GenTarget Inc

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Expression Lentivirus for Detection of Flp Recombination Reactions

Catalog Number	Product Name / Description	Amount
LVP1633	**FRT GFP/RFP ColorSwitch lentivirus (suCMV, Puro):** Lentivirus express "**FRT-GFP-Stop-FRT-RFP-Stop**" cassette under suCMV promoter, containing **puromycin** antibiotic selection.	**200ul** /vial x **(1 x10^8** IFU/ml, in PBS solution, premixed with 10 x Polybrene /60 ug/ml)
LVP1634	**FRT GFP/RFP ColorSwitch lentivirus (suCMV, Bsd):** Lentivirus express "**FRT-GFP-Stop-FRT-RFP-Stop**" cassette under suCMV promoter, containing **Blasticidin** antibiotic selection.	
LVP1635	**FRT GFP/RFP ColorSwitch lentivirus (EF1a, Puro):** Lentivirus express "**FRT-GFP-Stop-FRT-RFP-Stop**" cassette under **EF1a** promoter, containing **puromycin** antibiotic selection.	
LVP1636	**FRT GFP/RFP ColorSwitch lentivirus (EF1a, Bsd):** Lentivirus express "**FRT-GFP-Stop-FRT-RFP-Stop**" cassette under **EF1a** promoter, containing **Blasticidin** antibiotic selection.	

Storage: < -70 °C, avoid repeat freeze/thaw cycles. Stable for > 6 months.

Product Description:

1. Introduction:

1) GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into almost all kinds of mammalian cells, including stem cells, primary cells, and non-dividing cells both *in vivo* and *in vitro*. Lentiviral Particles stably integrate into the transduced cells' genome for long term expression, making it a great gene transfer agent.

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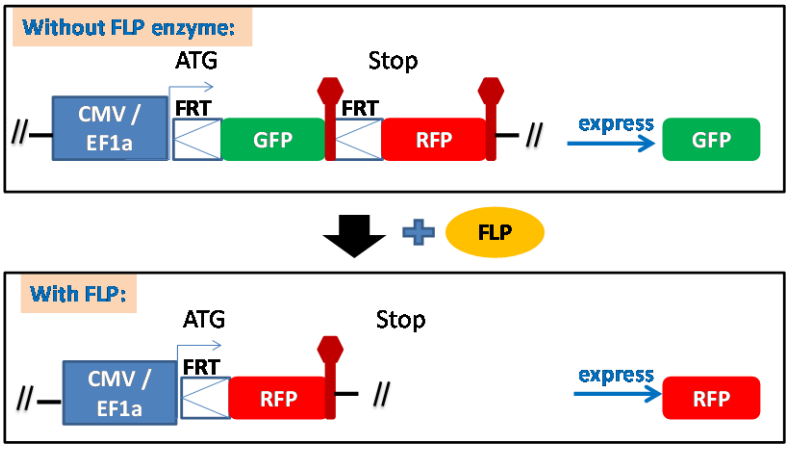
2) Flp-FRT recombination is a site-directed recombination technology. Flippase (Flp) is derived from the 2 μ plasmid of the yeast Saccharomyces cerevisiae. Flp recognizes and acts on DNA sequences known as Flippase Recognition Target (FRT) sites (see the sequence listed below) . Flp catalyzes site-specific recombination between two FRT sites. The recombination event involves the inversion or excision of the DNA segment located between the FRT sites, depending on their orientation. The Flp-FRT system provides a controlled means to manipulate and engineer DNA sequences.

FRT site: 5'gaagttcctattccgaagttcctattctctagaaagaataggaacttc3'

By inserting a "FRT-flanked expression target" into a host's genome, target expression can be controlled via Flp recombinase. Expression of FRT-flanked target occurs prior to the addition of Flp enzyme. When Flp is applied, it deletes the FRT flanked target segment and stops the target expression. Simultaneously, Flp-mediated recombination can activate expression of a second target downstream from the deleted segment.

