GenTarget Inc
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Premade Lentiviral Particles for Human 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
LVP003	h OCT4 (RFP-Bsd) inducible particles	200ul x (1×10^7 IFU/ml)
LVP004	h SOX2 (RFP-Bsd) inducible particles	200ul x (1×10^7 IFU/ml)
LVP005	h NANOG (RFP-Bsd) inducible particles	200ul x (1×10^7 IFU/ml)
LVP006	h LIN28 (RFP-Bsd) inducible particles	200ul x (1×10^7 IFU/ml)
LVP007	h Myc (RFP-Bsd) inducible particles	200ul x (1×10^7 IFU/ml)
LVP008	h Klf4 (RFP-Bsd) inducible particles	200ul x (1×10^7 IFU/ml)
LVP311	h OCT4 (Neo) inducible particles	200ul x (1×10^7 IFU/ml)
LVP312	h SOX2 (Neo) inducible particles	200ul x (1×10^7 IFU/ml)
LVP313	h NANOG (Neo) inducible particles	200ul x (1×10^7 IFU/ml)
LVP314	h LIN28 (Neo) inducible particles	200ul x (1×10^7 IFU/ml)
LVP315	h cMyc (Neo) inducible particles	200ul x (1×10^7 IFU/ml)
LVP316	h KLF4 (Neo) inducible particles	200ul x (1×10^7 IFU/ml)
LVP317	h OCT4 (EF1α) (puro) particles	200ul x (1×10^7 IFU/ml)
LVP318	h SOX2 (EF1α) (puro) particles	200ul x (1×10^7 IFU/ml)
LVP319	h NANOG (EF1α) (puro) particles	200ul x (1×10^7 IFU/ml)
LVP320	h LIN28 (EF1α) (puro) particles	200ul x (1×10^7 IFU/ml)
LVP321	h Myc (EF1α (puro) particles	200ul x (1×10^7 IFU/ml)
LVP322	h Klf4 (EF1α) (puro) particles	200ul x (1×10^7 IFU/ml)

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LVP588	h OCT4 (**EF1α**) (**RP**) particles	200ul x (1 x10^7 IFU/ml)
LVP589	h SOX2 (**EF1α**) (**RP**) particles	200ul x (1 x10^7 IFU/ml)
LVP590	h NANOG (**EF1α**) (**RP**) particles	200ul x (1 x10^7 IFU/ml)
LVP591	h LIN28 (**EF1α**) (**RP**) particles	200ul x (1 x10^7 IFU/ml)
LVP592	h Myc (**EF1α** (**RP**) particles	200ul x (1 x10^7 IFU/ml)
LVP593	h Klf4 (**EF1α**) (**RP**) particles	200ul x (1 x10^7 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.

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 **two sets of premade lentiviral particles** for human or mouse iPS genes. Each stem factor was natively expressed (without any tags) under either an **optional inducible suCMV promoter** (set#1) or enhanced **EF1a** promoter (set#2).

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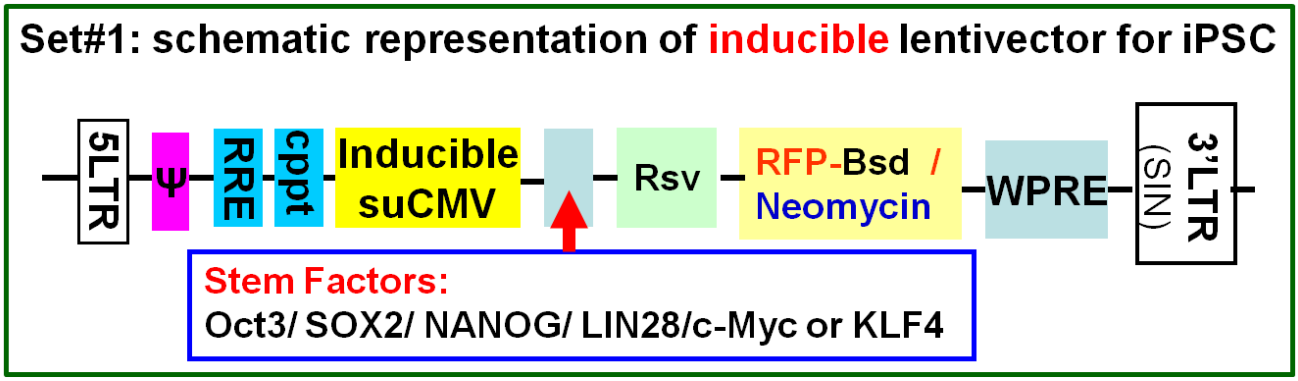
Utilizing our **Inducible Lentiviral Vector** system (see vector scheme below), GenTarget has generated high-titer inducible lentiviral particles for all six **human and mouse** stem cell factors. Each factor is fully sequence-verified and matched to the CDs in the NCBI database (see table below). High 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 stem factor 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
h Myc	NM_002467	526-1890
h Klf4	NM_004235	595-2034
h Oct3/4	NM_002701	55-1137
h SOX2	NM_003106	428-1381
h LIN28	NM_024674	115-744
h NANOG	NM_024865	217-1134

