

bioreprogramming



# Reprogramming for Stem Cell Biology

Unlock extraordinary potential with  
Reprogramming Lentiviruses from Sigma Life Science

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## Reprogramming Lentiviruses

# Stemgent® Reprogramming Lentiviruses for iPSCs

**Sigma® Life Science is pleased to offer Stemgent Reprogramming Lentiviruses for the creation of induced pluripotent stem cells!**

### Qualified by leaders in Stem Cell Biology

Sigma's Stemgent lentiviruses have been developed and qualified by experts and leaders in stem cell biology. With proven lentiviruses, increase your chances for successful iPSC creation and advance your research faster.

### Experienced reprogramming scientists ready to assist

As a Sigma reprogramming lentivirus customer, you will have access to a team of experienced reprogramming experts ready to provide assistance with any reprogramming challenge. With support from reprogramming experts, our customers are free to focus on their research rather than on tedious troubleshooting.

### Stringent quality testing

Sigma's application testing ensures the highest quality of reprogramming lentiviruses; all lots of lentivirus have been confirmed to successfully produce iPSC cells or to express their specific transcription factors. With proven viruses, our customers minimize the need to repeat experiments and maximize their efficiency.

### Lentiviral format with Dox-inducible option

By choosing a lentiviral format, you will maximize your chances for successful incorporation and increase your yields of iPSC cells. Those who opt for the Dox-inducible promoter will have added control over reprogramming factor expression.

### Broad assortment of formats, concentrations, and species

Sigma's broad offering enables researchers to select the viruses optimal to their research – no need to compromise.

Sigma Life Science and Stemgent have partnered to bring you proven reprogramming viruses for iPSC generation. Available in Dox-inducible, polycistronic and non-inducible formats for both human and mouse cells, Stemgent reprogramming viruses have been application tested and approved by leaders in the stem cell community. All six reprogramming factors are available as individual viruses and in functional sets.

- Oct4t
- Klf4
- cMyc
- Sox2
- Lin28
- Nanog

When you're ready to order, simply call your local Sigma Customer Service representative (800-325-3010 in the US) or visit the order center website at [sigma-aldrich.com](http://sigma-aldrich.com).

For technical service, email [techserv@sial.com](mailto:techserv@sial.com) or call 800-325-5832 in the US. A friendly, knowledgeable scientist will be glad to assist you!



To learn more about Sigma's Stemgent Reprogramming Lentiviruses, visit [sigma.com/ipsc](http://sigma.com/ipsc)

# Reprogramming Lentiviruses for Human Cells

## Stemgent® Dox Lentivirus Set: h4F2A (Cat. No ST000066)



Sigma Life Science and Stemgent are pleased to introduce the newest member of their Reprogramming Lentivirus family: the Dox-inducible human polycistronic lentivirus (h4F2A), which offers four human transcription factors (OCT4, SOX2, KLF4, c-MYC) within a

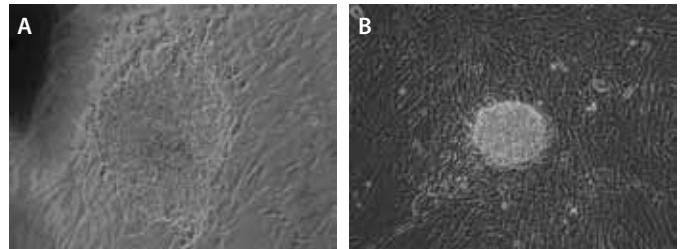
single, polycistronic expression cassette. Developed in the lab of Rudolph Jaenisch, M.D., at the Whitehead Institute at the Massachusetts Institute of Technology (MIT), the Stemgent Dox Lentivirus Set: h4F2A has been tested and proven to reprogram human somatic cells to induced pluripotent stem (iPS) cells. Introduction of the four factors from a single virus minimizes the number of random, proviral integrations required for successful reprogramming and reduces the risk of insertional mutagenesis effects in the resultant iPS cells, compared to those generated using four independent viruses.

- Advance your research faster with a proven technology, developed by a leader in Stem Cell Biology
- Save time, money, and precious samples with stringently tested tools of the highest quality
- Control expression of exogenous reprogramming factors with the Dox-inducible promoter
- Reduce the risk of insertional mutagenesis by delivering all four factors in one virus

The Stemgent Dox Lentivirus Set: h4F2A offers the four human transcription factors (OCT4, SOX2, KLF4, cMYC) within a single, polycistronic expression cassette, a separate constitutive rtTA lentivirus necessary for doxycycline (Dox)-inducible expression of the reprogramming factors, and a Dox-inducible Green Fluorescent Protein (tetO-GFP) lentivirus to serve as a transduction control. Each set contains an effective titer sufficient to perform two reprogramming reactions on 100,000 target cells in a 6-well plate format.

Each lot of the Stemgent Dox Lentivirus Set: h4F2A has been application tested to ensure functional reprogramming of human foreskin fibroblasts into iPS cells, confirmed by immunocytochemical (ICC) detection of markers of pluripotency on clonal iPS cell lines. Viral concentrations (TU/mL) have been determined through quantitative RT-PCR of transduced human cells.

