Mouse STEMCCA Cre-Excisable Constitutive Polycistronic (OKS) Lentivirus Reprogramming Kit

Catalog No. SCR518

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Not for use in diagnostic procedures.

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Introduction

Induced pluripotent stem (iPS) cells were first generated from somatic cells by the ectopic expression of the four Yamanaka transcription factors, Oct4, Klf4, Sox2, and c-Myc (OKSM)[1]. Four factor reprogrammed mouse iPSCs closely resemble mouse embryonic stem cells (ESC) in their morphology, proliferation, and globle gene expression profiles. In addition, fully reprogrammed mouse iPSCs have been shown to give rise to chimeric mice that are competent for germline transmission[2-4]. However, both the chimeras and progenies derived from mouse iPSC have an increased incidence of tumor formation, mainly due to the expression of the oncogene c-Myc[2]. Reprogramming is possible using three factors (OKS) without c-Myc; however the efficiency is extremely low and the kinetics of repgramming is significantly delayed compared to reprogramming with four factors[5]. Recent studies have shown that small molecule treatments, by modulating specifc signaling pathways and epigenetic status, can not only enhance reprogramming efficiency but can also replace one or more of the transcription factors[5,6]. A Cre-excisable polycistronic lentiviral vector[7,8] has been generated that allows for expression of a modified "stem cell cassette" or STEMCCA comprised of only three transcription factors (OKS) separated by the self-cleaving 2A peptide and IRES sequences. The elimination of c-Myc may provide less incidence of tumors in iPSCs. The Cre-Excisable Constitutive Polycistronic (OKS) Lentivirus **must** be used in combination with the small molecule medium supplements to generate mouse iPS colonies.

Product Description

EMD Millipore's STEMCCA Cre-Excisable Constitutive Polycistronic (OKS) Lentivirus Reprogramming Kit, which contains high titer Cre-excisable polycistronic (OKS) lentivirus, three small molecule reprogramming supplements, and Polybrene® transfection reagent, has been validated to generate mouse iPS colonies from mouse embryonic fibroblasts (MEFs). Mouse iPS cells obtained with this kit displayed characteristic ES cell-like morphology, stained positive for alkaline phosphatase, expressed the correct mouse ES cell marker phenotype (SSEA-1 and Sox-2) and can be rapidly expanded in normal mouse ES cell culture conditions. Following Cre-mediated excision, transgene-free mouse iPS cells can be further expanded and banked. The use of an excisable polycistronic lentiviral vector instead of three separate vectors for the derivation of iPS cells significantly reduces the risks of insertional mutagenesis and viral reactivation and is a step towards safer utilization of iPS technology for disease models and clinical therapies.

EMD Millipore's STEMCCA lentivirus has been tested to confirm the generation of iPS cells from p3 mouse embryonic fibroblasts. Cre-mediated excision of the reprogramming transgenes has been validated in the mouse system. Other cell types have not been tested and thus similar results can not be guaranteed.

Lentiviral particles were generated using the pPACKH1 Lentivector Packaging System at System Biosciences (SBI). www.systembio.com

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Kit Components

Mouse STEMCCA Constitutive LoxP (OKS) Lentivirus Kit (-20℃): (Part No. SCR518-1)

1. TGF-β RI Kinase Inhibitor II Supplement (1000X): (Part No. CS204458): One (1) vial containing 300 µL of the inhibitor in high quality DMSO. Store at -20℃. Aliquot into smaller working volumes. Avoid freeze thaw.
2. GSK3β Inhibitor Supplement (1000X): (Part No. CS204418): One (1) vial containing 300 µL of the inhibitor in high quality DMSO. Store at -20℃ . Aliquot into smaller working volumes. Avoid freeze thaw.
3. Methylation Modulator-A Supplement (1000X): (Part No. CS204419): One (1) vial containing 300 µL of the inhibitor in water. Store at -20℃. Aliq uot into smaller working volumes. Avoid freeze thaw.
4. Polybrene 10 mg/mL: (Part No. TR-1003-50UL) One (1) vial containing 50 µL of 10 mg/mL stock of Polybrene transfection reagent.

