



Pre-made Expression Lentivirus for Monitoring CRE Recombination Reactions

Catalog Number	Product Name / Description	Amount
LVP460-Puro & LVP460-Puro-PBS	LoxP GFP/RFP ColorSwitch lentivirus (Puro): Pre-made lentiviral particles expressing "LoxP-GFP-Stop-LoxP-RFP-Stop" cassette under suCMV promoter. It also contains a puromycin antibiotic selection marker under Rsv promoter.	200ul /per vial x (1x10 ⁷ IFU/ml) in DMEM medium with 10% FBS and 10x polybrene Or 200ul /per vial x (5x10 ⁷ IFU/ml) in PBS solution
LVP460-Neo & LVP460-Neo-PBS	LoxP GFP/RFP ColorSwitch lentivirus (Neo): Pre-made lentiviral particles expressing "LoxP-GFP-Stop-LoxP-RFP-Stop" cassette under suCMV promoter. It also contains a Neomycin antibiotic selection marker under Rsv promoter.	
LVP460-Bsd & LVP460-Bsd-PBS	LoxP GFP/RFP ColorSwitch lentivirus (Bsd): Pre-made lentiviral particles expressing "LoxP-GFP-Stop-LoxP-RFP-Stop" cassette under suCMV promoter. It also contains a Blasticidin antibiotic selection marker under Rsv promoter.	

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

Product Description

1. Instruduction:

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.

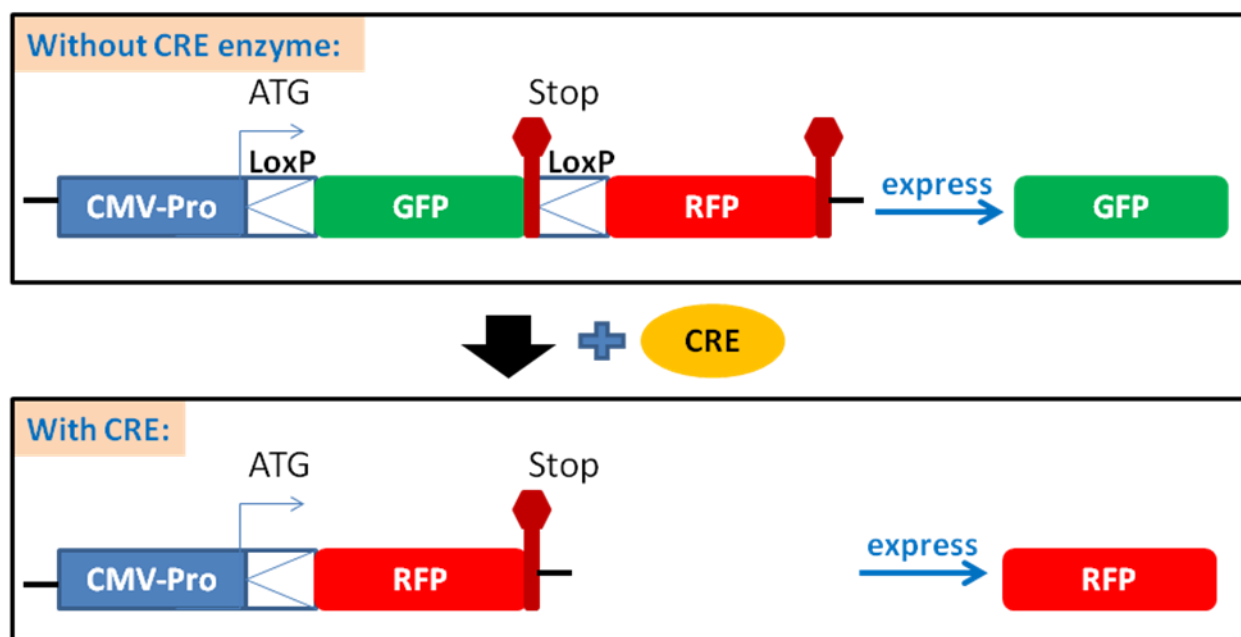
CRE recombinase, from bacteriophage P1, catalyzes recombination between 34 base-pair target sequences called lox sites and can join individual plasmids



containing lox sites. CRE recombination provides an excellent tool for conditional gene targeting in transgenic animal models by linking genotypic alterations to the biological outcomes (phenotypes).

By inserting a "LoxP-flanked expression target" into a host's genome, target expression can be controlled via CRE recombinase. Expression of LoxP-flanked target genes may occur prior to the addition of CRE enzyme; when CRE is applied, it deletes the LoxP flanked target segment and stops the target expression. Simultaneously, CRE-mediated recombination can activate expression of a second target downstream from the deleted segment.

