# Mouse STEMCCA Cre-Excisable Dox-Inducible Polycistronic (OKSM) Lentivirus Reprogramming Kit

Catalog No. SCR513

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## Introduction

Induced pluripotent stem (iPS) cells can be generated from somatic cells by the ectopic expression of the four Yamanaka transcription factors, Oct4, Klf4, Sox2, and c-Myc (OKSM)[1]. Induction of reprogramming has been achieved mostly through the co-infection of these factors in four separate expression vectors[1-5] Successful reprogrammings have required that a sufficient number of each virus deliver the four factors simultaneously to the same cell. This has raised concerns over the high number of integration sites that must arise from the random incorporation of four viruses to the genome and the difficulty in removing these viral integrations from genomic DNA. Moreover, the inability to predict whether cells receive one, two, three or all four factors has created heterogeneous cell populations, further complicating detailed study into the mechanism and timing of reprogramming.

To address these concerns, a cre-excisable, doxycycline (DOX)-inducible polycistronic reprogramming “stem cell cassette” or STEMCCA was generated which contains the four mouse transcription factors (OKSM) separated by the self-cleaving 2A peptide and IRES[7,11-14] sequences. The expression of the four factors are under the control of the doxycycline (DOX)-inducible tetO operator. Co-transfection with a lentivirus constitutively expressing reverse tetracycline transcriptional activator (rtTA) effectively reprogramed somatic cells to an embryonic stem (ES) cell-like state when cells are cultured in the presence of DOX. This single polycistronic cassette supported higher efficiency of reprogramming, reduced the number of viral integrations, enabled shut down of the viral reprogramming transgenes upon the withdrawal of doxycycline and facilitated subsequent Cre-mediated excision of the viral reprogramming transgenes. The results are iPS cells that are virtually free of the exogenous viral trangenes.

EMD Millipore’s Mouse STEMCCA Cre-Excisable Dox-Inducible Polycistronic (OKSM) Lentivirus Reprogramming Kit contains the following components.

- Two vials of Cre-Excisable, Dox-inducible polycistronic lentivirus expressing mouse transcription factors, Oct4, Klf4, Sox2 and c-Myc.
- Two vials of lentivirus containing constitutively expressed reverse tetracycline transcriptional activator (rtTA).
- 1 vial of Polybrene transfection reagent.

Advantages to the Mouse STEMCCA Cre-Excisable Dox-Inducible Polycistronic (OKSM) system include[12]:

- Single polycistronic lentivirus cassette minimizes viral integrations. Successfully transduced cells receive all four reprogramming factors simultaneously instead of heterogeneous populations of cells containing one, two, or three factors.
- Inducible expression allows for control of the reprogramming factors to be turned on or off as necessary via addition or removal of doxycycline. The system facilitates studies involving mechanism and timing of reprogramming.
- Ability to generate ‘secondary’ somatic cells that contain the reprogramming factors as defined doxycycline (DOX)-inducible transgenes through the creation of chimeric mice. ‘Secondary’ somatic cells allows accelerated efficient production of iPS cells upon exposure to DOX, obviating the need for lentivirus infection[12].
- Ability to excise viral reprogramming transgenes via Cre-Recombinase to produce iPS cells that are free of viral reprogramming transgenes.