Mouse STEMCCA Constitutive LoxP (OKS) Lentivirus Kit (-80℃): (Part No. SCR518-2)

5. EF1α-STEMCCA-LoxP (OKS) Lentivirus: (Part No. CS204448) Two (2) vials containing 15 µL of high titer lentivirus. For exact titer refer to the label on the front of manual.

Storage and Handling

- Small molecule supplements (components 1-3 above) are stable for at least 6 months when stored at -20℃. Upon first thaw, centrifuge the vial and gently mix the solution. Aliquot into smaller working volumes and freeze at -20℃ or -80℃. Upon addition to cell culture media, filter the supplemented media with a 0.22 µM filtration unit. Supplemented media is good for up to two weeks when stored at 2-8℃. **Important Safety Note: Wear personal protective equipment when using this product. Avoid skin contact or ingestion of all chemicals used in this protocol. GSK3β Inhibitor Supplement (1000X) and TGF-β RI Kinase Inhibitor II Supplement (1000X) contain DMSO; avoid contact with eyes and skin.**
- Polybrene reagent is stable for at least 1 year when stored at -20℃.
- Lentivirus is stable for at least 6 months when stored at -80℃. After first thaw, place immediately on ice and store in working aliquots to avoid further freeze thaws. Avoid freeze thaws as this will result in a decrease in the virus titer. **Important Safety Note: Replication-defective lentiviral vectors are not known to cause any diseases in humans or animals. However, lentiviruses can integrate into the host cell genome and thus pose some risk of insertional mutagenesis. Material is a risk group 2 and should be handled under BSL 2 controls.**

Materials Required but Not Provided

1. 6-well plates, culture flasks, dishes (TC grade)
2. MEF expansion medium (see page 3)
3. PMEF cells, not mitomycin-C treated (Cat. No. PMEF-CFL)
4. Phosphate Buffered Saline (1X PBS) (Cat. No. BSS-1005-B)
5. EmbryoMax® 0.1% Gelatin Solution (Cat. No. ES-006-B)

6. EmbryoMax Complete ES Cell Media w/15% FBS and mLIF (Cat. No. ES-101-B)
7. Trypsin-EDTA Solution (0.25% Trypsin & 1 mM EDTA) (Cat. No. SM-2003-C)
8. FibroGRO™ LS Complete Medium (Cat. No. SCMF002)
9. PMEF cells, growth-arrested, mitomycin-C treated (Cat. No. PMEF-CF)
10. Adenovirus expressing Cre recombinase and GFP (Vector Biolabs Cat. No. 1710)
11. DNeasy® Blood and Tissue Kit (QIAGEN Cat. No. 69504)

Reprogramming Mouse Somatic Cells

SECTION 1: REPROGRAMMING MOUSE EMBRYONIC FIBROBLASTS

Important note: The following protocol has been optimized using early passage primary mouse embryo fibroblasts (MEFs) and should be used only as a **guide** to further optimize reprogramming of other somatic cells derived from rodents.

Day 0: Seeding proliferating MEFs or target rodent cells

1. Coat a sterile 6-well plate with 0.1% gelatin solution (Cat. No. ES-006-B). Use 2 mL volume per well. Incubate for at least 30 minutes before using. Aspirate the gelatin solution just before seeding the MEFs or target rodent cells.

2. Make up 50 mL MEF Expansion Medium. Sterile filter with 0.22 µm filter.

Component	Quantity	Final Conc.	Millipore Cat. No.
DMEM High-Glucose Medium	44 mL	1X	SLM-021-B
Fetal Bovine Serum	5.0 mL	10%	ES-009-B
L-Glutamine (200 mM)	0.5 mL	2 mM	TMS-002-C
Penicillin Streptomycin Solution (100X)	0.5 mL	1X	TMS-AB2-C

3. Seed 1×10^5 actively proliferating p3 mouse embryonic fibroblasts (Cat. No. PMEF-CFL) in 3 mL MEF Expansion media into each well of a 0.1% gelatin coated 6-well plate. Incubate overnight in a 37℃, 5% CO_2 incubator. It is recommended to use early passage MEFs.

