GenTarget Inc

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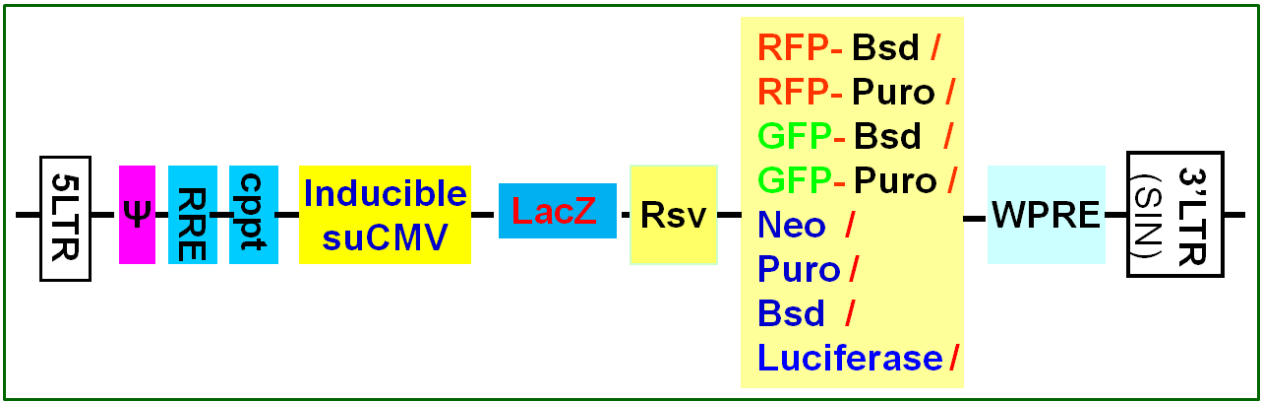
Pre-made lentivirus for β-Galactosidase expression

Cat#	Product Name	Amounts
LVP010	lacZ (RFP-Bsd) lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP301	lacZ (Puro) lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP333	lacZ (Neo) lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP334	lacZ (luciferase) lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP346	lacZ (Bsd) lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP347	lacZ (GFP-Bsd) lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP348	lacZ (RFP-puro) lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP021	GFP-lacZ (His) Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP021-PBS	GFP-lacZ (His) Concentrated Lentivirus in PBS	$5 x10^7$ IFU/ml x 200ul

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

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 integrate into the transduced cells' genome for long term expression, making it a great gene transfer agent.



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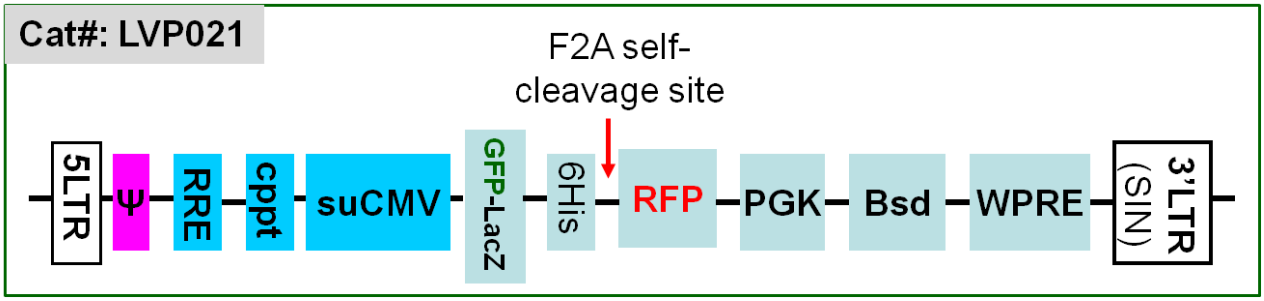
Pre-made **LacZ** lentiviral particles are generated from GenTarget's **Optional inducible lentiviral system** with different selection markers (see vector core structure scheme above). Full length **LacZ ORF** was fully verified by sequencing. VSV-G pseudotyped lentiviral particles are generated in 293T cell, and provided in **DMEM medium** with 10% FBS, 60ug/ml of polybrene, For more details about premade particles, please see **FAQ for pre-made lentiviral particles** (.pdf). (http://www.gentarget.com/pdf/FAQ-Premade-Lentiviral-particles.pdf)

For product Cat#: LVP021, the full-length Beta galactosidase (lacZ) was fusioned with N-term GFP protein and with C-term a 6His under an inducible CMV promoter. A RFP protein was bicistronically expressed under the same CMV promoter, mediated via a F2A element. (see vector map scheme below). So it has triple signals: GFP, RFP signals that can be visualized via microscope, and lacZ signal via staining. Please see the scheme for core lentivector structure:



About inducible expression:

LacZ was natively expressed (without any tags) under a tetracycline inducible suCMV promoter in which two tetracycline operator sequences was integrated. However, the particles can be used for regular constitutive high expression without requirements for tetracycline induction. It becomes inducible expression particles only when the tetracycline repressor protein (tetR) is present in advance. For inducible expression, the tetR must be expressed in advance to stop the transcription, and the expressed was activated by adding tetracycline. This inducible expression is tetracycline's dose dependent. In general, the amount of tetracycline is used at 1.0-10 ug/ml final concentration. The image below illustrates how the inducible expression works.

Gentarget provides "**premade tetR particles**" with different antibiotics for double selecting the transduced inducible expression cells.

