

amsbio

Lentivirus, Adenovirus & AAV

Viral Delivery Systems

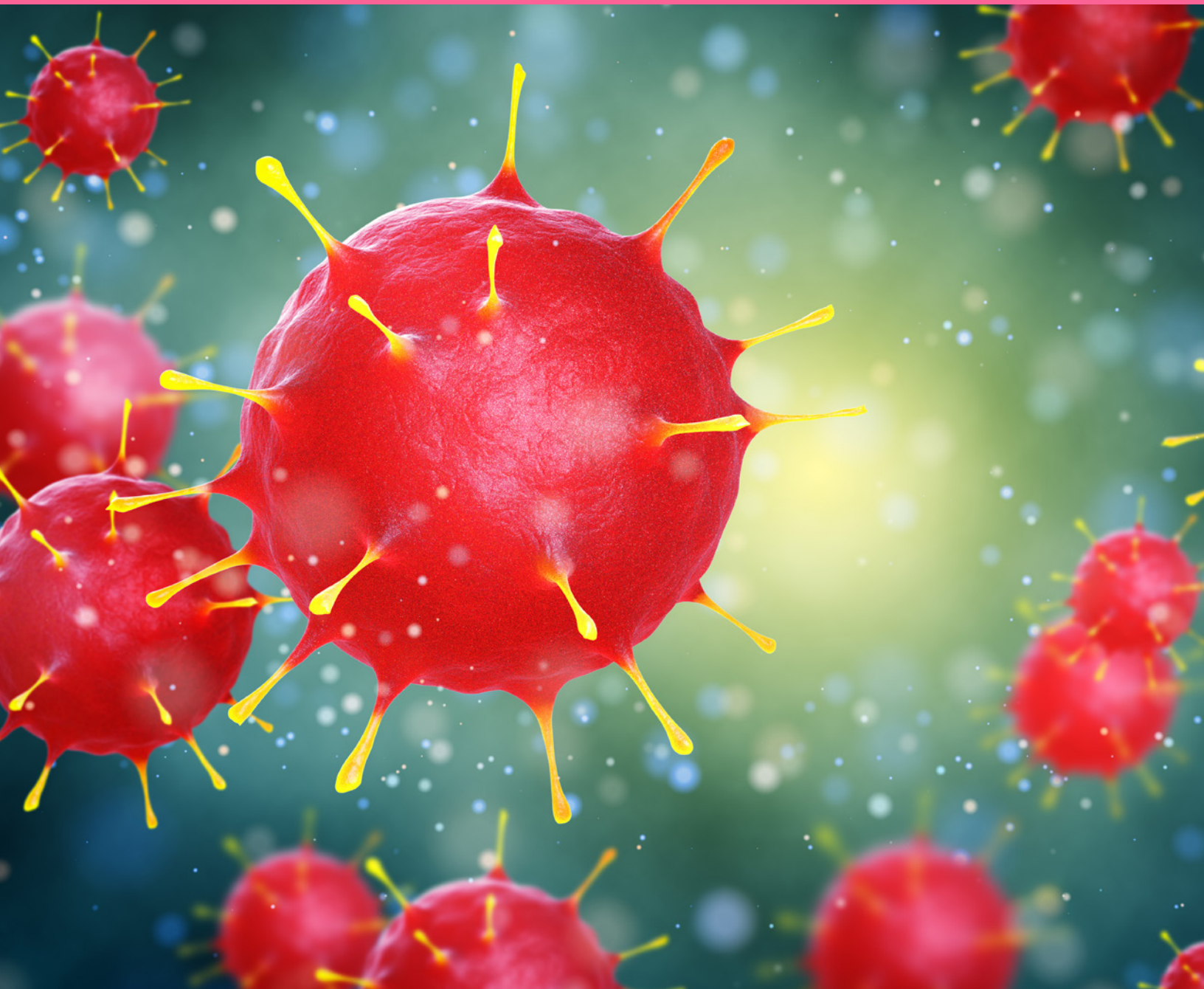
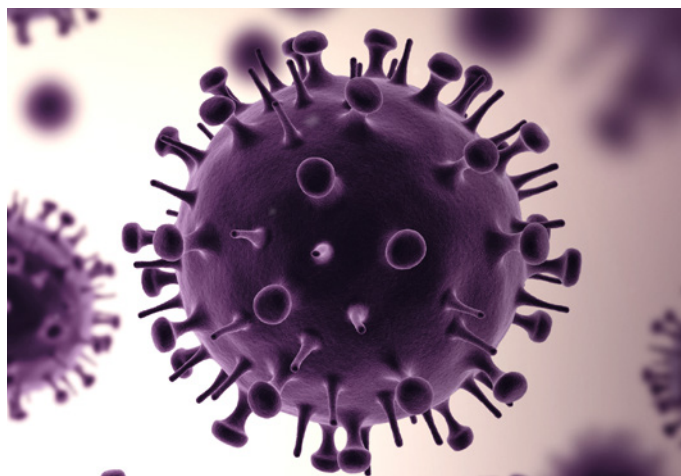


