Mammalian Expression Systems

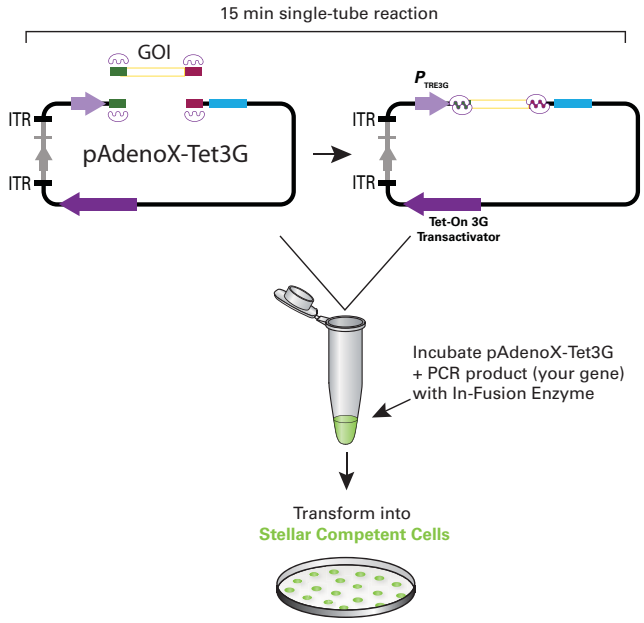
Tetracycline inducible expression using an all-in-one adenovirus vector

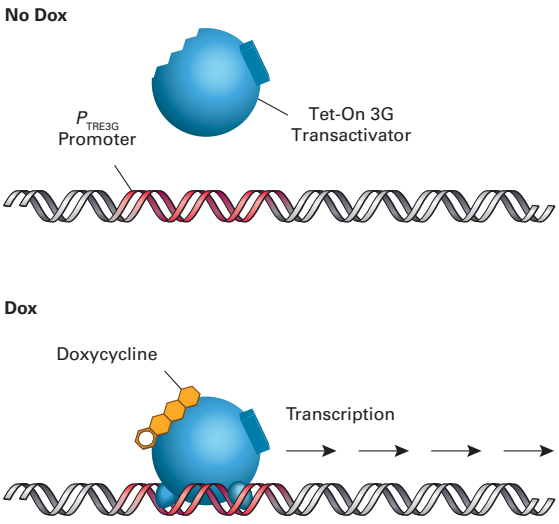
- **Very tight control of gene expression**
- **Simple-to-use, all-in-one tetracycline inducible system**
- **The most advanced adenoviral gene delivery technology**
- **Easiest adenoviral system to use; cloning is even simpler than standard plasmid cloning**

Clontech's **Adeno-X™ Adenoviral System 3 (Tet-On® 3G Inducible)** 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, the first choice for conditional expression.





The Adeno-X Adenoviral System 3 (Tet-On 3G Inducible) allows inducible gene expression only in the presence of doxycycline. The system includes In-Fusion® HD for cloning your gene of interest (GOI) directly into the easy-to-use, all-in-one pAdenoX-Tet3G expression vector.

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

What makes this system so easy to use?

1. **Simple & efficient cloning –** Each kit is supplied with a pre-linearized 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 1). 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 2-4).

