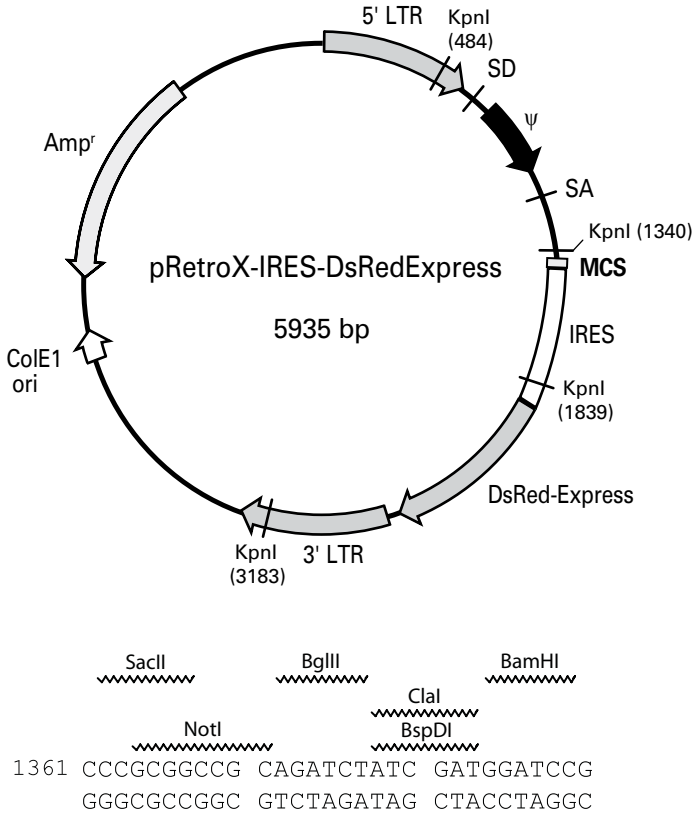
Vector Information

pRetroX-IRES-DsRedExpress Vector Information PT3965-5

Catalog No. 632521



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

Description

pRetroX-IRES-DsRedExpress is a bicistronic, fluorescent, retroviral vector that allows both a gene of interest and the DsRed-Express gene to be translated from a single bicistronic mRNA. pRetroX-IRES-DsRedExpress is designed for efficient delivery and selection (by flow cytometry or other methods) of stably transduced mammalian cells expressing the DsRed-Express fluorescent protein and the protein of interest. This vector can be used to obtain stable cell lines without time-consuming drug and clonal selection. DsRed-Express is a rapidly maturing variant of *Discosoma sp*. red fluorescent protein (DsRed) and is easily detected with standard Rhodamine/propidium iodide filter sets (1).

Bicistronic expression from this vector is facilitated by the encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES). This IRES facilitates cap-independent translation from an internal start site at the IRES/DsRed-Express junction (2). DsRed-Express contains nine amino acid substitutions that enhance its solubility, reduce its green emission, and accelerate its maturation (3). This retroviral vector is derived from the pMIN series of vectors (4, 5). These optimized vectors have the ability to produce high viral titers, express genes at high levels, and, due to the absence of retroviral coding sequences, exhibit improved safety profiles. The multiple cloning site (MCS) in pRetroX-IRES-DsRedExpress is between the 5′ MMLV LTR and the IRES sequence. Genes cloned into the MCS are expressed as a bicistronic message transcribed from the 5′ LTR.

pRetroX-IRES-DsRedExpress 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. For safety reasons, however, the vector lacks the structural genes (*gag*, *pol*, and *env*) necessary for retroviral particle formation and replication. pRetroX-IRES-DsRedExpress contains a ColE1 origin of replication, and an *E. coli* Amp^r gene for propagation and selection in bacteria.

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Use

pRetroX-IRES-DsRedExpress is designed to efficiently deliver and co-express your gene of interest and DsRed-Express in any mitotically-active mammalian cell. When cloning into pRetroX-IRES-DsRedExpress, your gene of interest should contain an initiation codon (ATG) and a stop codon. The vector can be infected or transfected into mammalian cells. If required, stable transformants can be selected by flow cytometry or limiting dilution.

In order to infect mammalian cells with pRetroX-IRES-DsRedExpress, 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 Expression System (631512), or Retro-X™ Universal Packaging System (631530). These cell lines package RNA from the vector into infectious, replication-incompetent, retroviral particles. Such retroviral particles can infect 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.

Location of features

- 5′ MMLV LTR: 1-592
- Ψ (packaging signal): 662-1067
- Splice Donor Site: 650-655
- Intron (containing Splice Acceptor Site): 1075-1308
- Multiple Cloning Site (MCS): 1361-1390
- EMCV IRES: 1392-1966
- *Discosoma sp*. red fluorescent protein (DsRed) gene: 1967-2644
- 3′ MMLV LTR: 2700-3291
- ColE1 origin of replication: 4144-4263
- Ampicillin resistance gene (β-lactamase): 4471-5328 (complementary)

Propagation in *E. coli*

- Suitable host strains: DH5α, Fusion Blue, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Copy number: high

Note:

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 retrovirus. Appropriate NIH, regional, and institutional guidelines apply.

References

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3. Bevis, B. J. & Glick, B. S. (2002) *Nat. Biotechnol.* **20**:83–87.
4. Yu S.S. *et al.* (2000) *Gene Ther.* **7**(9); 797-804.
5. Lee, J.T. *et al.* (2004) *Gene Ther.* **11**(1); 94-99.

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