Certificate of Analysis

Adeno-X™ Promoterless Vector Set

Contents

Catalog No.	Amount	Lot Number
632260 (Not sold separately)	10 rxns	Specified on product label.

Product Information

The Adeno-X Promoterless Vector Set is supplied with the Adeno-X Adenoviral System 3 (Universal) [Cat. No. 632266], which lets you an expression cassette of your choice in an adenoviral vector format. The system provides a perfect platform for the tissue-specific expression of your gene of interest, or for the efficient delivery of shRNA or microRNA. pAdenoX-PRLS is a prelinearized adenoviral vector that is ready for the insertion of an expression cassette by In-Fusion® HD PCR Cloning technology. Simply PCR-amplify your expression cassette and combine it with pAdenoX-PRLS 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-PRLS (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 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 User Manuals

User manuals for Clontech products are available for download at **www.clontech.com/manuals**
The following user manual applies to this product:

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

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(PA114269)

Vector Information

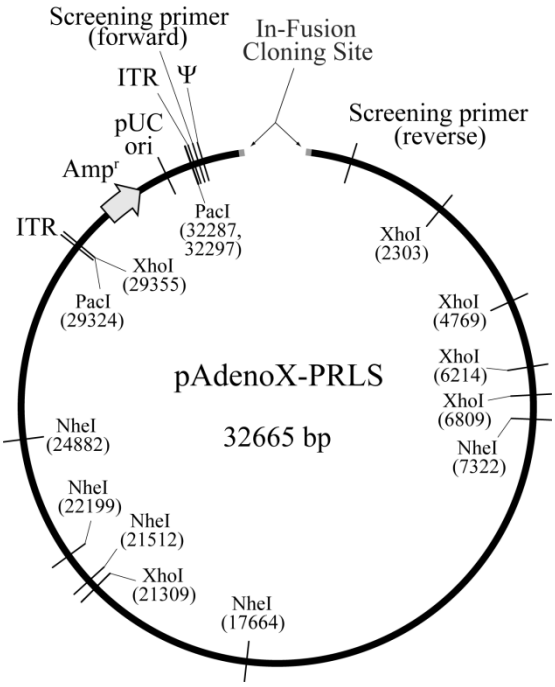

Figure 1. pAdenoX-PRLS (Linear) Vector Map.

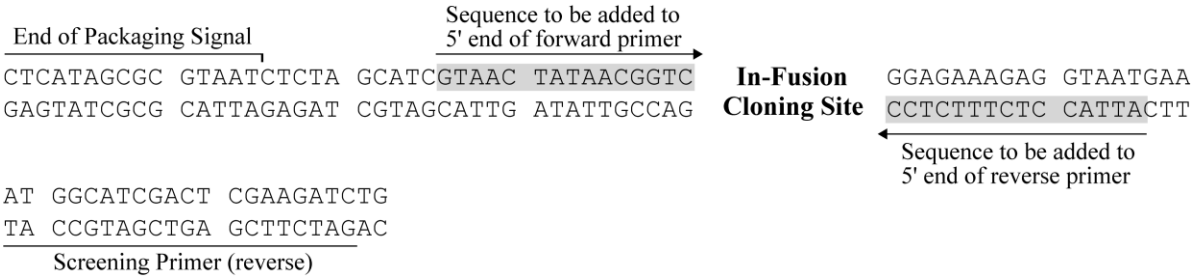

Figure 2. pAdenoX-PRLS (Linear) Vector In-Fusion Cloning Site. The shaded regions indicate the 15 nucleotides that need to be added to the 5′ ends of your gene-specific PCR primers in order to create regions of homology with the vector. The sequence at each end is different to allow for directional cloning.

Description

The pAdenoX-PRLS (Linear) Vector is a linearized adenoviral vector that lets you create your own gene expression systems in mammalian cells. The ends of the vector serve as the In-Fusion cloning site, allowing direct and rapid cloning of your gene system of interest. The vector contains no expression control elements—such as promoters or polyadenylation signals—flanking the cloning site, providing the perfect framework for the insertion of an entire expression cassette.

pAdenoX-PRLS contains a ΔE1/ΔE3, replication-deficient, type 5 adenovirus genome (Ad5) that is engineered for use in gene delivery and expression studies (1, 2). The Ad5 genome is flanked by inverted terminal repeats (ITR), which are necessary for the replication of adenoviral DNA. The vector also contains a pUC replication origin and an ampicillin resistance gene (Amp^r) for propagation and selection in *E. coli*.

Location of Features

- Screening Primer (reverse) [complementary]: 19–38
- ITR (inverted terminal repeat): 29251–29310
- Amp^r (ampicillin resistance gene; β-lactamase): 30211–31071
- pUC origin of replication: 31716–31889
- ITR (inverted terminal repeat): 32300–32359
- Screening Primer (forward): 32403–32427
- Ψ (packaging signal): 32492–32640

Additional Information

The pAdenoX-PRLS (Linear) Vector is provided as part of the Adeno-X Adenoviral System 3 (Universal) [Cat. No. 632266], and is designed for effortless cloning with In-Fusion cloning technology. Genes cloned into the vector must have a promoter, start and stop codons, and a polyA signal. In some cases, the addition of a Kozak consensus sequence (3) may improve expression levels.

pAdenoX-PRLS constructs are used to develop constitutive gene expression systems in mammalian cell lines. Before infecting cells with pAdenoX-PRLS constructs, however, it is necessary to linearize the constructs with PacI and transfect them into HEK 293 cells, where they will be packaged into viral particles.

Propagation in *E. coli*

- Recommended 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

1. Mizuguchi, H. & Kay, M. A. (1998) *Hum. Gene Ther.* **9**(17):2577–2583.
2. Mizuguchi, H. & Kay, M. A. (1999) *Hum. Gene Ther.* **10**(12):2013–2017.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**(20):8125–8148.

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	Enzymes	Fragment Sizes
pAdenoX-PRLS	NheI	687, 2683, 3848, 10342, & 15145 bp
	XhoI	595, 1445, 2466, 5653, 8046, & 14500 bp
	PacI	10, 2963, & 29732 bp

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

Functional Testing

The Adeno-X Adenoviral System 3 (Universal) 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.

Notice to Purchaser

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CATALOG NO.
632260

NOTICE TO PURCHASER:
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LICENSING STATEMENTS:

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

This document has been reviewed and approved by the Clontech Quality Assurance Department.

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