## Reprogramming Human Fibroblasts using the Dox Inducible Polycistronic Lentivirus Set: Human 4F2A LoxP



**Figure 1: Dox-independent iPS Cell Colonies** Two examples of iPS cell colonies post-dox removal. **A)** This colony exhibits compact cell morphology, a two-dimensional colony, and a clean border ideal for picking and expansion. **B)** Smaller emergent iPS colony with more three-dimensional structure that requires additional growth before manual picking.

### Ordering Information

Cat. No.	Product Description	Species
ST000066-1SET	Stemgent Dox Lentivirus Set: h4F2A	Human

# Reprogramming Lentiviruses for Human Cells

## Stemgent® Dox Lentivirus Set: hOKSM (Concentrated) (Cat. No. ST000037)



Each doxycycline-inducible lentivirus in this set is capable of expressing one of four human transcription factors (Oct4, Klf4, Sox2 or c-Myc) under the control of the doxycycline (Dox)-inducible tet(O) operator when transduced into mammalian cells. Each unique

virus is provided as a VSV-G pseudotyped and concentrated virus stock capable of infecting both dividing and non-dividing cells. The expression of these four transcription factors, along with the reverse tetracycline transcriptional activator (rtTA), has been shown to reprogram human adult fibroblasts to an embryonic stem (ES) cell-like state known as the induced pluripotent stem (iPS) cell.<sup>1</sup> The Dox-Inducible GFP-Lentivirus is included as a transduction control.

The Stemgent Dox Lentivirus Set: hOKSM was developed in the lab of Rudolf Jaenisch, M.D., at the Whitehead Institute at MIT.<sup>1</sup> Dr. Jaenisch is a recognized leader in the study of epigenetic regulation of gene expression with numerous publications focused on ES and iPS cellular mechanisms and techniques. Individual viruses also available.

### Components:

- Stemgent Dox Lentivirus hSox2 (concentrated), 100 µL (Cat. No. ST070036)
- Stemgent Dox Lentivirus hOct4 (concentrated), 100 µL (Cat. No. ST070035)
- Stemgent Dox Lentivirus hc-Myc (concentrated), 100 µL (Cat. No. ST070038)
- Stemgent Dox Lentivirus hKlf4 (concentrated), 100 µL (Cat. No. ST070037)
- Stemgent Dox Lentivirus GFP (concentrated)
- Stemgent Dox Lentivirus rtTA (concentrated)

## Stemgent Dox Lentivirus Set: hOKSM (Cat. No. ST000036)



Each doxycycline-inducible lentivirus in this set is capable of expressing one of four human transcription factors (Oct4, Klf4, Sox2 or c-Myc) under the control of the doxycycline (Dox)-inducible tet(O) operator when transduced into mammalian cells. Each

unique virus is provided as a VSV-G pseudotyped virus stock capable of infecting both dividing and non-dividing cells. The expression of these four transcription factors, along with the reverse tetracycline transcriptional activator (rtTA), has been shown to reprogram human adult fibroblasts to an embryonic stem (ES) cell-like state known as the induced pluripotent stem (iPS) cell.<sup>1</sup> The GFP-Lentivirus is included as a transduction control.

The Stemgent Dox Lentivirus Set: hOKSM was developed in the lab of Rudolf Jaenisch, M.D., at the Whitehead Institute at MIT.<sup>1</sup> Dr. Jaenisch is a recognized leader in the study of epigenetic regulation of gene expression with numerous publications focused on ES and iPS cellular mechanisms and techniques. Individual viruses also available.

### Components:

- Stemgent Dox Lentivirus hSox2, 1 mL (Cat. No. ST070032)
- Stemgent Dox Lentivirus hOct4, 1 mL (Cat. No. ST070031)
- Stemgent Dox Lentivirus hc-Myc, 1 mL (Cat. No. ST070034)
- Stemgent Dox Lentivirus hKlf4, 1 mL (Cat. No. ST070033)
- Stemgent Dox Lentivirus GFP (concentrated)
- Stemgent Dox Lentivirus rtTA (concentrated)

### Ordering Information

Cat. No.	Product Description	Species
ST000037-1SET	Stemgent Dox Lentivirus Set: hOKSM conc	Human
ST000036-1SET	Stemgent Dox Lentivirus Set: hOKSM	Human
ST070031-1ML	Stemgent Dox Lentivirus hOct4	Human
ST070032-1ML	Stemgent Dox Lentivirus hSox2	Human
ST070033-1ML	Stemgent Dox Lentivirus hKlf4	Human
ST070034-1ML	Stemgent Dox Lentivirus hc-Myc	Human
ST070035-100UL	Stemgent Dox Lentivirus hOct4 conc	Human
ST070036-100UL	Stemgent Dox Lentivirus hSox2 conc	Human
ST070037-100UL	Stemgent Dox Lentivirus hKlf4 conc	Human
ST070038-100UL	Stemgent Dox Lentivirus hc-Myc conc	Human

**Stemgent® Lentivirus Set: hOKSM****(Cat. No. ST000044)**

The expression of the four human transcription factors (TFs) (Oct4, Klf4, Sox2, and c-Myc) has been shown to reprogram human adult fibroblasts to an embryonic stem (ES) cell-like state known as the induced pluripotent stem (iPS) cell.<sup>2</sup> The Stemgent Dox Lentivirus Set: hOKSM consists of four

lentiviruses, each of which is capable of expressing one of these four factors when transduced into mammalian cells. Each unique virus is provided as a VSV-G pseudotyped virus stock capable of efficiently transducing a wide range cell types. Individual viruses also available.

