pRetroG-hOKSM Retroviral Vector

CATALOG NUMBER: RTV-700 **STORAGE:** -80ºC

QUANTITY AND CONCENTRATION: 100 µL of bacterial glycerol stock

Background

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.

pRetroG-hOKSM is a simple retroviral vector in which defined factors are in-frame fused into a single open reading frame (ORF) via self-cleaving 2A peptides. The human transcription factor ORF is followed by IRES-GFP as a reporter for viral transduction. This polycistronic expression vector, when packaged into a retrovirus, can efficiently reprogram human somatic cells into iPS cells in the presence of 0.5 mM sodium butyrate or other reprogram-enhancing reagents (See Ref. 1 for detail).

Retrovirus Production

Retroviral supernatant can be produced by co-transfecting 293T or 293RTV cells (Cat. #RV-100) with pRetroG-hOKSM along with plasmids coding for MMLV gag and pol genes (Cat. #RV-111) and VSVG (Cat. #RV-110). Alternatively pRetroG-hOKSM may be transfected into a retroviral packaging cell line such as Plat-A (Cat. #RV-102) or co-transfected with a VSVG-coding plasmid into Plat-GP (Cat. #RV-103). Supernatants can be used directly or purified/concentrated if needed. Supernatants should be used as soon as possible after retroviral production; long term storage is not recommended.

Post-Packaging Considerations

1. **Concentration and purification of your retrovirus**: Because of the latent nature of retrovirus, 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™ Retrovirus Concentration and Purification Kit (Cat. # VPK-130).

2. **Measure the titer of your retrovirus**: This is an important step to ensure consistent viral transduction into your host cell. Our QuickTiter™ Retrovirus Quantitation Kit (Cat. # VPK-120) allows you to determine the physical titer of your retroviral supernatant in about one hour.

3. **Use transduction reagents to increase infection efficiency:** Many cells are difficult to infect with retrovirus, 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™ Retrovirus Transduction Kit (Cat. # RV-200) form a super-complex with your virus to increase transduction efficiencies by promoting virus and cell interaction.

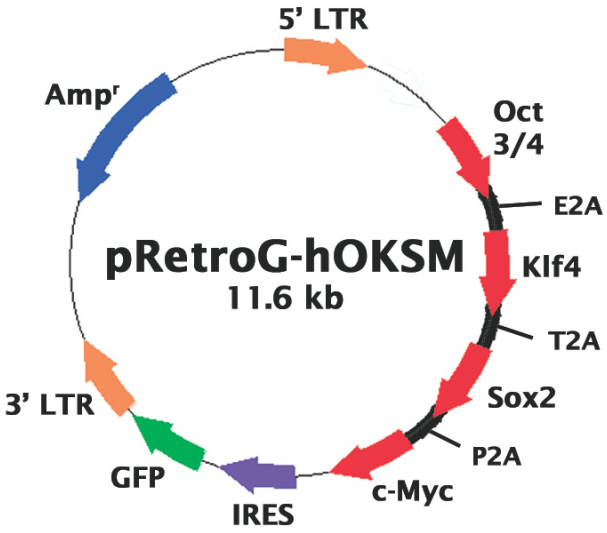

Figure 1. Schematic representation of pRetroG-hOKSM retroviral vector (11.6 kb). Digestion with NotI and BamHI produces 5.0 kb and 6.5 kb DNA fragments.

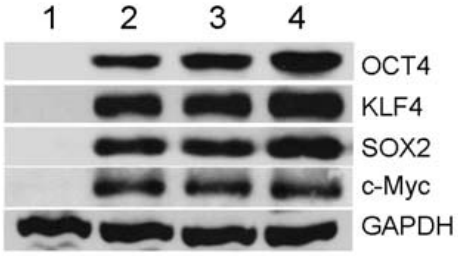

Figure 2. Expression of Stem Cell Factors and GFP. Western blot analysis of four transcription factors from the hOKSM fusion gene. **Lane 1**: Empty pRetroG plasmid (negative control). **Lanes 2 and 3**: pRetroG-hOKSM (two clones) plasmid was transfected transiently into U2OS cells and expression of each transcription factor was confirmed by Western blot with each corresponding antibody. **Lane 4**: Individual expression plasmids containing a single transcription factor as positive control.

