InvivoGen Insight

INNOVATION WITHIN REACH

SPRING 2010

Lentiviral vectors are increasingly becoming the tool of choice for gene delivery to difficult-to-transfect cells. Currently, production of high titers of lentiviral vectors is a time consuming, multi-step procedure with low reproducibility. To solve these problems, InvivoGen has developed LENTI-Smart™, a novel method to generate high titers of lentiviral vectors, simply, rapidly and efficiently. Depending on your needs, LENTI-Smart™ kits are available for the production of either integrating or non-integrating lentiviral (NIL) vectors. Integrating lentiviruses are best for stable transgene expression. NIL vectors are particularly useful for transient transgene expression in gene therapy protocols and stem cell modifications, where the risk of insertional mutagenesis is a safety concern. This newsletter also features innovative products for the generation of induced pluripotent stem (iPS) cells. These products comprise reprogramming enhancers, plasmids for the production of tagged cell-penetrating reprogramming factors and the multicistronic LENTI-Smart™ OSKM expression vector.

Inside this issue:

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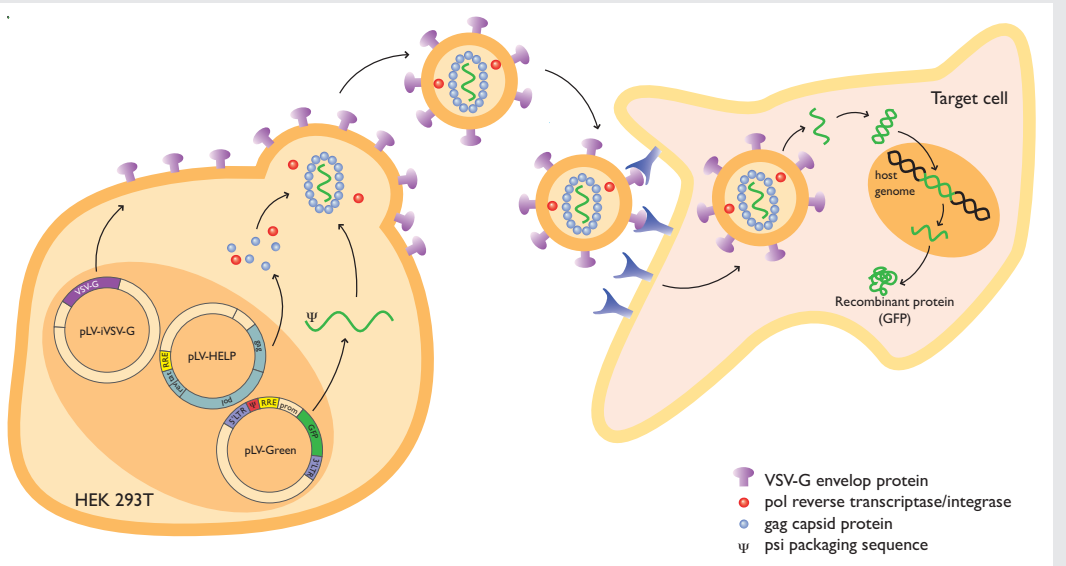
Lentiviral Vector Production and Cell Transduction

Lentiviral vectors derived from the human immunodeficiency virus (HIV-1) have become major tools for gene delivery in mammalian cells. The advantageous feature of lentivirus vectors is the ability to mediate potent transduction and stable expression into dividing and non-dividing cells both *in vitro* and *in vivo*. Lentiviral vectors are typically produced in HEK 293T cells. Essential lentiviral (HIV-1) genes must be expressed in these cells to allow the generation of lentiviral particles. These genes are usually expressed by several plasmids: (i) a lentiviral expression plasmid, such as pLV-Green, containing the *psi* (Ψ) packaging sequence and the transgene gene inserted between the lentiviral LTRs allow target cell integration, (ii) a packaging plasmid, such as pLV-HELP, encoding the *pol, gag, rev* and *tat* viral genes and containing the *rev*-response element (RRE); and (iii) a pseudotyping plasmid, such as pLV-iVSV-G, encoding the G protein of the Vesicular Stomatitis Virus (VSV-G) envelope gene. Unlike the HIV envelope, the VSV-G envelope has a broad cell host range extending the cell types that can be transduced by VSV-G-expressing lentiviruses. Two days after transfection of HEK 293T cells, the cell supernatant contains recombinant lentiviral vectors, which can be used to transduce the target cells. Once in the target cells, the viral RNA is reverse-transcribed, imported into the nucleus and stably integrated into the host genome. One or two days after the integration of the viral RNA, the expression of the recombinant protein can be detected.