**Components:**

- Stemgent Lentivirus hSox2, 1 mL (**Cat. No. ST070012**)
- Stemgent Lentivirus hOct4, 1 mL (**Cat. No. ST070013**)
- Stemgent Lentivirus hc-Myc, 1 mL (**Cat. No. ST070014**)
- Stemgent Lentivirus hKlf4, 1 mL (**Cat. No. ST070015**)

**Stemgent Lentivirus Set: hOSLN****(Cat. No. ST000005)**

The Stemgent Lentivirus Set: hOSLN provides the human transcription factors Oct4, Sox2, Lin28, and Nanog for use in reprogramming somatic cells into an embryonic stem (ES) cell-like state known as the induced pluripotent stem (iPS) cell.<sup>3</sup> Each virus is provided as a VSV-G pseudotyped

pan-tropic virus stock capable of infecting both dividing and non-dividing cells. The expression of the four transcription factors (Oct3/4, Sox2, Lin28, and Nanog) has been shown to direct a variety of differentiated cell types to become reprogrammed iPS cells.<sup>3</sup> Individual viruses also available.

**Components:**

- Stemgent Lentivirus hSox2, 1 mL (**Cat. No. ST070012**)
- Stemgent Lentivirus hOct4, 1 mL (**Cat. No. ST070013**)
- Stemgent Lentivirus hLin28, 1 mL (**Cat. No. ST070016**)
- Stemgent Lentivirus hNanog, 1 mL (**Cat. No. ST070017**)

