pLentG-KOSM Lentiviral Vector

STORAGE: -80ºC

QUANTITY AND CONCENTRATION: 100 µL of bacterial glycerol stock

Background

Lentiviral vectors based on the human immunodeficiency virus-1 (HIV-1) have become a promising tool for gene transfer studies. The advantageous feature of lentivirus vectors is the ability of gene transfer and integration into dividing and non-dividing cells. The pseudotyped envelope with vesicular stomatitis virus envelope G (VSV-G) protein broadens the target cell range. Lentiviral vectors have also proven to be effective in transducing brain, liver, muscle, and retina *in vivo* without toxicity or immune responses.

Induced pluripotent stem (iPS) cells can be generated from various somatic cells by the retrovirus- or lentivirus-mediated transfection of four transcription factors, namely Oct3/4, Sox2, c-Myc, and Klf4. iPS cells are indistinguishable from ES cells in morphology, proliferation, gene expression, and teratoma formation. Furthermore, when transplanted into blastocysts, iPS cells can give rise to adult chimeras, which are competent for germline transmission. However, reprogramming by viral infection of defined TFs is still inefficient (from 0.001% to 0.1 %) and requires very high transduction efficiency. Mouse embryo fibroblasts (MEFs) need at least 30% retrovirus transduction efficiency and an average of 15 different proviral copies to be reprogrammed into iPS cells. Although virus-free mouse iPS cells were recently generated by direct protein delivery, adenovirus-mediated gene delivery and DNA transfection approaches, efficiency of iPS cell generation is significantly lower (0.0001% - 0.0015%), compared with the retroviral or lentiviral infection approaches. Thus, lentivirus/retrovirus-mediated reprogramming methods are still major reprogramming approaches for generation of iPS cells, at least for basic research purposes.

pLentG-KOSM is a simple lentiviral vector in which defined factors are in-frame fused into a single open reading frame (ORF) via self-cleaving 2A peptides and are controlled by a CMV promoter. The mouse transcription factor ORF is followed by IRES-GFP as a reporter for viral transduction. This polycistronic expression system can efficiently reprogram mouse or human somatic cells into iPS cells; for example, in infected MEF (GFP^+), the reprogramming efficiency is around 1%, which is at least 10-fold higher than the reprogramming method using a pool of viruses in which each virus expresses a different stem cell factor gene. Notably, most of the iPS cells generated using the pLentG-KOSM system contain only a single copy of the viral vector (Ref. 1).

Lentivirus Production

Lentiviral supernatant can be produced by cotransfecting 293T cells (Cat.# LTV-100) with pLentG-KOSM and a second or third generation lentivirus packaging mix such as Cell Biolabs' ViraSafe™ Lentiviral Packaging System (Cat. # VPK-206). Supernatants can be used directly or purified/concentrated if needed. For long term storage, store supernatant at -80ºC in aliquots.

Post-Packaging Considerations

1. **Concentration and purification of your lentivirus**: Because of the latent nature of lentivirus, it is imperative that your virus be highly concentrated before infecting your host cell. Also, impurities from your viral supernatant can decrease the efficiency of infection. We recommend using Cell Biolabs' ViraBind™ Lentivirus Concentration and Purification Kit (Catalog # VPK-090).

2. **Measure the titer of your lentivirus**: This is an important step to ensure consistent viral transduction into your host cell. However, QPCR or stable clone counting can take as much as 1-2 weeks to perform. Traditional p24 ELISA kits can greatly overestimate your lentiviral titer. Our advanced p24 ELISA, QuickTiter™ Lentivirus Titer Kit (Catalog # VPK-107), uses exclusive technology that eliminates free p24 from your supernatant, giving you much more accurate lentiviral titers. Results are obtained in 6-18 hours.

3. **Use transduction reagents to increase infection efficiency:** Many cells are difficult to infect with lentivirus, and without supplemental reagents transduction efficiencies can be low. Reagents such as Polybrene® can help, but are often insufficient. Cell Biolabs' proprietary reagents in our ViraDuctin™ Lentivirus Transduction Kit (Catalog # LTV-200) form a super-complex with your virus to increase transduction efficiencies by promoting virus and cell interaction.