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Comparison Of Different Virus Types:

	Adenovirus	Lentivirus	AAV
Transfection efficiency	> 90%	~ 30%	30 – 40%
Host genome integration	No	Yes	Yes
Packaging capacity	Up to 34 kb	8.5 kb	4 kb
High viral titer	Yes	No	No

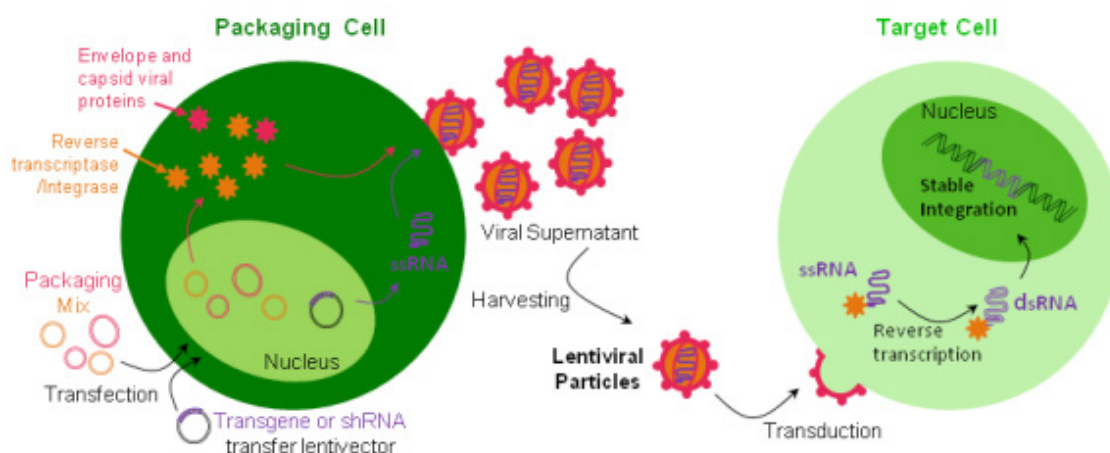
Lentivirus

What is Lentivirus?

Lentivirus is a subfamily of the retrovirus family. Lentiviruses can deliver significant amounts of genetic information into host cells and integrate it into the cellular genome. Genetically-engineered lentiviruses are therefore used as one of the most efficient tools of gene delivery. These lentiviruses contain a viral promoter which is used to control the expression of a transgene or shRNA but no virulence genes. This, together with several other security modifications makes them safe to use in the laboratory.

How does it work?

Co-transfection of packaging plasmids and a transfer vector into a packaging cell line allows efficient production of lentiviral particles which are released into the cells supernatant. Viral particles harvested from the cell supernatant can transduce a wide range of both dividing and non-dividing mammalian cell types. Upon infection with lentiviral particles, the single stranded RNA (ssRNA) is reverse-transcribed and the resulting double-stranded DNA (dsDNA) stably integrates into the genome of the host providing long term transcription of the gene or shRNA of interest.



Schematic diagram showing production of lentiviral particles and stable integration of the target DNA in the host genome.

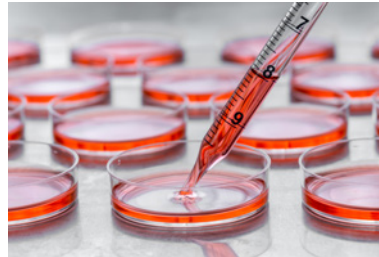
WHAT CAN YOU GET?

Over-expression of a transgene or miRNA
Knockdown of a gene via shRNA expression
Stable cell lines

Advantages of using Lentivirus:

- ▶ No transfection reagent needed
- ▶ Stable gene integration in the host genome (in contrast to adenovirus) for long-term expression
- ▶ Effectively transduce most mammalian cell lines including primary or stem cells
- ▶ Integrate into non-dividing cells; Unlike retrovirus, lentivirus does not require a mitotic event for integration into the host cell genome
- ▶ Infect 'difficult-to-transfect' cell lines
- ▶ Low immunogenicity when used *in vivo*

JUST ADD TO YOUR CELLS !



3rd generation lentivirus is self-inactivating boosting biosafety:

To prevent the generation of replication-competent viral particles, the genes encoding structural and other components required for packaging the viral genome are separated onto several different plasmids minimizing the threat of recombinant, replication-competent, virus production. None of the structural genes are present in the packaged viral genome, therefore no new replication-competent virus can be produced. Furthermore, a deletion in the U3 portion of the 3' LTR eliminates the promoter-enhancer region, further negating the possibility of viral replication.