Day 1: Virus Infection

4. Aspirate the MEF Expansion media and wash cells with 1X PBS buffer per well. Aspirate after the wash. Add 1 mL fresh FibroGRO™ LS Complete Medium (Cat. No. SCMF002) per well. Dilute 1 µL of Polybrene transfection reagent into 9 µL of sterile distilled water to create a 1:10 dilution. Add 5 µL of the diluted Polybrene transfection reagent to each well to be transduced. Final polybrene concentration should be 5 µg/mL. Set the plate aside in 37℃, 5% CO_2 incubator until ready to add the virus.

5. Using the equation below, determine the volume of virus required to achieve a multiplicity of infection (MOI) of at least 20. **Please make note of the titer as the viral titer may vary slightly from lot to lot.** When used in conjunction with the small molecule reprogramming supplements, an MOI of 20 will typically yield 3-7 mouse iPS cell colonies from p3 MEFs. Using an MOI < 20 is **not** recommended as the results are variable and may yield very few to no colonies.

$$\text{Virus volume } (\mu L) \text{ required} = \frac{\text{Number MEFs seeded (from step 3)}}{\text{Virus Titer (IFU/mL)}} \times \frac{\text{Desired MOI}}{1 \text{ mL}} \times 1000\ \mu L$$

Example: If the number of cells in the well at the time of transduction is 1×10^5, the viral titer is 3×10^8 IFU/mL, and a desired MOI is 20, then the volume of virus required is:

$$\frac{1 \times 10^5 \text{ cells}}{3 \times 10^8 \text{ IFU/mL}} \times \frac{20}{1 \text{ mL}} \times 1000\ \mu\text{L} = {}^{*}6.6\ \mu\text{L virus required for 1 well of a 6-well plate}$$

***Note:** Use the actual viral titer located on the label on the front of the manual in the equation above to determine the actual volume of virus to add.*

6. Thaw 1 vial of EF1α-STEMCCA-LoxP (OKS) Lentivirus (Part No. CS204448) at room temperature and quickly place the vial on ice after it is thawed. Quickly centrifuge the vial to spin down the contents. Keep the virus on ice and proceed immediately to the next step.
7. Add the required volume of thawed virus directly to the wells containing the attached MEFs (from Step 4). Gently rock the plate from side to side to thoroughly mix the virus onto the MEFs. Incubate overnight in a 37℃, 5% CO_2 incubator.

Day 2: Addition of Small Molecule Supplements

8. Exchange the media with 3.0 mL Complete ES Cell Media with 15% FBS and LIF (Cat. No. ES-101-B). Add 3 µL **each** of TGF-β RI Kinase Inhibitor II Supplement (1000X) (Part No. CS204458), GSK3β Inhibitor Supplement (1000X) (Part No. CS204418) and Methylation Modulator-A Supplement (1000X) (Part No. CS204419).

   Each well should contain the following:

   3 mL Complete ES Cell Media with 15% FBS and LIF (Cat. No. ES-101-B)

   3 µL TGF-β RI Kinase Inhibitor II Supplement (1000X) (Part No. CS204458)

   3 µL GSK3β Inhibitor Supplement (1000X) (Part No. CS204418)

   3 µL Methylation Modulation-A Supplement (1000X) (Part No. CS204419)

   ~3.009 mL Total Volume

   ***Important Note:** The addition of small molecule supplements are absolutely critical to generate mouse iPS colonies from the Cre-excisable (OKS) lentivirus.*

Day 4 through Day 12-14: Exchange small molecule supplemented medium every other day

9. Exchange the media with 3 mL fresh Complete ES Cell Media with 15% FBS and LIF (Cat. No. ES-101-B) containing 3 µl **each** of the small molecule supplements every other day for a total of 10-13 days. Mouse iPS cell colonies start to emerge around day 7-10.
10. Mouse iPS cell colonies can be selected and clonally expanded (typically around Day 12-14) when they reach an approximate size where the colony fits into the frame of a 10X Magnification view (please refer to Figure 2D for an example of an iPS colony that can be picked).

