GenTarget Inc

7930 Arjons Drive, Suite B
San Diego, CA 92126
Phone: (858) 6788683
Fax: (800)3804198
Email: orders@gentarget.com

Pre-made Lentiviral Particles for Nuclear Permeant CRE Recombinase Expression

Cat#	Product Name	Amounts
LVP336	CRE (Bsd), CMV lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP336-PBS	CRE (Bsd), CMV lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP339	CRE (Puro), CMV lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP339-PBS	CRE (Puro), CMV lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP297	CRE (Neo), CMV lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP297-PBS	CRE (Neo), CMV lentivirus, in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP013	CRE-2A-RFP (Bsd), CMV lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP013-PBS	CRE-2A-RFP (Bsd), CMV lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP338	CRE-2A-RFP (Puro), CMV lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP338-PBS	CRE-2A-RFP (Puro), CMV lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP027	CRE-2A-RFP (Neo), CMV lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP027-PBS	CRE-2A-RFP (Neo), CMV lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP337	CRE-2A-GFP (Bsd), CMV lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP337-PBS	CRE-2A-GFP (Bsd), CMV lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP407	CRE-2A-GFP (Puro), CMV lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP407-PBS	CRE-2A-GFP (Puro), CMV lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS

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LVP408	CRE-2A-GFP (Neo), CMV lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP408-PBS	CRE-2A-GFP (Neo), CMV lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP804	CRE-2A-GFP, CMV lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP804-PBS	CRE-2A-GFP, CMV lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP805	CRE-2A- RFP, CMV lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP805-PBS	CRE-2A- RFP, CMV lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP519	CRE (Bsd), EF1a lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP519-PBS	CRE (Bsd), EF1a lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP520	CRE (Puro), EF1a lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP520-PBS	CRE (Puro), EF1a lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP521	CRE (Neo), EF1a lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP521-PBS	CRE (Neo), EF1a lentivirus, in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP522	CRE-2A-RFP (Bsd), EF1a lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP522-PBS	CRE-2A-RFP (Bsd), EF1a lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP523	CRE-2A-RFP (Puro), EF1a lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP523-PBS	CRE-2A-RFP (Puro), EF1a lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP524	CRE-2A-RFP (Neo), EF1a lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP524-PBS	CRE-2A-RFP (Neo), EF1a lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP525	CRE-2A-GFP (Bsd), EF1a lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS

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LVP525-PBS	**CRE**-2A-**GFP** (**Bsd**), EF1a lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP526	**CRE**-2A-**GFP** (**Puro**), EF1a lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP526-PBS	**CRE**-2A-**GFP** (**Puro**), EF1a lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP527	**CRE**-2A-**GFP** (**Neo**), EF1a lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP527-PBS	**CRE**-2A-**GFP** (**Neo**), EF1a lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP573	**CRE** (**Puro**), CAG lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP573-PBS	**CRE** (**Puro**), CAG lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP574	**CRE** (**Bsd**), CAG lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP574-PBS	**CRE** (**Bsd**), CAG lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP575	**CRE** (**Neo**), CAG lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP575-PBS	**CRE** (**Neo**), CAG lentivirus, in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP576	**CRE** (**GFP-Puro**), CAG lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP576-PBS	**CRE** (**GFP-Puro**), CAG lentivirus in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP577	**CRE** (**RFP-Bsd**), CAG lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP577-PBS	**CRE** (**RFP-Bsd**), CAG lentivirus in PBS	5 x10^7 IFU/ml x 200ul in PBS
LVP578	**CRE** (**RFP-Puro**), CAG lentivirus	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP578-PBS	**CRE** (**RFP-Puro**), CAG lentivirus in PBS	5 x10^7 IFU/ml x 200ul iin PBS
LVP304	**Luciferase**-2A-**CRE** (**Bsd**) lentiviral particles	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP304-PBS	**Luciferase**-2A-**CRE** (**Bsd**) lentiviral particles, in PBS	5 x10^7 IFU/ml x 200ul in PBS

