GenTarget Inc

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# Premade Lentiviral Particles for Mouse iPS Stem Factors

**For generating induced pluripotent stem (iPS) cells or other applications.**
FOR RESEARCH USE ONLY, not for diagnostic or therapeutic use.

| Cat# | Product Name | amounts |
|---|---|---|
| LVP003m | m OCT4 (RFP-Bsd) inducible particles | 200ul x ($1x10^8$ IFU/ml) |
| LVP004m | m Sox2 (RFP-Bsd) inducible particles | 200ul x ($1x10^8$ IFU/ml) |
| LVP005m | m NANOG (RFP-Bsd) inducible particles | 200ul x ($1x10^8$ IFU/ml) |
| LVP006m | m LIN28 (RFP-Bsd) inducible particles | 200ul x ($1x10^8$ IFU/ml) |
| LVP007m | m Myc (RFP-Bsd) inducible particles | 200ul x ($1x10^8$ IFU/ml) |
| LVP008m | m Klf4 (RFP-Bsd) inducible particles | 200ul x ($1x10^8$ IFU/ml) |
| LVP311m | m OCT4 (Neo) inducible particles | 200ul x ($1x10^8$ IFU/ml) |
| LVP312m | m Sox2 (Neo) inducible particles | 200ul x ($1x10^8$ IFU/ml) |
| LVP313m | m NANOG (Neo) inducible particles | 200ul x ($1x10^8$ IFU/ml) |
| LVP314m | m LIN28 (Neo) inducible particles | 200ul x ($1x10^8$ IFU/ml) |
| LVP315m | m Myc (Neo) inducible particles | 200ul x ($1x10^8$ IFU/ml) |
| LVP316m | m KLF4 (Neo) inducible particles | 200ul x ($1x10^8$ IFU/ml) |

**Storage:** < -70 °C, avoid repeat freeze/thaw cycles. Products stable for 6 month.

## 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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Conversion of fully differentiated mouse or human somatic cells into embryonic-like cells (so called induced Pluripotent Stem Cells or iPSCs) has attracted enormous attention. Multiple reports have demonstrated that iPS cells can be generated by using a set of transcription factors or stem cell factors delivered via expression virus or by expressed proteins. The main stem cell factors are: OCT3/4, SOX2, NANOG, LIN28, c-Myc, and KLF4, although the combination of reprogramming factors may be slightly different. iPSCs hold the promise of curing many human diseases and accelerating stem cell research.

GenTarget provides **premade lentiviral particles** for mouse iPS genes. Each stem factor was natively expressed (without any tags) under an **optional inducible suCMV promoter**

Utilizing our **Inducible Lentiviral Vector** system (see vector scheme below), GenTarget has generated high-titer inducible lentiviral particles for all six **mouse** stem cell factors. Each factor is fully sequence-verified and matched to the CDs in the NCBI database (see table below). High titer lentiviral particles/ supernatant were produced in 293T packaging cells (Cat# **TLV-C**) with a packaging mix (Cat# **HT-pack**). They are pseudotyped with VSV-G glycoprotein. They are packaged in DMEM medium (containing 10% FBS and 10x polybrene as ready-to-use status), and supplied as 200 µl/per vial at ~ 1x $10^7$ IFU/ml.

All six mouse stem factors were sequencing verified. Their sequences fully match to the CD region according to the NCBI's database (see table below).

| Target | NCBI ID | Matched ORF position |
|---|---|---|
| m Myc | NM_010849.4 | 582--1946 |
| m Klf4 | NM_010637.3 | 605--2056 |
| m Oct3/4 | NM_013633.2 | 62--1120 |
| m SOX2 | NM_011443.3 | 412--1371 |
| m LIN28 | NM_145833.1 | 76--705 |
| m NANOG | NM_028016.2 | 216--1133 |

Includes a tetracycline inducible **suCMV promoter** to drive iPS gene expression and contains a **RFP-Bsd** fusion dual or **Neomycin** selection marker under an **RSV promoter** (see vector map scheme below).