The advantage of AMSBIO Lentiviruses:

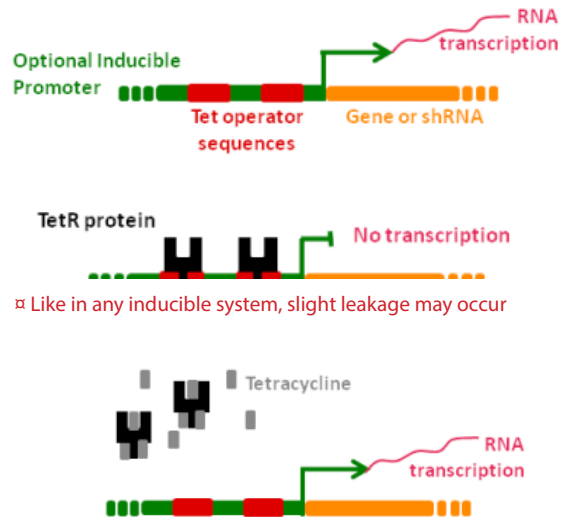
- ▶ Lentiviral particles are VSV-G pseudotyped, and so can transduce virtually any cell type
- ▶ **High titer** (from 1×10^7 to 1×10^9 Infectious Unit/ml)
- ▶ Lentiviral particles are available for *in vivo* use (concentrated in PBS and without FBS)
- ▶ Choice of native or poly-histidine tagged proteins for easier purification of the expressed protein
- ▶ Many carry a non-fused fluorescent gene allowing **visualisation of transduced cells**
- ▶ Rapid delivery of the ready-to-use pre-made lentiviral particles
- ▶ Choice of **constitutive or optional inducible promoter** for custom particles

You control when your target is expressed!

► Our optional inducible promoters have two copies of the tetracycline (Tet) operator sequence integrated. This does not affect the efficiency of the promoters, and without further intervention will drive regular high constitutive expression of your gene or shRNA of interest.

► By transducing one of the Tet repressor (TetR) lentiviral particles, the transcription of the transgene or shRNA will be repressed by the binding of TetR to the Tet operator sequences of the promoter.

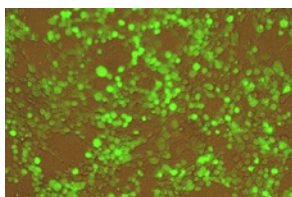
► Whenever expression is desired, tetracycline (or doxycycline, a tetracycline derivative) can be added to the medium of the transduced cells. It will bind and inhibit the TetR protein, allowing high expression of the target.



AMSBIO offers more than 30,000 pre-made Lentiviral particles and we are adding more ones every week

A wide range of pre-made lentiviral particles are available off-the-shelf. Different fluorescent and/or antibiotic resistance markers as well as optional inducible or constitutive promoters are available.

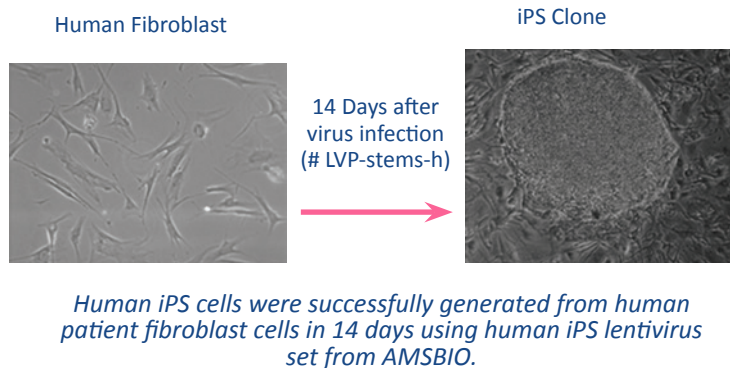
- More than 20,000 lentiviruses expressing human or mouse genes
- Lentiviral particles expressing fluorescent proteins: GFP, RFP, CFP and YFP



GFP expression in HeLa cells after transduction of Pre-made GFP lentivirus (#LVP001)

- Lentiviruses expressing the Tetracycline Repressor (TetR) Protein to allow the inducible feature of our optional inducible systems.
- Lentivirus expressing several Key enzymes like the CRE recombinase, Beta-lactamase, Beta-galactosidase (LacZ)...

- ▶ **Lentiviruses expressing Firefly, Renilla or Cypridina luciferase proteins**
- ▶ **Lentiviral particles for inducing pluripotent stem cells (iPSC) from mouse or human cells.** Those particles express one or several iPSC key stem cell transcription factors (OCT3/4, SOX2, NANOG, LIN28, cMYC and KLF4) to convert differentiated mouse or human somatic cells into embryonic-like cells.



