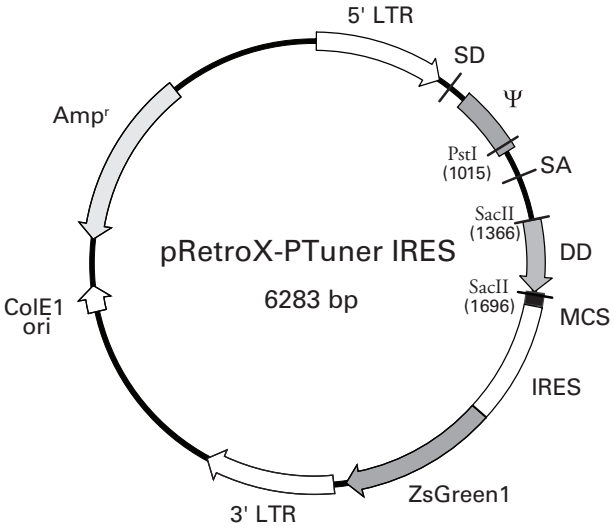
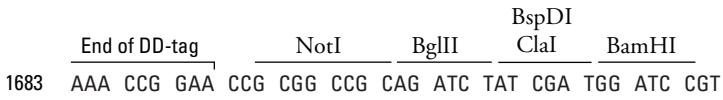
Vector Information

pRetroX-PTuner IRES Vector Information PT4039-5

Catalog No. 632167





pRetroX-PTuner IRES Vector Map and Multiple Cloning Site (MCS).

Description

pRetroX-PTuner IRES is a bicistronic, retroviral expression vector that allows you to precisely regulate the amount of your protein of interest in stably transduced mammalian cells. The vector encodes a 12 kDa, FKBP (L106P) destabilization domain (DD; 1) that is expressed as an N-terminal tag on your protein of interest; this domain causes the rapid degradation of any protein to which it is fused. Once expressed, the amount of DD-tagged protein present in the cell can be rapidly increased by the addition of Shield1 stabilizing ligand to the medium. Shield1 is a membrane permeable molecule that binds to the DD tag, 'shielding' the fusion protein from proteasomal degradation. pRetroX-PTuner IRES allows the simultaneous expression of your DD-tagged protein of interest and ZsGreen1 fluorescent protein from the same bicistronic mRNA transcript. Because ZsGreen1 is unaffected by the DD tag, it can be used as an indicator of transfection efficiency, as well as a marker for selection and cell sorting.

ZsGreen1 is a human codon-optimized variant of the reef coral *Zoanthus sp.* green fluorescent protein, ZsGreen (2). Bicistronic expression of ZsGreen1 and the DD-tagged protein of interest is facilitated by the encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES). This IRES allows cap-independent translation of ZsGreen1 from an internal start site at the IRES/ZsGreen1 junction (3). A gene cloned into the multiple cloning site (MCS), located between the DD and the IRES sequences, is expressed as a bicistronic message transcribed from the 5′ LTR.

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(PR113807; published 17 January 2011)

pRetroX-PTuner IRES is derived from the pMIN series of retroviral vectors (4, 5). These optimized vectors have the ability to produce high viral titers, and high levels of recombinant protein. In addition, because they lack retroviral structural genes (gag, pol, and env) necessary for retroviral particle formation and replication, these vectors exhibit improved safety profiles. pRetroX-PTuner IRES contains all of the necessary viral RNA processing elements; these include the 5′ and 3′ LTRs, the packaging signal (Ψ), and the tRNA primer-binding site. pRetroX-PTuner IRES also contains a ColE1 origin of replication, and an *E. coli* Amp^r gene for propagation and selection in bacteria.

Use

pRetroX-PTuner IRES is available in the RetroX-ProteoTuner Shield System N (w/ ZsGreen1) [Cat. No. 632167]. It is designed to efficiently deliver and co-express your DD-tagged protein of interest and ZsGreen1 in any mitotically active mammalian cell. In order to create your DD-tagged protein of interest, your gene of interest must be cloned into the MCS in the same reading frame as the DD tag sequence, and it must contain a stop codon at the end of its coding sequence.

In order to infect mammalian cells with pRetroX-PTuner IRES, the vector must be transfected into a packaging cell line, such as the RetroPack™ PT67 Cell line (631510), AmphoPack™ -293 (631505), EcoPack™ 2-293 (631507), Pantropic Retroviral Expression System (631512), or Retro-X™ Universal Packaging System (631530). These cell lines package RNA transcribed from the vector into infectious, replication-incompetent, retroviral particles. Such retroviral particles can transduce target cells and transmit the gene of interest, but cannot replicate within these cells due to the absence of viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimizes the chance of producing replication-competent virus due to recombination events during cell proliferation.

Stably transduced mammalian cells express ZsGreen1, the brightest commercially available green fluorescent protein. The presence of this protein allows the selection of stable transductants by flow cytometry (or other detection methods) with standard FITC filter sets (ZsGreen1 has an excitation maximum of 493 nm and an emission maximum of 505 nm).

When cells expressing a DD-tagged protein of interest are grown in medium containing Shield1, the ligand binds to the DD tag and protects the fusion protein from degradation. As a result, the protein quickly accumulates inside the cells in amounts that are directly proportional to the concentration of Shield1 in the medium. If the cells are subsequently grown in medium lacking Shield1, the DD tag is no longer stabilized, and the fusion protein is rapidly degraded. Because the effects of Shield1 are concentration-dependent and reversible, it is possible to fine-tune the amount of fusion protein present in the cells simply by adjusting the concentration of Shield1 in the medium (1).

Location of Features

- 5' MMLV LTR: 1–592
- SD (splice donor site): 650–655
- Ψ (packaging signal): 662–1067
- SA (intron containing splice acceptor site): 1075–1308
- DD (FKBP-L106P destabilization domain): 1368–1691
- MCS (multiple cloning site): 1692–1719
- IRES (EMCV): 1722–2296
- ZsGreen1 (*Zoanthus sp.* green fluorescent protein): 2297–2992
- 3' MMLV LTR: 3048–3639
- ColE1 origin of replication: 4492–4611
- Amp^r (ampicillin resistance gene; β-lactamase): 4819–5608 (complementary)

Propagation in *E. coli*

- Suitable host strains: Stellar™ Competent Cells.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Copy number: high

Notes:

The viral supernatants produced by this retroviral vector could, depending on your cloned insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant lentivirus. Appropriate NIH, regional, and institutional guidelines apply.

References

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2. BD Living Colors pIRES2-ZsGreen1 Vector (January 2005) *Clontechniqes* **XX**(1):8.
3. Jang, S. K. *et al.* (1988) *J. Virol.* **62**(8):2636–2643.
4. Yu S.S. *et al.* (2000) *Gene Ther.* **7**(9):797-804.
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