LENTI-Smart™ - Integrating / Non-Integrating Lentiviral Vectors Made Easy

Lentiviral vectors are major tools for gene delivery in mammalian cells, with the ability to mediate potent transduction and stable expression into dividing and non-dividing cells both *in vitro* and *in vivo*. LENTI-Smart™ is a lyophilizate of optimized packaging plasmids combined with a DNA transfection reagent which upon rehydration serves as a "carrier" for your favorite lentiviral expression plasmid. LENTI-Smart™ kits are available for the production of either integrating or non-integrating lentiviral (NIL) vectors.

- **LENTI-Smart™ (INT)** allows the generation of integrating lentiviruses for long term expression in dividing and non-dividing cells.
- **LENTI-Smart™ NIL** is the first kit designed for the generation of non-integrating lentiviral vectors, allowing transient transgene expression in dividing cells and long-term expression in non-dividing cells.

Description

LENTI-Smart™ is a ready-to-use product that allows for rapid and reliable production of high titers of second generation lentiviral particles in HEK 293T cells. LENTI-Smart™ combines a mix of optimized packaging plasmids precomplexed to a transfection reagent, LyoVec™, selected for its high transfection efficiency and low cell toxicity. This lyophilized complex is provided with a control lentiviral expression plasmid.

Packaging Plasmids

The two packaging plasmids forming the LENTI-Smart™ lyophilizate provide the structural and replication proteins *in trans* that are required for the production of the lentiviral particles.

- **pLV-iVSV-G** expresses the G glycoprotein gene from Vesicular Stomatitis Virus (VSV-G) to allow production of a pseudotyped lentiviral vector with a broad host range.
- **pLV-HELP,** in the LENTI-Smart™ (INT) kit, contains the viral *gag*, *pol*, *rev* and *tat* genes and the *rev*-responsive element (RRE).
- **pLV-HELP-NIL D64,** in the LENTI-Smart™ NIL kit, expresses a mutant integrase (D64V) resulting in the generation of lentiviral vectors that are integration defective[1,2].

Control Lentiviral Expression Plasmid

The LENTI-Smart™ kit includes a control lentiviral expression plasmid designed to optimize virus production and cell transduction.

- **pLV-Green** expresses a green fluorescent protein (GFP) gene and contains key viral elements for lentivirus production and safety:

Contents and Storage

LENTI-Smart™ (INT) and LENTI-Smart™ NIL are available in 2 sizes, either 5 vials or 10 vials. Each vial allows the transfection of HEK 293T cells with a lentiviral expression plasmid in a 10-cm culture plate or a 75 cm^2 flask.

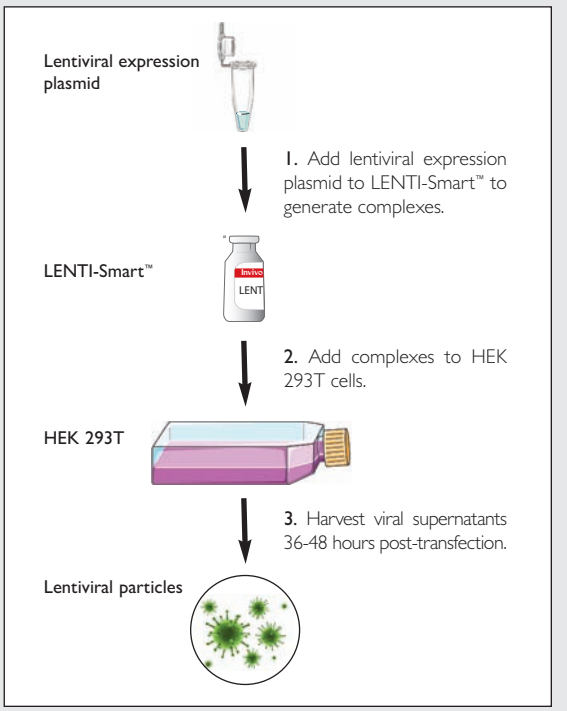
A LENTI-Smart™ Starter Kit is also available to allow the generation of both integrating and non-integrating lentiviral vectors. It contains 5 vials of LENTI-Smart™ (INT) and 5 vials of LENTI-Smart™ NIL.

All LENTI-Smart™ kits are provided with a vial of the control lentiviral expression plasmid. The LENTI-Smart™ vials are provided lyophilized, the control plasmid is provided as a liquid. Products are shipped at room temperature and should be stored at -20°C.

