Certificate of Analysis

Adeno-X™ Tet-On® 3G Vector Set

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Catalog No.	Amount	Lot Number
631179 (Not sold separately)	10 rxns	Specified on product label.

Product Information

The Adeno-X Tet-On 3G Vector Set is supplied with the Adeno-X Adenoviral System 3 (Tet-On 3G Inducible) [Cat. No. 631180]. This system provides the tight control and high sensitivity of our Tet-On 3G tetracycline-inducible expression systems in an all-in-one adenoviral vector format. pAdenoX-Tet3G is a prelinearized, adenoviral vector that is ready for the insertion of your gene using In-Fusion® HD PCR Cloning technology. Simply PCR-amplify your gene of interest 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.

Package Contents

- 10 µl pAdenoX-Tet3G (Linear) Vector (200 ng/µl)
- 50 µl Adeno-X Screening Primer Mix 3 (10 µM)
- 20 µl Adeno-X Control Fragment (50 ng/µl)

Storage Conditions

- Store plasmids at –20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Shipping Conditions

- Dry ice (–70°C)

Product Documents

Documents for our products are available for download at **takarabio.com/manuals**

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The following documents apply to this product:

- Adeno-X Adenoviral System 3 User Manual (PT5177-1)

Vector Information

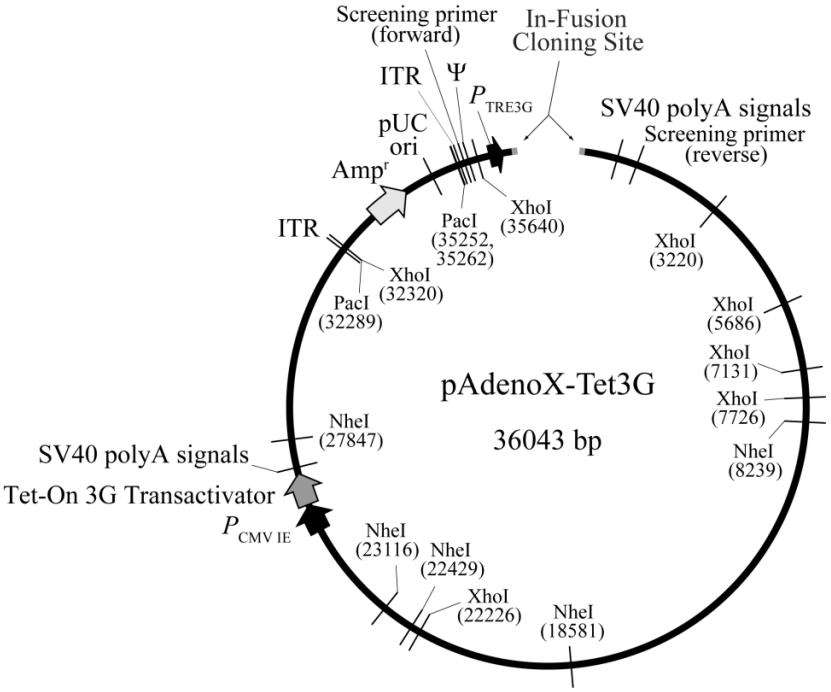

Figure 1. pAdenoX-Tet3G (Linear) Vector Map.

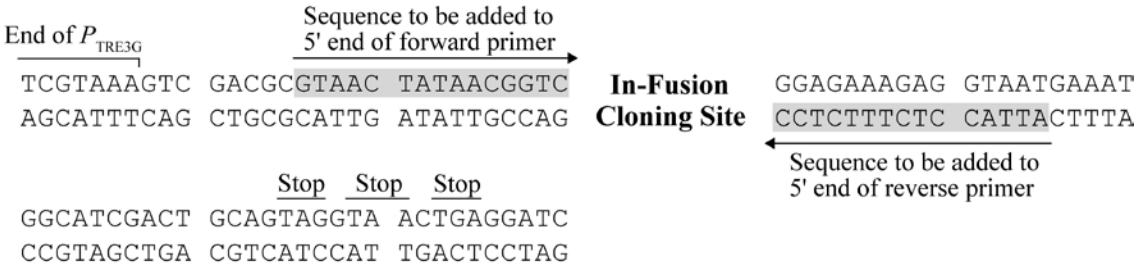

Figure 2. pAdenoX-Tet3G (Linear) Vector In-Fusion Cloning Site. The shaded regions indicate the 15 nucleotides that need to be added to the 5′ ends of your gene-specific PCR primers in order to create regions of homology with the vector. The sequence at each end is different to allow for directional cloning.

Description

The pAdenoX-Tet3G (Linear) Vector is a linearized, all-in-one tetracycline (Tet)-inducible, adenoviral expression vector designed to express a gene of interest under the control of the Tet-responsive promoter P_{TRE3G}. P_{TRE3G} exhibits exceptionally low basal activity; expression is induced by the binding of Tet-On 3G but is virtually silent in its absence (Loew et al. 2010).

The vector also expresses Tet-On 3G, a tetracycline-controlled transactivator that exhibits high activity in the presence of the inducer doxycycline (Dox), and exceptionally low activity in its absence. This 3rd generation Tet-On transactivator

demonstrates significantly increased sensitivity to Dox compared to its predecessors (Zhou et al. 2006). Expression of Tet-On 3G is driven by the human cytomegalovirus immediately early promoter ($P_{CMV\ IE}$).

pAdenoX-Tet3G contains a ΔE1/ΔE3, replication-deficient, type 5 adenovirus genome (Ad5) that is engineered for use in gene delivery and expression studies (Mizuguchi and Kay 1998; Mizuguchi and Kay 1999). The vector also includes a pUC origin of replication and an ampicillin resistance gene for propagation and selection in *E. coli*.