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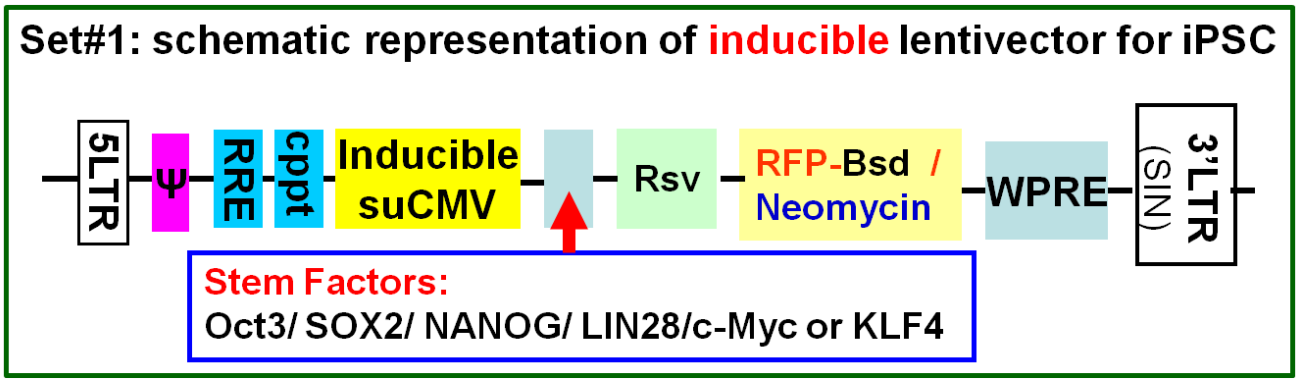