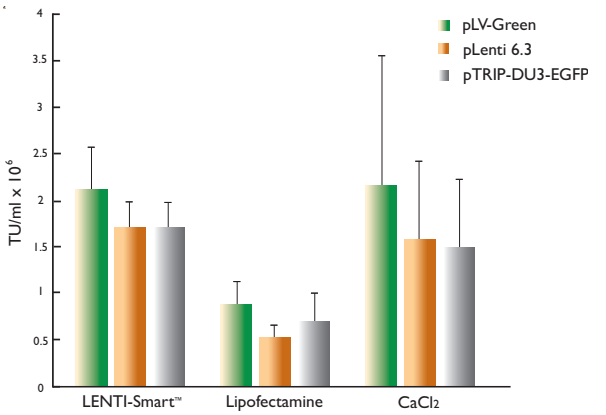
User manual and simplified maps are available on our website:
www.invivogen.com/lentismart
Sequences of the plasmids are provided upon request.

PRODUCT	QTY	CAT. CODE
LENTI-Smart™ (INT)	5 vials 10 vials	ltsint-5 ltsint-10
LENTI-Smart™ NIL	5 vials 10 vials	ltsnil-5 ltsnil-10
LENTI-Smart™ Starter Kit	10 vials	lts-str

LENTI-Smart™ Procedure



The unique formulation of LENTI-Smart™ allows to prepare lentiviral expression plasmid / packaging plasmids complexes by simply rehydrating the lyophilizate with the lentiviral expression plasmid solution. There is no need for a transfection reagent as it is included in the LENTI-Smart™ lyophilizate. Transfection of HEK 293T cells is readily performed by adding the complexes to the cells. Lentiviral particles can be collected 2 days after transfection.

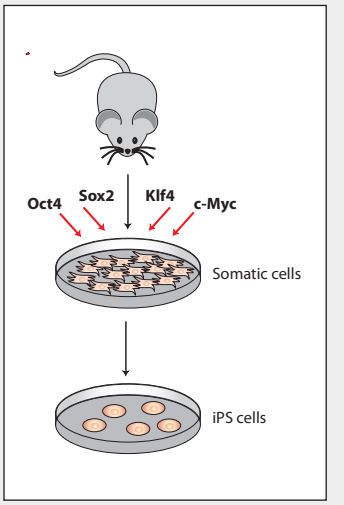


Viral titers obtained using LENTI-Smart™ and other transfection reagents with different commercially available lentiviral expression plasmids. Reproducibility is superior with LENTI-Smart™ (reduced standard deviation, n=5).

Generation of iPS Cells

Generation of induced pluripotent stem (iPS) cells by reprogramming adult somatic cells can be achieved by ectopic expression of specific transcription factors. The most widely used set of "reprogramming" factors, Oct4, Sox2, Klf4 and c-Myc, was identified initially by screening 24 pre-selected factors in mouse embryonic fibroblasts (MEFs) by Takahashi and Yamanaka[1]. This cocktail of transcription factors, OSKM, was shown to work for different types of somatic cells and for different species, including rhesus monkey[2] and human cells[3]. Further studies have demonstrated that Oct4 and Sox2 are indispensable whereas Klf4 and c-Myc are dispensable (although the efficiency of iPS cell formation is significantly lower)[4]. The generation of iPS cells is usually achieved by genetic transduction of the reprogramming genes using retroviral or lentiviral vectors. However, the use of integrating viral vectors represent an obstacle to the therapeutic translation of iPS cells as this technology can produce insertional mutagenic lesions that are potentially tumorigenic. Two recent publications detail the use of polycistronic lentiviral vectors delivering the OSKM quartet to somatic cells in a single lentiviral construct reducing the number of genomic insertions[5, 6]. Alternative approaches to deliver the reprogramming factors with minimal or total absence of genetic modifications have been developed. These approaches include the use of LoxP sites and Cre-induced excision and piggyBac transposon excision of integrated reprogramming vector sequences[7, 8], and the use of an oriP/EBNA1-based episomal vector[9]. Non-integrating lentiviral vectors may also represent a promising approach[10]. One possible strategy to entirely replace gene delivery is protein transduction. Previous studies have demonstrated that various proteins can be delivered into cells by conjugating them with a short peptide that mediates cell penetration, such as poly-arginine[11]. Zhou *et al.* have designed and purified poly-arginine tagged Oct4, Sox2, Klf4 and c-Myc proteins that were found to readily enter cells and translocate into the nucleus[12]. After several cycles of protein supplementation, iPS cells were successfully generated from MEFs. Using a similar approach, *Kim et al.* obtained protein-induced pluripotent stem cells from human newborn fibroblasts after several rounds of treatment with cell extracts of HEK293 cell lines expressing poly-arginine tagged OSKM genes[13]. Substantial research and development is still required before iPS cells are ready for therapeutic applications.

