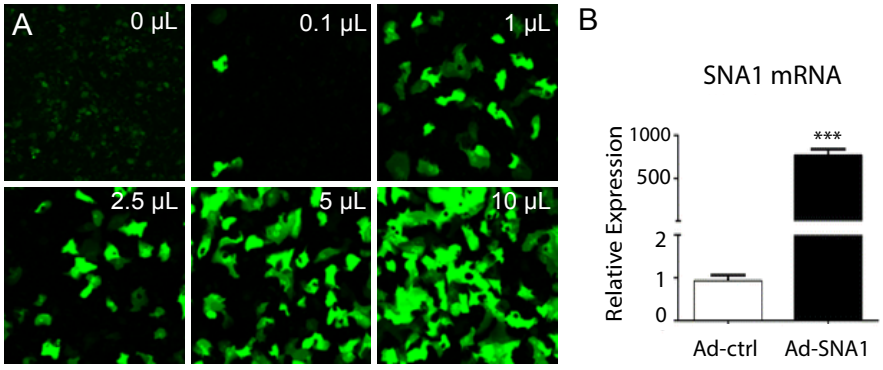
Traditionally, cloning into a recombinant adenovirus vector can be a difficult process. However, Vigene has pioneered the pEnter entry vector system to allow for ORFs to be easily shuttled into a large panel of destination vectors or recombined with a recombinant viral vector for virus production.



pEnter Advantages

- Easily shuttle ORFs into over 30 destination vectors with a variety of promoter and reporter options
- Quickly transfer an ORF using simple cloning techniques in just 2-3 hours
- Accommodates large inserts and toxic genes
- Large collection of pre-packaged and ready-to-ship ORFs (see page 10 for Vigene's product catalog)

Application Spotlight



Adenovirus-GFP expression in hepatocytes. (A) Hepatocytes 42h after infection with Ad-GFP at a titer of $6x10^9$ vp/mL. (B) qRT-PCR showing mRNA expression level was significantly increased in HUVECs after Ad-SNAI1 infection compared to Ad-ctrl. Figures adapted from: (A) Vigene Biosciences; (B) Sun, J.-X. et al. *Angiogenesis* **21**, 635–652 (2018).

LENTIVIRUS

Advantages

- Integrates into host genome
- Infects both dividing and non-dividing cells
- Strong and stable transgene expression
- Large packaging capacity (~6 kb)
- Can be used to make stable cell lines

Limitations

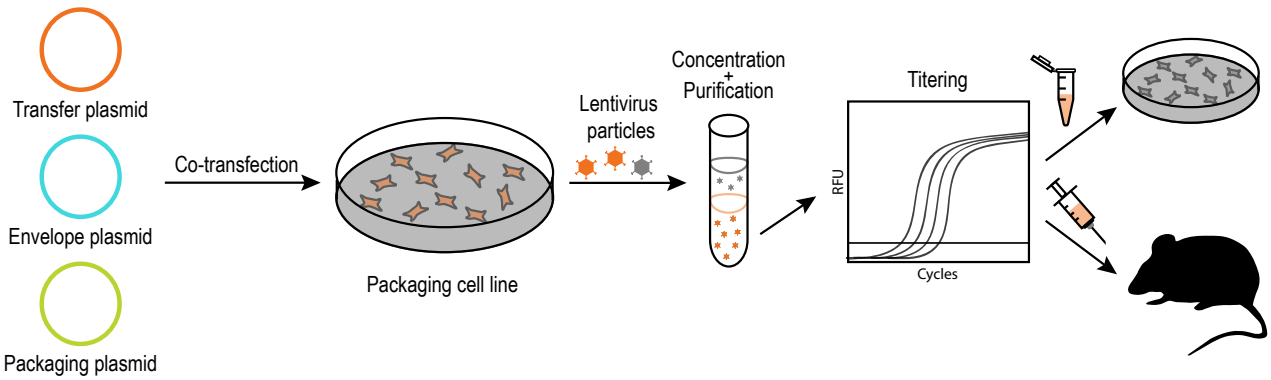
- Potential for insertional mutagenesis due to random integration
- Titer highly sensitive to viral genome size
- Small probability of generating replication competent lentivirus

You can send us your plasmid to package into lentivirus or work with our team of scientists to design a construct for you. See page 9 for a full list of Vigene's services!