Location of Features

- SV40 polyA signals: 106–903
- Screening Primer (reverse) [complementary]: 936–955
- $P_{CMV\ IE}$ (human cytomegalovirus promoter): 25329–26010
- Tet-On 3G Transactivator: 26097–26843
- SV40 polyA signals: 26858–27312
- ITR (inverted terminal repeat): 32216–32275
- Amp^r (ampicillin resistance gene; β-lactamase): 33176–34036
- pUC origin of replication: 34681–34854
- ITR (inverted terminal repeat): 35265–35324
- Screening Primer (forward): 35368–35392
- Ψ (packaging signal): 35457–35605
- P_{TRE3G} (3^{rd} generation Tet-responsive promoter): 35645–36020

Additional Information

The pAdenoX-Tet3G (Linear) Vector is provided as part of the Adeno-X Adenoviral System 3 (Tet-On 3G Inducible) (Cat. No. 631180) and is designed for effortless cloning with In-Fusion cloning technology. Genes cloned into the vector must have a start codon. In some cases, the addition of a Kozak consensus sequence (Kozak 1987) may improve expression levels; however, many genes have been efficiently expressed in Tet gene regulation systems without the addition of a Kozak sequence. Before infecting cells with pAdenoX-Tet3G constructs, it is necessary to linearize the constructs with PacI and transfect them into HEK 293 cells, where they will be packaged into viral particles.

pAdenoX-Tet3G constructs are used to develop stable, Tet-inducible gene expression systems in mammalian cell lines. The addition of Dox to the system causes Tet-On 3G to undergo a conformational change that allows it to bind to P_{TRE3G}, activating transcription of the gene of interest in a highly dose-dependent manner. Additional information can be found in the Adeno-X Adenoviral System 3User Manual (PT5177-1).

Propagation in *E. coli*

- Recomended host strain: Stellar™ Competent Cells
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

NOTE: The viral supernatants produced by transfecting HEK 293 cells with recombinant pAdeno-X Viral DNA could, depending on your DNA insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant adenovirus. **The user is strongly advised not to create adenoviruses capable of expressing known oncogenes.** Appropriate NIH, regional, and institutional guidelines apply, as well as guidelines specific to other countries. NIH guidelines require that adenoviral production and transduction be performed in a Biosafety Level 2 facility. For more information, see appropriate HHS publications.

References

Kozak, M. An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* **15,** 8125–48 (1987).

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

Mizuguchi, H. & Kay, M. A. Efficient construction of a recombinant adenovirus vector by an improved in vitro ligation method. *Hum. Gene Ther.* **9,** 2577–83 (1998).

Mizuguchi, H. & Kay, M. A. A simple method for constructing E1- and E1/E4-deleted recombinant adenoviral vectors. *Hum. Gene Ther.* **10,** 2013–7 (1999).

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).

Quality Control Data

Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Vector	Enzyme(s)	Fragment(s)
pAdenoX-Tet3G	NheI	687, 3848, 4731, 10342, & 16475 bp
	XhoI	595, 1445, 2466, 3320, 3663, 10094, & 14500 bp
	PacI	10, 2963, & 33110 bp

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280}: 1.8–2.0

Functional Testing of Linear Markers

The Adeno-X Adenoviral System 3 (Tet-On 3G Inducible) was tested using the control fragment (*lacZ*) according to the protocol described in the Adeno-X Adenoviral System 3 User Manual (PT5177-1). Chemically competent Stellar *E. coli* cells were transformed with 1.5 µl of the In-Fusion reaction mixture. After 60 min at 37°C in SOC medium, the cells were plated on agar containing 100 µg/ml ampicillin. Transformants were grown at 37°C for 24–30 hrs. PCR colony screening with the Adeno-X Screening Primer Mix revealed that >50% of the resultant colonies contained recombinant adenoviral DNA.

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

Notice to Purchaser

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CATALOG NO.

631179

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STATEMENT 25

This product is covered by U.S. Patent No. 6,303,362.

STATEMENT 42

Use of the Tetracycline controllable expression systems (the "Tet Technology") is covered by a series of patents including U.S. Patent # 7541446, # 8383364, # 9181556 , European patents EP # 1200607, # 1954811, #2352833 and corresponding patent claims outside these regions which are proprietary to TET Systems GmbH & Co. KG. Academic research institutions are granted an automatic license with the purchase of this product to use the Tet Technology only for internal, academic research purposes, which license specifically excludes the right to sell, or otherwise transfer, the Tet Technology or its component parts to third parties. Notwithstanding the above, academic and not-for profit research institutions whose research using the Tet Technology is sponsored by for profit organizations, which shall receive ownership to any data and results stemming from the sponsored research, shall need a commercial license agreement from TET Systems in order to use the Tet Technology. In accepting this license, all users acknowledge that the Tet Technology is experimental in nature. TET Systems GmbH & Co. KG makes no warranties, express or implied or of any kind, and hereby disclaims any warranties, representations, or guarantees of any kind as to the Tet Technology, patents, or products. All others are invited to request a license from TET Systems GmbH & Co. KG prior to purchasing these reagents or using them for any purpose. Takara Bio USA, Inc. is required by its licensing agreement to submit a report of all purchasers of the Tet-controllable expression system to TET Systems.

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