    ***Note:** Once the mouse iPS cell colonies are established (typically day 12-14), it is no longer necessary to add the Mouse iPS Cell Boost Supplement to the media. Passage colonies as normal without the addition of the small molecule supplements.*

SECTION 2: CLONAL EXPANSION OF MOUSE iPS COLONIES

At approximately Day 14 or when the mouse iPS colonies are of sufficient size (see step 10), they can be picked for clonal expansion, freezing, and subsequent excision with Adeno-Cre recombinase.

Day before passaging

11. One day prior to passaging the iPS colonies, prepare a fresh 6-well plate with inactivated MEFs to support the expansion of the mouse iPS cells as follows.

a. Coat each well of a fresh sterile 6-well plate with 2 mL of 0.1% gelatin solution (Cat. No. ES-006-B). Incubate for 30 minutes at 37°C. Set aside until ready to receive inactivated MEFs.

b. Aspirate the 0.1% gelatin coating solution from each well before seeding the inactivated MEFs. Thaw inactivated MEFs (Cat. No. PMEF-CF). Count the number of thawed MEFs and seed 4 x 10^5 cells per well of a 6-well dish. Use MEF Expansion medium to culture the cells (see page 3). Total volume per well should be 3 mL. Incubate overnight in a 37°C, 5% CO_2 incubator.

Day of passaging

12. On the day of passaging add 100 µL of 0.25% Trypsin-EDTA (prewarmed to 37°C) to a 15 mL conical tube for each mouse iPS colony to be picked. For example, for 10 iPS colonies, prepare 10 separate conical tubes containing 0.25% trypsin-EDTA. Set aside.

13. Under a dissecting microscope in a laminar flow hood, scrape and pipette up one iPS colony and deposit the pieces into the 15 mL conical tube containing 0.25% trypsin-EDTA. Repeat this step for each iPS colony to be clonally expanded, being careful to keep each iPS colony in separate conical tubes.

14. Incubate the 15 mL conical tubes at room temperature for 5-10 minutes to allow the trypsin to dissociate the cell colonies.

15. Add 5 mL fresh Complete ES Cell Media with 15% FBS and LIF to each 15 mL conical tube containing the dissociated cell colonies.

16. Using a 5 or 10 mL pipette, slowly pipette up and down to break apart any remaining cell clumps.

17. Discard the media from the plate of inactivated MEFs (from Step 11) and add the dissociated mouse iPS colony from each 15 mL conical tube (approximately 5 mL volume) into separate wells of the 6-well dish containing inactivated MEFs. Incubate the 6-well dish in a 37°C, 5% CO_2 incubator.

    Note: *It is important to avoid cross-colony contamination between mouse iPS clones. Therefore, each well of the 6-well dish should only contain dissociated cells from one mouse iPS colony.*

18. Replace with fresh Complete ES Cell Media with 15% FBS and LIF the following day and every other day thereafter for two weeks or until the mouse iPS colonies are 80% confluent.

19. When mouse iPS clones are 80% confluent, they can be further expanded and frozen. Freezing back vials of each mouse iPS clone before proceeding with the Adeno-Cre recombinase excision is strongly recommended.

20. After freezing back multiple vials of cells per clone, each mouse iPS clone can be grown in a T25 flask to 80% confluency for subsequent excision analysis.

SECTION 3: EXCISION OF EF1α-STEMCCA-LoxP VIRAL TRANSGENES

Important Note: *Excision has been demonstrated for mouse iPS clones only. A full demonstration of excision requires a lengthy protocol of cloning and subcloning iPS colonies and will require an average of 30-50 days to complete.*

One Day Prior to Excision Analysis:

21. Prepare a 10-cm plate with inactivated MEFs to support the expansion of the mouse iPS clones. Refer to step 11 for exact protocol. Please note that for 10-cm plates, inactivated MEFs should be plated at 1 x 10^6 cells.