Recombinant Lentivirus Production

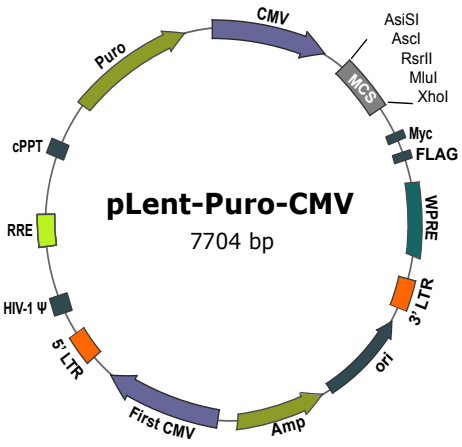
Recombinant lentiviruses are derived from HIV-1, so there are certain precautions that must be taken for its safe use for gene delivery. The biggest concern is the possibility of generating replication-competent lentivirus through crossover events between elements in the viral vector and the packaging cell line. To improve safety, essential viral elements, such as gag, pol, and env, are supplied *in trans* via packaging and envelope plasmids and the gene of interest (GOI) is delivered separately on a transfer plasmid. In addition, non-essential viral components for viral propagation are removed. To produce lentivirus particles, the transfer plasmid containing the GOI, the envelope plasmid, and the packaging plasmid are co-transfected into the packaging cell line.

The lentivirus particles are collected from the cell lysate and purified using sucrose gradient centrifugation to remove impurities for safe use *in vivo*. Viral titer is then determined using quantitative PCR with primers to the long-inverted terminal repeats present in the viral genome. The viral particles are then ready to use for *in vitro* or *in vivo* research.

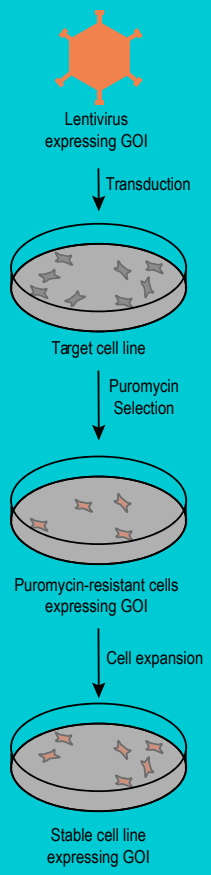


Generating Stable Cell Lines

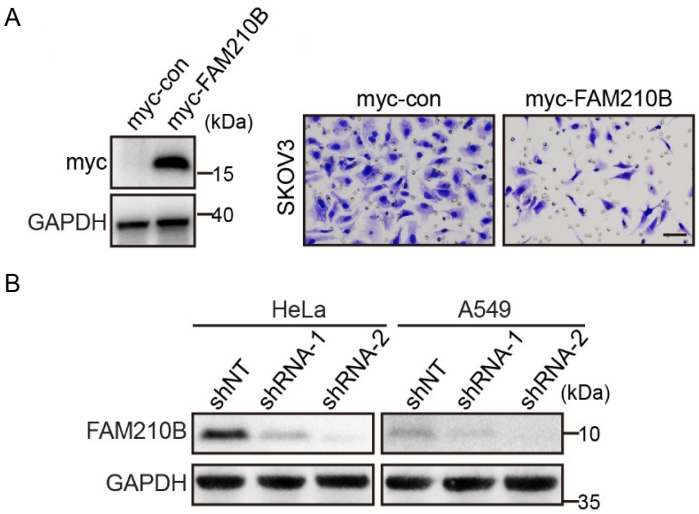
In contrast to using transient transfection methods or other viruses, lentivirus can be used to generate stable cell lines expressing a gene of interest to study long-term protein expression. Lentiviral vectors randomly integrate their genetic cargo into the host genome (with a preference for actively transcribed genes) without transferring sequences that encode for proteins derived from the packaging virus. Vigene's lentiviral vectors contain a puromycin-resistance gene that allows for selection of transduced cells.



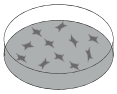
Selecting for Transduced Cells



Application Spotlight



Lentivirus overexpressing or silencing FAM210B. (A) Immunoblot of SKOV3 cells 24h after infection with lentivirus expressing myc-control or myc-FAM210B and crystal violet stain 48 hr after infection. (B) Cells infected with lentivirus expressing non-targeting shRNA or shRNA against FAM210B. Figure adapted from: (A, B) Sun, S. et al. *Cell Death & Disease* **8**, e2870 (2017).