- ▶ **Organelle targeting lentiviral particles for sub-cellular localization analysis (nucleus, cytoplasm, ER, Golgi, mitochondria, nuclear membrane, peroxisome, plasma membrane, microtubule, histone, lysosome, endosome...).**
- ▶ **Find all the pre-made lentiviral particles that we provide on www.amsbio.com/Lentivirus.aspx**

IF YOU CAN'T FIND THE LENTIVIRAL PARTICLES YOU WANT IN OUR LIST
Try our transgene and shRNA expression lentivirus custom service!

AMSBIO Lentivirus Services

Detail your requirements --> Let us do the work --> We ship ready to use reagents

We can do it all for you, from the shRNA design or gene template acquisition to the lentiviral particles (or the stable cell line) generation.

We will provide you with high titer lentiviral particles guaranteed. You will receive 0.5ml of ready-to-use lentivirus packaged in medium with FBS or concentrated in PBS (serum-free).

Ready-to-use: Just add the lentivirus to the medium of your cells and you can visualise the infection of your cells in 48-72 hours.

The AMSBIO service advantages:

- ▶ **Engineered lentivector for highly efficient DNA integration into cell genome**
- ▶ **Guaranteed high titer lentivirus**
- ▶ **Our experts with years of experience in lentiviral cloning and expression**
- ▶ **Competitive price and quality**
- ▶ **Flexible service, contact info@amsbio.com for any particular requirement**

shRNA Lentivirus Service

► We can produce ready-to-use shRNA lentiviral particles for any specific gene. Either you provide us the shRNA sequence or we will design it for you.



Ψ: Encapsidation signals
RRE: Rev-Response Element
Cppt: Central polypurine tract/central termination sequence element
WPRE: Woodchuck hepatitis virus Post-transcriptional Regulatory Element

GFP-Blasticidin
 GFP-Puromycin
 RFP-Blasticidin
 RFP- Puromycin
 Blasticidin
 Puromycin

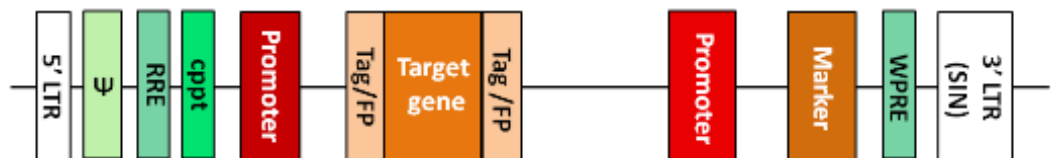
AMSBIO will:

- Clone a defined shRNA target sequence into our lentiviral shRNA vector
- Design 3-4 shRNA sequences against your gene of interest
- Produce the shRNA lentiviral particles
- Deliver the guaranteed high titer ready-to-use lentivirus to you in just 2-3 weeks
- Guarantee at least 75% knockdown

Over-Expression Lentivirus Service

We can create the target gene lentivirus of your choice. Either you provide us the gene template or we synthesize it or obtain it from our vast cDNA collection of human and mouse genes.

Schematic representation of gene expression lentivector



suCMV,
 Optional inducible suCMV*,
 EF1A,
 Another promoter

No tag,
 Tag 6His, HA, Myc, V5...,
 a fusion GFP/RFP/CFP,
 in C-term or N-term

RSV,
 PGK,
 SV40,
 Or ask for a
polycistronic
system

Blasticidin,
 Neomycin,
 Puromycin,
 Firefly Luciferase,
 GFP-Blasticidin,
 GFP-Puromycin,
 RFP-Blasticidin,
 RFP-Puromycin,
 Another marker

Ψ : Encapsidation signals
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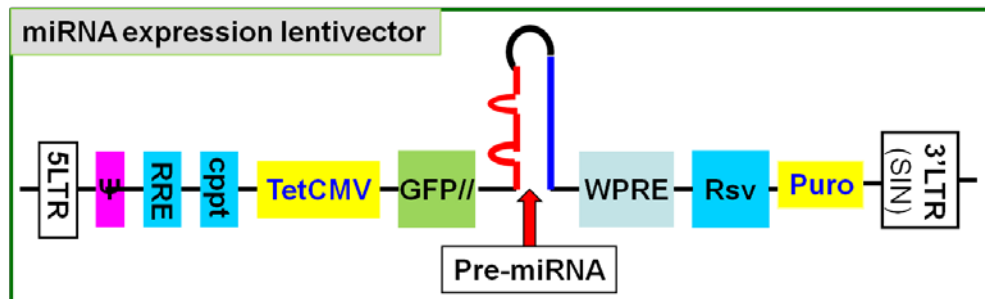
AMSBIO will:

- Sub-clone your selected gene into one of our expression lentivectors
- Produce the lentiviral particles
- Deliver the guaranteed high titer ready-to-use lentivirus to you in just 2-3 weeks

You can have your protein of interest just 72 hours after delivery of the particles.

miRNA Expression Lentivirus Service

Human or mouse microRNA (miRNA) precursors and their native context sequences (upstream and downstream flanking genomic sequences) have been PCR amplified, and cloned into a pLenti-TetCMV (GFP-Stop-3UTR/miRNA)-Rsv(Puro) lentivector. The GFP and pre-miRNA are co-transcribed under the same promoter: the optional inducible CMV promoter. The GFP provides a convenient indicator for miRNA expression levels, whilst the puromycin antibiotic selection marker provides the selection method for long term stable expression. We can construct an expression lentivector and produce ready-to-use lentivirus for the precursor miRNA expression of any human or mouse miRNA listed in miRBase database. See the scheme below for the core vector structure.

