Fastest, easiest adenoviral system ever

Adeno-X Adenoviral System 3

- Cloning into adenovirus is as simple and rapid as into any plasmid
- Clone directly into the pAdenoX vector, no shuttle vector required
- Transduce dividing and non-dividing cells
- Very high titer, high expression, broad host range
- Highly flexible formats

Introduction

Why choose adenoviral gene delivery?

Adenoviral gene transfer is one of the most reliable methods for introducing genes into mammalian cells. Because infection by adenovirus is not cell-cycle dependent, you can deliver your gene to primary as well as transformed cell lines. Adenoviruses are ideal tools for protein production in mammalian cells because following infection, your target gene is transiently expressed at very high levels since many cells receive multiple copies of the recombinant genome. Additionally, adenoviruses are capable of infecting a wide variety of proliferating and quiescent cell types from many different animal species including humans, non-human primates, pigs, rodents, mice, and rabbits.

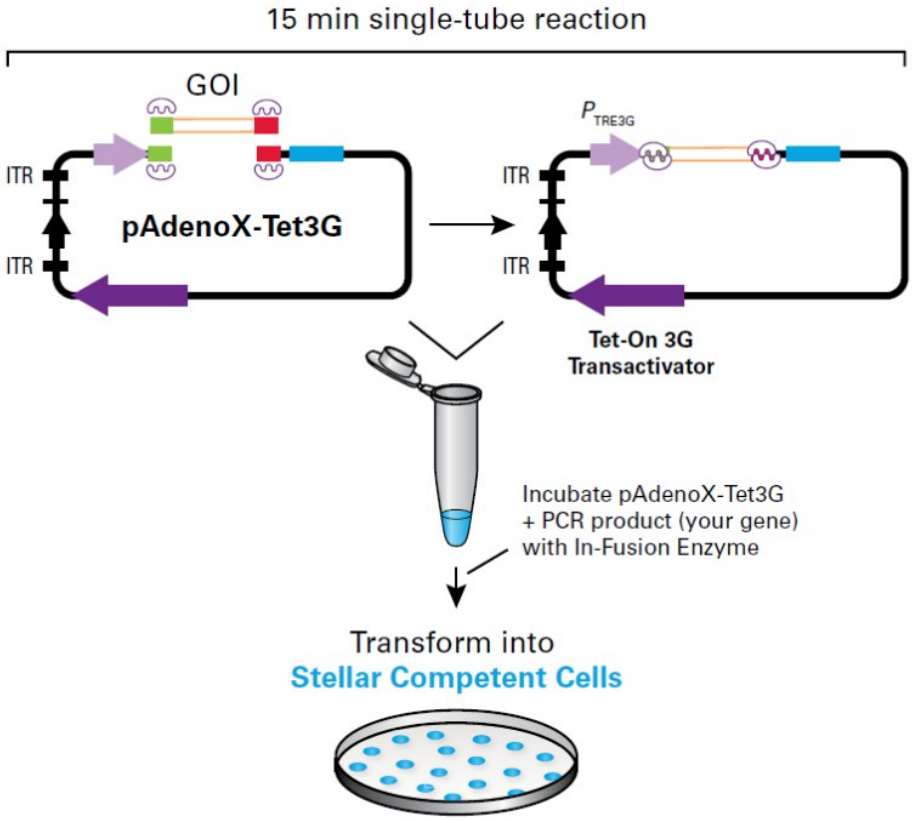


Figure 1. The Adeno-X Adenoviral System 3 is the most advanced commercially available adenoviral gene delivery system. It provides by far the simplest, fastest, and most efficient method for constructing recombinant adenoviral vectors.

Adeno-X System 3 features	
Feature	**Description**
No shuttle vector required	Clone directly into the adenoviral vector using In-Fusion HD Cloning
Easy to use	As easy as any plasmid cloning system
Fast	2–3 days for cloning (others take at least 8 days)
Super high-efficiency cloning	100s of colonies, 9/10 clones are correct
Highly flexible formats	Use an existing expression cassette or create one without additional subcloning
Best technologies offered	Tet-On 3G inducible expression, fluorescent reporters, multiple fragment cloning

