Tetracycline-inducible expression using an all-in-one adenovirus vector

Adeno-X Adenoviral System 3 (Tet-On 3G Inducible)

- Very tight control of gene expression
- Simple-to-use, all-in-one tetracycline-inducible system
- The most advanced adenoviral gene delivery technology
- Easiest adenoviral system to use; cloning is even simpler than standard plasmid cloning

Introduction

The Adeno-X Adenoviral System 3 (Tet-On 3G Inducible) from Takara Bio combines the tightest and most sensitive control of gene expression with the most advanced commercially available adenoviral vector system. With this system, tightly-controlled inducible expression is as easy as constitutive expression, and cloning into an adenoviral vector is as straightforward as cloning into any plasmid.

How does the Tet-On 3G inducible system work?

Target cells that express the Tet-On 3G transactivator protein and contain a gene of interest (GOI) under the control of a TRE3G promoter (*P*TRE3G) will express high levels of your GOI, but only when cultured in the presence of doxycycline (Dox), a tetracycline analog. When bound by Dox, the Tet-On 3G protein undergoes a conformational change that allows it to bind to tet operator (tetO) sequences located in *P*TRE3G (Figure 1). In contrast to TetR-based systems, Tet-On technologies activate rather than repress transcription, a critical difference which results in far lower basal expression, higher maximal expression, a more rapid response time—and ultimately, makes them the first choice for conditional expression.

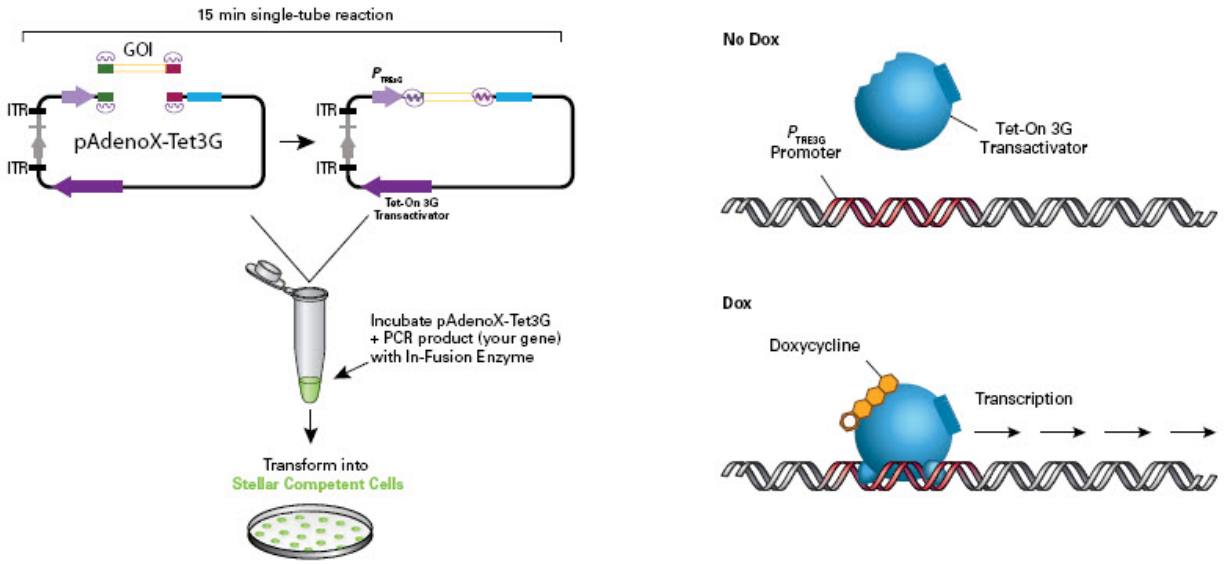

Figure 1. The Adeno-X Adenoviral System 3 (Tet-On 3G Inducible). The system includes In-Fusion HD Cloning for cloning your gene of interest (GOI) directly into the easy-to-use, all-in-one pAdenoX-Tet3G expression vector. Your cloned gene of interest (GOI)is under the control of the TRE3G promoter and will express high levels of your GOI, but only when cultured in the presence of Dox.

Protocol overview

What makes this system so easy to use?