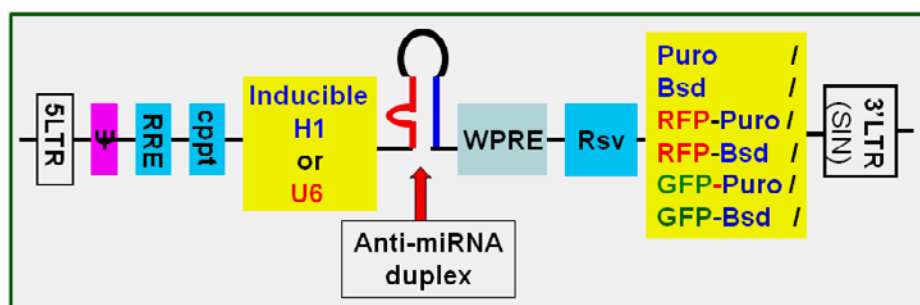


AMSBIO will:

- ▶ Sub-clone your selected gene into one of our expression lentivectors
- ▶ Produce the lentiviral particles
- ▶ Deliver the guaranteed high titer ready-to-use lentivirus to you in just 2-3 weeks

Anti-miRNA expression lentivirus service

Anti-miRNA down-regulates miRNA activity through a blocking mechanism. The anti-miRNA oligonucleotide tags are reverse complements specifically synthesised to bind to their target miRNA. They therefore prevent the miRNA being used for the RNA interference process. The anti-miRNA lentivirus can be used for miRNA sectional functional analysis by down-regulating miRNA activity. Other possible uses include analysis of miRNA target sites, identification and validation of these sites, and screening for miRNAs that regulate gene expression or affect cellular processes. We offer anti-miRNA lentiviruses for all miRNAs listed in the miRBase database. They are expressed under optional inducible H1 promoter or constitutive U6 promoter. We also offer several markers, including fluorescent markers, and negative control lentivirus. See the scheme below for the core vector structure.



AMSBIO will:

- ▶ Sub-clone your selected gene into one of our expression lentivectors
- ▶ Produce the lentiviral particles
- ▶ Deliver the guaranteed high titer ready-to-use lentivirus to you in 2-3 weeks

Stable Cell Line Service

AMSBIO can generate your stable cell line of interest expressing shRNA or transgene (each available constitutive or inducible), in a very cost-effective and timely manner.

Either you provide us with the host cell line and the template or we procure them for you.

AMSBIO will:

- ▶ Clone your shRNA or transgene into our lentiviral vector and generate the lentiviral particles
- ▶ Transduce the cell line of your interest
- ▶ Select the stably transduced, highly expressing cells (Validate the genomic integration via genomic PCR and the high-expression clone by Western Blot if applicable)
- ▶ Deliver two cryogenically preserved vials of stable cells (1×10^6 cells/each) in around 2 months

**ALL OUR LENTIVIRUS PRODUCTS ARE FOR
RESEARCH USE ONLY**

LENTIVIRUS Biosafety considerations:

Please note that although our lentiviral vectors contain all necessary biosafety features, work with lentiviral particles should be carried out under Biological Safety Level 2 (BL2) or higher. Please conduct a thorough risk assessment for your project and contact your health and safety facilities for local guidelines and regulations.

Manipulator Safety warning:

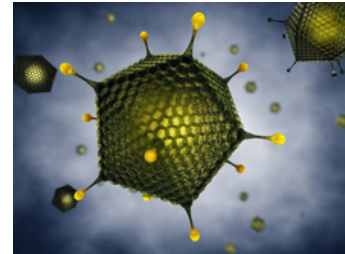
Even though our lentiviral particles are self-inactivating, they can infect the manipulator. Wear gloves all the time, and use extra caution when using and handling them!

⇒ Learn more about AMSBIO Lentivirus at: www.amsbio.com/lentivirus.aspx

ADENOVIRUS

What is An Adenovirus?