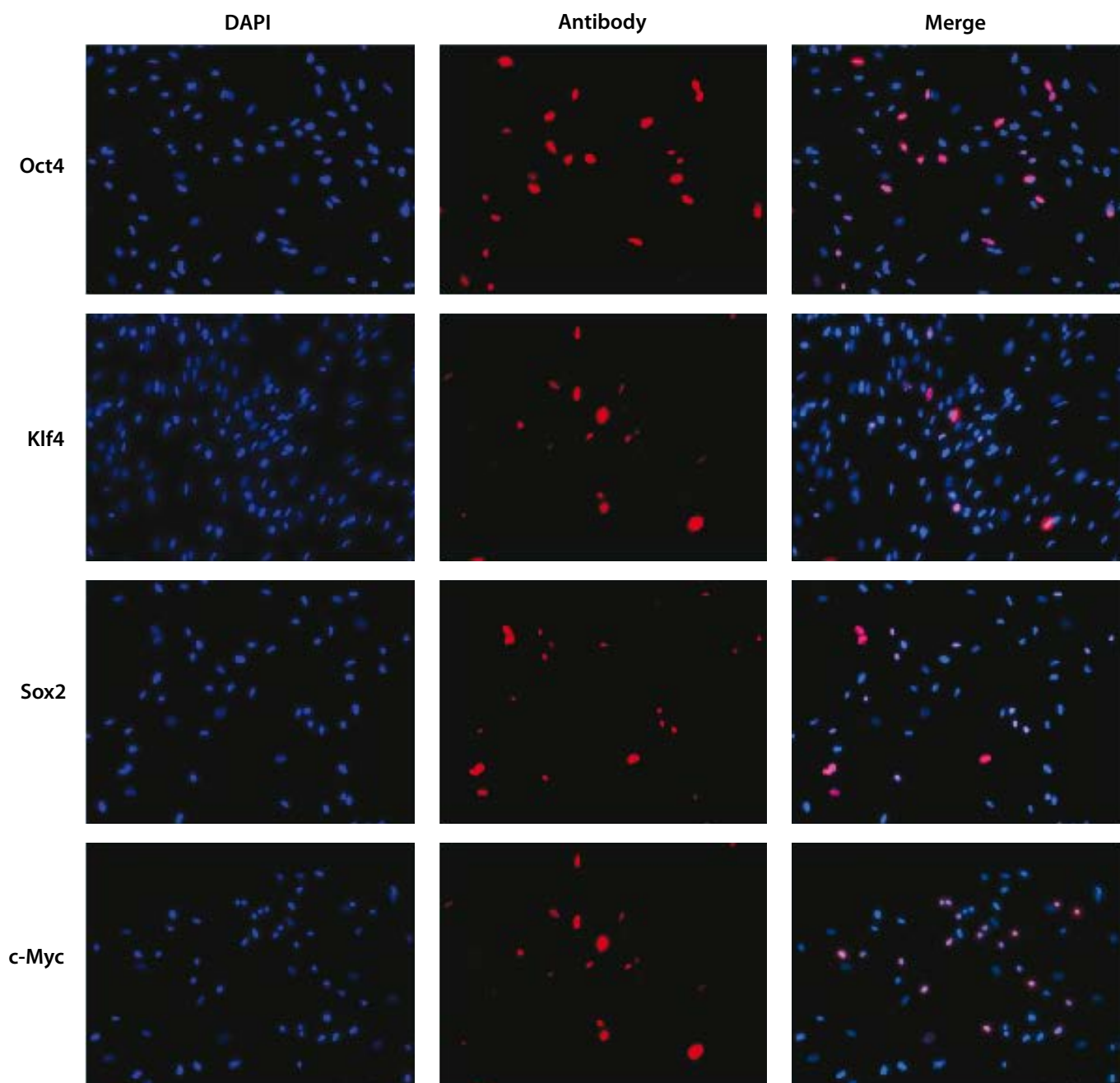
**References**

- (1) Hockemeyer, D., Soldner, F., Cook, E.G., Gao, Q., Mitalipova, M., and Jaenisch, R. (2008) A drug-inducible system for direct reprogramming of human somatic cells to pluripotency. *Cell Stem Cell* 3, 346-353.
- (2) Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861-872.
- (3) Yu et al., *Science* 318:1917-1920 (2007).