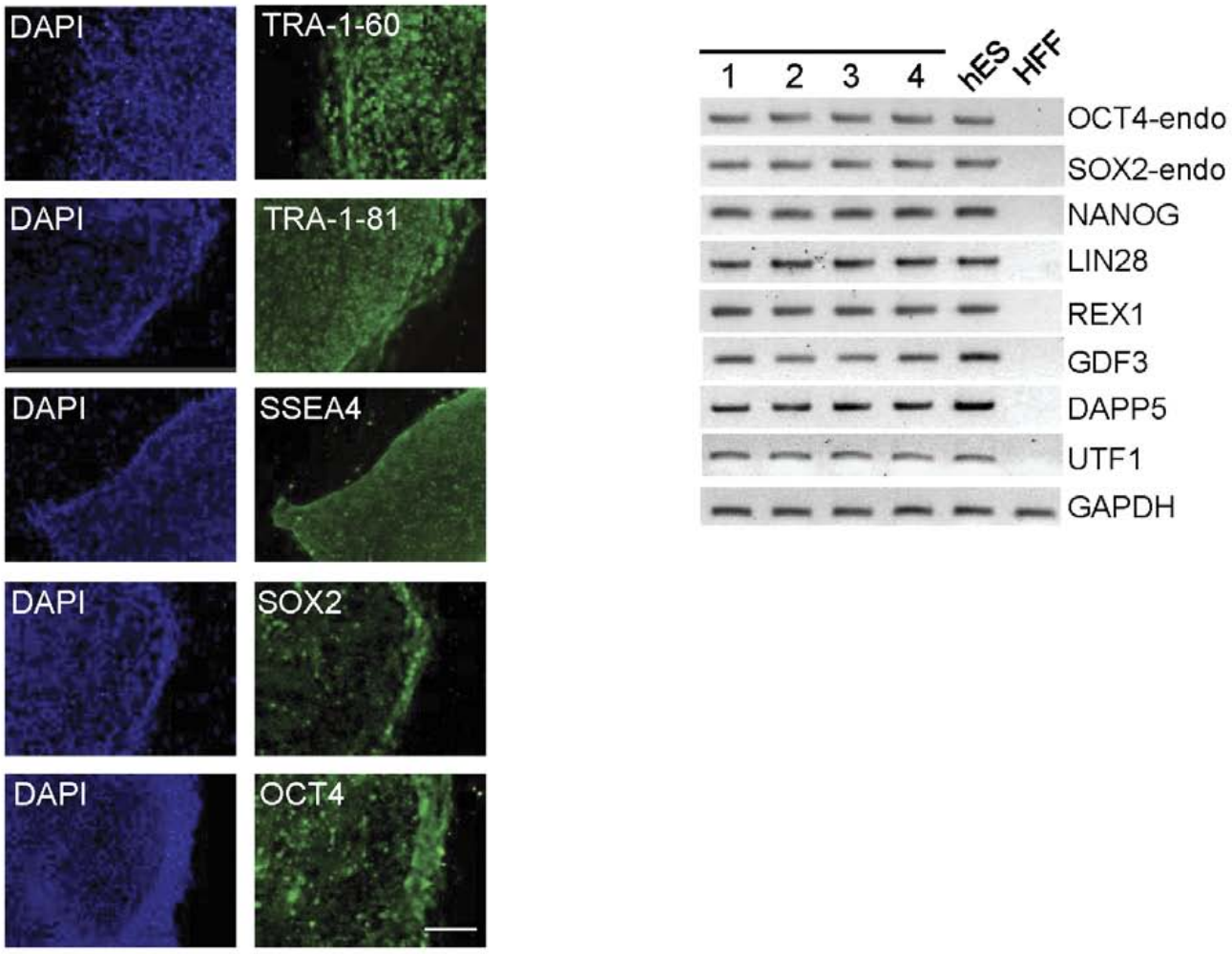

Figure 3. Characterization of Human iPS Cells Generated from Human Fibroblasts using Retroviruses Expressing OKSM Fusion Gene and One or Three Small Molecules. Left: Immunofluorescence staining of pluripotency markers in human iPS cells. **Right**: Semi-quantitative RT-PCR analysis of pluripotency markers in human iPS cells.

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. Zhang, Z, et al., (2011) *PLoS one* **6**, e26592.
2. Shao, L, *et al*., (2009) *Cell Research* **19**, 296-306.
3. Carey, B, *et al.*, (2009) *Proc. Natl. Acad. Sci. U. S. A.* **106**, 157-162.
4. Okita, K; Ichisaka, T; Yamanaka, S. (2007) *Nature* **448**:313–317.
5. Takahashi, K; Yamanaka, S. (2006) *Cell* **126**:663–676.
6. Takahashi, K; Tanabe, K; Ohnuki, M; Narita, M; Ichisaka, T, et al. (2007) *Cell* **131**:861–872.

License Information

The pRetroG-hOKSM vector is licensed from the Maine Medical Research Institute. Purchase of this product is for research use only by the purchaser. Resale of this product to a third party is strictly prohibited.

Appendix: pRetroG-kKOSM Sequence and Key Features

19-5031:	Human OKSM
19-1098:	Oct3/4
1180-2613:	Klf4
2689-3636:	Sox2
3715-5031:	c-Myc
5059-5622:	IRES
5623-6354:	GFP
6456-6970:	3' LTR
8269-9129:	Ampicillin Resistance
10,142-10,658:	5'LTR
10,797-11,546:	Retroviral Psi packaging element (Ψ+)

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