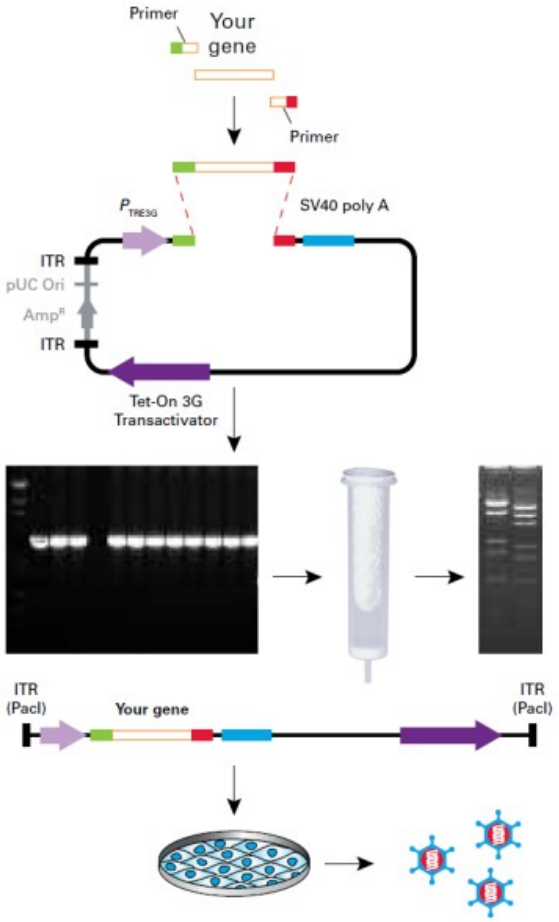
Clone into adenovirus just like any other plasmid

Until now the main drawback of commercially supplied adenoviral vector systems has been the need to use complex cloning procedures to overcome the difficulties with cloning into large plasmids (~34 kb). Procedures have included precloning into shuttle vectors and subcloning through multiple steps and multiple different strains of *E. coli*, all of which increase hands-on time and leave more room for error. At Takara Bio, our Adeno-X virologists thought "wouldn't it be great if you could clone directly into the adenoviral plasmid just like any plasmid?" They then harnessed the power of In-Fusion HD Cloning technology to make this happen.

Protocol overview

There is no simpler adenoviral expression system

An overview of the procedure for creating recombinant adenovirus using the Adeno-X Adenoviral System 3 is shown in Figure 2. The system relies upon the ability of the In-Fusion HD enzyme to precisely recognize and fuse 15 bp of homology between two linear DNA molecules. To generate short regions of homology to the prelinearized pAdeno-X vector, simply add 15 bp of additional sequence to the primers you use to PCR-amplify your gene of interest. Then, combine the DNA together with the In-Fusion HD enzyme in a 15-min reaction and transform Stellar Competent Cells. Cloning is always directional and 90% of the clones contain the correct insert. The kit includes a control cloning fragment, primers for colony PCR verification of positive clones, and a NucleoBond Xtra kit for transfection-grade plasmid purification.



1. Amplify your gene with 15 bp of homology to pAdenoX

2. Clone using In-Fusion technology and Stellar Competent Cells

3. Perform colony PCR to verify correct clone(s)

4. Purify pAdenoX-GOI using NucleoBond Xtra

5. Digest with XhoI/NheI to verify integrity

6. Digest with PacI (to linearize)

7. Transfect HEK 293 cells

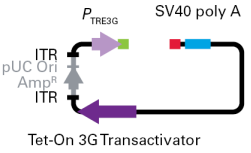
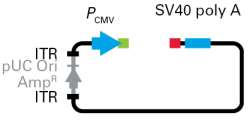
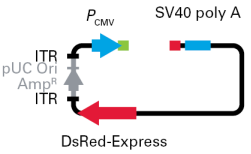
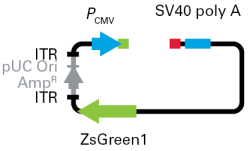
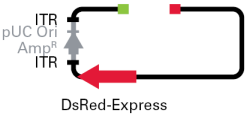
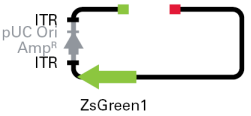
8. Harvest recombinant adenovirus

Figure 2. Constructing recombinant adenovirus with In-Fusion technology. DNA sequences can be rapidly transferred as PCR products to any pAdenoX vector using the In-Fusion cloning method. In this example, your gene of interest is amplified with 15-bp extensions that are homologous to the ends of the linearized adenoviral vector. The PCR product is then purified and mixed with the linearized adenoviral vector of choice in the In-Fusion reaction. Following the reaction, a portion of the mixture is transformed into Stellar Competent Cells and screened. Once a PCR-positive clone is identified, the recombinant pAdenoX vector is amplified, purified, and subsequently linearized with the restriction enzyme PacI, then transfected into HEK 293 cells for viral rescue and amplification.

Multiple formats are available

Adeno-X Adenoviral System 3 is available in seven formats, including the most advanced tetracycline-inducible expression system, constitutive expression systems with or without fluorescent reporters, and universal systems that allow you to clone and express any entire expression cassette of your choice (Table 1).

