Certificate of Analysis

Lenti-X™ CherryPicker™ Cell Capture (IRES) Vector Set

Catalog No.	Amount	Lot Number
632581 (Not sold separately) Sold as a part of 632575.	Each	Specified on product label.

Product Information

The Lenti-X CherryPicker Cell Capture (IRES) Vector Set allows you to identify, monitor, and capture mammalian cells expressing your protein of interest. The vector set contains a bicistronic, lentiviral expression vector (pLVX-CherryPicker2) that allows simultaneous, constitutive expression of a protein of interest and a membrane-targeted red fluorescent protein (CherryPicker) that is displayed on the cell surface. Cells expressing CherryPicker can be captured on magnetic beads via a CherryPicker-specific antibody. As a result, co-expression of your protein of interest and CherryPicker allows you to easily: (1) monitor cells expressing the protein of interest; (2) capture and analyze cells expressing the protein of interest; and (3) culture the captured cells as a more homogeneous population. The lentiviral expression system allows you to generate high-titer lentivirus for transducing virtually any dividing or nondividing mammalian cell type, including primary and stem cells. The vector set includes pLVX-CherryPicker2 (described above) and a control vector (pLVX-CherryPicker Control) that constitutively express CherryPicker in mammalian cells.

Package Contents

- 20 µl pLVX-CherryPicker2 Vector (500 ng/µl)
- 20 µl pLVX-CherryPicker Control Vector (500 ng/µl)

Storage Conditions

- Store plasmids 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.

Storage Buffer

- 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)

Concentration

- 500 ng/µl

Shipping Conditions

- Dry ice (–70°C)

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Product Documents

Documents for our products are available for download at **takarabio.com/manuals**
The following documents apply to this product:

- CherryPicker Assay Kit Protocol-At-A-Glance
- pLVX-CherryPicker2 Vector Information
- pLVX-CherryPicker Control Vector Information

pLVX-CherryPicker2 and pLVX-CherryPicker Control Vector Information

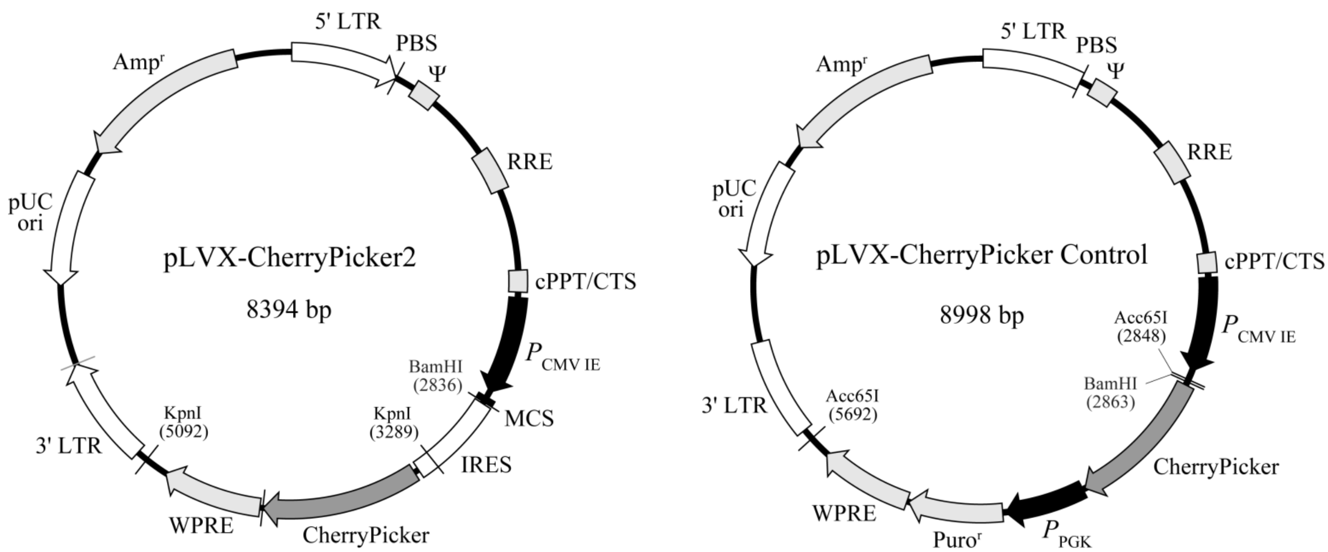

Figure 1. pLVX-CherryPicker2 and pLVX-CherryPicker Control Vector Maps.