Services and Applications	AAV	Adenovirus	Lentivirus
Small-scale Packaging 	>5E12 GC/mL 500 uL $650 Estimated Timeline: 2-3 weeks	>1E10 VP/mL 500 uL $590 Estimated Timeline: 3-4 weeks	>2E8 IFU/mL 100 uL $1,200 Estimated Timeline: 2-3 weeks
Large-scale Packaging 	>1E13 GC/mL 500 uL $1,815 Estimated Timeline: 2-3 weeks	>1E12 VP/mL 500 uL $1,510 Estimated Timeline: 5-6 weeks	>1E9 IFU/mL 200 uL $1,950 Estimated Timeline: 2-3 weeks
Preclinical Packaging 	Total yield up to 1E16 GC Please inquire for price and estimated timeline	Total yield up to 1E16 VP Please inquire for price and estimated timeline	Total yield up to 1E12 IFU Please inquire for price and estimated timeline
cGMP Production 	Total yield up to 1E17 GC Please inquire for price and estimated timeline	Total yield up to 1E15 VP Please inquire for price and estimated timeline	Total yield up to 1E12 IFU Please inquire for price and estimated timeline
Cloning Services	We offer a range of custom molecular cloning services: • Mutagenesis • Gene synthesis • CRISPR/Cas9 • shRNA cloning We also have an extensive selection of promoters and reporters for your research needs. Promoter options will be on next page. Reporters GFP | DIO-GFP | RFP | DIO-RFP mCherry | DIO-mCherry | Luciferase		

NOTE: AAV titer is measured in genome copies, adenovirus titer is measured in viral particles, and lentivirus titer is measured in infectious units.

VECTOR PROMOTER OPTIONS

Vector	Promoter	Promoter Description	Expression
pAV-FH	CMV (508 bp)	Human cytomegalovirus immediate early enhancer/promoter	Ubiquitous
pAV-CAG	CAG (944 bp)	CMV early enhancer fused to modified chicken β-actin promoter	Ubiquitous
pAV-EF1a	EF1a (1,190 bp)	Human eukaryotic translation elongation factor 1 α1 promoter	Ubiquitous
pAV-PGK	PGK (399 bp)	Phosphoglycerate kinase 1 promoter	Ubiquitous
pAV-UBC	UBC (1,137 bp)	Ubiquitin C promoter	Ubiquitous
pAV-ALB	ALB (2351 bp)	Albumin Promoter	Liver
pAV-CaMKIIa	CaMKIIa (1,291 bp)	α-calcium-calmodulin dependent kinase II promoter	Neurons
pAV-GFAP	GFAP (2,040 bp)	Glial fibrillary acidic protein promoter	Astrocytes
pAV-GFAP104	GFAP104 (845 bp)	Hybrid of EF1a and GFAP	Astrocytes
pAV-MBP	MBP (1,311 bp)	Myelin basic protein promoter	Oligodendrocytes
pAV-MCK	MCK (1,359 bp)	Muscle Creatine Kinase Promoter	Muscle
pAV-3xEnhancer MCK	3X enhancer MCK (728 bp)	Muscle Creatine Kinase Promoter with 3 enhancers	Muscle
pAV-aMHC	aMHC (372 bp)	α-cardiac myosin heavy chain promoter	Heart
pAV-NSE	NSE (1,321 bp)	Neuron-specific enolase promoter	Neurons
pAV-PDX1	PDX1 (854 bp)	Pancreatic and duodenal homeobox 1 promoter	Pancreas
pAV-Rpe65	Rpe65 (718 bp)	Retinoid isomerohydrolase Promoter	Retina
pAV-Syn	Syn (471 bp)	Synapsin 1 promoter	Neurons
pAV-cTnT	cTnT (702 bp)	Cardiac troponin T promoter	Heart

Product	AAV	Adenovirus	Lentivirus	Plasmid	Retrovirus
Controls	✓	✓	✓		✓
ORFs		✓	✓	✓	
miRNA		✓		✓	
Zika ORFs	✓	✓		✓	
Biosensors	✓				
PD Research Tools	✓				

Don't see the product you want? Contact us with your project details!
Custom-made products are available upon request.

Additional Resources

Visit our website to read more about packaging into AAV, adenovirus, and lentivirus.

- www.vigenebio.com/aav-packaging/
- www.vigenebio.com/adenovirus-packaging/
- www.vigenebio.com/lentivirus-packaging/

Please visit our **Technical Resource page** for more information, including product manuals and our AAV Biosensors handbook.

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