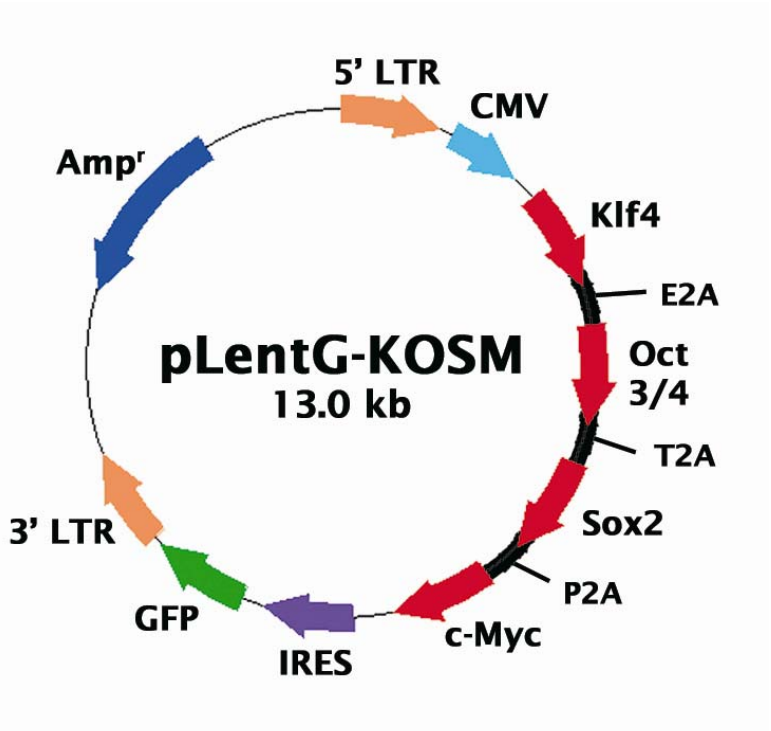

Figure 1. Schematic representation of pLentG-KOSM lentiviral vector (13 kb). Digestion with NotI and RsrII produces 3 kb and 10 kb DNA fragments.

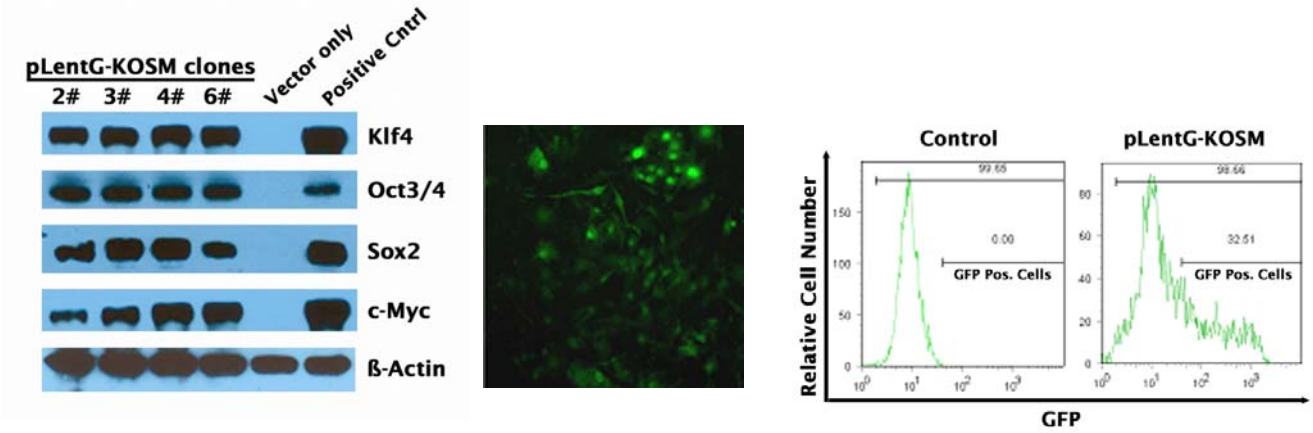

Figure 2. Expression of Stem Cell Factors and GFP. (Left) Expression of *KOSM* fusion gene in 293T cells. pLentG-KOSM vector was transiently transfected into 293T cells. Correct processing of each factor was confirmed by western blot analysis. Expression vectors containing *KLF4*, *OCT3/4*, *SOX2*, and *c-MYC* cDNA were included as positive controls. (Middle) Representation of GFP image in MEFs. GFP fluorescence was observed 3 days after infection with lentiviruses containing the *KOSM* fusion gene. (Right) Representation of flow cytometry analysis of GFP-expressing MEFs 3 days after infection.

