pAdenoX-ZsGreen1

Catalog No.
632261 (Not sold separately)
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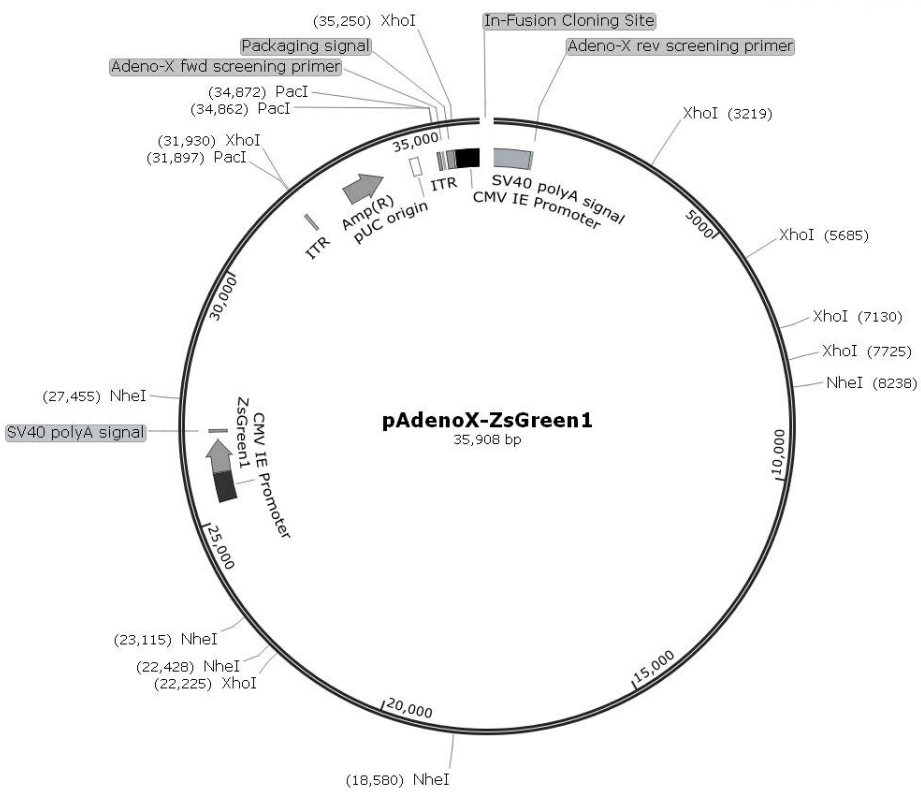

End of $P_{CMV\ IE}$ — Sequence to be added to 5' end of forward primer

```
CAGATCCGTC GACGCGTAAC TATAACGGTC   In-Fusion    GGAGAAAGAG GTAATGAAAT
GTCTAGGCAG CTGCGCATTG ATATTGCCAG   Cloning Site CCTCTTTCTC CATTACTTTA
```

Sequence to be added to 5' end of reverse primer

```
          Stop  Stop  Stop
GGCATCGACT GCAGTAGGTA ACTGAGGATC
CCGTAGCTGA CGTCATCCAT TGACTCCTAG
```

Figure 1. pAdenoX-ZsGreen1 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-ZsGreen1 Vector is a linearized adenoviral expression vector designed to constitutively express a gene of interest and ZsGreen1 in mammalian cells. The ends of the vector serve as the In-Fusion cloning site, allowing direct and rapid cloning of your gene of interest. Expression of the gene of interest and ZsGreen1 is driven by independent human cytomegalovirus immediately early promoters ($P_{CMV\ IE}$).

ZsGreen1 is a human codon-optimized variant of the reef coral *Zoanthus sp.* green fluorescent protein (ZsGreen) that has been engineered for brighter fluorescence (excitation and emission maxima: 493 and 505 nm, respectively (Matz et al. 1999; Haas et al. 1996). Constitutive expression of ZsGreen1allows you to observe individual virus-producing cells during the entire adenoviral production process.

pAdenoX-ZsGreen1 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, 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

- SV40 polyA signals: 106–903
- Screening Primer (reverse) [complementary]: 936–955
- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 25352–25940
- ZsGreen1: 25965–26657
- SV40 polyA signals: 26813–26863
- ITR (inverted terminal repeat): 31825–31884
- Amp^r (ampicillin resistance gene; β-lactamase): 32787–33647
- pUC origin of replication: 34292–34465
- ITR (inverted terminal repeat): 34876–34935
- Screening Primer (forward): 34979–35003
- Ψ (packaging signal): 35068–35216
- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 35256–35845

Additional Information

The pAdenoX-ZsGreen1 (Linear) Vector is provided as part of the Adeno-X Adenoviral System 3 (CMV, Green) [Cat. No. 632267] 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.

pAdenoX-ZsGreen1 constructs are used to develop constitutive expression systems in mammalian cell lines. Before infecting cells with pAdenoX-ZsGreen1 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 ZsGreen1 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, ZsGreen1 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 ZsGreen1

- Excitation maximum = 493 nm
- Emission maximum = 505 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

Haas, J., Park, E. C. & Seed, B. Codon usage limitation in the expression of HIV-1 envelope glycoprotein. *Curr. Biol.* **6,** 315–24 (1996).

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

Matz, M. V. *et al.* Fluorescent proteins from nonbioluminescent Anthozoa species. *Nat. Biotechnol.* **17**, 969–973 (1999).

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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