1. Takahashi K. & Yamanaka S., 2006. Cell. 126:663-76. **2. Liu H. *et al.*, 2008.** Cell Stem Cell. 3:587-90. **3. Takahashi K. *et al.*, 2007.** Cell. 131:861-72. **4. Nakagawa M. *et al.*, 2008.** Nat Biotechnol. 26:101-6. **5. Sommer CA. *et al.*, 2009.** Stem Cells. 27:543-9. **6. Chang CW. *et al.*, 2009.** Stem Cells. 27:1042-9. **7. Soldner F. *et al.*, 2009.** Cell. 136:964-77. **8. Kaji K. *et al.*, 2009.** Nature. 458:771-5. **9. Yu J. *et al.*, 2009.** Science. 324:797-801. **10. Sarkis C. *et al.*, 2008.** Curr Gene Ther. 8:430-7. **11. Ogawa T. *et al.*, 2007.** Stroke. 38: 1354 - 1361. **12. Zhou H. *et al.*, 2009.** Cell Stem Cell. 4:381-4. **13. Kim D. *et al.*, 2009.** Cell Stem Cell. 4:472-6.



Induction of pluripotent stem cells: Somatic cells are obtained from adult organism. The reprogramming factors are introduced into the cultured somatic cells The cells are grown under ES cells conditions. After 2-3 weeks, iPS cells emerge. These induced pluripotent stem cells may be differentiated into various cell types for regenerative medicine applications.

LENTI-Smart™ OSKM - Generation of iPS Cells

Induced pluripotent stem (iPS) cells are generated from differentiated somatic cells overexpressing a set of specific transcription factors called reprogramming factors. Typically four reprogramming factors are chosen, *Oct4*, *Sox2*, *Klf4* and *c-Myc* (OSKM), which are introduced into the target cells through genetic transduction using retroviral or lentiviral vectors. iPS cells offer exciting possibilities in stem cell research and regenerative medicine. To facilitate the generation of iPS cells, InvivoGen has developed LENTI-Smart™ OSKM for the production of lentiviral vectors expressing the **human or murine OSKM reprogramming genes**.

Description

LENTI-Smart™ OSKM comprises the complexing lyophilizate, formed by the packaging plasmids and the transfection reagent, and the lentiviral plasmid expressing the OSKM genes of human or mouse origin.

Single Reprogramming Expression Cassette

The four reprogramming genes are expressed from a single multicistronic transcript[1]. This reprogramming cassette contains the coding sequences of Oct4, Sox2, Klf4 and c-Myc separated by three different "self-cleaving" 2A peptides (E2A, P2A, and T2A, respectively)[2].

Integrating or Non-Integrating Lentiviral Vectors

Two LENTI-Smart™ OSKM kits are available allowing the generation of integrating or non-integrating lentiviral vectors:

- **LENTI-Smart™ (INT) OSKM** features pLV-HELP which expresses the wild-type integrase for the production of integrating lentiviral vectors
- **LENTI-Smart™ NIL OSKM** features pLV-HELP-NIL which expresses a mutant integrase for the production of non-integrating lentiviral (NIL) vectors, designed for transient transgene expression.

1. Carey BW. *et al.*, 2009. PNAS 106:157-62. **2. Donnelly ML. *et al.*, 2001.** J Gen Virol. 82:1027-41.

Schematic representation of the "reprogramming cassette"

Contents and Storage

LENTI-Smart™ (INT) OSKM and LENTI-Smart™ NIL OSKM are available in 2 sizes, either 5 vials or 10 vials. Each vial allows the transfection of HEK 293T cells with the lentiviral OSKM expression plasmid in a 10-cm culture plate or a 75 cm^2 flask. The LENTI-Smart™ vials are provided lyophilized, the OSKM plasmid is provided as a liquid. Products are shipped at room temperature and should be stored at -20°C.