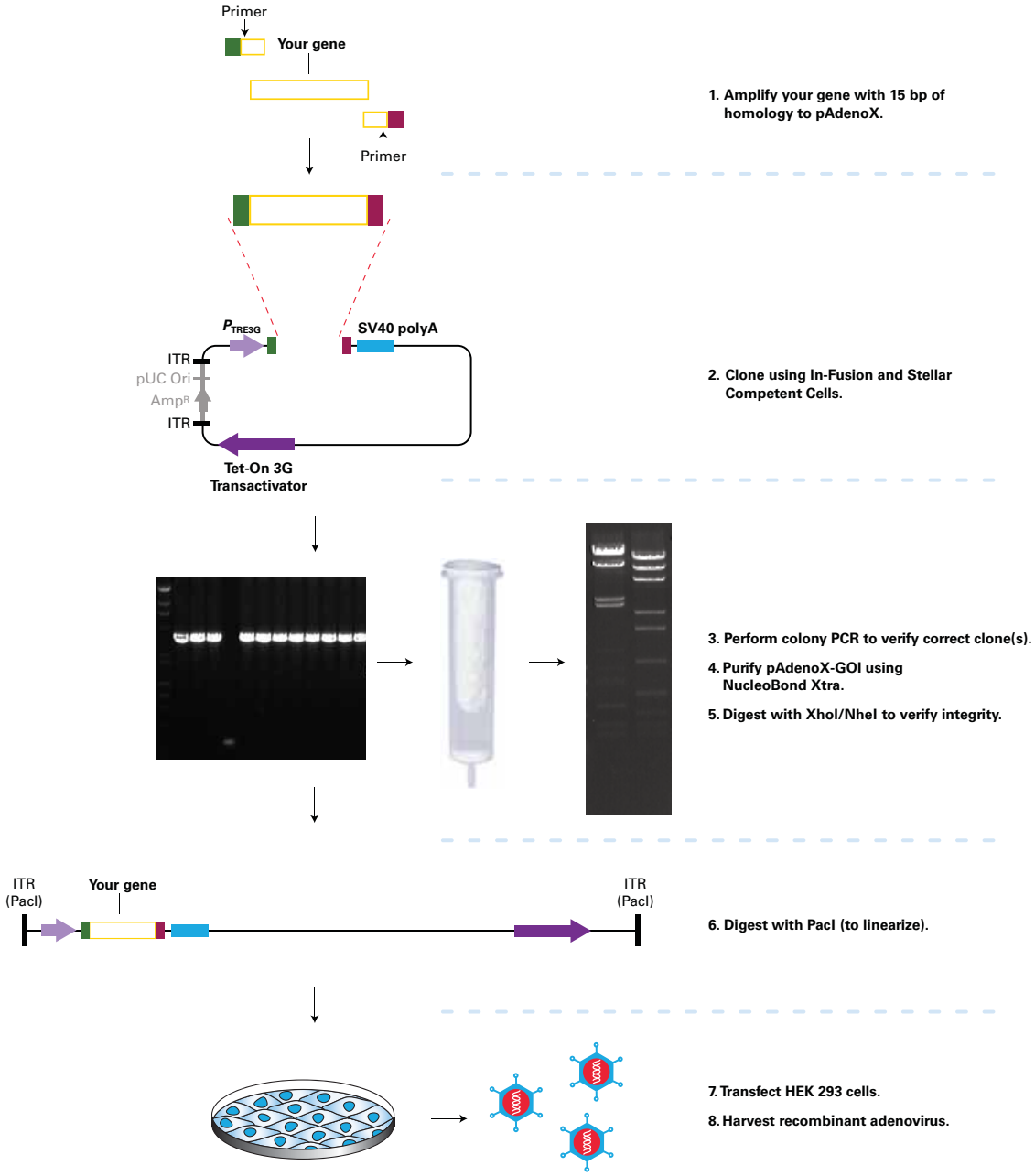

Figure 1. Constructing recombinant adenovirus with In-Fusion technology. DNA sequences can be rapidly transferred as PCR products to any pAdenoX vector using the In-Fusion cloning method. In this example, your gene of interest is amplified with 15 bp extensions that are homologous to the ends of the 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.

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

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 (1).

2. Tet-On 3G transactivator protein—compared to early generations mutations have significantly increased its sensitivity to the inducer doxycycline (2).

When the two elements are combined, not only can you detect high expression of your protein after exposure to Dox, you can control the level of expression by titration of the Dox concentration (Figure 2) and you can generate very high fold induction, up to 3000 fold difference between the induced and uninduced states (Figure 3). Maximum expression level can be manipulated by increasing the amount of virus per cell (Figure 4).

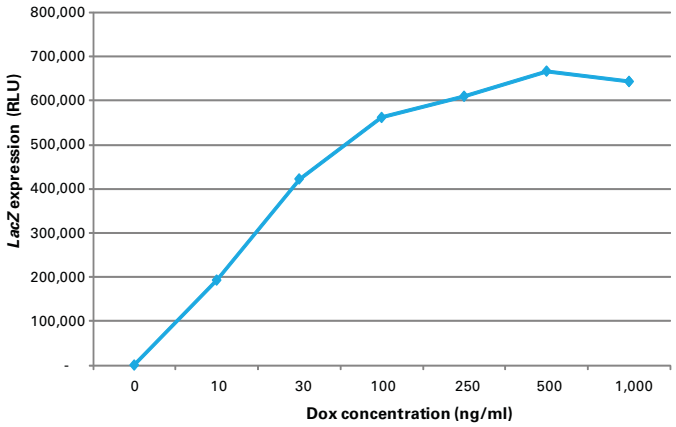

Figure 2. The Adeno-X Tet-On 3G Systems are highly inducible. Using equal amounts of high-titer supernatants, HeLa cells cultured at the indicated concentrations of Dox were infected with Adeno-X Tet-On 3G LacZ virus. Cultures were harvested and assayed for β-galactosidase activity using the Luminescent β-gal Reporter System 3 (Cat. No. 631713).

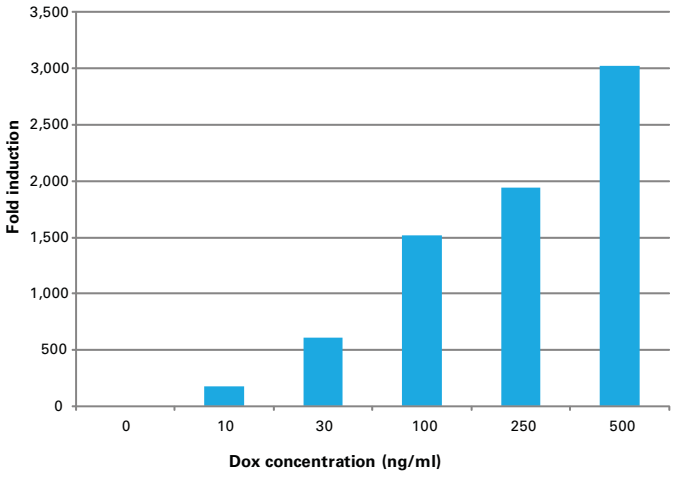

Figure 3. The Adeno-X Tet-On 3G Systems generate very high-fold induction, with up to 3000 fold difference between induced and uninduced states. Using equal amounts of high-titer supernatants, HeLa cells cultured at the indicated concentrations of Dox were infected with Adeno-X Tet-On 3G Luciferase virus. Cultures were harvested and assayed for luciferase activity.