1. **Set #1**

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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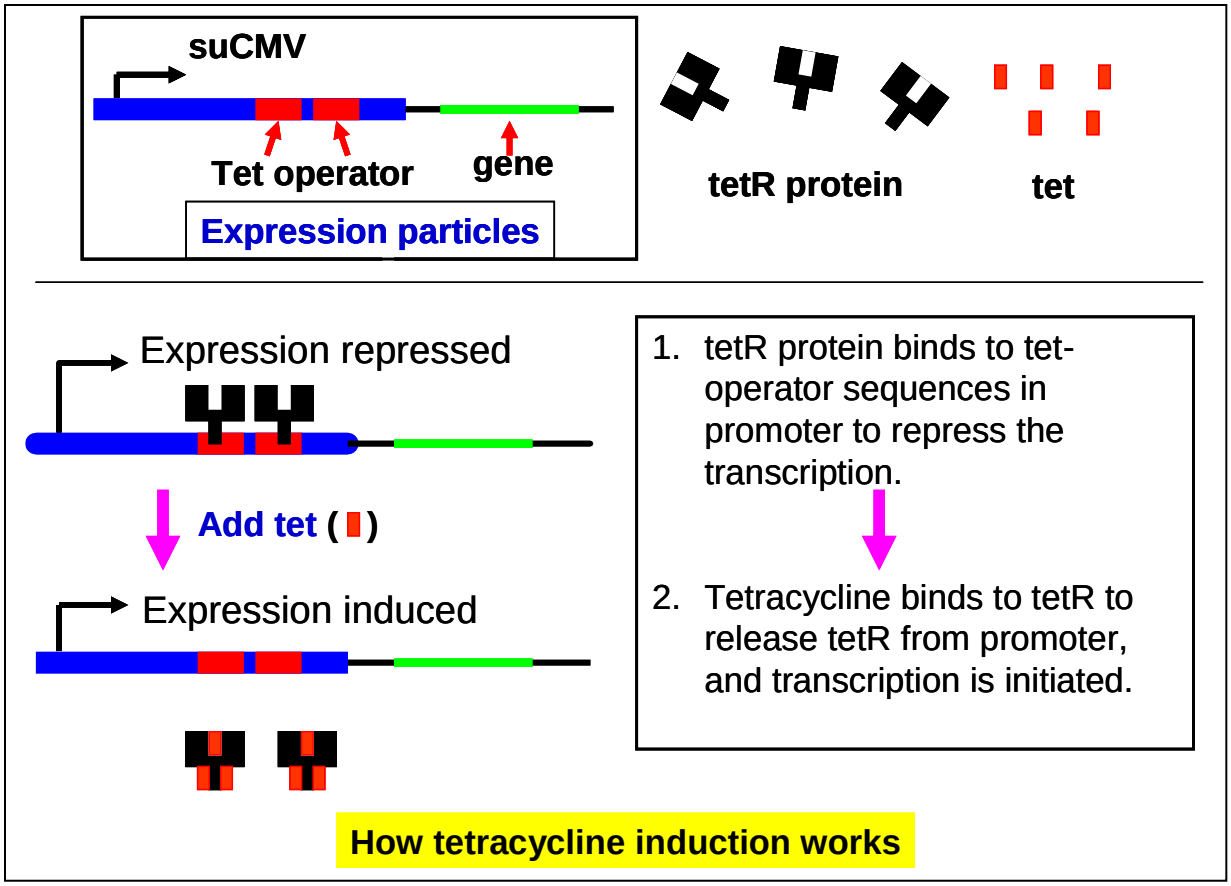

The particles in Set 1 can be used for regular constitutive high expression, or optionally for tetracycline-induced expression when the tetracycline regulator protein (tetR) is present in advance. For inducible expression, TetR must be expressed in advance to stop transcription; expression is then activated 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 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.