## Product Description

EMD Millipore's Mouse STEMCCA Cre-Excisable Dox-Inducible Polycistronic (OKSM) Lentivirus Reprogramming Kit contains high titer Cre-excisable, DOX- inducible polycistronic (OKSM) lentivirus, constitutive reverse tetracycline transcriptional activator (rtTA) lentivirus, and Polybrene® transfection reagent. The kit has been validated for the generation of mouse iPS cells from mouse embryonic fibroblasts (MEFs). Mouse iPS cells display characteristic ES cell-like morphology, stained positive for alkaline phosphatase, expressed the correct mouse ES cell marker phenotype (Oct-4, SSEA-1, 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, inducible polycistronic lentiviral vector instead of four 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 exicsion 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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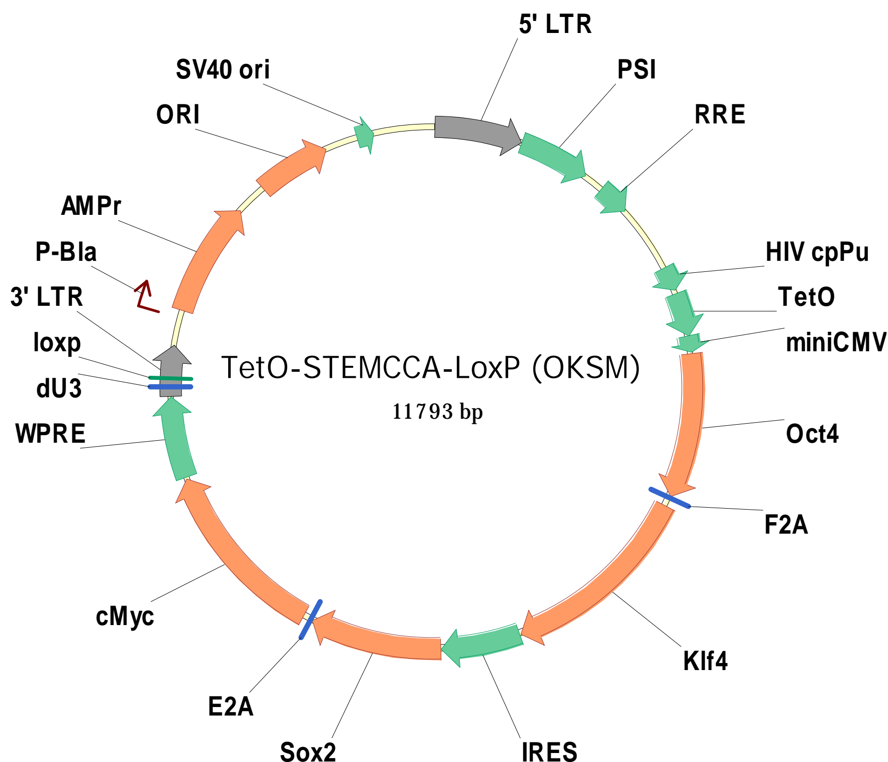


**Figure 1.** Schematic map of TetO-STEMCCA-LoxP (OKSM) lentiviral vector.

## Kit Components

**Catalog number SCR513:**

1. TetO-STEMCCA-LoxP (OKSM) Lentivirus: (Part number CS204442) Two (2) vials, each contains 15 μL of high titer lentiviruses. For exact titer refer to the label on the front of the manual.
2. rtTA Lentivirus: (Part number CS204506) Two (2) vials, each contains 15 μL of high titer constitutively expressed reverse tetracycline transcriptional activator (rtTA) lentiviruses. For exact titer refer to the label on the front of the manual.
3. Polybrene 10 mg/mL: (Part number TR-1003-50UL) One (1) vial containing 50 μL of 10 mg/mL stock of Polybrene transfection reagent.

## Storage and Handling

Lentiviruses are stable for at least 6 months when stored at -80°C. 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. Polybrene reagent is stable for at least 1 year when stored at -20°C.

**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. FibroGRO™ LS Complete Medium (Cat. No. SCMF002)
2. ESGRO-2i Medium (Cat. No. SF016-100 or SF016-200)
3. EmbryoMax Complete ES Cell Media w/ 15% FBS and mLIF (Cat. No. ES-101-B)
4. MEF expansion medium (see page 4)
5. Doxycycline, Hyclate (DOX) (Cat. No. 324385)
6. Rodent somatic cells or PMEF cells, not mitomycin-C treated (Cat. No. PMEF-CFL)
7. PMEF cells, growth-arrested, mitomycin-C treated (Cat. No. PMEF-CF)
8. EmbryoMax® 0.1% Gelatin Solution (Cat. No. ES-006-B)
9. ESGRO (LIF) (Cat. No. ESG1107)
10. Trypsin-EDTA Solution (0.25% Trypsin & 1 mM EDTA) (Cat. No. SM-2003-C)
11. Accutase™ Cell Dissociation Solution (Cat. No. SCR005)
12. Phosphate Buffered Saline (1X PBS) (Cat. No. BSS-1005-B)
13. Adenovirus expressing Cre recombinase and GFP (Vector Biolabs Cat. No. 1710)
14. DNeasy® Blood and Tissue Kit (QIAGEN Cat. No. 69504)
15. 6-well plates, culture flasks, dishes (TC grade)

# 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 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 at room temperature 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. | EMD 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 x $10^5$ 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°C, 5% $CO_2$ incubator. It is recommended to use early passage MEFs. ***Do not use MEFs that are beyond p3.***

### Day 1: Virus Infection

4. Aspirate MEF Expansion media and wash cells with 1X PBS buffer, 3 mL 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°C, 5% $CO_2$ incubator until ready to add the virus.