GenTarget provides **pre-made CRE reporting lentivirus** for easy, fast and convenient testing and monitoring of CRE recombination efficiency *in vivo* and *in vitro*. This lentivirus has been engineered to constitutively express the "**LoxP-GFP-stop-LoxP-RFP-Stop**" cassette under a super CMV promoter. (See the expression cassette scheme below). It reports the occurrence of CRE-mediated recombination events via a "color switch" mechanism, thereby providing an easy, fast and continual monitoring for the presence of CRE or CRE recombination event.



2. Multiple Selection Markers

GenTarget offers the CRE reporting lentivirus with a broad variety of antibiotic selection markers including **puromycin, neomycin, or blasticidin**. The selection markers are expressed under an RSV promoter (not shown in the schematic above).



These markers allow easy selection for transduction positive cells (those expressing the CRE detection cassette) by antibiotic resistance.

3. Two Formats

The LoxP ColorSwitch particles are provided as at **200 µl/vial** in two formats:

- DMEM medium with 10 % FBS and 60 µg/ml polybrene (10x)
- PBS solution, which is best for *in vivo* applications, cell cultures requiring serum-free conditions, or hard-to-infect cells.

4. How ColorSwitch Reporting Works

The CRE reporting lentivirus is used to monitor or confirm the efficiency of CRE recombination *in vivo*. It is an easy and very effective tool for verifying the performance of CRE-loxP systems (CRE expression plasmids, pre-made CRE lentiviral particles, and purified CRE enzyme) *in vivo*, and can serve as a control indicator for the same systems.

The CRE reporting lentivirus demonstrates a strong GFP fluorescence after infection into mammalian cells but does not show an RFP fluorescence signal. Once the CRE protein is present in the nucleus, CRE excises/deletes the DNA fragment between two loxP sites. As a result, the GFP is removed and RFP is expressed, with a resulting switch to RFP fluorescence. The ratio of RFP-expressing to GFP-expressing cells can be easily monitored via fluorescence cell sorting, visualized by microscopy, or the fluorescence intensity may be measured by a fluorometer with GFP and RFP filter sets. See the sample results below.

Notes:

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

** It is not possible for CRE to be delivered into all cells; therefore, some GFP positive cells may still be detected even after the application of CRE.

***Also, because some cells may integrate multiple copies of the LoxP-GFP-LoxP-RFP cassette, and since 100% CRE recombination is not possible for all sites in every cell, there will be many cells demonstrating both GFP and RFP signals after the addition of CRE recombinase. The important observation is the dramatic increase in RFP positive cells following addition of CRE. And the RFP/GFP intensity ratio reflects the CRE recombination rate.



Application protocol (for reference only):

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 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.)
For example, seed Hela cells at $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$ 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 $37^\circ\text{C}/\text{CO}_2$ 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 -80°C for future use. But virus titer will decrease by ~10% for each re-thaw.)

Day 3: At ~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 CRE 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.).

CRE 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 CRE recombination activity.

- **Apply the CRE enzyme into the cells** (which can be achieved by infected cell with CRE expression lentivirus, or by regular lipid-transfection of a CRE expression plasmid, or even simply by adding purified penetrating CRE protein enzyme. (**Note:** Gentarget provides ready-to-use [CRE expression lentivirus](#) with different antibiotic selection marker for CRE delivery into cells).
- Put cells in normal culture conditions for **48-72 hours**.
- **Detect CRE recombination reaction:** The RFP signal will gradually showed up and peaked at 48 hours or longer times (dependent upon CRE delivery methods) after the CRE delivery. The RFP/GFP cell population ratio or the RFP signal intensity reflects the CRE-LoxP recombination efficiency (rate). You can sort the cell by FACS machine, other meters, or visualize the RFP positive cell under fluorescent signal.