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LVP409	**Luciferase**-2A-**CRE** (**Puro**) lentiviral particles	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP409-PBS	**Luciferase**-2A-**CRE** (**Puro**) lentiviral particles, in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP410	**Luciferase**-2A-**CRE** (**Neo**) lentiviral particles	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP410-PBS	**Luciferase**-2A-**CRE** (**Neo**) lentiviral particles, in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP411	**Luciferase**-2A-**CRE** (**GFP**-**Bsd**) lentiviral particles	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP411-PBS	**Luciferase**-2A-**CRE** (**GFP**-**Bsd**) lentiviral particles, in PBS	5 x10^7 IFU/ml x 200ul in PBS
LVP412	**Luciferase**-2A-**CRE** (**GFP**-**Puro**) lentiviral particles	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP412-PBS	**Luciferase**-2A-**CRE** (**GFP**-**Puro**) lentiviral particles, in PBS	5 x10^7 IFU/ml x 200ul in PBS
LVP413	**Luciferase**-2A-**NLS-CRE** (**RFP**-**Bsd**) lentiviral particles	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP413-PBS	**Luciferase**-2A-**CRE** (**RFP**-**Bsd**) lentiviral particles, in PBS	5 x10^7 IFU/ml x 200ul In PBS
LVP414	**Luciferase**-2A-**CRE** (**RFP**-**Puro**) lentiviral particles	1x10^7 IFU/ml x 200ul in DMEM with 10% FBS
LVP414-PBS	**Luciferase**-2A-**CRE** (**RFP**-**Puro**) lentiviral particles, in PBS	5 x10^7 IFU/ml x 200ul In PBS

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

1. Product Description:

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

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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 studies in transgenic animal models by linking genotypic alterations to biological outcomes (phenotypes). GenTarget provides premade, expression-ready CRE lentiviral particles for *in vivo* and *in vitro* use. CRE expressed by these particles contains the **nuclear localization signal** (**NLS**), PKKKRKV from the SV40 Large T-antigen at its N-terminus, allowing penetration of the nuclear membrane and thereby increasing the number of *in vivo* recombination events.

GenTarget's **CRE recombinase** is expressed under either an optional inducible CMV promoter (TetCMV), an enhanced constitutive EF1a promoter, or a CAG promoter with **a variety of fluorescent markers, antibiotic markers, or fluorescent-antibiotic fusion dual markers**. We also provide CRE-expressing lentivirus "**triple-labeled**" with luciferase, an antibiotic resistance marker, and a fluorescent protein. Some of the lentiviruses express CRE and a marker bicistronically under the same promoter as individual proteins (rather than fusion proteins) through a translation skipping mechanism that mediated by a self-cleavage (F2A) element. Some product (CAT#:LVP804) does not contain any antibiotic marker.

Please see the vector schemes below for each expression vector structure. Pre-made CRE lentiviruses are generated from GenTarget's **Optional Inducible Lentiviral System, or SureTiter™ Lentiviral System**.

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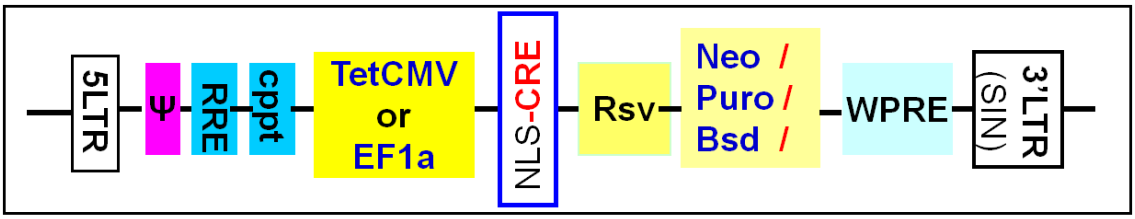