Adenoviruses are double-stranded DNA viruses that can infect a broad range of cell types including dividing and non-dividing cells and are, therefore, widely used vehicles for gene delivery. Our adenoviral expression vector is derived from human adenovirus type5 with the E1 and E3 genomic region deleted. Since E1 is essential for the assembly of the virus particles, our adenovirus system produces only replication-incompetent adenovirus. Adenovirus can be used to transfect a wide range of cells including primary cells and stem cells as well animal models, such as monkeys, mouse and human cells. Adenoviral transfection efficiency is often very high, and can regularly reach 100%.



Adenovirus advantages

It is easy to obtain a very high titer (1×10^{11} VP/ml, and concentrated to 10^{13} VP/ml) when using the AMSBIO adenovirus. Once an initial stock is generated, the adenovirus can be easily amplified in HEK293 cells to achieve very high titer. Each mammalian cell can produce on average 10,000 adenoviruses.

► Adenoviral vector is replication deficient and safe

Adenoviruses are commonly found in the human body. Since the E1 and E3 regions have been deleted, the recombinant adenoviruses are unable to replicate within the human body. Nonetheless, they should be treated like other recombinant DNA materials, just like plasmid clones.

► Transient expression in mammalian cells

Unlike lentivirus, adenoviruses do not insert into host genome which inactivates other genes and activate oncogenes. Recombinant adenovirus remains epichromosomal in host cells, making them ideal for in vivo studies such as human gene therapy.

► Adenoviruses are not toxic to host cells

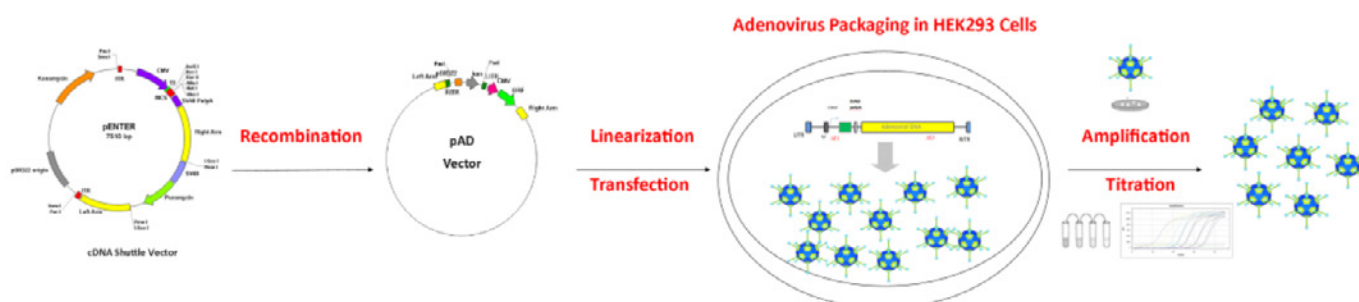
Post-transfection viability of the host cells is almost 100%; as it is well tolerated in a wide range of cells. Unlike plasmid transfection, when toxic chemicals have to be used during transfection.

► Accommodate large transgenes

Our E1 and E3 deletion adenoviral vector can hold a gene insertion of up to 8kb, whilst our E1, E3 and E4 deletion adenoviral vector can accommodate a transgene insertion of up to 10.5kb. Gutless adenoviral vector can hold transgene as large as 34kb.

► Adenovirus is relatively stable

Recombinant adenoviruses can be stored at fridge for weeks, -20°C freezers for months and -80°C freezers for years. The stable nature of the adenoviruses makes purification and long-term storage possible, making them suitable for human gene therapy and pharmaceutical product development.



AMSBIO offers large collection of adenovirus clones

Key Features

- 17,000 human full-length ORF cDNA clones in shuttle vector and ready to be cloned
- More than 20 different destination vectors available for different fluorescent and affinity tags at either N-terminus or C-terminus
- The entry vector and all destination vectors are designed for adenovirus production
- Custom cloning of your DNA or shRNA to one of your desired shuttle vectors
- Fast delivery of the primary virus stock with minimum titer of 1×10^8 VP/ml
- Primary virus stocks can be amplified and purified upon request

Adenoviral miRNA Clones and Virus

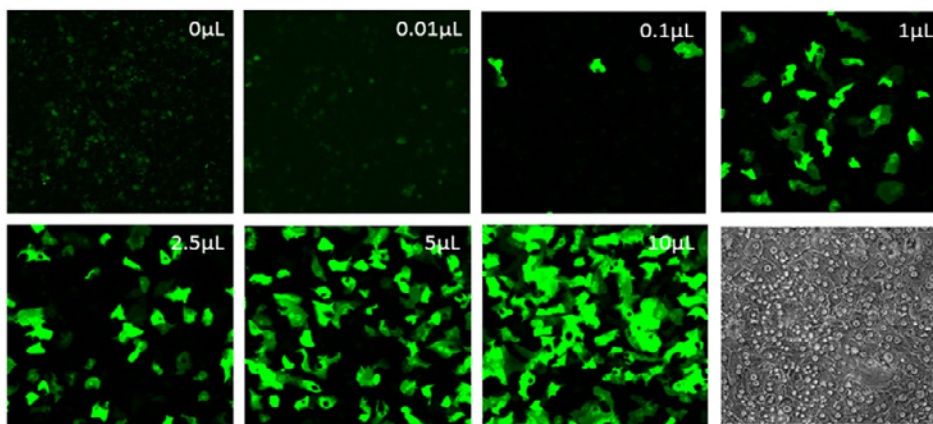
Adenoviral miRNA clones are human miRNA expression plasmids with a GFP reporter cloned into the 34kb adenoviral genome.