Table 1. Adeno-X Adenoviral System 3 formats

Cat. No.	Product	Description	Vector Map
631180	Adeno-X Adenoviral System 3 (Tet-On 3G Inducible)	• Tightly-controlled, doxycycline-inducible expression system	
632269	Adeno-X Adenoviral System 3 (CMV)	• Constitutive expression from a CMV promoter	
632268	Adeno-X Adenoviral System 3 (CMV, Red)	• Constitutive expression from a CMV promoter • Red fluorescent protein to easily monitor transfection and transduction	
632267	Adeno-X Adenoviral System 3 (CMV, Green)	• Constitutive expression from a CMV promoter • Green fluorescent protein to easily monitor transfection and transduction	
632266	Adeno-X Adenoviral System 3 (Universal)	• Use any promoter and any polyA sequence • Ideal for tissue-specific expression or expression of shRNA or miRNA	
632265	Adeno-X Adenoviral System 3 (Universal, Red)	• Use any promoter and any polyA sequence • Ideal for tissue-specific expression or expression of shRNA or miRNA • Red fluorescent protein to easily monitor transfection and transduction	
632264	Adeno-X Adenoviral System 3 (Universal, Green)	• Use any promoter and any polyA sequence • Ideal for tissue-specific expression or expression of shRNA or miRNA • Green fluorescent protein to easily monitor transfection and transduction	

Results

Tetracycline-inducible expression

When you clone your gene into pAdenoX-Tet3G, you are creating a system with the tightest and most sensitive control of gene expression. Tightly-controlled, doxycycline-induced expression is as easy as constitutive expression, since the Tet-On 3G transactivator protein and the P_{TRE3G}-controlled gene of interest are present on the same adenoviral vector. Up to 3,000-fold induction can be achieved using this system (Figure 3).

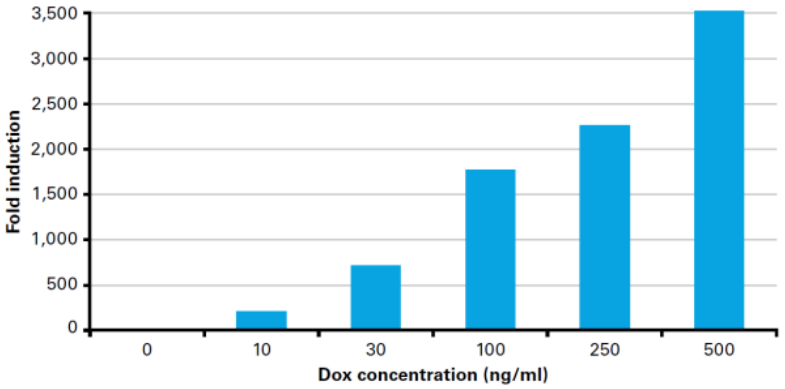


Figure 3. The Adeno-X Tet-On 3G Systems generate very high-fold induction, with up to a 3,000-fold difference between induced and uninduced states. Using equal amounts of high-titer supernatants, HeLa cells cultured at the indicated concentrations of Dox were infected with Adeno-X Tet-On 3G Luciferase virus. Cultures were harvested and assayed for luciferase activity.

Clone any expression cassette into the universal vectors

You are not limited to using a CMV expression system—we have created universal systems with vectors that lack a promoter and polyA signal in the cloning site. Simply amplify an entire expression cassette (from promoter to polyA) from a pre-existing construct and clone using In-Fusion HD (Figure 4, Panel A). Universal systems can be used for expression from alternative promoters that are better suited to your target cell type, such as EF1-alpha or tissue-specific promoters. Alternatively, you may wish to transfer your shRNA or miRNA expression cassette from a pre-existing plasmid to one of the universal pAdenoX vectors in order to create a high-efficiency RNAi delivery system. Even if your expression cassette does not exist, you can create one using multiple fragment cloning (Figure 4, Panel B), with only a small loss in cloning efficiency (Table 2).