1. **Simple & efficient cloning**—Each kit is supplied with a prelinearized adenoviral plasmid (pAdenoX-Tet3G) and a complete In–Fusion HD Cloning Kit. Simply amplify your gene of interest using primers that contain 15 bp of homology to the vector insertion site and fuse the two linear DNA molecules using In-Fusion (Figure 2). This system makes cloning into the 34-kb pAdenoX-Tet3G vector as simple as cloning into any plasmid.
2. **All-in-one vector**—The Tet-On 3G transactivator gene has been pre-cloned into the E3 region of the adenoviral genome and is expressed constitutively from a CMV promoter. Clone your gene of interest using In-Fusion HD at the E1 region of the adenovirus between the tightly regulated P_{TRE3G} promoter and an SV40 polyA signal. Because the two regions are widely separated, interference from the CMV promoter cannot affect basal expression from P_{TRE3G} and so a very low basal expression and high fold inducibility are retained (Figures 3–5).

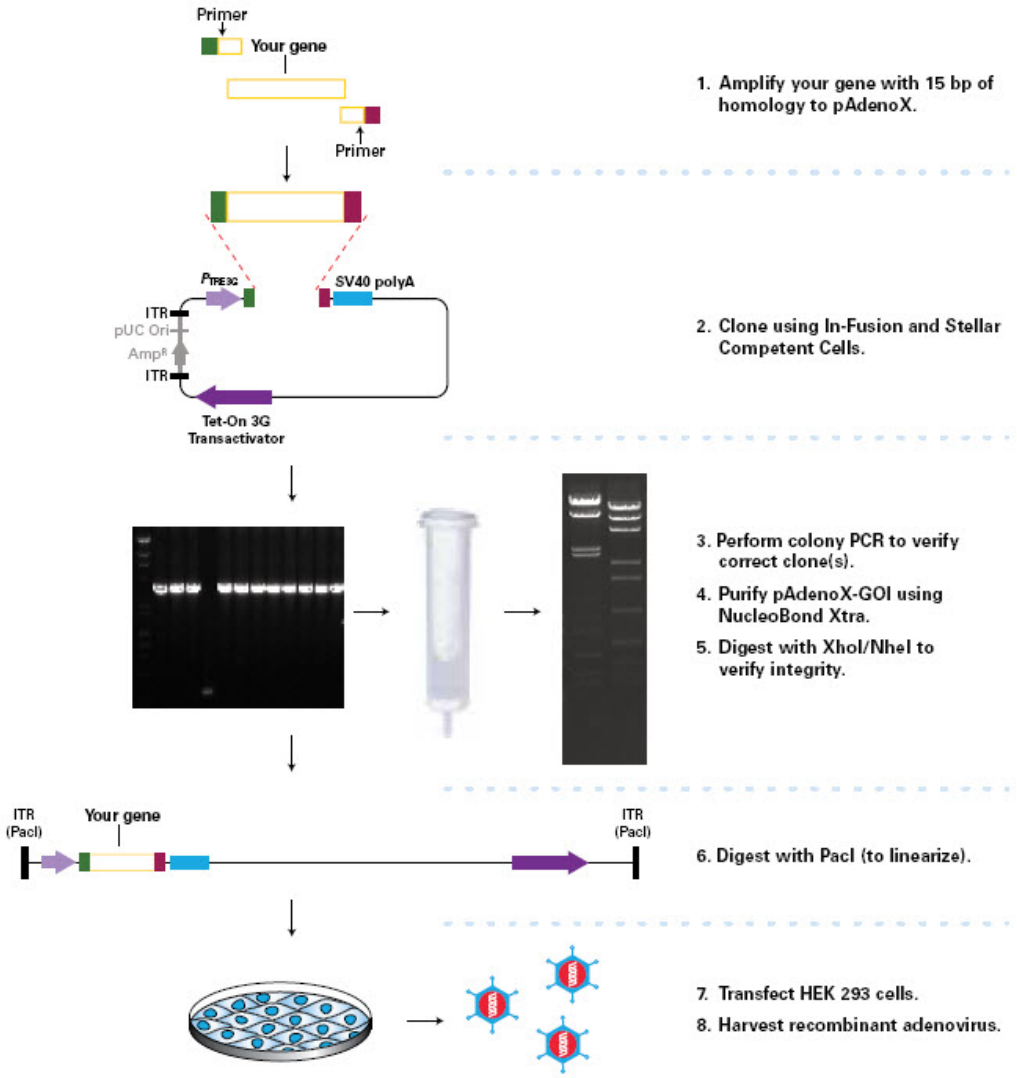

Figure 2. Constructing recombinant adenovirus with In-Fusion technology. DNA sequences can be rapidly transferred as PCR products to any pAdenoX vector using the In-Fusion cloning method. In this example, your gene of interest is amplified with 15-bp extensions that are homologous to the ends of the 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.

Results

Lowest-ever background, highest sensitivity

The combination of two optimized elements makes Tet-On 3G the highest performing inducible expression system.