PRODUCT	QTY	CAT. CODE
LENTI-Smart™ (INT) hOSKM	5 vials 10 vials	ltsint-hoskm-5 ltsint-hoskm-10
LENTI-Smart™ (INT) mOSKM	5 vials 10 vials	ltsint-moskm-5 ltsint-moskm-10
LENTI-Smart™ NIL hOSKM	5 vials 10 vials	ltsnil-hoskm-5 ltsnil-hoskm-10
LENTI-Smart™ NIL mOSKM	5 vials 10 vials	ltsnil-moskm-5 ltsnil-moskm-10

Poly-Arginine-HA Tagged Reprogramming Factors

Description

Poly-arginine-HA tagged reprogramming factors allow the production of recombinant cell-penetrating reprogramming factors. They correspond to the four transcription factors, Oct4, Sox2, Klf4, and c-Myc (OSKM), fused at their C terminus to a poly-arginine (i.e. 11R) peptide in tandem with 3 motifs of the hemaglutinine (HA) tag. The poly-arginine peptide enables the recombinant proteins to readily enter the cells and have been shown to allow their translocation into the nucleus[1, 2]. The HA tag is useful for their detection by Western blot or their purification by affinity chromatography.

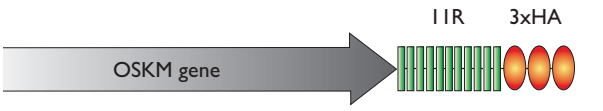
Poly-arginine-HA tagged reprogramming factors are cloned in the pUNO1 plasmid within a mammalian expression cassette comprising the EF-1α/HTLV composite promoter and the SV40 poly adenylation sequence. pUNO1 plasmids are selectable in *E. coli* and mammalian cells with blasticidin.

1. Zhou H. *et al.*, 2009. Cell Stem Cell. 4:381-4. **2. Kim D. *et al.*, 2009.** Cell Stem Cell. 4:472-6.

PRODUCT	QTY	CAT. CODE (HUMAN)	CAT. CODE (MOUSE)
pUNO1-OCT4-11RHA	*E. coli*	puno1rha-hoct4	puno1rha-moct4
pUNO1-SOX2-11RHA	*E. coli*	puno1rha-hsox2	puno1rha-msox2
pUNO1-KLF4-11RHA	*E. coli*	puno1rha-hklf4	puno1rha-mklf4
pUNO1-cMYC-11RHA	*E. coli*	puno1rha-hmycb	puno1rha-mmycb

Applications

Poly-arginine-HA tagged reprogramming factors are designed for the generation of protein-induced pluripotent cells. Following their transfection into mammalian cell lines, such as HEK293, the cell extracts can be used crude to treat the target cells or processed to purifiy the poly-arginine-HA-tagged proteins on an anti-HA affinity column.



Schematic representation of a poly-arginine-HA tagged reprogramming gene

For native reprogramming factors in the pUNO1 plasmid, see www.invivogen.com/ipsc

Contents and Storage

Each pUNO1 plasmid is provided as a lyophilized transformed *E. coli* strain on a paper disk. Transformed strains are shipped at room temperature and should be stored at -20°C.
Each pUNO1 is provided with 4 pouches of *E. coli* Fast-Media® Blas (2 TB and 2 Agar).

Reprogramming Enhancers

Direct reprogramming of somatic cells is currently a slow and inefficient process, in particular when the *c-Myc* oncogene is omitted in an effort to reduce tumorigenicity. Several chemicals have recently been reported to either enhance reprogramming efficiencies or substitute for specific reprogramming factors. Among the reported chemicals, some are known to affect chromatin modifications while others influence signal transduction pathways.

PRODUCT	DESCRIPTION	WORKING CONCENTRATION	QUANTITY	CATALOG CODE
5-Aza-cytidine	DNA methyltransferase inhibitor	2 µM	100 mg	inh-aza
Bix-01294	G9a histone methyltransferase inhibitor	1 µM	2 mg	inh-bix
PD0325901	MEK inhibitor	0.5 µM	2 mg	inh-pd32
SB431542	TGF-β receptor inhibitor	2 µM	5 mg	inh-sb43
Valproic Acid	HDAC inhibitor	2 mM	5 g	inh-vpa

Small molecules that modulate chromatin modifications include the DNA methyltransferase inhibitor (5-azacytidine), the histone deacetylase inhibitor (valproic acid) and a G9a histone methyltransferase (Bix-01294)[1, 2, 3]. The MEK inhibitor PD035901 and the TGF-β receptor inhibitor SB431542 potentiate reprogramming by targeting signaling pathways[4].

1. Shi Y *et al.*, 2008. Cell Stem Cell. 3:568-74. **2. Huangfu D. *et al.*, 2008.** Nat Biotechnol.;26:795-7. **3. Durcova-Hills G. *et al.*, 2008.** PLoS One. 3:e3531. **4. Lin T. *et al.*, 2009.** Nat Methods. 6:805-8.

Contents and Storage

Each product is provided as a solid and shipped at room temperature. Store at room temperature, 4°C or -20°C according to the product label.

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