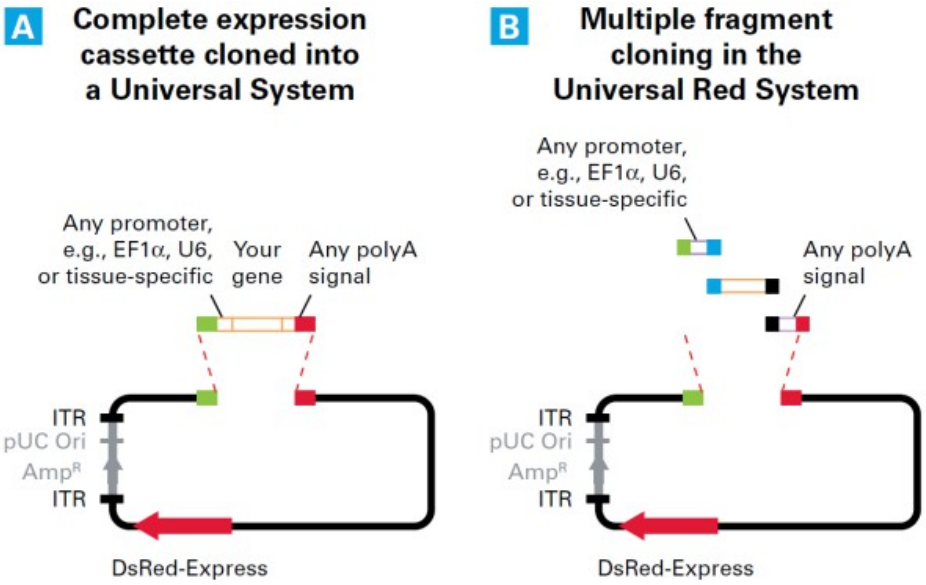


Figure 4. The Universal Adeno-X Expression Systems contain vectors that lack a promoter and polyA signal in the cloning site. You can either clone an expression cassette from a pre-existing construct into the vector (**Panel A**) or create a new one using multiple fragment In-Fusion HD cloning (**Panel B**).

Very high titer, easily amplified, very stable

Recombinant adenoviruses such as Adeno-X are lytic only in packaging cells that provide the essential E1 protein in *trans* (such as HEK 293 cells). This lytic mechanism of amplification means that virus particles produced by one cell can reinfect adjacent packaging cells to produce a cascade of virus production and ultimately far higher titers (>10^9 IFU/ml) than can be achieved with any recombinant lentivirus system (Table 3). Moreover, it is very simple to reamplify and make more virus particles. Unlike lentivirus production, there is no need to optimize transfection conditions; you simply reinfect HEK 293 cells with your existing adenovirus prep and wait for the packaging cells to produce more virus. Adenovirus can be stored frozen in high-titer aliquots for long-term studies.

Table 2. Multiple fragment cloning efficiency with the Adeno-X System 3		
	Number of colonies (1/10th plated)	**%correct**
pAdenoX + 1 insert	200–300	90
pAdenoX + 2 inserts	200–300	60
pAdenoX + 3 inserts	25–40	25

Table 3. Adenoviral gene delivery vs. lentiviral gene delivery		
	Lentivirus	**Adenovirus**
Infects many different human cell types	Yes	Yes
Infects both dividing and non-dividing cells	Yes	Yes
Non-integrating virus	No	Yes
High level of protein expression (up to 10–20% total protein)	No	Yes
Ability to accommodate long inserts (up to 8 kb)	No	Yes
Easy to scale-up/amplify	No	Yes
Easy to get titers >10^9 IFU/ml	No	Yes
Easy to get a multiplicity of infection >25 copies per cell	No	Yes

Conclusions

The Adeno-X Adenoviral System 3 provides a simple, rapid method for gene delivery into mammalian cells by allowing direct cloning into adenoviral vectors using In-Fusion HD Cloning technology. The system is available in seven different formats, including a tetracyline-inducible system which generates very high-fold induction, and universal systems that may be used to clone any expression cassette or create a new expression cassette using multiple fragment cloning. The Adeno-X Adenoviral System 3 produces very high titers of recombinant retroviruses that are easily amplified and remain stable when frozen.

Related Products

Cat. #	Product	Size	License	Quantity	Details
632269	Adeno-X™ Adenoviral System 3 (CMV)	10 Rxns		*	

Adeno-X Adenoviral System 3 (CMV) provides the components necessary to generate a constitutive expression system in an adenoviral vector format. pAdenoX-CMV (included in the vector set) is a prelinearized, adenoviral vector that is ready for the insertion of your gene of interest via our In-Fusion HD PCR Cloning technology. Simply PCR-amplify your gene and combine it with pAdenoX-CMV in an In-Fusion HD Cloning reaction. In-Fusion HD Cloning is fast, simple, precise and efficient, making Adeno-X Adenoviral System 3 the most advanced commercially available adenoviral gene delivery tool.

Documents	Components	You May Also Like	Image Data	Resources

632267	Adeno-X™ Adenoviral System 3 (CMV, Green)	10 Rxns		*	
632268	Adeno-X™ Adenoviral System 3 (CMV, Red)	10 Rxns		*	
631180	Adeno-X™ Adenoviral System 3 (Tet-On® 3G Inducible)	10 Rxns		*	
632266	Adeno-X™ Adenoviral System 3 (Universal)	10 Rxns		*	
632264	Adeno-X™ Adenoviral System 3 (Universal, Green)	10 Rxns		*	
632265	Adeno-X™ Adenoviral System 3 (Universal, Red)	10 Rxns		*	

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