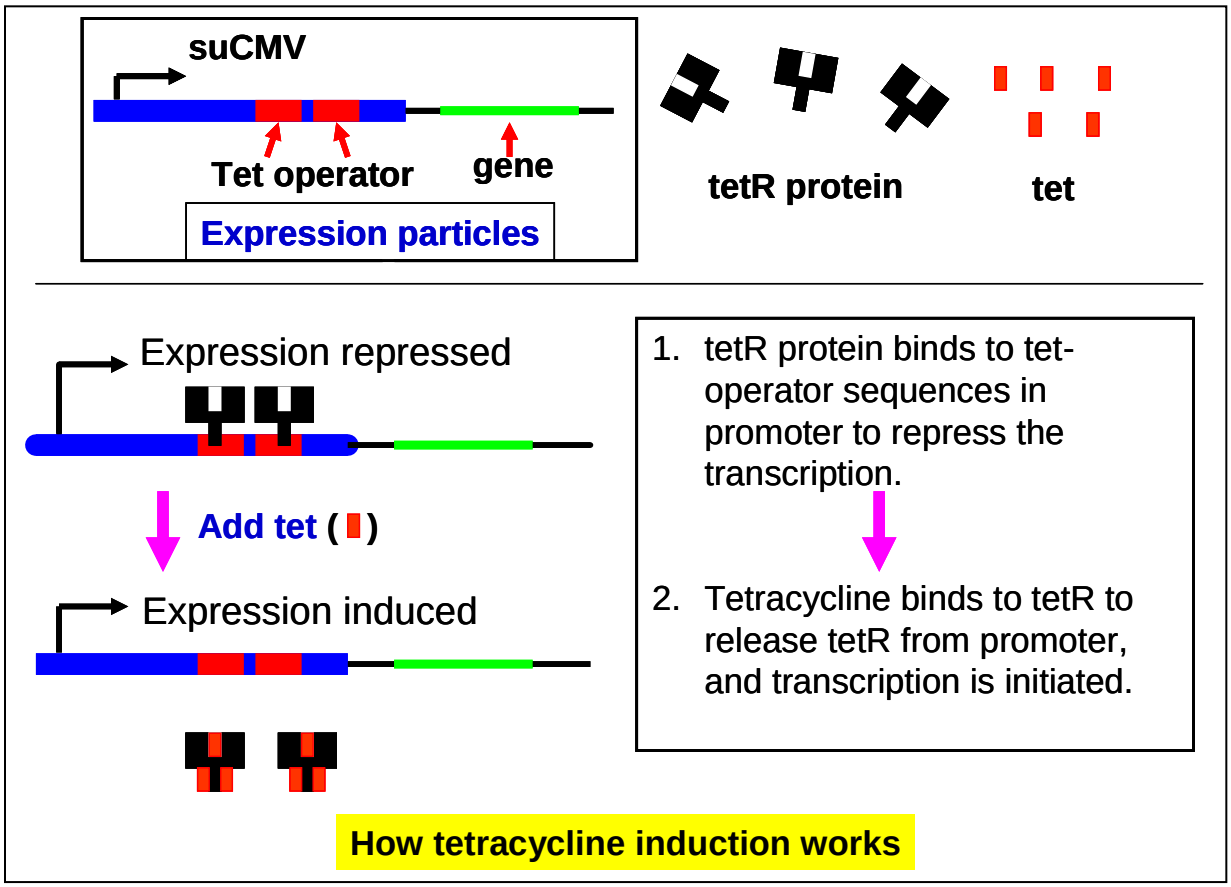


The particles can be used for regular constitutive high expression, or optionally for tetracycline-induced expression when the tetracycline regulator protein (tetR) is present in advance. For inducible expression, TetR must be expressed in advance to stop transcription; expression is then activated by adding tetracycline. This inducible expression is tetracycline dose dependent; in general, tetracycline is used at a final concentration of 1 µg/ml. Please see

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the schematic above for the mechanism of inducible expression, and see our website for more details about our Inducible lentiviral system. GenTarget provides premade lentivirus expressing TetR with a variety of antibiotic markers. For general information about lentiviral particles, please refer to FAQ about premade lentiviral particles.

## 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.

## Attachment:

### iPS cell generation procedure for the Dox inducible system

(*For reference only*)

**Day 0: Seed the parent cells:**

- Seed human fibroblast cells at 1 × $10^5$ cells/well in a 6-well plate, culture in 5ml of growth medium
- Incubate overnight at 37°C with 5% CO2

**Day 1: Viral Transduction:**

- Remove medium, add 2.5 ml of pre-warmed fibroblast growth medium, and then add 500µl of iPS lentivirus. Gentle mix for even distribution.
- Incubate overnight at 37°C with 5% CO2. [Note: set up inducible GFP positive control wells by adding 200ul/per well of GFP control particles]

**Day 2: Change Medium**

- At about 24 hours post-transduction, change to 5 ml growth medium.
- Incubated overnight at 37°C with 5% CO2.

**Day 3: Re-plate the transduced cells to feeder cells**

- At three days post-transduction, trypsinize cells and centrifuge at 200 x g for 5 minutes
- Resuspend in Fibroblast Cell Growth Medium
- Re-plate in a 150mm MEF Feeder Dish
- Incubate overnight at 37 °C with 5% CO2

**Day 4: Induce Reprogramming using Dox**

- At 24 hours after re-seeding, replace Fibroblast Cell Growth Medium with 2.0 ml Dox-Induction Medium containing 2µg/ml Dox. [**Note:** set up a negative control well without Dox.]
- Incubated Cells overnight at 37°C with 5% CO2.

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**Day 5+: Change Induction Medium**

- Change Dox-Induction Medium every 48 hours
- Continue to pass the cells until they show typical human ES cell morphology