Key Advantages

- ▶ 1,272 Human miRNAs in pAD-MIR adenoviral vector
- ▶ Fully sequence-verified by NextGen Sequencing
- ▶ Adenovirus production ready and saves subcloning time
- ▶ 100% gene delivery in most cell types, ideal for hard-to-transfect cells
- ▶ Premade adenoviruses come pre-packaged and ready-to-transfect

Technical Features

- ▶ High Safety - pAD Vector (serotype 5) contains E1/E3 deletion to eliminate self-replication
- ▶ Each adenoviral vector contains only one copy of the miRNA sequence
- ▶ Easy amplification as regular plasmids in E. coli
- ▶ miRNA precursor contains miRNA hairpin sequence and 150-200bp flanking sequence
- ▶ Strong CMV promoter-driven transcription
- ▶ Contains kanamycin as selectable marker
- ▶ Built-in GFP tag allows simultaneous transfection monitoring
- ▶ Unique vector designs accommodate large inserts (up to 8.5kb-30kb)



- GFP adenovirus
- High transfection efficiency with adenoviral transfection
- 42 hr post transfection with GFP adenovirus (6×10^9 VP/ml) on 12 well plate (0.75×10^6 cells/well) in hepatocytes cells

⇒ Learn more about AMSBIO adenovirus at : www.amsbio.com/adenovirus.aspx

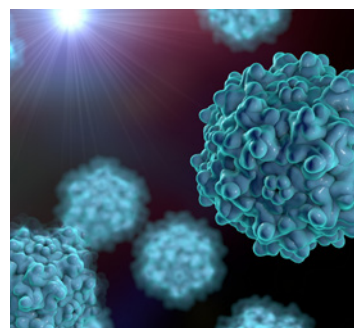
ADENO-ASSOCIATED VIRUS

What is an ADENO-ASSOCIATED VIRUS?

Adeno-associated virus (AAV) is a non-enveloped, single-stranded DNA virus which is approximately 20nm in size and can infect both dividing and non-dividing cells.

AAV does not cause disease and elicits a very mild immune response. Being able to infect both dividing and non-dividing cells, it incorporates its genome into that of the host cells and only replicates in the presence of a helper virus; most commonly adenovirus or herpes simplex virus.

- ▶ Transfects dividing & non-dividing cells
- ▶ Easy to produce at high viral titre
- ▶ Very mild immune response in vivo
- ▶ Only replicates in presence of helper virus



What services amsbio offers:

We offer the highest quality recombinant AAV vectors and the most complete AAV expression systems that can be used to express shRNA, human ORF and more.

AAV Cloning Service:

- ▶ AAV Human cDNA ORF cloning
- ▶ AAV shRNA cloning
- ▶ AAV CRISPR

AAV Packaging Service:

- ▶ Small Scale Crude AAV Packaging service
- ▶ Large Scale Custom AAV Packaging service

You'll get reliable, reproducible, high purity, high titre viral stock every time you order.

Cloning Services

- ▶ Production of AAV without helper adenovirus
- ▶ Nonpathogenic with minimal immune response
- ▶ Multiple Serotypes (AAV1, AAV2, AAV5, AAV6, AAV7, AAV8, & AAV9)
- ▶ Superior safety features
- ▶ Ideal for mammalian ORF expression
- ▶ Competitive price
- ▶ Different promoters and reporters available

Packaging Services

We have the platform to suit all your needs when it comes to combining scientific development and processing advancements in the field of gene therapy. With our robust AAV production system, using AAV as a therapeutic vehicle for a broad range of diseases is now a simple task.

AAV Serotype	Muscle	Hepatocyte	Pulmonary	Brain	Retinal pigmented epithelium	Pancreas	Kidney
AAV 1	X			Neurons and glial cells	X	X	
AAV 2							X
AAV 5			Lung alveolar cells	Neurons and glial cells	X		
AAV 6	X		X				
AAV 7	X			Neurons	X		
AAV8	X	X		Neurons	X	X	
AAV9	X	X	X		X	X	X

⇒ Learn more about AMSBIO adeno-associated virus at : www.amsbio.com/adeno-associated-virus.aspx

READY TO USE AAV BIOSENSORS

Biosensors are genetically engineered fluorescent proteins (FP) attached to an additional protein sequence which makes them sensitive to small biomolecules (e.g. Ca^{2+}). Adeno-associated virus (AAV) Biosensor products come as ready-to-use AAV virus with a choice of promoter and the ability to include the Cre inducible (FLEX-ON) expression. The viruses encode your chosen biosensor, either calcium or glutamate sensor and are ready for in vivo injection. We have packaged these indicators into the most commonly used AAV serotypes (AAV8 and AAV9).

