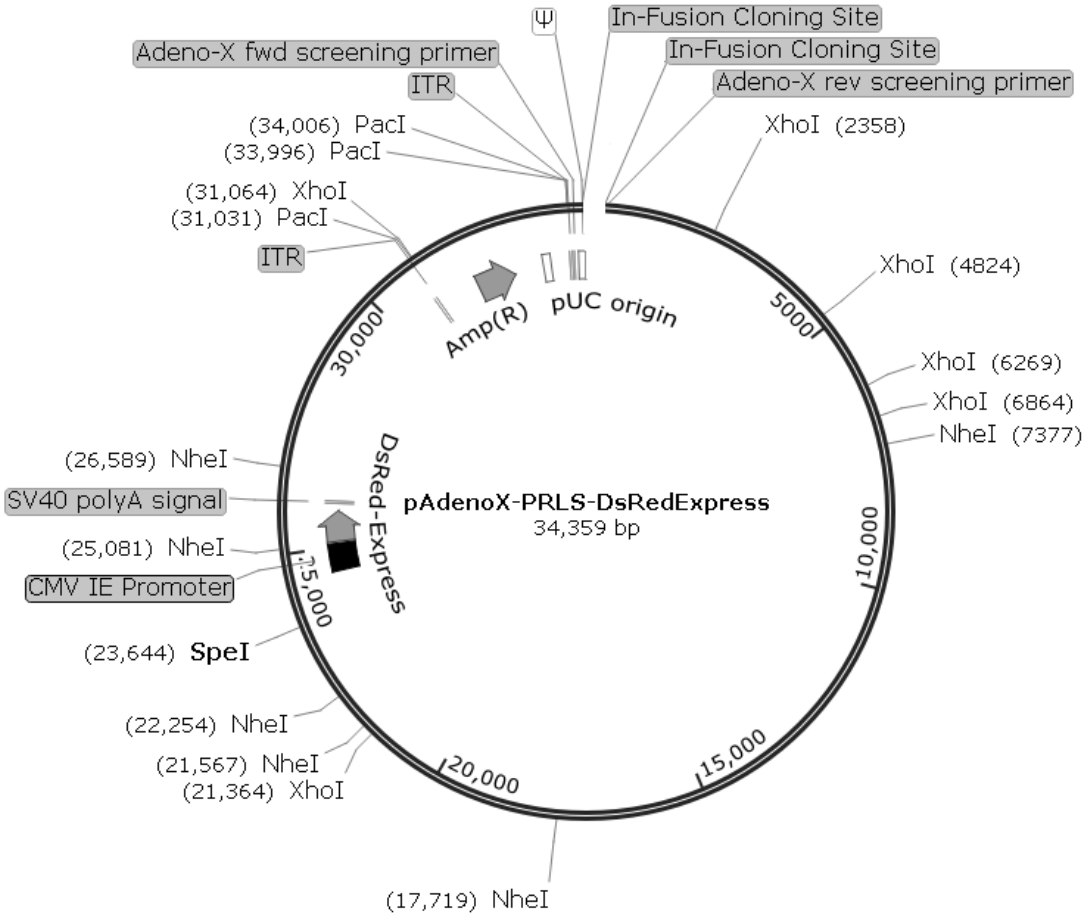
pAdenoX-PRLS-DsRedExpress

Catalog No.

632259 (Not sold separately). Sold as part of 632265.



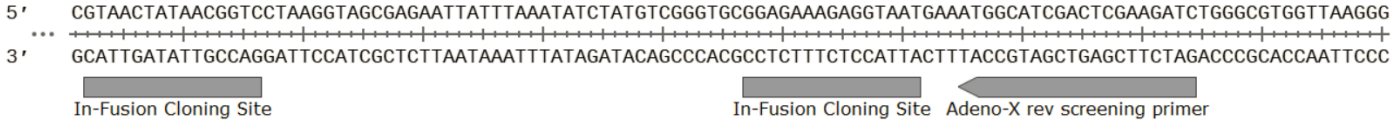


Figure 1. pAdenoX-PRLS-DsRedExpress vector map and In-Fusion cloning site. The shaded regions indicate the 15 nucleotides that need to be added to the 5′ ends of your gene-specific PCR primers in order to create regions of homology with the vector. The sequence at each end is different to allow for directional cloning.