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                                          NotI
               XhoI      SpeI       XbaI              BamHI
2801 GTGAATTCCT CGAGACTAGT TCTAGAGCGG CCGCGGATCC
     CACTTAAGGA GCTCTGATCA AGATCTCGCC GGCGCCTAGG
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Figure 2. pLVX-CherryPicker2 Vector Multiple Cloning Site (MCS).

Description

pLVX-CherryPicker2 is a bicistronic, HIV-1-based, lentiviral expression vector that allows the simultaneous, constitutive expression of a protein of interest and a membrane-targeted red fluorescent protein (CherryPicker) that is displayed on the cell surface. CherryPicker is a fusion protein composed of the fluorescent protein mCherry and the transferrin receptor membrane-anchor domain (Shaner et al. 2004; Winnard et al. 2007). Expression of the fusion protein can be monitored using fluorescence microscopy or flow cytometry. The excitation and emission maxima of mCherry are 587 nm and 610 nm, respectively. Because the membrane-anchor domain causes the fusion protein to be localized on the cell surface, cells expressing the fusion—and your protein of interest—can be bound by CherryPicker-specific antibodies and captured on magnetic beads. Both the antibody and the magnetic beads are provided in the CherryPicker Assay Kit (Cat. No. 632570 & 632571).

Constitutive expression of the bicistronic transcript is driven by the human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$), located just upstream of the multiple cloning site (MCS). Simultaneous expression of the protein of interest and

CherryPicker is made possible by the presence of an encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES; Jang et al. 1988) positioned between the MCS and the CherryPicker coding sequence. The IRES facilitates cap-independent translation of CherryPicker from an internal start site at the IRES/CherryPicker junction (Jang et al. 1988).

pLVX-CherryPicker2 contains all of the viral processing elements necessary for the production of replication-incompetent lentivirus, as well as elements to improve viral titer, transgene expression, and overall vector function. The woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) promotes RNA processing events and enhances nuclear export of viral and transgene RNA (Zufferey et al. 1999), leading to increased viral titers from packaging cells, and enhanced expression of your gene of interest in target cells. In addition, the vector includes a Rev-response element (RRE), which further increases viral titers by enhancing the transport of unspliced viral RNA out of the nucleus (Cochrane, Chen, and Rosen 1990). Finally, pLVX-CherryPicker2 also contains a central polypurine tract/central termination sequence element (cPPT/CTS). During target cell infection, this element creates a central DNA flap that increases nuclear import of the viral genome, resulting in improved vector integration and more efficient transduction (Zennou et al. 2000). In addition to lentiviral elements, pLVX-CherryPicker2 contains a pUC origin of replication and an *E. coli* ampicillin resistance gene (Amp^r) for propagation and selection in bacteria.

pLVX-CherryPicker Control is a control vector that constitutively expresses the CherryPicker fusion protein from the human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$). This vector was designed to allow confirmation of: (1) proper CherryPicker expression and localization; and (2) successful capture of transfected cells onto magnetic beads via a CherryPicker-specific antibody. The vector contains a puromycin resistance gene ($Puro^r$) under the control of the murine phosphoglycerate kinase promoter (P_{PGK}) for the selection of stable transductants. pLVX-CherryPicker Control is not intended to be used as a cloning vector.