⇒ Choose your perfect AAV biosensor:

1. Decide which biosensor is most suitable for your research question (calcium or glutamate; consider color, kinetics, SNR).
2. Decide whether you would like to include FLEX.
3. Choose a promoter (universal promoter-CAG, or tissue specific neuron specific-synapsin promoter-Syn).
4. Choose the AAV vector serotype (AAV8 or AAV9). Other serotypes are available upon request.

Biosensor	Description	FLEX (Y/N)	Promoter	AAV	Product Code
Calcium	CaMPARI: Higher-throughput assays with cultured cells	N	Syn	AAV8	BS10-NORAAV8
				AAV9	BS10-NORAAV9
				AAV8	BS10-NXRAAV8
		Y	CAG	AAV9	BS10-NXRAAV9
				AAV8	BS10-CXRAAV8
				AAV9	BS10-CXRAAV9
	GCaMP6s: Improved SNR, slower kinetics; Green indicator	N	Syn	AAV8	BS1-NOSAAV8
				AAV9	BS1-NOSAAV9
				AAV8	BS1-NXSAAV8
		Y	CAG	AAV9	BS1-NXSAAV9
				AAV8	BS1-CXSAAV8
				AAV9	BS1-CXSAAV9
	GCaMP6m: Improved SNR, intermediate kinetics; Green indicator	N	Syn	AAV8	BS2-NOMAAV8
				AAV9	BS2-NOMAAV9
				AAV8	BS2-NXMAAV8
		Y	CAG	AAV9	BS2-NXMAAV9
				AAV8	BS2-CXMAAV8
				AAV9	BS2-CXMAAV9
	GCaMP6f: Improved SNR, faster kinetics; Green indicator	N	Syn	AAV8	BS3-NOFAAV8
				AAV9	BS3-NOFAAV9
				AAV8	BS3-NXFAAV8
		Y	CAG	AAV9	BS3-NXFAAV9
				AAV8	BS3-CXFAAV8
				AAV9	BS3-CXFAAV9
	GCaMP3: Green indicator	N	CAG	AAV8	BS4-CX3AAV8
				AAV9	BS4-CX3AAV9
	GCaMP5: Green indicator	N	CAG	AAV8	BS5-PXAAV8
				AAV9	BS5-PXAAV9
Calcium	jRCaMP1a: Red, improved SNR, slower kinetics, bright	N	Syn	AAV8	BS6-NOAAV8
				AAV9	BS6-NOAAV9
				AAV8	BS6-NXAAV8
		Y	CAG	AAV9	BS6-NXAAV9
				AAV8	BS6-CXAAV8
				AAV9	BS6-CXAAV9
	jRCaMP1b: Red, improved SNR, improved dynamic range, bright	N	Syn	AAV8	BS7-NOBAAV8
				AAV9	BS7-NOBAAV9
				AAV8	BS7-NXBAAV8
		Y	CAG	AAV9	BS7-NXBAAV9
				AAV8	BS7-CXBAAV8
				AAV9	BS7-CXBAAV9
	jRGECO1a: Red, improved SNR	N	Syn	AAV8	BS8-NOAAV8
				AAV9	BS8-NOAAV9
				AAV8	BS8-NXAAV8
		Y	CAG	AAV9	BS8-NXAAV9
				AAV8	BS8-CXAAV8
				AAV9	BS8-CXAAV9
	jRGECO1b: Red, improved SNR	N	Syn	AAV8	BS9-NOBAAV8
				AAV9	BS9-NOBAAV9
				AAV8	BS9-NXBAAV8
		Y	CAG	AAV9	BS9-NXBAAV9
				AAV8	BS9-CXBAAV8
				AAV9	BS9-CXBAAV9
Glutamate	iGLuSnFR: Rapid detection, improved SNR; direct visualization of synaptic release (as opposed to Ca^{2+} imaging)	N	CMV	AAV8	BS11-COG-AAV8
				AAV9	BS11-COG-AAV9

⇒ Learn more about AMSBIO AAV biosensors at : www.amsbio.com/aav-biosensors.aspx

AAV Biosensors are covered under US Patents #14/350,199; #8,629,256, #14/800,814, #14/800,814, #14/941,406, #14/974,483, 14/941,406, 14/974,483, 14/452,428 and foreign equivalents and licensed from Janelia Research Campus, HHMI, Janelia, Virginia, USA.

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