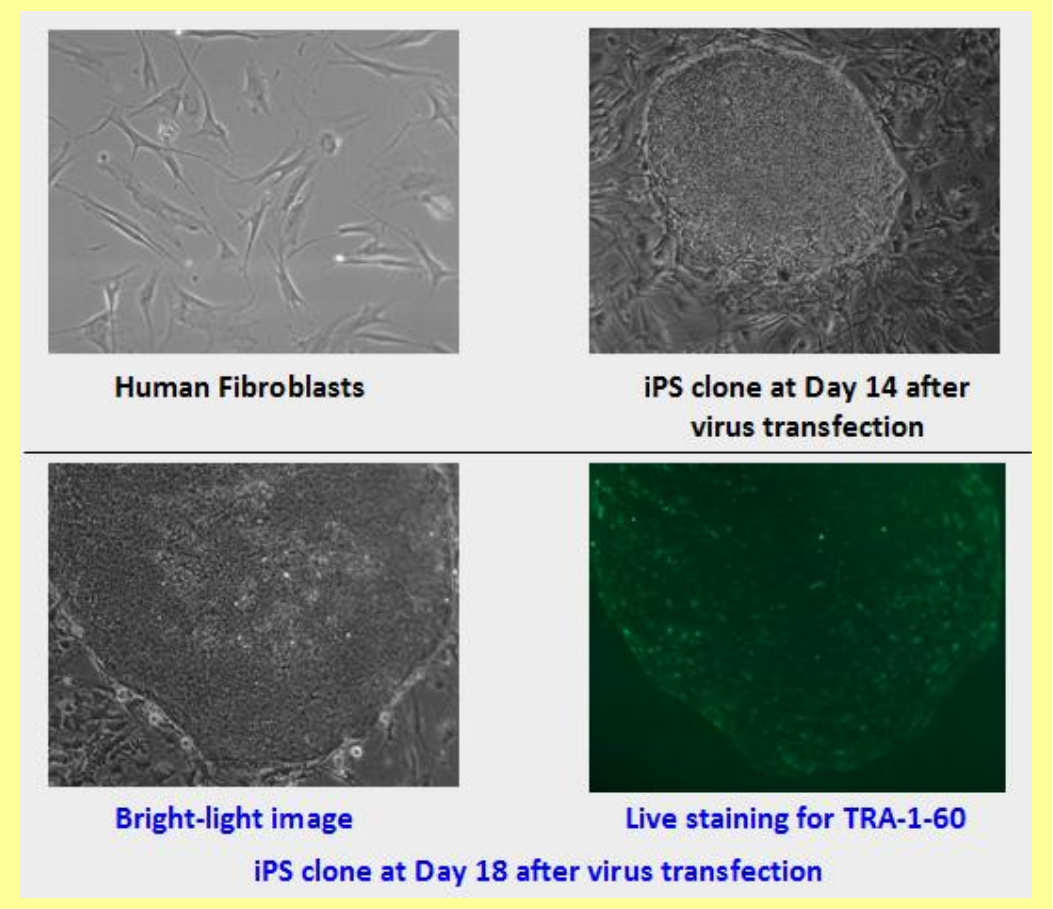
**Day 14++: Select iPS cell colonies**

- Pick the iPS cell colonies that conform to proper cell morphology using a sterile glass picking tool.
- Trypsinize each individually isolated iPS cell colony and pass into each well of a 24-well feeder plate.

**Passage, and Expansion of iPS cell colonies**

- Incubate a 24-well plate at 37°C and 5% CO2,
- Replace culture medium with fresh medium without Dox every 48 hours.
- Passage into an appropriate size plate for iPS cell expansion (the process takes about 6-10 days).
- Monitor iPS cell colony growth and morphology, and validate the iPS colonies. Save iPS cells in cryogenic vials.

**iPS cell sample images:**



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## References:

1. NIH stem cell training program (Link).
2. Masaki Ieda, Ji-Dong Fu, et al. (2010). Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors. Cell 142, 375-386.
3. Takahashi, K. and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663-676.
4. Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, R., Slukvin, I.I., and Thomson, J.A. (2007). Induced pluripotent stem cell lines derived from human somatic cells. Science 318, 1917-1920.
5. Park, I.H., et al., Reprogramming of human somatic cells to pluripotency with defined factors. Nature, 2008. 451(7175): p. 141-6.
6. Shao, L., et al., Generation of iPS cells using defined factors linked via the self-cleaving 2A sequences in a single open reading frame. Cell Res., 2009. 19(3): p. 296-306.
7. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors. (Link).
8. CDC guidelines for Lab Biosafety levels (Link).

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

| Product Category | Product Description (please click into each category's page) |
|---|---|
| Pathway Reporter | Lentivirus for all kinds of pathway 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-T, TCR-T, Assay cell lines, and Cell Antigens & Receptors. |
| CRISPR Gene Editing | Preamde lentivirus express humanzied wild-type **Cas9** endonuclease, the **dCas9**, gRNAs, **CRISPR** gene editing research |
| 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, |

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| | |
|---|---|
| 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 |
| 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. |
| LoxP ColorSwitch | Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE 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) |

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| | |
|---|---|
| | 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. |
| | |