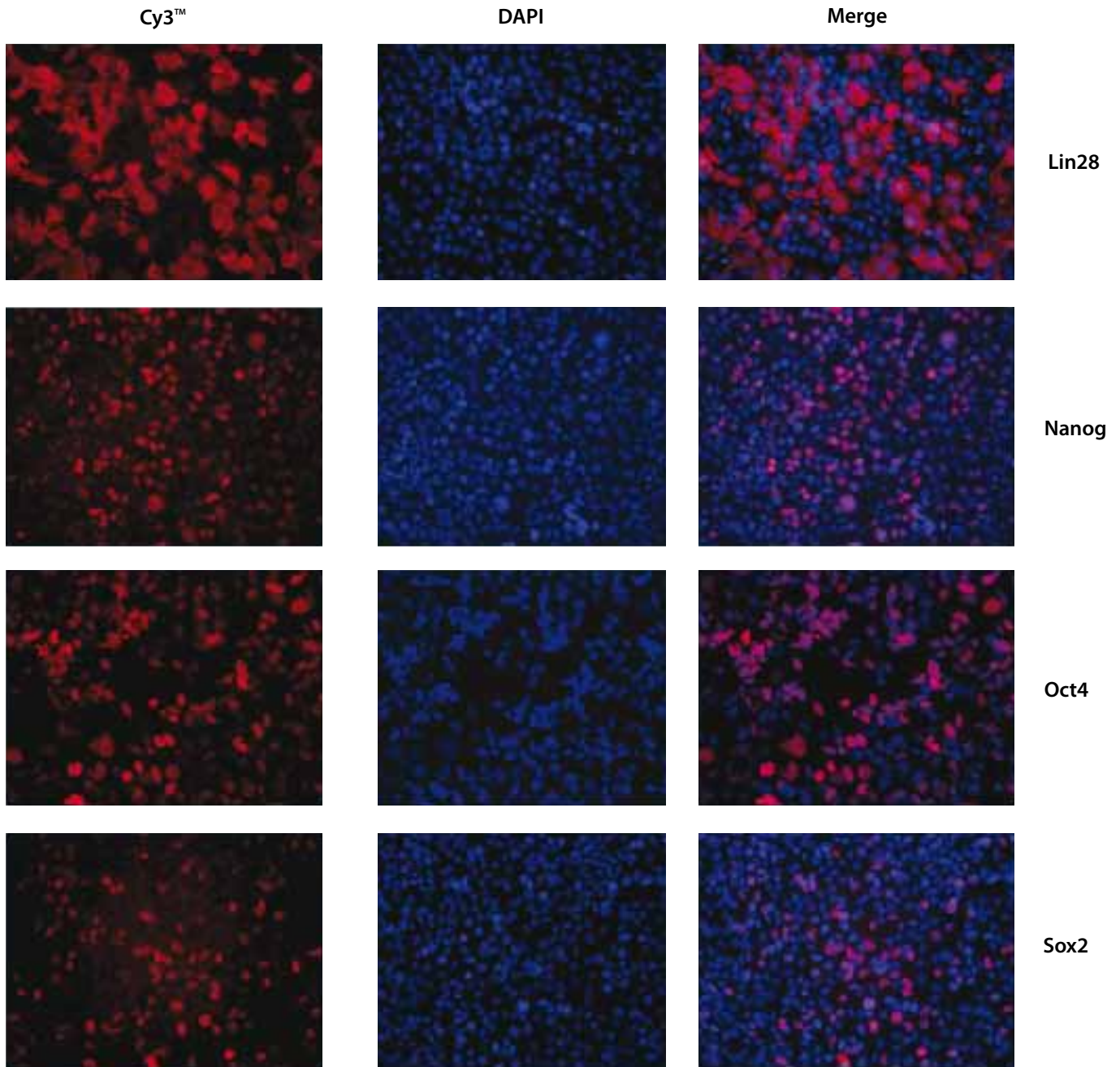
**Ordering Information**

Cat. No.	Product Description	Species
ST000005-1SET	Stemgent Lentivirus Set: hOSLN	Human
ST000044-1SET	Stemgent Lentivirus Set: hOKSM	Human
ST070012-1ML	Stemgent Lentivirus hSox2	Human
ST070013-1ML	Stemgent Lentivirus hOct4	Human
ST070014-1ML	Stemgent Lentivirus hc-Myc	Human
ST070015-1ML	Stemgent Lentivirus hKlf4	Human
ST070016-1ML	Stemgent Lentivirus hLin28	Human
ST070017-1ML	Stemgent Lentivirus hNanog	Human

# Reprogramming Lentiviruses for Human Cells



**Figure 2:** Immunocytochemistry (ICC) analysis 48 hours post-doxycycline (Dox) induction to monitor transduction efficiency (200x magnification) of the **Stemgent® Dox Lentivirus Set: hOKSM (Concentrated) (Cat. No. ST000037)**. BJ human foreskin fibroblast cells ( $\sim 2 \times 10^5$  cells in a single well of 6-well plate) were co-transduced with 30 ng of each of the four lentiviruses in the Stemgent Dox Lentivirus Set: hOKSM (Concentrated) and 30 ng of concentrated reverse tetracycline transcriptional activator (rtTA) lentivirus. Expression of rtTA is required for doxycycline-inducible expression of the four reprogramming factors. The left column represents DAPI-stained nuclei, the middle column represents Oct4-, Klf4-, Sox2-, or c-Myc-specific antibody staining, and the right column represents the merged images of the DAPI- and antibody-stained cells.



**Figure 3:** Immunocytochemistry (ICC) detection of proteins ectopically expressed by **Stemgent® Lentivirus Set: hOSLN (ST000005)**. HEK293-AD cells were transduced with the indicated lentiviruses (Sox2, Oct4, Nanog, and Lin28) at M.O.I. = 10. The cells were fixed 72 hours post-transduction, stained with corresponding antibodies, and visualized by Cy3-conjugated secondary antibodies. Cell nuclei were counter-stained with DAPI.

# Reprogramming Lentiviruses for Mouse Cells

## Stemgent® Dox Lentivirus Set: mOKSM (Cat. No. ST000021)



The Stemgent Dox Lentivirus Set: mOKSM + RG provides an affordable inducible method for generating iPS cells. The set includes mOct4, mSox2, mc-Myc, and mKlf4 transcription factors along with reverse tetracycline transcriptional activator (rtTA) and GFP, prepackaged as lentivirus

particles tested and proven to reprogram mouse somatic cells. Each unique virus is provided as a VSV-G pseudotyped virus stock capable of infecting both dividing and non-dividing cells. The expression of the four transcription factors, in conjunction with rtTA, has been shown to direct a variety of differentiated cell types to become reprogrammed iPS cells.<sup>1</sup> Individual viruses also available.

### Components:

- Stemgent Dox Lentivirus mSox2, 1 mL (Cat. No. ST070007)
- Stemgent Dox Lentivirus mOct4, 1 mL (Cat. No. ST070008)
- Stemgent Dox Lentivirus mc-Myc, 1 mL (Cat. No. ST070009)
- Stemgent Dox Lentivirus mKlf4, 1 mL (Cat. No. ST070010)
- Stemgent Dox Lentivirus GFP (concentrated)
- Stemgent Dox Lentivirus rtTA

## Stemgent Dox Lentivirus Set: mOKSM (Concentrated) (Cat. No. ST000014)



Each doxycycline-inducible lentivirus in this set is capable of expressing one of four mouse transcription factors (Oct4, Sox2, Klf4 or c-Myc) under the control of the doxycycline (Dox)-inducible tetO operator when transduced into mammalian cells. Each unique virus is provided as a VSV-G pseudotyped and concentrated virus stock capable of infecting both dividing and non-dividing

cells. The expression of these four transcription factors, along with the reverse tetracycline transcriptional activator (rtTA), has been shown to reprogram a variety of mouse cell types including fibroblasts, B cells, T cells, and neural precursor cells to an embryonic stem (ES) cell-like state known as the induced pluripotent stem (iPS) cell.<sup>1</sup> Proper isolation and subsequent injection of these iPS cells into blastocysts can generate mice that carry all four inducible viruses, which can be reactivated upon the addition of Dox.<sup>2</sup>

The Dox-Inducible GFP-Lentivirus (Concentrated) is included as a transduction control. The Stemgent Dox Lentivirus Set: mOKSM was developed in the lab of Rudolf Jaenisch, M.D., at the Whitehead Institute at MIT.<sup>1</sup> Dr. Jaenisch is a recognized leader in the study of epigenetic regulation of gene expression with numerous publications focused on ES and iPS cellular mechanisms and techniques. Individual viruses also available.