Day of Excision Analysis:

22. To each T25 flask containing approximately 80% confluent mouse iPS clones (from step 20), add 3-5 mL of 0.25% trypsin-EDTA and incubate in a 37°C incubator for 3 minutes.
23. Inspect the flask and ensure the complete detachment of cells by gently tapping the side with the palm of your hand. Confirm under the microscope that the cells are in single-cell suspension.

    Note: *It is critical to obtain a single cell suspension of each mouse iPS colony before initiating the adenoviral Cre recombinase infection.*

24. Apply 10 mL Complete ES Cell Media with 15% FBS and LIF (pre-warmed to 37°C) to each flask. Pipette up and down several times to ensure single cell suspension and collect the cell suspension to a 15 mL conical tube.
25. Centrifuge for 5 minutes at 300 xg to pellet the cells. Remove the supernatant and resuspend with 10 mL fresh Complete ES Cell Media with 15% FBS and LIF (pre-warmed to 37°C). Pipette up and down several times to ensure single cell suspension.
26. MEF depletion: Transfer the singly dissociated cell suspension to a fresh gelatin coated T75 flask. Incubate in a 37°C, 5% CO_2 incubator for 45 minutes to allow any MEFs to adhere to the flask.
27. After 45 minutes, collect the floating mouse iPS cells into 15 mL conical tube.

    Note: *It is critical to remove as many MEFs as possible before initiating the adenoviral Cre recombinase infection to ensure that the adenovirus is not diluted by the MEFs. If necessary, repeat the MEF depletion (steps 26-27).*

28. Centrifuge at 300 xg for 10-15 minutes to pellet the cells.
29. Remove supernatant and resuspend the cell pellet in a small volume (1 mL) of Complete ES Cell Media with 15% FBS and LIF. Pipette up and down, being careful not to introduce bubbles, to ensure that cells are singly dissociated. If cells are not singly dissociated, use a fire-polished Pasteur pipette to pipette to a single cell suspension.
30. Count the number of cells using a hemocytometer.
31. Aliquot 1 x 10^5 singly dissociated cells to a microcentrifuge tube.
32. Thaw the Adeno-Cre recombinase (Vector Biolabs Cat. No. 1710) on ice until ready to use.
33. Using the equation provided below, determine the volume of virus required to achieve a multiplicity of infection (MOI) of 3000.

$$\text{Virus volume } (\mu\text{L}) \text{ required} = \frac{\text{Number iPS cells (from step 31)}}{\text{Virus Titer (IFU/mL)}} \times \frac{\text{Desired MOI}}{1 \text{ mL}} \times 1000\ \mu\text{L}$$

Example: If the number of cells being transduced is 1×10^5, the viral titer is 1×10^{10} IFU/mL, and the desired MOI is 3000, then the volume of virus required is:

$$\frac{1 \times 10^5 \text{ cells}}{1 \times 10^{10} \text{ IFU/mL}} \times \frac{3000 \text{ MOI}}{1 \text{ mL}} \times \frac{1000 \mu\text{L}}{} = 30 \mu\text{L virus required}$$

34. Add the calculated volume of Adeno-Cre recombinase (from step 33) to the microcentrifuge tube containing the singly dissociated cells (from step 31).

35. Bring the final volume in the microcentrifuge tube to 250 µL by adding Complete ES Cell Media with 15% FBS and LIF.

36. Incubate the microcentrifuge tube in a 37°C, 5% CO_2 incubator for 6 hours. During this incubation time, pipette the cell mixture up and down every two hours to ensure that the cells remain in a single cell suspension.

37. After the 6 hour incubation, add the entire cell suspension (approximately 250 µL) to the 10 cm plate containing inactivated MEFs from step 21.