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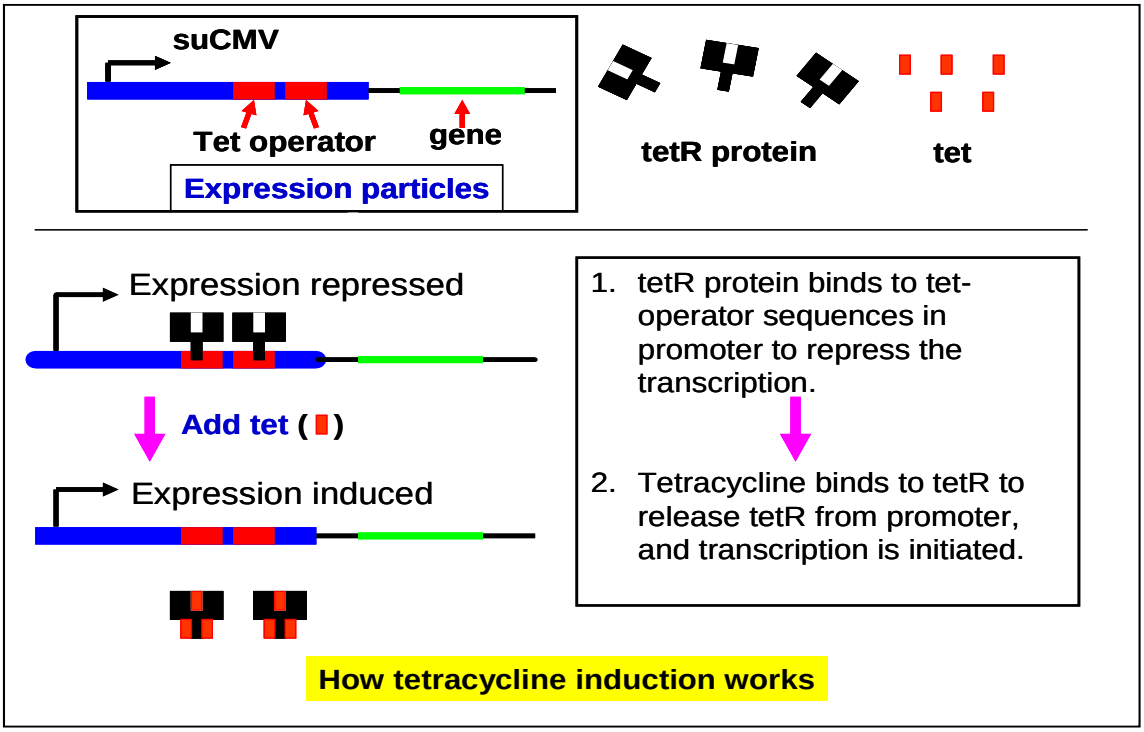

How tetracycline induction works

ession

- Particles are used in a tetR expression stable cell line that constantly express tetR protein in advance;
- Transfect a tetR expression plasmid before transduce lentiviral particles;
- Co-transduce both the tetR repressor particles and the gene expression particles into the sample cells (with equal MOI) and the double transduced cells can be selected by both antibiotics, and then used for inducible expression.

Key features:

1. High LacZ expression level and high viral titer;
2. Easy transduction monitoring via the RFP or GFP fluorescent signal under microscope (not available for all particles);
3. Dual markers: transduced cells can be sorted via a fluorescent signal or selected via antibiotics (not available for all particles);
4. **The lentivirus are ready and easy to use, simply add 50ul into your cell culture in 24-well plate (see sample image below).** (**Note**: dependent upon your specific needs, you may design the transduction with different MOI for different levels of expression.)

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Transduction sample image:

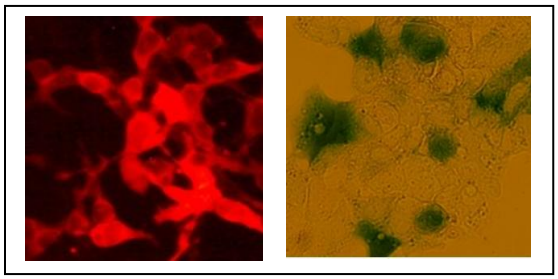

Figure 1: 50 ul of pre-made lentiviral particles (Cat#: LVP010) added into 293HEK cells. At 72 hours after transduction, image was taken under microscope with RFP filter (Left image), then cells washed with PBS and stained with lacZ staining kit for 10min, take the bright light image to see lacZ stained cells (Right image) (**note**: prolonged staining time should show all lacZ positive cells which is not showed here).

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:

- 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. Do nothing.
  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 48hr~72hr (Depend upon cell type) 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, or select by antibiotic killing. 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:

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

Measure cell density (not grow over 3 million/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. Continue growing cells in CO2 incubator.

Day 3+:

At 48 hour to 72 hours (Depend upon cell type) after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Pass cells into 0.5 million/ml density in completed medium containing the

corresponding antibiotic (**Note:** amount of antibiotic depends on cell type. A killing curve must pre-established). Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

Safety Precaution:

Please use extra caution when using lentiviral particles. Remember. Ware glove all the time at handling Lentiviral particles! Please refer CDC and NIH's links (see references) for more details regarding to safety issues.

References:

1. Molecular Therapy (2003) 7, 460–466; doi: 10.1016/S1525-0016(03)00024-8
2. Annu Rev Microbiol. 1994;48:345-69.
3. Microbiol Mol Biol Rev. 2005 Jun;69(2):326-56.
4. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors. (Link).
5. CDC guidelines for Lab Biosafety levels (Link).

Warranty:

This product is warranted to meet its quality as described when used 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;

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Product Category	Product Description (please click into each category's page)
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 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.

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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 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

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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.