### Components:

- Stemgent Dox Lentivirus mSox2 (concentrated), 100 µL (Cat. No. ST070002)
- Stemgent Dox Lentivirus mOct4 (concentrated), 100 µL (Cat. No. ST070003)
- Stemgent Dox Lentivirus mc-Myc (concentrated), 100 µL (Cat. No. ST070004)
- Stemgent Dox Lentivirus mKlf4 (concentrated), 100 µL (Cat. No. ST070005)
- Stemgent Dox Lentivirus GFP (concentrated)
- Stemgent Dox Lentivirus rtTA (concentrated)

### References

- (1) Brambrink, T., Foreman, R., Welstead, G.G., Lengner, C.J., Wernig, M., Suh, H., and Jaenisch, R. (2008) Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. *Cell Stem Cell* **2**, 151-159.
- (2) Wernig, M., Lengner, C.J., Hanna, J., Lodato, M.A., Steine, E., Foreman, R., Staerk, J., Markoulaki, S., and Jaenisch, R. (2008) A drug-inducible transgenic system for direct reprogramming of multiple somatic cell types. *Nat Biotechnol* **26**, 916-924.

### Ordering Information

Cat. No.	Product Description	Species
ST000014-1SET	Stemgent Dox Lentivirus Set: mOKSM conc	Mouse
ST000021-1SET	Stemgent Dox Lentivirus Set: mOKSM	Mouse
ST070002-100UL	Stemgent Dox Lentivirus mSox2 conc	Mouse
ST070003-100UL	Stemgent Dox Lentivirus mOct4 conc	Mouse
ST070004-100UL	Stemgent Dox Lentivirus mc-Myc conc	Mouse
ST070005-100UL	Stemgent Dox Lentivirus mKlf4 conc	Mouse
ST070007-1ML	Stemgent Dox Lentivirus mSox2	Mouse
ST070008-1ML	Stemgent Dox Lentivirus mOct4	Mouse
ST070009-1ML	Stemgent Dox Lentivirus mc-Myc	Mouse
ST070010-ML	Stemgent Dox Lentivirus mKlf4	Mouse

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### Stemgent® Dox Lentivirus Set: m4F2A (Concentrated) (Cat. No. ST000043)



The polycistronic 4F2A lentivirus expresses each of the four mouse transcription factors (Oct4, Sox2, Klf4, or c-Myc) contained within a single expression cassette under the control of the doxycycline (Dox)-inducible tetO operator. The inclusion of three unique 2A peptide (P2A, T2A, and

E2A) sequences (one between each transcription factor) results in "ribosomal skipping", which allows for the equivalent expression of all four transcription factors from a single vector.<sup>1</sup> Introduction of the four factors from a single virus minimizes the number of proviral integrations required for successful reprogramming. This reduces the risk of insertional mutagenesis effects in the resultant induced pluripotent stem (iPS) cells when compared to those generated using four independent viruses. The 4F2A virus is capable of generating iPS cells from both embryonic and adult murine somatic cells, as well as neonatal human foreskin keratinocytes (NHFK).<sup>2</sup>

The virus is provided as a VSV-G pseudotyped and concentrated virus stock capable of infecting both dividing and non-dividing cells. The individual expression of these four transcription factors, along with the reverse tetracycline transcriptional activator (rtTA), has been shown to reprogram a variety of mouse cell types including fibroblasts, B cells, T cells, as well as neural precursors to the embryonic stem (ES) cell-like state known as the iPS cell state. Proper isolation and subsequent injection of these iPS cells into blastocysts has been shown to generate mice that carry all four inducible viruses, which can be reactivated upon the addition of doxycycline.

The Dox-Inducible Mouse 4F2A Lentivirus was developed in the lab of Rudolf Jaenisch, M.D., at the Whitehead Institute at MIT.<sup>1</sup> Dr. Jaenisch is a recognized leader in the study of epigenetic regulation of gene expression with numerous publications focused on ES and iPS cellular mechanisms and techniques.

#### Components:

- Stemgent Dox Lentivirus m4F2A (concentrated), 1 mL (Cat. No. ST070029)
- Stemgent Dox Lentivirus GFP (concentrated)
- Stemgent Dox Lentivirus rtTA (concentrated)