1. P_{TRE3G} promoter—mutations have reduced background expression from the inducible promoter to very low levels compared to previous generations of the Tet-On System (Loew et al. 2010).
2. Tet-On 3G transactivator protein—compared to early generations, mutations have significantly increased its sensitivity to the inducer doxycycline (Zhou et al. 2006).

When the two elements are combined, not only can you detect high expression of your protein after exposure to Dox, but you can control the level of expression by titration of the Dox concentration (Figure 3) and you can generate very high fold induction, up to a 3,000-fold difference between the induced and uninduced states (Figure 4). The maximum expression level can be manipulated by increasing the amount of virus per cell (Figure 5).

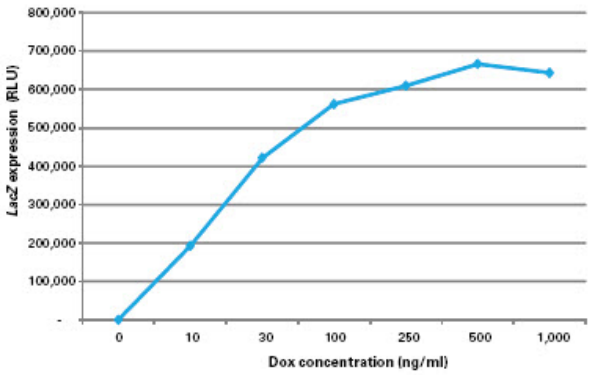

Figure 3. The Adeno-X Tet-On 3G systems are highly inducible. Using equal amounts of high-titer supernatants, HeLa cells cultured at the indicated concentrations of Dox were infected with Adeno-X Tet-On 3G lacZ virus. Cultures were harvested and assayed for β-galactosidase activity using the Luminescent β-gal Reporter System 3 (Cat. # 631713).

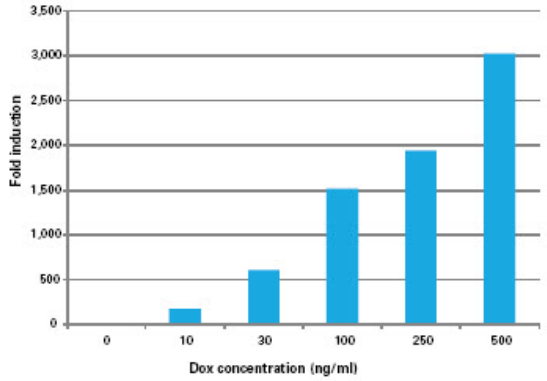

Figure 4. The Adeno-X Tet-On 3G systems generate very high-fold induction, with up to 3,000-fold difference between induced and uninduced states. Using equal amounts of high-titer supernatants, HeLa cells cultured at the indicated concentrations of Dox were infected with Adeno-X Tet-On 3G luciferase virus. Cultures were harvested and assayed for luciferase activity.

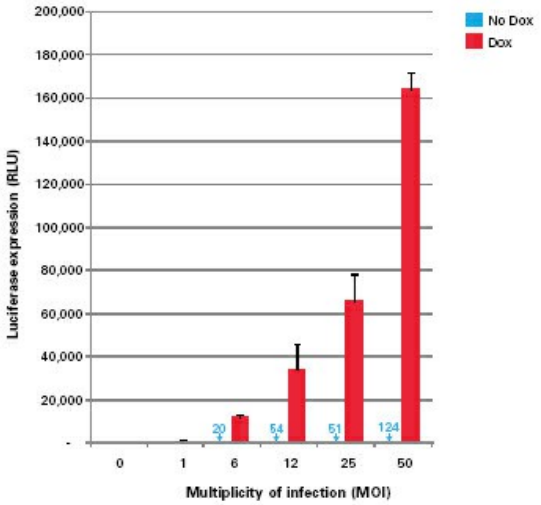

Figure 5. Expression increases with higher MOIs. HeLa cells were infected with varying MOIs of pAdenoX Tet-On 3G adenovirus that expresses luciferase. After four hours, the media was replaced with fresh media +/- doxycycline (1 µg/ml). Cultures were harvested and assayed for luciferase activity. Maximal expression increases with increasing MOI, which also results in a slight increase in background expression.

Unlike the leading competitor, the Adeno-X system really is easy

Compared to the leading competitor system, which requires eight days or more for a cloning procedure that involves cloning into a shuttle vector and transformation of two different *E. coli* strains, the Adeno-X system really is easy and allows you to finish cloning with high efficiency in just two to three days. The Adeno-X system does not use a shuttle vector, so no subcloning is needed. A high-performance *E. coli* strain (Stellar Competent Cells) is included with the kit (see Table I).

