



GenTarget Inc

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Pre-made Stem factors (mouse set) lentiviral particles for iPS

For generating induced pluripotent stem (iPS) cells or other applications.

RESEARCH USE ONLY, not for use in diagnostics or therapeutics

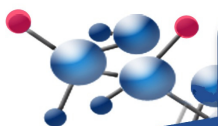
Cat#	Product Name	amounts
LVP003m	m OCT3/4 inducible lentiviral particles	200ul x (1x10 ⁸ IFU/ml)
LVP004m	m SOX2 inducible lentiviral particles	200ul x (1x10 ⁸ IFU/ml)
LVP005m	m NANOG inducible lentiviral particles	200ul x (1x10 ⁸ IFU/ml)
LVP006m	m LIN28 inducible lentiviral particles	200ul x (1x10 ⁸ IFU/ml)
LVP007m	m Myc inducible lentiviral particles	200ul x (1x10 ⁸ IFU/ml)
LVP008m	m KLF4 inducible lentiviral particles	200ul x (1x10 ⁸ IFU/ml)
LVP-stems-m	a full set of pre-made serum-free inducible lentiviral particles for Six mouse stem factors: OCT3/4, SOX2, NANOG, LIN28, c-Myc and KLF4	200ul/ea x 6

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

Product Description:

Lentiviral system is a gene delivery tool using lentivector for gene expression or knockdown. Lentivector is HIV-1 (Human Immunodeficiency Virus 1) derived plasmid. It produces lentiviral particles (lentivirus) that are capable of infect (or transduce) into broad range of mammalian cell types or organs, including primary cells and non-dividing cells both in vivo and in cell culture system. The lentivector construct can stably integrates into the transduced cell's genome, independent of cell cycle, for long-term expression or knockdown. Therefore, lentivirus holds unique promise as gene transfer agents.

Converting fully differentiated mouse or human somatic cells into embryonic-like cells (so called induced Pluripotent Stem Cell: iPSC) has attracted enormous attention in stem cell research. Multiple reports have demonstrated that iPS cells were generated by using a set of transcription factors or stem cell factors that delivered as expression virus or expressed proteins. Although the combination of reprogramming factors may slightly



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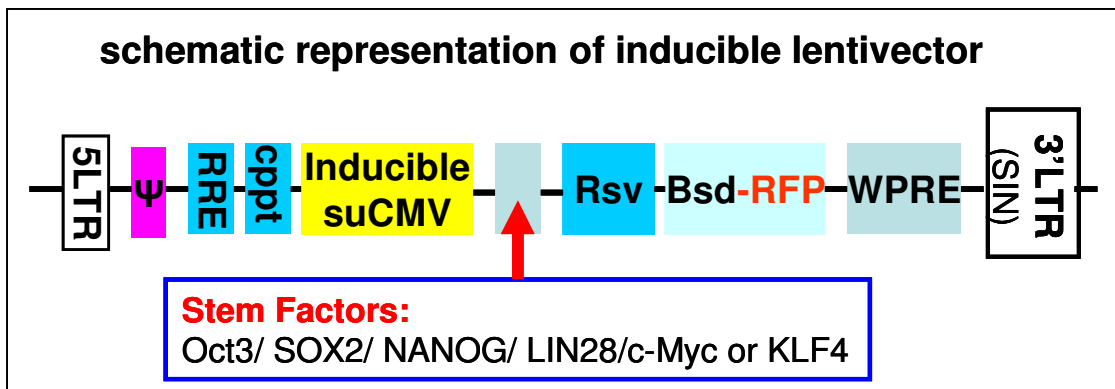
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different, the main stem cell factors are: OCT3/4, SOX2, NANOG, LIN28, c-Myc and KLF4.

AMSBIO/GenTarget's pre-made lentiviral particles for iPSC are generated from its proprietary lentiviral vector system (see vector map scheme below). Six **mouse** stem cell factors were first individually cloned into lentivector. Then, lentivectors were co-transfected with a packaging mix (Cat# **HT-pack**) into a 293T packaging cells (cat# **TLV-C**). The pre-made lentiviral particles are VSV-G pseudotyped virus, packaged in **serum-free** medium with 60ug/ml polybrene, and supplied as 200ul/per vial at $> 1 \times 10^7$ IFU/ml. (Note: Polybrene was reported to enhance virus transduction at 6-8ug/ml final concentration. But Polybrene could be toxic to some cell types.)