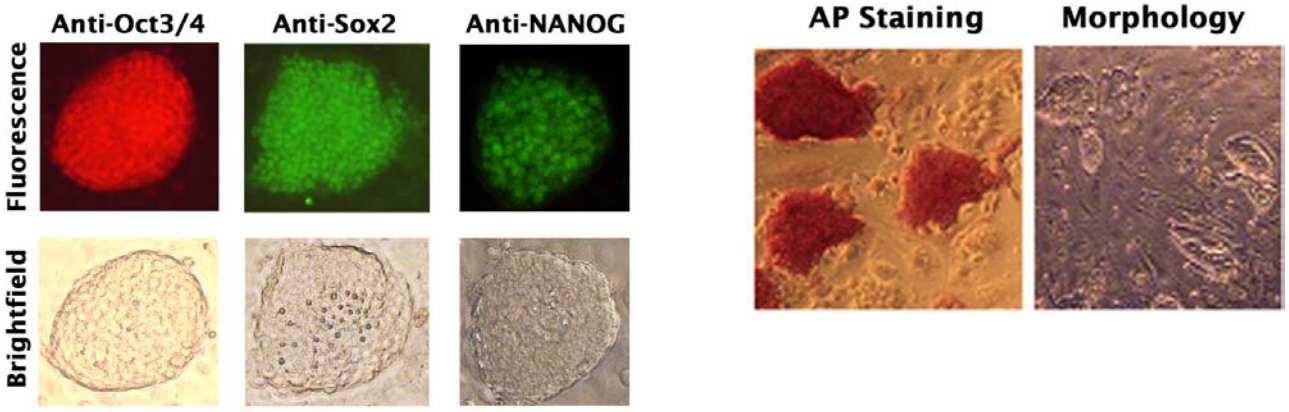

Figure 3. Characterization of induced iPS cell colonies generated from MEFs infected with the lentiviruses containing KOSM fusion gene. (Left) Staining of pluripotency markers (OCT3/4, SOX2, and NANOG) in induced cell colonies (200× magnification). (Right) AP staining (left panel, 100× magnification) and morphology (right panel, 40× magnification) in induced ES cell-like cell colonies.

Safety Consideration

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. Always wear gloves, use filtered tips and work under a biosafety hood.

References

1. Shao, L, *et al*., (2009) *Cell Research* **19**, 296-306.
2. Carey, B, *et al.,* (2009) *Proc. Natl. Acad. Sci. U. S. A.* **106**, 157-162.

3. Okita, K; Ichisaka, T; Yamanaka, S. (2007) *Nature* **448**:313–317.
4. Takahashi, K; Yamanaka, S. (2006) *Cell* **126**:663–676.
5. Takahashi, K; Tanabe, K; Ohnuki, M; Narita, M; Ichisaka, T, et al. (2007) *Cell* **131**:861–872.

License Information

Appendix

1. **Sequence for KOSM fusion gene**
   Blue: Mouse Klf4; Red: Mouse Oct-3; Green: Mouse Sox2; Violet: Mouse c-Myc
   Other: Self-cleaving 2A Peptides

ATGGCTGTCAGCGACGCTCTGCTCCCGTCCTTCTCCACGTTCGCGTCCGGCCCGGCGGGAAGGGAGAAGACACTGCGTCCAGCAG
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2. **Sequence for KOSM fusion protein**
   Blue: Mouse Klf4; Red: Mouse Oct-3; Green: Mouse Sox2; Violet: Mouse c-Myc
   Other: Self-cleaving 2A Peptides

MAVSDALLPSFSTFASGPAGREKTLRPAGAPTNRWREELSHMKRLPPLPGRPYDLAATVATDLESGGAGAACSSNNPALLARRET
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