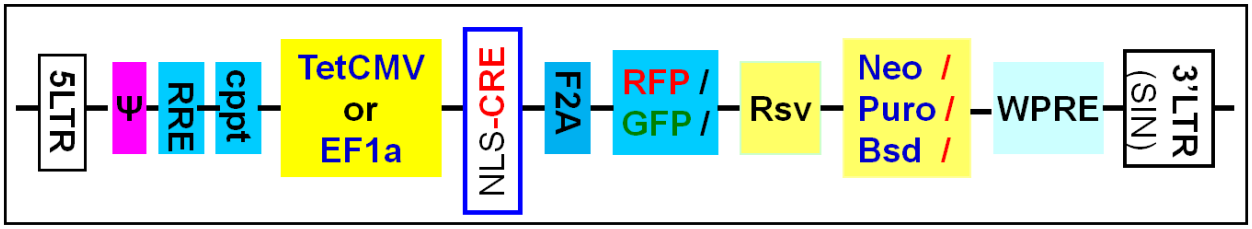

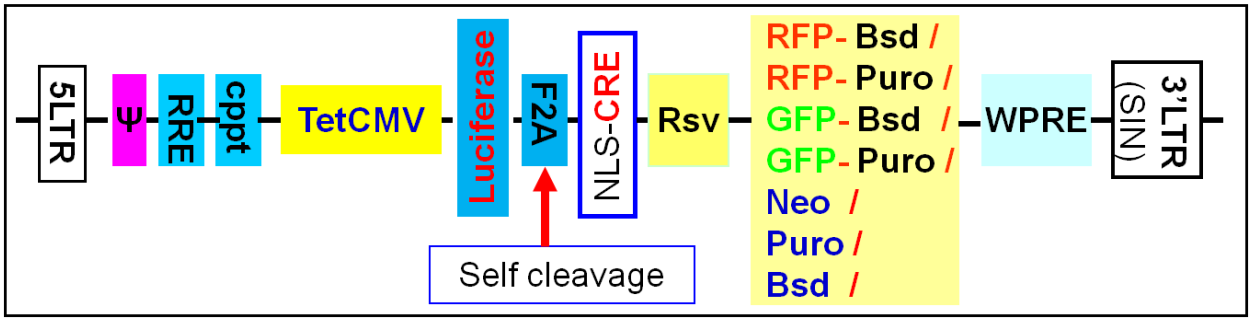

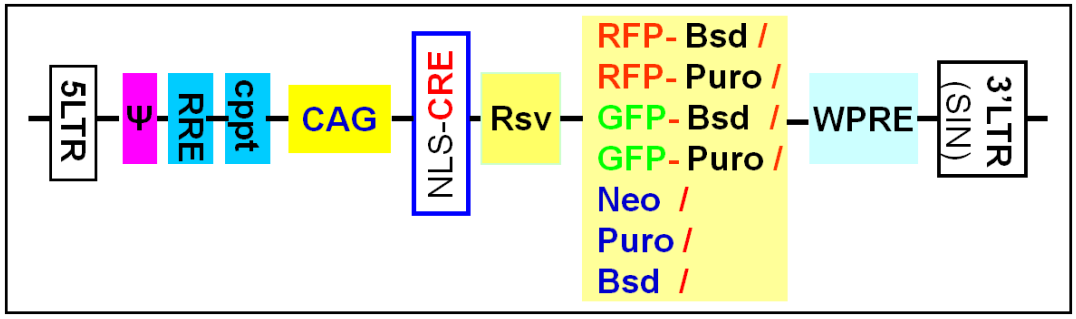

VSV-G pseudotyped particles are generated from 293T cells and passed through a 0.45 mm filter. Titer is validated for each lot.

Ready-to-use CRE particles are provided in two formats in 200 µl aliquots:

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

For more details, please see **FAQs for pre-made lentiviral particles** (.pdf).

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2. About the promoters:

EF1a is strong promoter in all cell types, and has a low likelihood of being silenced in long term cell culture. It is **not** an inducible promoter; it constitutively expresses CRE in all cell types.

The optional inducible CMV promoter (**TetCMV**) can also constitutively express high levels of CRE without any induction. It is the strongest promoter in most cell lines; however, it may be silenced after long-term culture in some lines. The TetCMV promoter is embedded with two copies of the repressor binding sequence (TetO). Consequently, the TetCMV can be used for **optional** tetracycline-induced expression. For inducible expression, the TetR repressor protein must be expressed in advance to stop CMV-driven expression. Expression can then be activated by the addition of tetracycline (see the picture below for details). Inducible expression is tetracycline dose-dependent; in general, a final tetracycline concentration of 1.0~5.0 µg/ml is used. The image below illustrates how inducible expression works.

The **CAG** promoter is a combination of the cytomegalovirus (CMV) early enhancer element and the chicken beta-actin promoter. It is the strongest promoter in embryonic stem (ES) cells and is frequently used to drive high level gene expression in mouse ES cells.