Location of Features

pLVX-CherryPicker2 Vector

- 5′ LTR (5′ long terminal repeat): 1–635
- PBS (primer binding site): 636–653
- Ψ (packaging signal): 685–822
- RRE (Rev-response element): 1303–1536
- cPPT/CTS (central polypurine tract/central termination sequence): 2028–2151
- $P_{CMV\ IE}$ (cytomegalovirus immediate early promoter): 2185–2787
- MCS (multiple cloning site): 2803–2840
- IRES (internal ribosome entry site): 2842–3419
- CherryPicker (mCherry-transferrin receptor membrane-anchor domain fusion): 3417–4346
- WPRE (woodchuck hepatitis virus posttranscriptional regulatory element): 4363–4954
- 3′ LTR (3′ long terminal repeat): 5157–5793
- pUC origin of replication: 6262–6935 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 7080–8076 (complementary)

pLVX-CherryPicker Control Vector

- 5′ LTR (5′ long terminal repeat): 1–635
- PBS (primer binding site): 636–653
- Ψ (packaging signal): 685–822
- RRE (Rev-response element): 1303–1536

- cPPT/CTS (central polypurine tract/central termination sequence): 2028–2151
- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 2185–2787
- CherryPicker (mCherry-transferrin receptor membrane-anchor domain fusion): 2885–3811
- P_{PGK} (phosphoglycerate kinase promoter): 3825–4333
- Puro^r (puromycin resistance gene): 4354–4953
- WPRE (woodchuck hepatitis virus posttranscriptional regulatory element): 4967–5558
- 3′ LTR (3′ long terminal repeat): 5761–6397
- pUC origin of replication: 6866–7539 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 7684–8680 (complementary)

Additional Information

Genes cloned into the MCS must include an initiation codon (ATG). Before either of the pLVX-CherryPicker vectors can be transduced into target cells, it must be packaged into viral particles in HEK293T cells, using our Lenti-X™ HTX Packaging System (Cat. Nos. 631247 and 631249). This packaging system allows the safe production of high titer, infectious, replication-incompetent, VSV-G pseudotyped lentiviral particles that can infect a wide range of cell types, including nondividing and primary cells (Wu et al. 2000).

Cells expressing the gene of interest can be quickly identified by screening for mCherry fluorescence. When CherryPicker is expressed in mammalian cell cultures, red-emitting cells can be detected by flow cytometry or fluorescence microscopy 24 hr after transfection. Cells expressing your protein of interest and CherryPicker can be captured on magnetic beads via a CherryPicker-specific antibody. The resulting homogenous cell population can be analyzed immediately or cultured further.

Caution!
The viral supernatants produced by this lentiviral vector could 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.

Propagation in *E. coli*

- Recommended host strain: DH5α™, XL1 Blue, and other general-purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

Excitation and emission maxima of mCherry & CherryPicker

- Excitation maximum = 587 nm
- Emission maximum = 610 nm

References

Cochrane, A. W., Chen, C. H. & Rosen, C. A. Specific interaction of the human immunodeficiency virus Rev protein with a structured region in the env mRNA. *Proc. Natl. Acad. Sci. U. S. A.* **87,** 1198–1202 (1990).

Jang, S. K. *et al.* A segment of the 5’ nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. *J. Virol.* **62,** 2636–43 (1988).

Shaner, N. C. *et al.* Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat. Biotechnol.* **22,** 1567–1572 (2004).

Winnard, P. T., Kluth, J. B., Kato, Y., Artemov, D. & Raman, V. Development of novel chimeric transmembrane proteins for multimodality imaging of cancer cells) Development of novel chimeric transmembrane proteins for multimodality imaging of cancer cells. *Cancer Biol. Ther.* **6,** 1889–1899 (2007).

Wu, X. *et al.* Development of a novel trans-lentiviral vector that affords predictable safety. *Mol. Ther.* **2,** 47–55 (2000).

Zennou, V. *et al.* HIV-1 genome nuclear import is mediated by a central DNA flap. *Cell* **101,** 173–85 (2000).

Zufferey, R., Donello, J. E., Trono, D. & Hope, T. J. Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of transgenes delivered by retroviral vectors. *J. Virol.* **73,** 2886–92 (1999).

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	Enzyme(s)	Fragment(s)
pLVX-CherryPicker2	BamHI	8.4 kb
	KpnI	1.8 & 6.6 kb
pLVX-CherryPicker Control	BamHI	9.0 kb
	Acc65I	2.8 & 6.2 kb

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

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

Notice to Purchaser

Lenti-X™ CherryPicker™ Cell Capture (IRES) Vector Set

CATALOG NO.

632581

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11/13/2019

Notice to Purchaser

STATEMENT 55

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