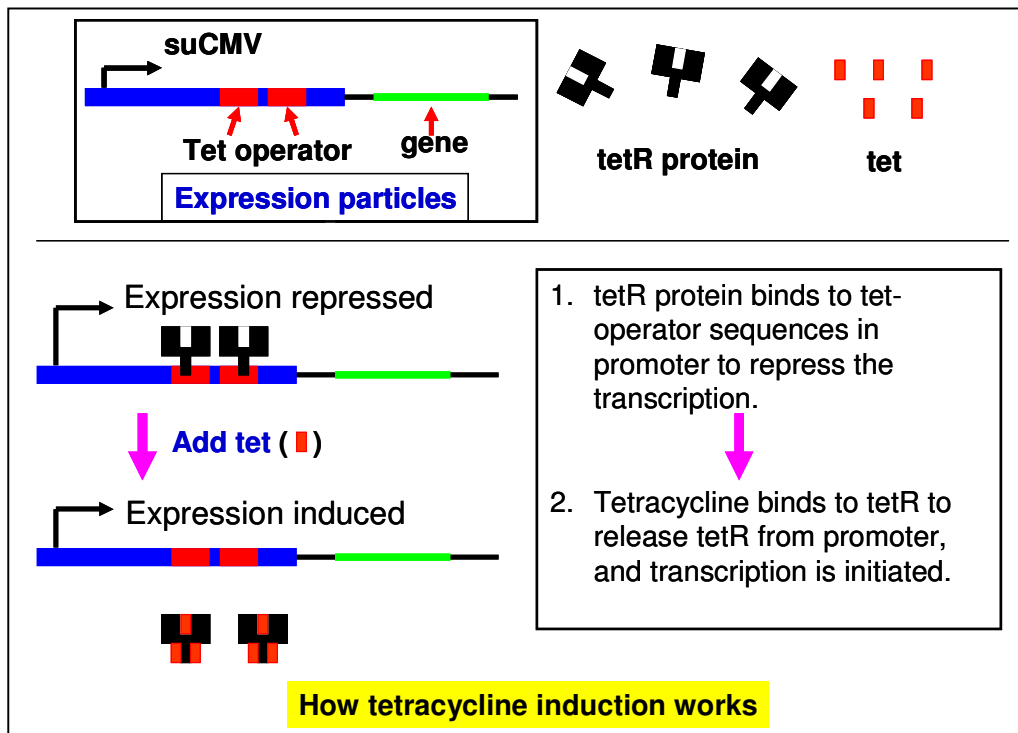
All six stem factor were sequencing verified. Their sequences fully match to the CD region according to the NCBI's database (see table below). Lentiviral particles contain blasticidin-RFP fusion marker, which allow to select the transduced cells by either fluorescence sorting or Blasticidin selection.

Target	NCBI ID	Matched ORF position
m Myc	NM_010849.4	582 ~ 1946
m Klf4	NM_010637.3	605 ~ 2056
m Oct4	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

Each stem factor was natively expressed (without any tags) under a tetracycline inducible suCMV promoter in which two tetracycline operator sequences was integrated. The particles can be used for regular constitutive high expression. And the same particles can also used as tetracycline induced expression when the tetracycline regulator 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 1ug/ml final concentration. Please see the schematic instruction (below) for the mechanism of inducible expression, and see our website for more details about **Inducible lentiviral system**. For particles general information, please refer to **FAQ about premade lentiviral particles**.



Experimental Protocols for generating iPS cells (for your reference only):

(Note: for the purpose other than generating iPS cells, please follow our web-link: general transduction protocols under premade lentiviral particles)

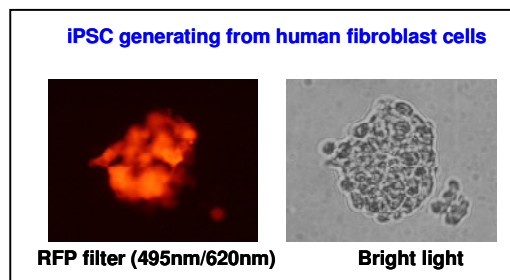
1. Seed the desired parent cells at 1×10^5 cells/well in 24-well plate, incubated overnight;
2. Add 50ul of each lentiviral particle for iPSC (Oct3/4, Sox2, NANOG, LIN28, c-Myc and Klf4)

Notes:

- You can set up your own factor combination dependent upon your cell types).
- Optional: to check the transduction ability of your cells, add 50ul of **GFP(His) Control Lentiviral particles** (LVP002) into cells. Check the expression level by GFP signal and transduction efficiency by RFP signal under fluorescent microscope.



3. Change medium into completed medium at 12hr or longer time point after transduction (please be noticed longer transduction time resulted more cell dead as we observed the over-expressed Klf4, SOX2 and c-Myc caused cell death), and incubated again overnight. **Notes: you may use the serum-free particles without the worry about the unwanted differentiation)**
4. Check cell viability: you may observe some cells are dying (floating up). From now on change medium with serum-free medium every two days.
5. Check transduction efficiency (at 72 hours after transduction) by vitalizing RFP signal under florescent microscope using RFP filter (filer: Ex~550 / Em620nm). You may observe majority cell are dead, but there are some living cells showing good RFP signal.
6. At day 8~10, split transduced live cells into feeder cells using your defined medium, continue incubate for 24~48hrs,
7. Change medium with hES medium, continue incubate and change hES medium everyday;
8. At day 13 ~ 18: you will observe cell's morphology changes, the ES like colonies forming (see sample image below).



Safety Precaution:

Please use extra caution when using lentiviral particles. Remember, you are dealing with transduction particles which can infection human cells. **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.

Related Product		
Premade lentiviral particles for six human stem factors: Cat#: LVP003, LVP004, LVP005, LPV006, LVP007, LVP008 and LVP-stems		
SC015	h Oct3/4 stable cells	2 x 10 ⁶ cells
SC016	h LIN28 stable cells	2 x 10 ⁶ cells
SC017	h NANOG stable cells	2 x 10 ⁶ cells

References:

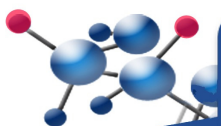
1. [NIH stem cell training program \(Link\)](#).



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2. 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.
3. 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.
4. Park, I.H., et al., Reprogramming of human somatic cells to pluripotency with defined factors. *Nature*, 2008. 451(7175): p. 141-6.
5. 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.
6. NIH Guidelines for [Biosafety Considerations for Research with Lentiviral Vectors](#). (Link).
7. [CDC guidelines for Lab Biosafety levels \(Link\)](#).



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