If inducible expression is desired, repressor regulator (TetR) expression must be delivered in advance of or at the same time as transduction. The presence of TetR can be achieved by the following methods:

- **TetR stable cell lines** that constitutively express the TetR protein
- **Co-transfection** with a TetR expression plasmid and a target-inducible expression vector
- **Co-transduction** with TetR lentiviral particles and inducible gene expression lentiviral particles

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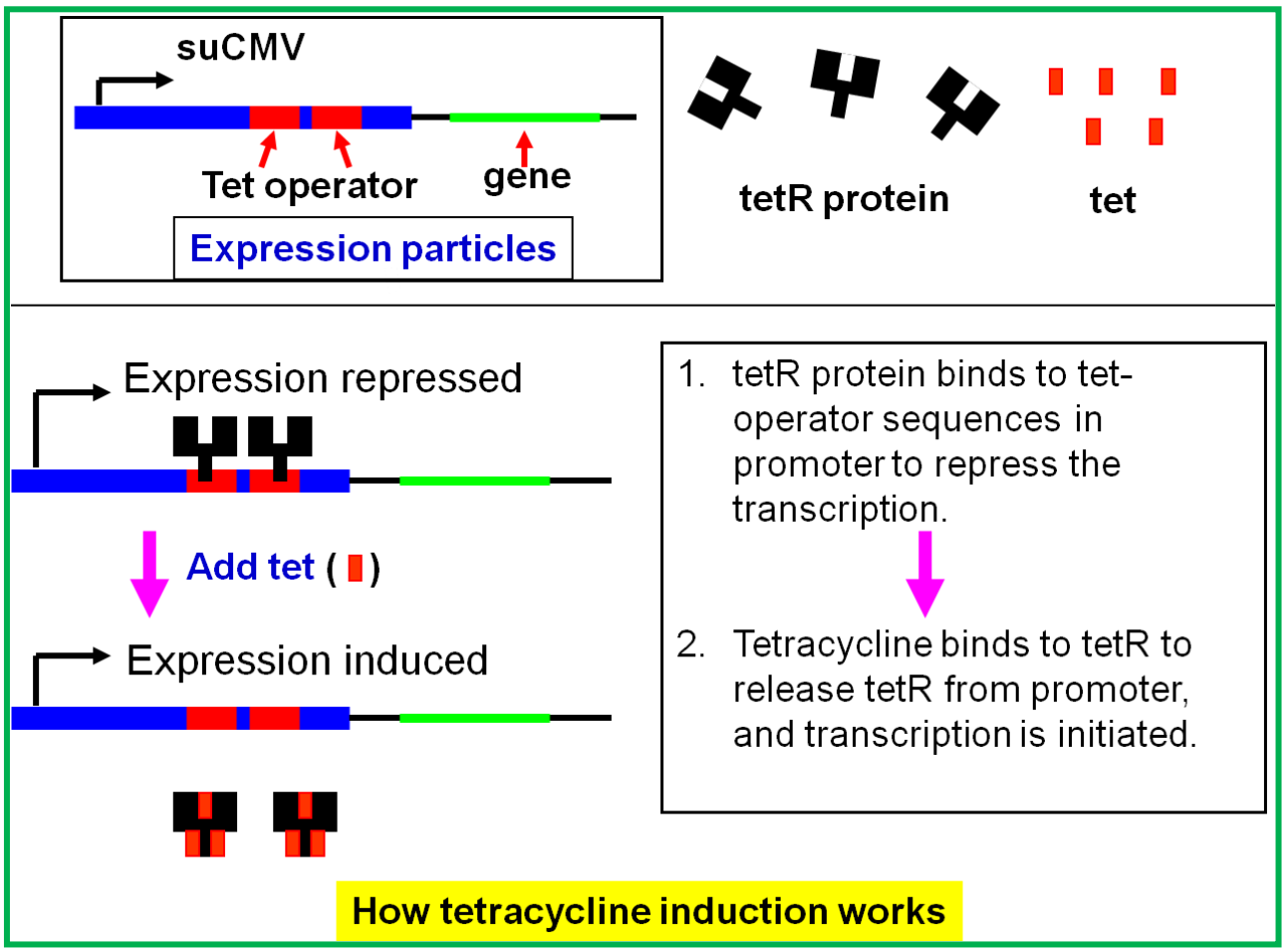

GenTarget provides "**premade TetR particles**" with different antibiotics for double selecting the transduced cells.