GenTarget provides **Flp reporting lentivirus** for easy, fast and convenient testing and monitoring / detecting of Flp recombination efficiency *in vivo* and *in vitro*. This lentivirus has been engineered to constitutively express the "**FRT-GFP-stop-FRT-RFP-Stop**" cassette under either an enhanced CMV promoter or an enhanced EF1a promoter. CMV promoter provides the highest expression level in most cell types, EF1a promoter tends not to get silenced in long-term culture. (See the expression cassette scheme below).



Those products detect the occurrence of Flp-mediated recombination events via a "color switch" mechanism, thereby providing an essay, fast and continual monitoring for the presence of Flp or Flp recombination event.

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2. Desired Promoter and Selection Marker:

(1) The color-switch cassette is driven by either **CMV** promoter or **EF1a** promoter. You can pick the best promoter suitable for your cell types. The CMV give the highest expression level in most cell types. The EF1a promoter has medium to high express level and not subject to promoter silence.

(2) Those Flp reporting lentivirus contains an antibiotic selection of **puromycin (Puro), or Blasticidin (Bsd)**. The selection is expressed under an RSV promoter (not shown in the schematic above). These markers allow easy selection for transduction positive cells by antibiotic killing.

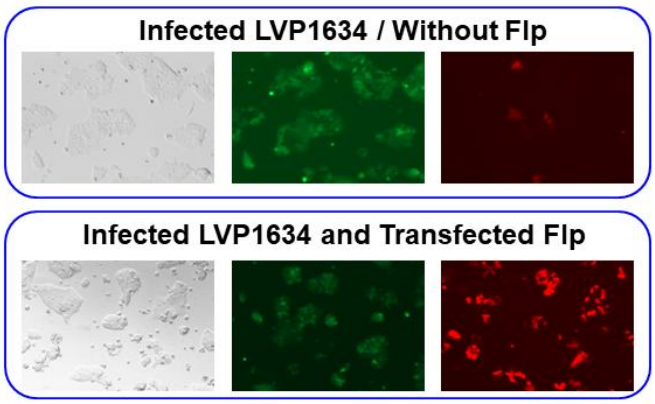
3. How ColorSwtich Reporting Works:

The Flp reporting lentivirus is used to monitor or detect the efficiency of Flp recombination *in vivo*. It is an easy and effective tool for verifying the performance of Flp-FRT system *in vivo*.

The Flp reporting lentivirus demonstrates the strong GFP fluorescence after infection into mammalian cells, but does not show the RFP fluorescence signal (or with minimal leaking RFP expression in some cells). Once the Flp protein is present in the nucleus (which can be delivered by Flp expression lentivirus (CAT#: LVP1624, or lipid transfection of a Flp expression plasmid).

Flp enzyme excises / deletes the DNA fragment between two FRT sites. As a result, the GFP is removed and the downstream RFP is expressed. You will observe the increases in RFP fluorescence positive cell percent and RFP signal intensity. The RFP signal can be easily monitored via fluorescence cell sorting, visualized by microscopy, or the fluorescence intensity measurement by fluorometer. See the sample results below.

Sample images of Flp-FRT recombination detection:



(Note: GFP filter wavelength: Ex450-490 ~Em525; RFP filter: ~Ex558/~Em583).

Top panel / without Flp: FLP reporter cell line (Cat#: SC096-Bsd) was cultured in a 24-well-plate. This cell line was generated by transduction of Flp reporting lentivirus (CAT#: LVP1634). Images were taken with a GFP filter set (Ex 490nm/Em 525nm) and an RFP filter set (Ex 535nm/Em 583nm).

Bottom panel / with Flp: FLP reporter cell line (Cat#: SC096-Bsd) was cultured in a 24-well-plate. Flp expression lentivirus (CAT#: LVP1624) was applied to cells. Images were taken at 48 hours post lentivirus transduction.