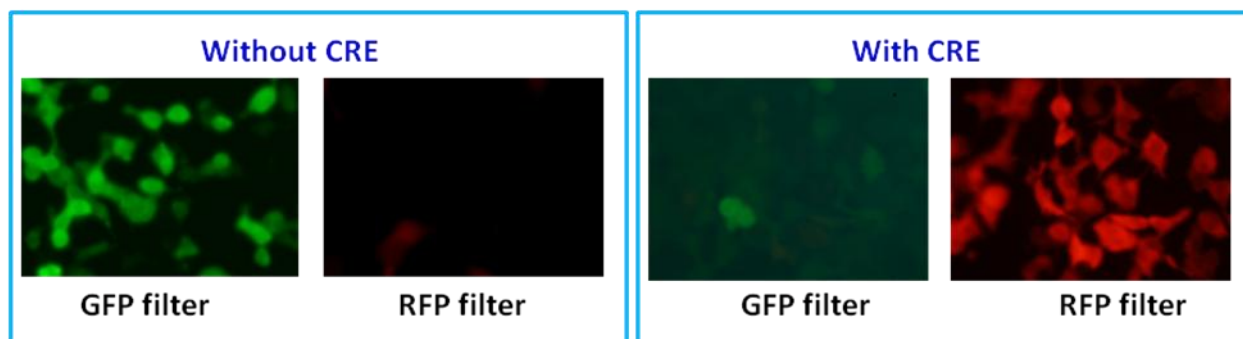


2. Suspension cells transduction Protocols:

1. Grow your cell in your completed suspension culture medium, shaking in flask in CO² incubator if necessary;
2. Measure cell density. When cell grow to $\sim 3 \times 10^6$ cell/ml, measure cell viability (should be > 90%), then diluted cells into 1×10^6 cell/ml in completed medium;
3. Transduction: thaw lentiviral particles at room temperature. Simply add premade lentiviral particle into the diluted cells at ratio of: **50 to 100ul virus per 0.5 ml of cells** (Note: depending on the cell types; you may need to use more or less viruses). Grow cells in flask, shaking in CO₂ incubator.
4. At 24 hours after transduction, add equal amount of fresh medium containing related antibiotics (Note: each particles contain an antibiotic marker and the antibiotic amounts to use depends upon cell types). Grow cell in CO₂ incubator.
5. At 72 hours after transduction, check fluorescence under microscope or calculate the transduction efficiency using cell sorting machine (like FACS or Guava machine).
6. You can sort the fluorescent positive cells, and maintain the antibiotic selection to generate stable cell lines.

(Note: GFP filter wavelength: Ex450-490 ~Em525; RFP filter: ~Ex545/~Em620).

Sample images of CRE-loxP recombination detection:



Left panel / without CRE: CRE reporter cell line (Cat#: [SC018-Bsd](#)) was created by LoxP460-Neo particles, cultured in a 24-well-plate. Images were taken with a GFP filter set (Ex 490nm/Em 525nm) and an RFP filter set (Ex 545nm/Em 620nm).

Right panel / with CRE: CRE reporter cell line (Cat#: [SC018-Bsd](#)) was created by LoxP460-Neo particles, cultured in completed in 24-well plate. 50 μ l of CRE expression lentiviral particle (Cat#: [LVP339](#)) was added into the cells in one well. Images were taken at ~ 72 hours after the addition of CRE expression lentivirus.



Safety Precaution:

GenTarget lentiviral particles adapt 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. Sauer, B. (1987) "Functional expression of the Cre-Lox site-specific recombination system in the yeast *Saccharomyces cerevisiae*", *Mol Cell Biol* **7**: 2087-2096
2. Stanislaw J. Kaczmarczyk and Jeffrey E. Green. *Nucleic Acids Res.* 2001 June 15; 29(12): e56.

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.

Related Products: GenTarget's Pre-made lentivirus Products

Product Category	Product Description (please click category name to see product's pages)
Human, mouse or rat ORFs	Premade lentivirus expressin a human, mouse or rat gene with RFP-Blastididin fusion dual markers.
Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, GFP / RFP / CFP / BFP / YFP .
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression: firefly and Renilla with different antibiotic selection markers.
CRE recombinase	Premade lentivirus for expressing nuclear permeant CRE recombinase with different flurescent and antibiotic markers.
LoxP ColorSwitch	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
CRISPR /hu CAS9	Preamde lentivirus express humanzied wild-type Cas9 endonuclease for genomic editing with CRISPR
TetR inducible expression repressor	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.



iPS factors	Premde lentivirus for human and mouse iPS (Myc, NANOG, OCT4, SOX2, FLF4) factors with different fluorescent and antibitoic markers
T-antigen Expression	Express SV40 large T antigen with different selection markers
Cell Organelle imaging	Premade lentivirus for cell organelle imaging. The fluorescent marker GFP/RFP/CFP was sub-cellular localized in different cell organelle for living cell imaging.
LacZ expression	Express different full length β- galactosidase (lacZ) with different selection markers
Anti-miNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
Fluorescent-ORF fusion	Pre-made lentivirus expression a " GFP/RFP/CFP-ORF " fusion target.
Pre-made shRNA lentivirus	Premade shRNA lentivirus for knockdown a specific genes (P53, LacZ, Luciferase and more).
microRNA and anti-microRNA lentivirus	Premade lentivirus expression human or mouse precursor miRNA . And anti-miRNA lentivector and virus for human and mouse miRNA.
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 specific enzymes with different selection markers.