Table I. Comparison of the Adeno-X System 3 to the leading competitor's "easy" system		
	Adeno-X System 3	**Competitor's system**
Cloning time	• 2–3 days	• 8 days
Cloning procedure	• Simple • 30 min hands-on time	• Complicated • Lots of hands-on time
Cloning technology	• In-Fusion HD Cloning	• Homologous recombination in bacteria
Subcloning into shuttle vector	• Not required	• Clone into a shuttle vector first
Viral DNA yield	• High	• Low for the recombination strain
***E.coli* strain**	• Stellar chemically competent cells (supplied)	• 2 strains required
Cloning efficiency	• 9/10 clones correct	• 1/10 to 3/10 correct
Screening	• PCR-based	• Miniprep followed by restriction digestion
Inducible expression	• Tightest control with Tet-On 3G technology	• Technology not available
Monitor using fluorescent proteins	• Red and green • Bright and consistent	• Green only • Less bright
Multiple-fragment cloning	• Single-step cloning	• Clone in multiple steps

Supreme flexibility—create mutations or fusion proteins in a single reaction

The power of In-Fusion Cloning technology enables you to directly join the pAdenoX-Tet3G vector to one or more PCR fragments in a single reaction. This means that in a single cloning reaction you can, for example, fuse your gene of interest to a fluorescent protein/tag or create a point mutation within your gene of interest (Zhu et al. 2007). To do so, you simply amplify 2 PCR products each with 15 bp homology to the pAdenoX- Tet3G vector at one end and 15 bp homology to each other at the other end. The central overlapping region will be at the junction of the fusion protein or at the region of the point mutation (Figure 6).

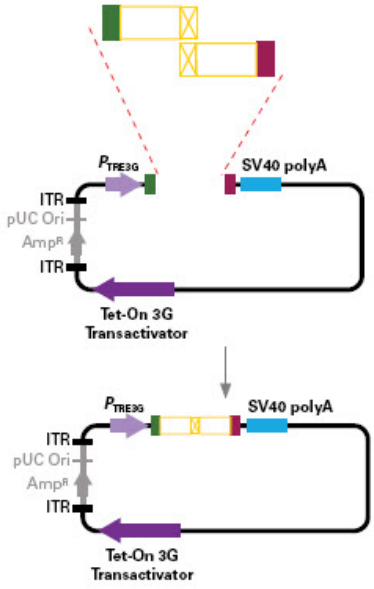

Figure 6. Create a point mutation and clone in one step using In-Fusion Cloning.

Conclusions

The Adeno-X Adenoviral System 3 (Tet-On 3G Inducible) provides tightly-controlled gene expression in an easy-to-use tetracycline-inducible system. An all-in one adenoviral vector constitutively expresses the Tet-On 3G transactivator and allows simple, efficient In-Fusion HD Cloning of a gene of interest under the control of a P_{TRE3G} promoter. This optimized transactivator/promoter combination provides exceptionally low background and high sensitivity, resulting in a highly inducible system that generates very high fold-induction and maximal expression levels that increase with increasing MOI. The Adeno-X Adenoviral System 3 (Tet-On 3G Inducible) is easier to use than a leading competitor system because it does not use a shuttle vector and thus requires no subcloning. This system is also quite flexible, allowing the creation of mutations or fusion proteins in a single reaction.

References

Loew, R., Heinz, N., Hampf, M., Bujard, H. & Gossen, M. Improved Tet-responsive promoters with minimized background expression. *BMC Biotechnol.* **10,** 81 (2010).

Zhou, X., Vink, M., Klaver, B., Berkhout, B. & Das, A. T. Optimization of the Tet-On system for regulated gene expression through viral evolution. *Gene Ther.* **13,** 1382–90 (2006).

Zhu, B., Cai, G., Hall, E. O. & Freeman, G. J. In-fusion assembly: seamless engineering of multidomain fusion proteins, modular vectors, and mutations. *Biotechniques* **43,** 354–9 (2007).

Related Products

Cat. #	Product	Size	License	Quantity	Details
631180	Adeno-X™ Adenoviral System 3 (Tet-On® 3G Inducible)	10 Rxns		*	

Adeno-X Adenoviral System 3 (Tet-On 3G Inducible) provides the components necessary to generate a tightly controlled, highly sensitive, inducible expression system in an all-in-one adenoviral vector format. pAdenoX-Tet3G (included in the vector set) is a prelinearized, adenoviral vector that is ready for the insertion of your gene of interest with our In-Fusion HD PCR Cloning technology. Simply PCR-amplify your gene and combine it with pAdenoX-Tet3G 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.

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631106	Tet System Approved FBS	500 mL	*	

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