Notes:

1) Like any mammalian pol II promoter, the CMV promoter seek any possible ORFs, and in some cell types, it can slightly express the 2nd ORF (the RFP in this case) which is considered the basal or leaking RFP signal.

2) The efficiency of recombination mediated by the Flp-FRT systems can vary depending on the specific experimental conditions and the context in which they are used, such as the specific DNA sequences surrounding the recombination sites, the expression levels of the recombinase enzymes (Flp), and the experimental design.

3) Why you still observe the GFP signal after apply Flp enzyme on cells?
The reasons are:
(1) The weak recombination excise GFP cassette only in a subset of cells;
(2) Since each cell genome may be inserted multiple copies of FRT GFP/RFP cassettes, not all copies of GFP was excised in the cells. Therefore, in those cells, you will observe both GFP and RFP signal.
(3) The same Flp enzyme can catalyze the reverse reaction, restoring the original genomic configuration (i.e the excused GFP was reinstalled).
(4) The important observation is the dramatic increase in RFP positive cells following addition of Flp.

Application protocol:

1. **Adhesive cells Transduction Protocols:**

Note: A quick transduction protocol is: add 50ul virus into one well in 24-well-plate where cell density is at 50% ~ 75%. At 48hr to 72 hours after virus added (no need to change medium), visualize the positive rate under fluorescent microscope. For stable cell line generation, pass cell into antibiotic containing medium, or sort the cells via fluorescent signal. Or simply select the cells by antibiotics.

Day 0: Seed the desired cells in complete medium at appropriate density incubate overnight. (Note: at the time of transduction, it grows to 50% ~75% confluent.)

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For example, seed Hela cells at 0.5 x 10^5/ml x 0.5ml in a well of a 24-well plate;

Day 1: Remove the culture medium. Add fresh, warmed, complete medium (0.5ml). Thaw the Pre-made lentiviral stock at room temperature. Add appropriate amount of virus stock to obtain the desired MOI. Return cells to 37°C/CO^2 incubator. (Try to avoid thaw and freeze cycles for pre-made lentivirus. But if you cannot use all virus in one time, you still can re-freeze the virus at -80oC for future use. But virus titer will decrease by ~10% for each re-thaw.)

Day 3: At 48~72hr after transduction, check the transduction rate *via* fluorescence image with a suitable filter under fluorescent microscope, or calculate the exact transduction rate via Flow Cytometry System (FACS) or any flow cytometry (such as Guava machine). Note: You should only see GFP signal at this stage before you apply FLP enzyme to the cells.

Day 3 + : Transduced cells can be sorted out via FACS, selected by its specific antibiotics. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line. (Refer to any literatures about How to generate stable cell lines.).

Flp enzyme delivery: The selected cell should demonstrate strong GFP signal and should have no RFP signal. After the cell selection, the cells are ready used as an indicator cell line for Flp recombination activity.

- **Apply the Flp enzyme into the cells** (which can be achieved by infected cell with Flp expression lentivirus, CAT#: LVP1624, or by regular lipid-transfection of a Flp expression plasmid, or even simply by adding purified neu-clear penetrating Flp protein enzyme.
- Put cells in normal culture conditions for **48-72 hours**.
- **Detect Flp recombination reaction**: The RFP signal will gradually showed up and peaked at 48 hours or longer times (dependent upon Flp delivery methods) post the Flp delivery. The RFP signal intensity reflects the FLP-FRT recombination efficiency (rate). You can sort the cell by FACS machine, other meters, or visualize the RFP positive cell under fluorescent signal.