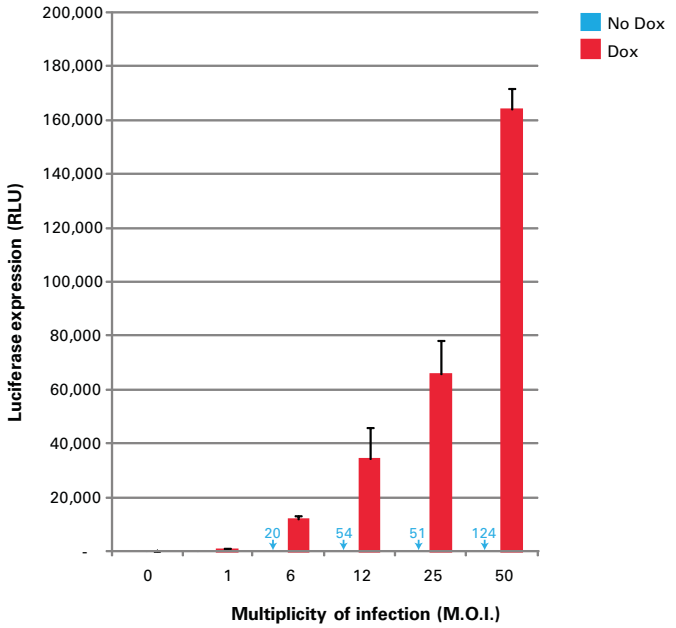

Figure 4. Expression increases with higher MOIs. HeLa cells were infected with varying M.O.I.s of pAdenoX Tet-On 3G adenovirus that expresses luciferase. After 4 hours, the media was replaced with fresh media +/- doxycycline (1 µg/ml). Cultures were harvested and assayed for luciferase activity. Maximal expression increases with increasing M.O.I., which also results in a slight increase in background expression.

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

Unlike the leading competitor, Adeno-X really is easy

Compared to the leading competitor system, which requires 8 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 2-3 days. Adeno-X uses no shuttle vector so requires no subcloning, and a single high-performance *E.coli* strain (Stellar) is included with the kit (see Table I).

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 (3). 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 5).

References

1. Löw, R. *et al.* (2010) *BMC Biotechnology* **10**:81.
2. Zhou, X. *et al.* (2006) *Gene Ther.* **13**(19):1382–1390.
3. Zhu, B. *et al.* (2007) *BioTechniques* **43**(3):354–359.

Table I: Comparison of the Adeno-X System 3 to the Leading 'Easy' Competitor

	Adeno-X	Competitor
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	• 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	• Mini-prep 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

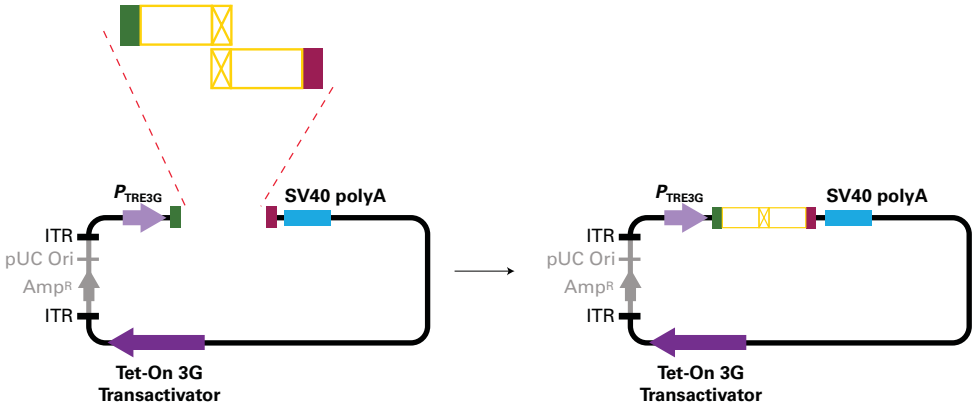

Figure 5. Create a point mutation and clone in one step using In-Fusion.

Product	Size	Cat. No.
Adeno-X Adenoviral System 3 (Tet-On 3G Inducible)	each	631180
Adeno-X Rapid Titer Kit	100 titrations	632250
Tet System Approved FBS	500 ml	631106
Doxycycline	5 g	631311