38. Inspect the plate under a microscope to ensure that the iPS cells are in single cell suspensions after plating onto MEF feeder layer.

39. Replace media the following day and every other day thereafter with fresh Complete ES Cell Media with 15% FBS and LIF until colonies start to appear. Colonies will become apparent after 2-3 weeks.

40. After 2-3 weeks, manually pick as many individual colonies as possible from the 10-cm plate. Select smaller colonies that are spaced apart as these colonies have more likely arisen from single cells. Follow the protocol outlined in Section 2, steps 11 – 19 to expand individual colonies from the 10-cm plate to separate wells of 6-well plate for subsequent expansion of clones for freezing and genomic DNA isolation for PCR analysis of excision. It is important to avoid cross-colony contamination as this will affect the PCR excision analysis.

    Note: *Freezing back multiple vials of each mouse iPS clone that has undergone the Cre recombinase excision reaction before proceeding with PCR analysis is strongly recommended.*

SECTION 4: PCR ANALYSIS OF GENOMIC DNA

41. Isolate genomic DNA from mouse iPS colonies that have undergone the excision process (from step 40). As a control, also isolate genomic DNA from mouse iPS colonies that have not undergone the excision process (from step 19). Using a commercial kit, follow the manufacturer's instructions regarding the isolation of genomic DNA. For example, the DNeasy Blood and Tissue Kit from QIAGEN (Cat. No. 69504) may be used to obtain genomic DNA from a confluent T25 flask of each mouse iPS clone (typical cell yield = $5 \times 10^5 – 1 \times 10^6$ cells).

42. Use the following primers (not provided) to set up PCR reactions for the analysis of the excision reaction in the iPS clones. ***Note:*** *The WPRE primer set is specific to the viral genome while the GAPDH primer set is used to normalize for cDNA template between PCR reactions.*

Marker	Primer Sequence	Product Size
WPRE	Forward 5' - ACG AGC ACA AGC TCA CCT CT - 3'	350 bp
	Reverse 5' - TCA GCA AAC ACA GTG CAC ACC - 3'	
GAPDH	Forward 5' - CCT TCA TTG ACC TCA ACT AC - 3'	500 bp
	Reverse 5' - GGA AGG CCA TGC CAG TGA GC -3'	

43. For each iPS clone, prepare the following PCR reaction mix for each primer set (WPRE and GAPDH) in separate, clean RNAse-free PCR tubes.

Component	Per Reaction
10X PCR Reaction Buffer	5.0 µL*
10 mM dNTPs	1.0 µL
50 mM $MgCl_2$	1.5 µL*
10 µM primer mix (WPRE or GAPDH)	1.0 µL
Taq Platinum DNA Polymerase	0.4 µL*
200 ng genomic DNA template	x µL
Sterile distilled water	To final 50 µL

****Note****: The volumes of the 10X PCR reaction buffer, $MgCl_2$ and DNA polymerase should be based upon the instructions provided with the DNA polymerase enzyme.*

44. Thoroughly mix the reaction mixture by pipetting up and down.

45. Briefly centrifuge PCR tubes.

46. Transfer the reactions to a PCR machine and perform PCR using the following optimized cycling parameters:

    a. Initial denaturation at 94℃ for 2 minutes.

    b. 30 cycles of:

        95℃ 30 seconds

        65℃ 45 seconds

        72℃ 45 seconds

    c. Final extension at 72℃ for 10 minutes.

47. After the PCR reactions have terminated, the samples can be stored at 4℃. For long term storage, samples should be stored at -20℃.

48. Analysis of Results: Following the PCR reaction, prepare a 1.5% agarose gel containing ethidium bromide and directly load 10 to 25 µL of each PCR reaction mix containing an appropriate amount of 5X loading dye to each well. Include a molecular weight marker with bands between 100 bp to 1000 bp in an adjacent well. The gel can be analyzed when the red dye has migrated to 2/3 distance from the loading point.