#### References

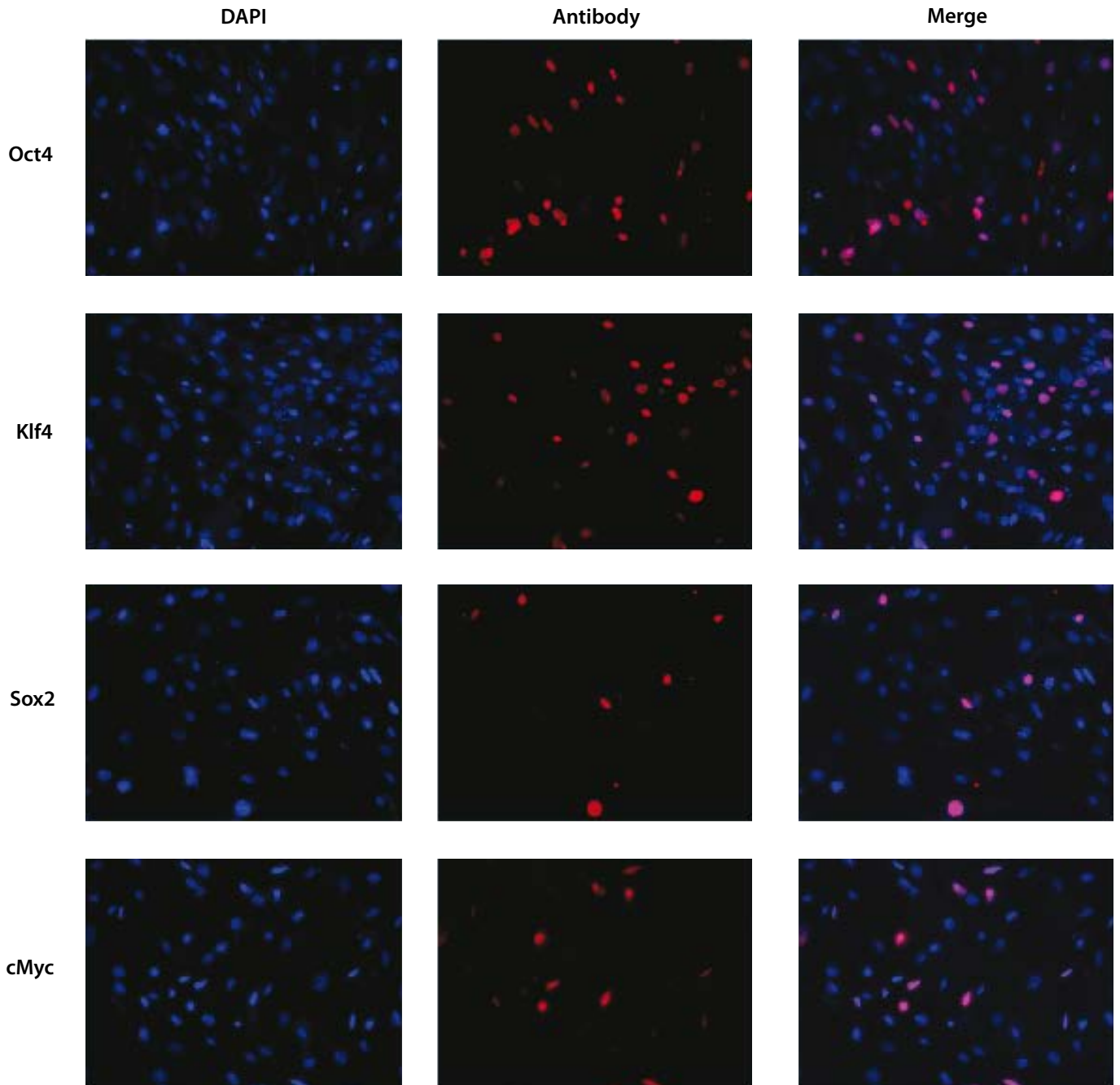
- (1) Szymczak, A.L., Workman, C.J., Wang, Y., Vignali, K.M., Dilioglou, S., Vanin, E.F., and Vignali, D.A. (2004) Correction of multi-gene deficiency in vivo using a single 'self-cleaving' 2A peptide-based retroviral vector. *Nat Biotechnol* 22, 589-594.
- (2) Carey, B.W., Markoulaki, S., Hanna, J., Saha, K., Gao, Q., Mitalipova, M., and Jaenisch, R. (2009) Reprogramming of murine and human somatic cells using a single polycistronic vector. *Proc Natl Acad Sci USA* 106, 157-162.