2. **Set #2**

Uses the enhanced constitutive **EF1α** promoter to drive iPS gene expression (see vector map scheme below) with the option of using either a **RFP-puromycin** fusion dual marker or the **puromycin** marker alone.

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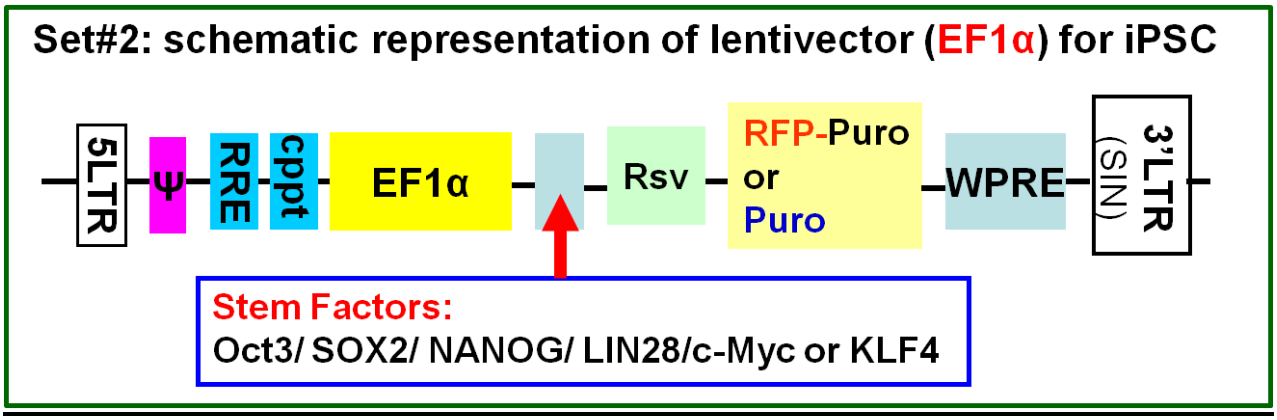

Safety Precaution:

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.

Related Products: GenTarget's pre-made lentivirus product category.

Lentivirus Category (click to see)	**Product Description**
Target Expression	Premade lentivirus express a **human, mouse or rat** gene with Fluorescent-Antibiotic fusion dual selection.
Luciferase expression	Premade lentivirus express all kinds of luciferase**: firefly; Renilla; Cypridina; Red-Luc; Nano-Luc,** with different fluorescent and antibiotic selection.
Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, **GFP / RFP/ CFP/ BFP / YFP/niRFP /unstable GFP, etc.**
Cytoskeleton Imaging	Fluorescent (GFP / RFP/ CFP) labelled cell skeleton protein (Actin; Tubulin; Paxillin; Vimentin)
Cell Organelle imaging	Premade lentivirus for cell organelle imaging. The fluorescent labelled cell organelle lentivirus for living cell imaging.
CRISPR /hu CAS9	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease for genomic editing by **CRISPR**