Safety Precaution:

Gentarget lentiviral particles adapts must advanced lentiviral safety features (using the third generation vectors with self-inactivation SIN-3UTR), and the premade lentivirus is replication incompetent. However, please use extra caution when using lentiviral particles. Use the lentiviral particles in Bio-safety II cabinet. Wear glove all the time when handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

References:

1. Senecoff JF, Rossmeissl PJ, Cox MM (May 1988). "DNA recognition by the FLP recombinase of the yeast 2 mu plasmid. A mutational analysis of the FLP binding site". Journal of Molecular Biology. 201 (2): 405–21. doi:10.1016/0022-2836(88)90147-7

2. Buchholz F, Angrand PO, Stewart AF (July 1998). "Improved properties of FLP recombinase evolved by cycling mutagenesis". Nature Biotechnology. 16 (7): 657–62.
3. Golic MM, Rong YS, Petersen RB, Lindquist SL, Golic KG (September 1997). "FLP-mediated DNA mobilization to specific target sites in Drosophila chromosomes". Nucleic Acids Research. 25 (18): 3665–71

Warranty:

This product is for research use only. It is warranted to meet its quality as described when used in accordance with its instructions. GenTarget disclaims any implied warranty of this product for particular application. In no event shall GenTarget be liable for any incidental or consequential damages in connection with the products. GenTarget's sole remedy for breach of this warranty should be, at GenTarget's option, to replace the products.

Attachment: GenTarget's pre-made lentivirus product categories.

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Repoter Lentivirus for all kinds of pathway screening assays
Cell Immortalization	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.
ImmunoOncology Research	Lentivirus products for immuno therapy research: CAR and TCR; Assay Cell Lines for T-cell targeted killing assay and other cell-based assays; over-expression lentivirus products for the immune response targets; Cell surface antigens (CDs); immune checkpoint / Receptors; CRISPR gene Repair and knock-IN lentivirus; CRISPR knockout lentivirus;
CAR-T, TCR Lentivirus	**CARs** Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; **TCRs**: MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRαV3-F2A-TRβV5-6;
CRISPR Gene Editing	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease, the **dCas9**, gRNAs, **CRISPR** gene editing research
Epigenomic: CRISPRi and CRISPRa	"**dCas9-Protein**" fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
Cell-Specific Reporter	a set of reporter lentiviruses to express a luminescence or fluorescent reporter (firefly Luciferase, Renilla luciferase, RFP or GFP fluorescent marker) under a

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Product Category	Product Description (please click into each category's page)
	tissue specific promoter
Infectious Antigens	Llentivirus that express all kinds of infectious antigens with C-term 6His-tag.
Virus Like Particles (VLP)	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
Non-integrating LV	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.
shRNA Knockdown	Knockdown verifeid and customized shRNA lentivirus for target knockdown,
microRNA lentivirus	Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA.
Anti-miNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
Human and mouse ORFs	Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin fusion dual markers.
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression**: firefly and Renilla, Red-Luc and more,** with different antibiotic selection markers.
Fluorescent Markers	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, niRFP, unstable GFP and others.
Luminescent Imaging	Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms
Sub-cellular Imaging	Lentivirus contain a well-defined organelle targeting signal fusioned to a fluorescent protein, great tools for live-cell imaging and for dynamic investigation of sub-cellular signal pathways.
Cytoskeleton Imaging	A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure
Unstable GFP	Lentivirus express the the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway

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Product Category	Product Description (please click into each category's page)
	assay and in knockdown / knockout detection
near-infrared RFP	The near-infrared Red fluorescent (niRFP) expression Lentiviurs provides the whole-body images with better contrast and brighter images
Fluorescent-ORF fusion	Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target.
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.
CRE, Flp ColorSwtich	Lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" or "FRT-GFP-Stop-FRT-RFP" cassette, used to monitor the CRE or Flp recombination event in vivo.
SEAP Reporter	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),
TetR Repressor	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
rtTA Expression	rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF inducible system.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic markers
LacZ expression	Express different full length **β- galactosidase (lacZ)** with different selection markers
Negative control lentiviruses	Premade **negative control lentivirus with different markers**: serves as the negative control of lentivurs treatment, for validation of the specificity of any lentivirus target expression effects.
Other Enzyme expression	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.
Ultra titer lentivirus	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.