#### Ordering Information

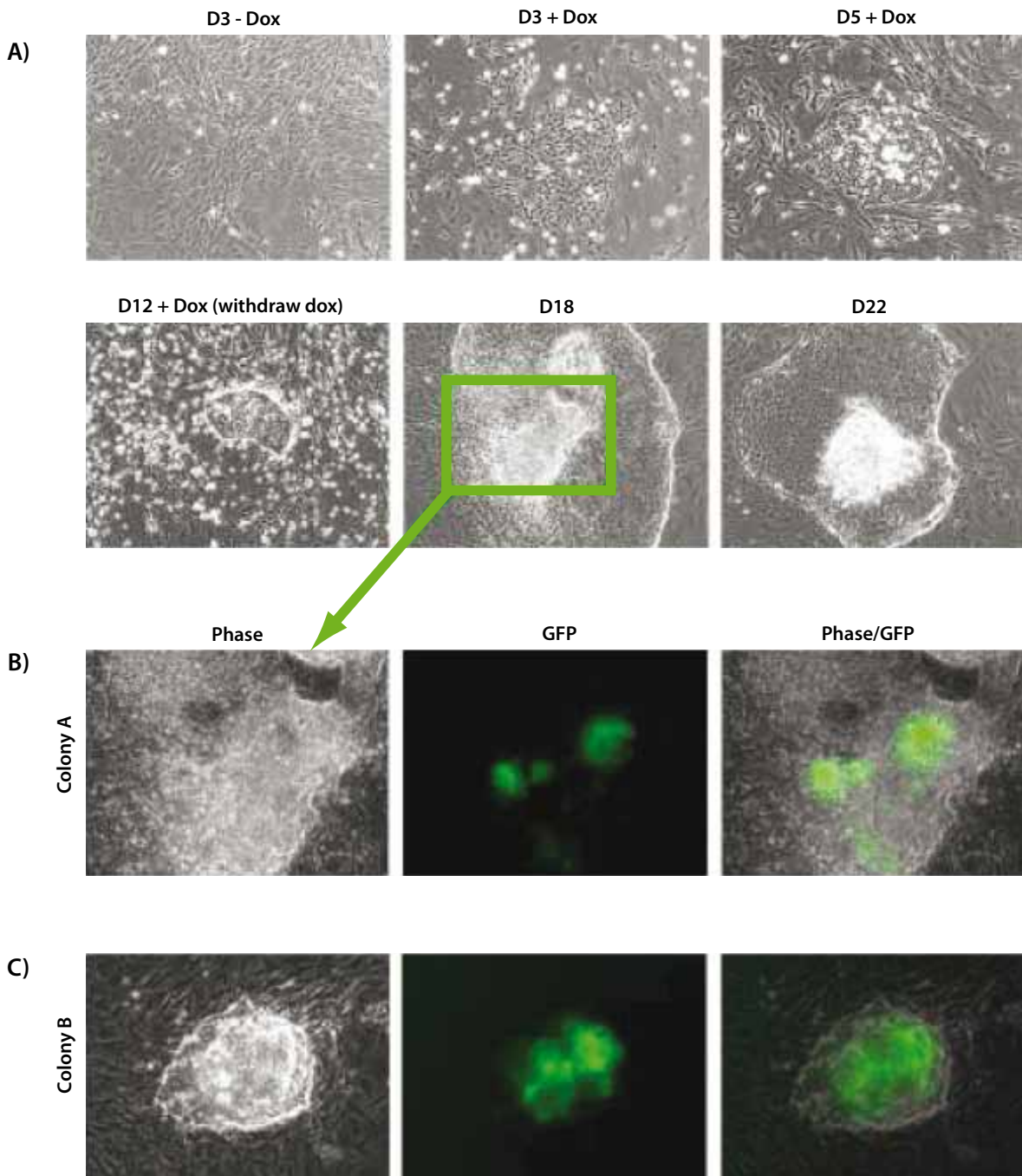
Cat. No.	Product Description	Species
ST000043-1SET	Stemgent Dox Lentivirus Set: m4F2A	Mouse

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# Reprogramming Lentiviruses for Mouse Cells



**Figure 4:** Immunocytochemistry (ICC) analysis 48 hours post-doxycycline (Dox) induction to monitor transduction efficiency (200x magnification) of the **Stemgent® Dox Lentivirus Set: mOKSM (concentrated) (Cat. No. ST000014)**. Nanog-GFP; rtTA mouse embryonic fibroblasts (MEFs) ( $2 \times 10^5$  cells in a single well of 6-well plate) were co-transduced with 30 ng of each of the four lentiviruses included in the Stemgent® iPSC Lentivirus Set. The Nanog-GFP/rtTA MEFs contain the GFP cDNA inserted at the Nanog locus as well as a reverse tetracycline transcriptional activator (rtTA) expression cassette which is required for Dox-inducible expression. The left column represents DAPI-stained nuclei (blue), the middle column represents Oct4-, Klf4-, Sox2-, or c-Myc-specific antibody staining (red), and the right column represents the merged images of the DAPI- and antibody-stained cells.



**Figure 5:** Morphological conversion of Nanog-GFP/rtTA MEFs to the induced Pluripotent Stem (iPS) cell state. Nanog-GFP/rtTA MEFs transduced with all four viruses contained in the **Stegment® Dox Lentivirus Set: (Concentrated) (Cat. No. ST000014)** were visually monitored for changes in morphology and GFP expression following Dox induction. A) 100x phase-contrast imaging of cells from the 100 mm Reprogramming Efficiency and iPS Cell Colony Isolation Dishes demonstrating the compaction and conversion of the Nanog-GFP/rtTA MEFs into iPS cell colonies. Dox removed from 100 mm Reprogramming Efficiency dishes on Day 12. Upper left panel: 20x negative control image from (-) Dox control plate. B) 200x phase-contrast and GFP fluorescence images of highlighted day 18 post-Dox induction iPS cell colony. C) 200x phase-contrast and GFP fluorescence images of additional day 18 post-Dox induction iPS cell colony. GFP expression reflects the endogenous Nanog expression level and is used for monitoring the pluripotent state. GFP fluorescence became visible beginning on day 18 post-Dox induction.

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