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Fluorescent Fusion target	Lentivirus express the "**Fluorescent-Target**" fusion proteins. A desired target is fused to Green, Blue, Red, or Cyan Fluorescent Protein, demonstrating the target's functionality and localization
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	**SEAP** (Secreted Embryonic Alkaline Phosphatase) secreted expression lentivirus under different promoter.
TetR repressor expression	Premade lentivirus expressin **TetR** (tetracycline regulator) protein, the repressor protein for the inducible expression system.
rtTA Expression	Lentivirus express the reverse tetraccycline transcription activator gene, rtTA-M2 with different selection.
Pathway Reporter	Different Report lentivirus (**Luc, RFP, GFP, SEAP**) under a pathway specific response promoter.
Cell Immortalization	Comprehesive lentivirus for cell immortalization, for different cell types.
Cell Specific reporter	Different Report lentivirus driven by cell specific promoter.
Infectious Antigens	Lentivirus express all kinds of infectious antigens.
Viral Like Particle (VLP)	Lentiviral particles pseudo-typed with high density of surface envelope protein.
Immuno Therapy	Lentivirus products for Immuno Therapy application.
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
Anti-miNA lentivirus	Pre-made lentivirus expression a specific **anti-miRNA** cassette.
Pre-made shRNA lentivirus	Premade shRNA lentivirus for knockdown a specific genes (**P53, LacZ, Luciferase** and more).
microRNA and	Premade lentivirus expression human or mouse

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anti-microRNA lentivirus	**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	Ready-to-use lentivirus, expressing **specific enzymes** with different selection markers.

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.

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

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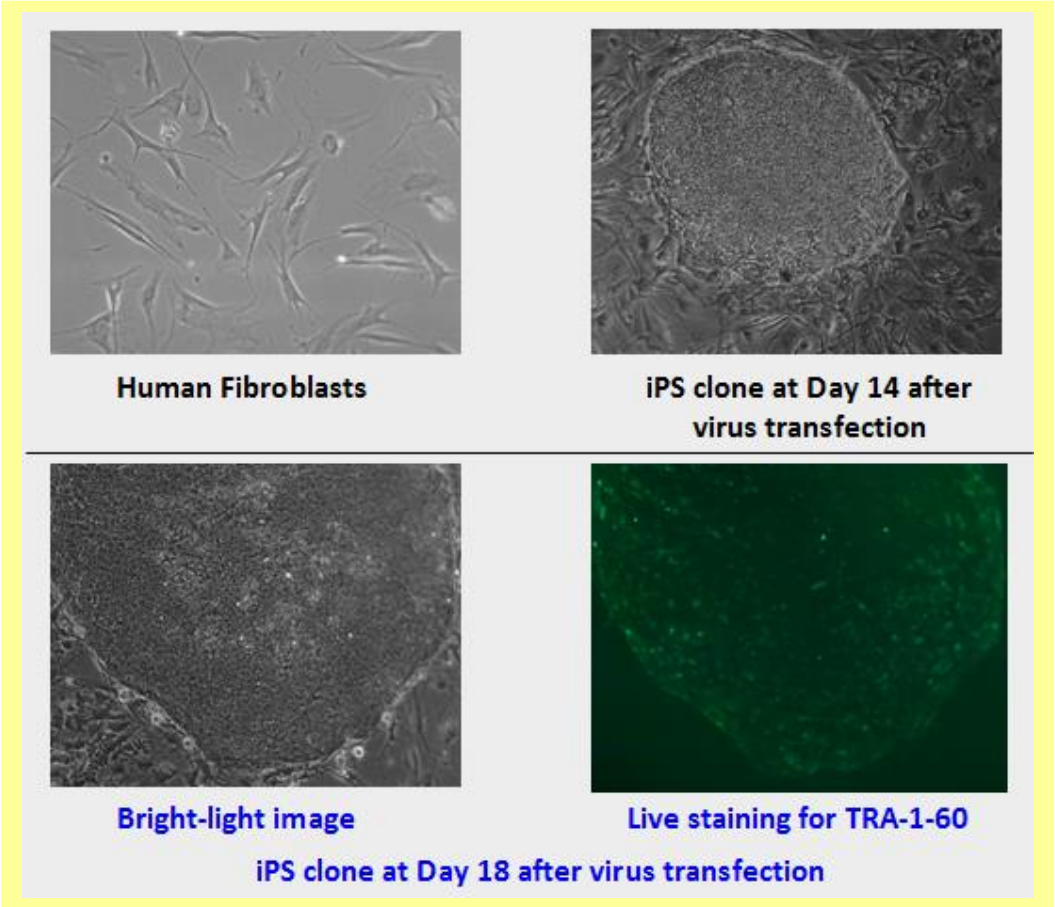
Day 14++: Select iPS cell colonies

- 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).