    The GAPDH band is used to normalize for gel loading. Ideally a band corresponding to 500 bp (GAPDH) should be present at comparable intensity in all the PCR samples.

    Presence of a 350 DNA band, corresponding to the WPRE transcript indicates that the viral transgene has not been excised in the particular mouse iPS clone.

    Absence of a 350 DNA band indicates that the viral STEMCCA transgene has been successfully excised in the particular mouse iPS clone.

Representative Results

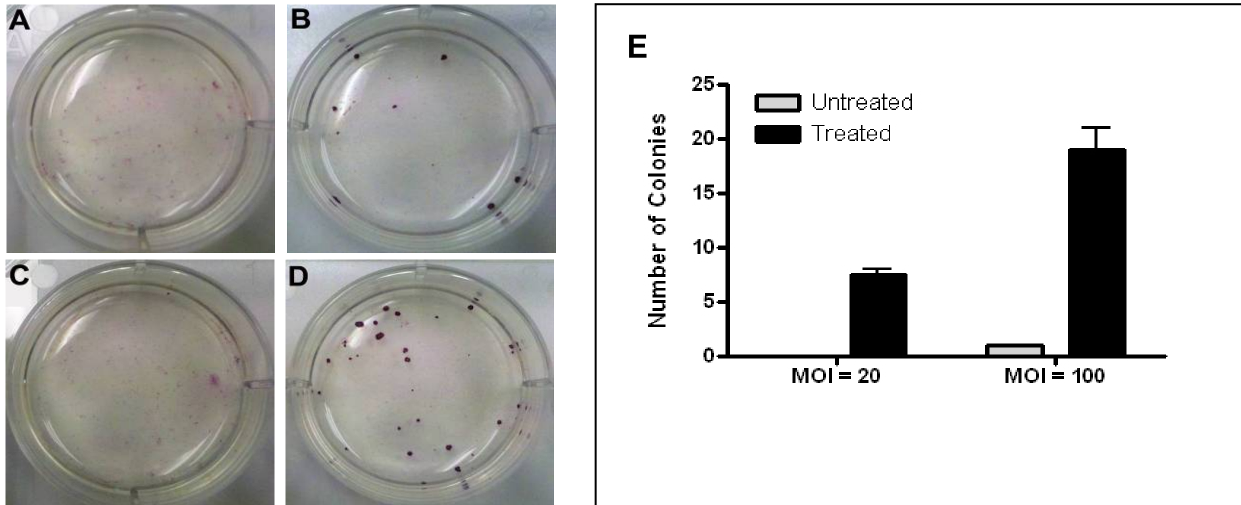

Figure 1. Mouse iPS colonies were generated from proliferating MEFs (passage 3) infected with the STEMCCA Cre-Excisable Constitutive Polycistronic (OKS) Lentivirus. Lentiviral infection was performed with an MOI of 20 (**A, B**) or 100 (**C, D**) and 5 µg/mL polybrene reagent. A combination of small molecule supplements were applied one day after infection (**B**, **D**) and maintained throughout the reprogramming process (i.e. day 12-14). Cells were fixed on day 12 after infection and were stained using the Alkaline Phosphatase Detection Kit (Cat. No. SCR004). AP positive colonies were only observed in wells containing small molecule supplements (**E**).