3. Key features of CRE expression lentivirus:

- **High expression levels** of nuclear permeant CRE and high viral titer
- **A selection of promoters** to meet your needs
- **Easy transduction monitoring** of the GFP or RFP signal by fluorescence microscopy (not all products)
- **Dual markers:** transduced cells can be sorted via a fluorescent signal or selected for antibiotic resistance (not for all products)
- **Ready to use:** simply add 50 µl into your cell culture in a 24-well plate. (**Note**: depending upon your specific needs, you may transduce with different MOIs for different levels of expression.)

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4. Transduction Protocols:

1) Transduction Protocol for Adhesive cells :

Note: Pre-made lentivirus is provided ready to use, so it can be simply added into your cell culture; the amount of virus to add depends on cell type. For quick transduction, add 50 µl of virus into each well of 24-well-plate where cell density is 50% to 75%. After 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy. For stable cell line generation, pass cells into medium containing antibiotic or perform fluorescence cell sorting followed by antibiotic selection.

Day 0:

Seed cells in complete medium at the appropriate density and incubate overnight.

Note: at the time of transduction, cells should be 50%-75% confluent. 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 and add 0.5ml fresh, warm, complete medium.
- Thaw the pre-made lentiviral stock at room temperature and add the appropriate amount of virus stock to obtain the desired MOI.
- Return cells to 37°C, CO_2 incubator.

  Note: Try to avoid freezing and thawing. If you do not use all of the virus at one time, you may re-freeze the virus at -80 °C for future use; virus titer will decrease by ~10% for each freeze/thaw cycle.

Day 3:

At ~72hr after transduction, check the transduction rate by fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS or Guava).

Day 3 + (optional):

Sort transduced cells by FACS, and select for antibiotic resistance. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line (refer to the pertinent literature on generation of stable cell lines).

2) Transduction Protocol for Suspension Cells:

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Grow cells in complete suspension culture medium; use a shaking flask in a CO^2 incubator if necessary.

Measure cell density. When density has reached ~3 x 10^6 cells/ml, measured viability should be > 90%. Dilute cells into 1 x 10^6 cell/ml in complete medium.

Day 1:

- Thaw lentiviral particles at room temperature.
- Add premade lentiviral particles into the diluted cells at a ratio of: 50 to 100 µl virus per 0.5 ml of cells (Note: depending on cell type, you may need to use more or less virus).
- Grow cells in a shaking flask in a CO2 incubator.

Day 2:

At 24 hours after transduction, add an equal amount of fresh medium containing relevant antibiotics. **Note:** amount of antibiotic depends on cell type. Continue growing cells in CO2 incubator.

Day 3:

At 72 hours after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

Note: Filter wavelength settings:

BFP filter: ~Ex380 ~Em460;
CFP filter: ~Ex436 ~Em480;
GFP filter: ~Ex450-490 ~Em525;
YFP filter: ~Ex500 ~Em535;
RFP filter: ~Ex545 ~Em620;
iRFP filter: ~Ex690 ~Em715

5. 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. Ware glove all the time at handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

6. References:

1. Molecular Therapy (2003) 7, 460–466; doi: 10.1016/S1525-0016(03)00024-8
2. Nucleic Acids Research, 2001, V29, No12 e56;

3. Annu Rev Microbiol. 1994;48:345-69.
4. Microbiol Mol Biol Rev. 2005 Jun;69(2):326-56.
5. NIH Guidelines for Bio-safety Considerations for Research with Lentiviral Vectors. (Link).
6. CDC guidelines for Lab Bio-safety levels (Link).

7. 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.

8. Related products:

GenTarget's pre-made lentivirus product category.

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.
Pathway Reporter	Premade lentivirus reporter express a luminescence or fluorescent report (firefly **Luciferase**, **Renilla** luciferase, **RFP** or **GFP** fluorescent marker) under a pathway specific promoter.
Cell-Specific Reporter	Premade lentivirus reporter for targeting expression of a luminescence or fluorescent report (firefly **Luciferase**, **Renilla** luciferase, **RFP** or **GFP** fluorescent marker) under a cell type specific promoter.
Cell Immortalization	Premade different set lentivirus for primary cell immortalization, including **SV40 larget T antigen**, human **TER**T, siRNA-P53, EBV genes, HpV16 E6, Adenovial E1A, HOSA9, CDK4 cMyc KRas and more
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**

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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
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.