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Description

The pAdenoX-PRLS-DsRedExpress (Linear) Vector is a linearized adenoviral expression 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.

The vector expresses DsRed-Express, a rapidly maturing variant of the *Discosoma* sp. red fluorescent protein (DsRed). Constitutive expression of DsRed-Express—driven by the human cytomegalovirus immediately early promoter ($P_{CMV\ IE}$)—allows you to observe individual virus-producing cells during the entire adenoviral production process. DsRed-Express is easily detected with standard rhodamine/propidium iodide filter sets (excitation and emission maxima are 557 nm, and 583 nm, respectively; Bevis & Glick, 2002).

pAdenoX-PRLS-DsRedExpress also contains a ΔE1/ΔE3, replication-deficient, type 5 adenovirus genome (Ad5) that is engineered for use in gene delivery and expression studies (Mizuguchi & Kay, 1998, 1999). The Ad5 genome is flanked by inverted terminal repeats (ITR), which are necessary for the replication of adenoviral DNA. The vector also includes a pUC origin of replication and an ampicillin resistance gene for propagation and selection in *E. coli*.

Location of Features

- Screening Primer (reverse) [complementary]: 75–94
- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 24491–25079
- DsRed-Express: 25118–25795
- SV40 polyA signals: 25947–25997
- ITR (inverted terminal repeat): 30921–32781
- Amp^r (ampicillin resistance gene; β-lactamase): 31921–32781
- pUC origin of replication: 33426–33599
- ITR (inverted terminal repeat): 34010–34169
- Screening Primer (forward): 34113–34137
- Ψ (packaging signal): 34140–34288

Additional Information

The pAdenoX-PRLS-DsRedExpress (Linear) Vector is provided as part of the Adeno-X Adenoviral System 3 (Universal, Red) [Cat. No. 632265], 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 (Kozak, 1987) may improve expression levels.

pAdenoX-PRLS-DsRedExpress constructs are used to develop gene expression systems in mammalian cell lines. Before infecting cells with pAdenoX-PRLS-DsRedExpress 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.

Constitutive expression of DsRed-Express lets you directly monitor and optimize your initial transfection efficiency. In addition, the fluorescent protein lets you detect the virus as it begins to replicate within the packaging cells, allowing you to identify the best time to harvest adenoviral stocks during amplification, regardless of whether you are plaque-purifying the virus or collecting a population of viruses. Finally, DsRed-Express expression permits the detection of infected target

cells, e.g., the viral titer can be determined within 24–48 hr by counting infected cells visualized by fluorescence microscopy.

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

Excitation and emission maxima of DsRed-Express

- Excitation maximum = 557 nm
- Emission maximum = 583 nm

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

Bevis, B. J. & Glick B. S. Rapidly maturing variants of the Discosoma red fluorescent protein (DsRed) *Nat. Biotechnol*. **20,** 83–87 (2002).

Kozak, M. An analysis of 5'-noncoding sequences from 699 vertebrate messenger rNAS. *Nucleic Acids Res.* **15**, 8125–8148 (1987).

Mizuguchi, H. & Kay, M. A. Efficient Construction of a Recombinant Adenovirus Vector by an Improved In Vitro Ligation Method. *Hum. Gene Ther.* **9,** 2577–2583 (1998).

Mizuguchi, H. & Kay, M. A. A simple method for constructing E1- and E1/E4-deleted recombinant adenoviral vectors. *Hum. Gene Ther.* **10,** 2013–2017 (1999).

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