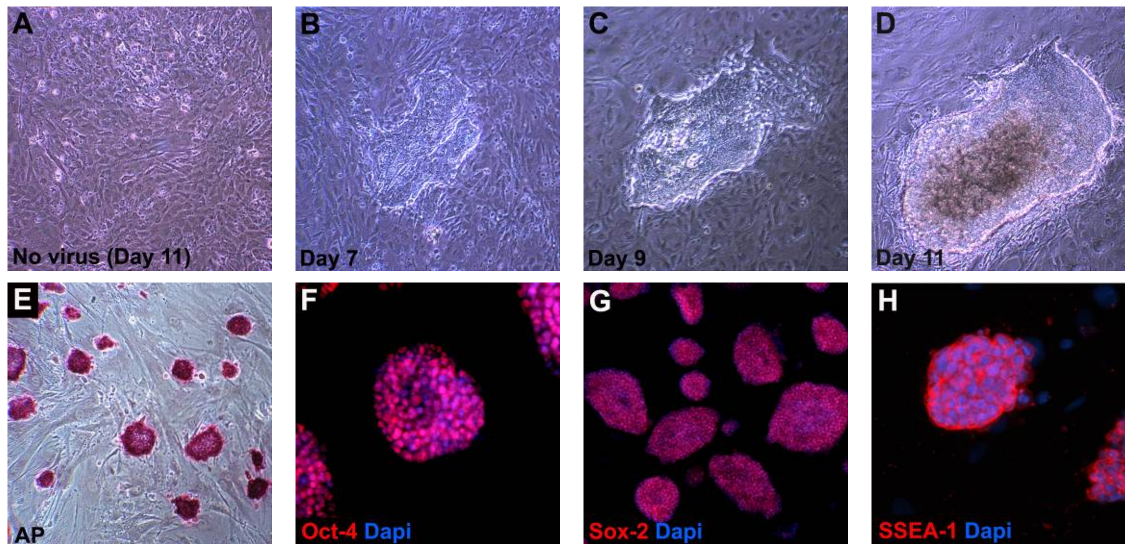

Figure 2. Mouse iPS cells derived from MEFs (passage 3) infected with the STEMCCA Cre-Excisable Constitutive Polycistronic (OKS) Lentivirus display cell morphology and staining characteristics of mouse ES cells. Lentiviral infection was performed with an MOI of 20 and 5 µg/mL Polybrene reagent. After 12-14 days, non-infected MEFs remained in a monolayer culture with no ES cell-like colonies observed (**A**) while infected MEFs formed multilayered, tightly packed cells with well-defined borders in the presence of small molecule supplements (**B-D**). Three factor (OKS) derived mouse iPS cells exhibit high alkaline phosphatase activity (**E**, Cat. No. SCR004) and expressed high levels of Oct-4 (**F**, Cat. No. MAB4419), Sox-2 (**G**, Cat. No. AB5603), and SSEA-1 (**H**, Cat. No. MAB4301). Cell nuclei were counterstained with DAPI (blue).

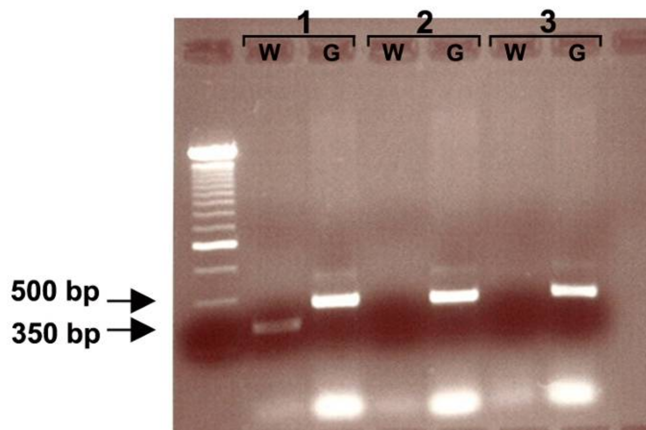

Figure 3. PCR analyses of mouse iPS clones that have undergone Cre recombinase excision. In this example, excision was demonstrated on mouse iPS clones generated from EF1α-STEMCCA-LoxP (OKSM) lentivirus. Presence of a 350 bp PCR product, corresponding to the WPRE transcript specific to the STEMCCA lentiviral genome indicates that the viral transgene **has not** been excised in the particular mouse iPS clones (clone 1). The absence of a 350 bp PCR product indicates that the viral STEMCCA transgene has been successfully excised (clones 2, 3). PCR reactions were normalized using GAPDH. A 500 bp PCR product corresponding to GAPDH is present at comparable intensity in all PCR samples. W = WPRE; G = GAPDH.

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