5. Using the equation provided below, determine the volume of virus required to achieve a multiplicity of infection (MOI) of 10 – 20. **Please make note of the titer as the viral titer may vary slightly from lot to lot.** Two viruses (TetO-STEMCCA-LoxP (OKSM) and rtTA lentiviruses) with equal MOI will be used to infect the cells. An MOI of 10 – 20 each for TetO-STEMCCA-LoxP (OKSM) and rtTA lentiviruses will typically yield 5-10 mouse iPS cell colonies from p3 MEFs. Using an MOI < 10 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 x $10^5$, the viral titer is 3 x $10^8$ IFU/mL, and a desired MOI is 20, then the volume of virus required is:

   $$\frac{1 \times 10^5 \text{ cells}}{3 \times 10^8 \text{ IFU/mL}} \times \frac{20}{1 \text{ mL}} \times 1000\ \mu L = {}^*6.6\ \mu L$$

   **each** of TetO-STEMCCA-LoxP (OKSM) and rtTA lentiviruses are 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 the requisite amount of vial(s) (1 vial = 15 μL) each of TetO-STEMCCA-LoxP (OKSM) and rtTA lentiviruses at room temperature and quickly place the vials on ice after they are thawed. Quickly centrifuge the vials to spin down the contents. Keep the viruses on ice and proceed immediately to the next step.

7. Pre-mix the required volume of thawed viruses (TetO-STEMCCA-LoxP (OKSM) and rtTA) in a sterile eppendorf tube and immediately add the viral mixture to the wells containing the attached MEFs (from Step 4). Gently rock the plate from side to side to thoroughly mix the viruses onto the MEFs. Incubate overnight in a 37°C, 5% $CO_2$ incubator.

**Day 2: Addition of Mouse ES Cell Media containing Doxycycline (DOX)**

8. Prepare stock solutions of doxycycline (10 mM) by dissolving DOX in sterile water. Filter sterilize the solution. DOX stock solutions can be stored in the dark at 4°C for up to 4 weeks without noticeable changes in activity.

a. Prepare Mouse ES Cell Media containing 2 μM DOX by adding 0.6 μL DOX (10 mM stock) to every 3 mL of Complete ES Cell Media with 15% FBS and LIF (Cat. No. ES-101-B). Scale up medium volumes according to experimental design.

b. Exchange media in each well with 3 mL Complete ES Cell Media with 15% FBS and LIF (Cat. No. ES-101-B) containing 2 μM DOX.

***Note:** Mouse iPS Cell Boost Supplement (SCM087) should not be used as it does not exert an effect on the inducible system.*

**Day 4 – Day 13: Exchange with Mouse ES Cell Media containing DOX 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 2 μM DOX every other day for a total of 10 – 13 days. Mouse iPS cell colonies start to emerge around day 7 – 10.

10. Continue to monitor the growth of the mouse iPS cell colonies daily. Look for colonies that are compact and have defined borders (refer to Figure 2). Mouse iPS cell colonies can be selected and clonally expanded (typically around Day 10 – 12) when they reach an approximate size where the colony fits into the frame of a 10X Magnification view (please refer to Figure 2C for an example of an iPS colony that can be picked).

## SECTION 2: CLONAL EXPANSION OF MOUSE iPS COLONIES

At approximately Day 11 or when the mouse iPS colonies are of sufficient size (see step 10), they can be picked for clonal expansion in serum free ESGRO-2i Medium (without DOX). Once colonies are established, it is no longer necessary to add the DOX to the expansion medium.

***Note:** Avoid expansion media that contains serum as this may result in mixed cultures containing full and partial reprogrammed (i.e. flat morphologies) colonies.* ***Use of ESGRO-2i medium (Cat. No. SF016-100 or SF016-200) is highly recommended to obtain good ES cell-like morphology.*** *Good ES-cell like morphologies are typically observed after the 3rd to 5th passage in ESGRO-2i medium. If making own serum-free based media, KOSR can be used to replace FBS.*

**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 4). 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 serum free ESGRO-2i Medium 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 serum free ESGRO-2i Medium 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 TetO-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 $1 \times 10^6$ 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 $1 \times 10^5$ 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 $1 \times 10^5$, the viral titer is $1 \times 10^{10}$ IFU/mL, and the desired MOI is 3000, then the volume of virus required is:

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

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°C for 2 minutes.

    b. 30 cycles of:

        95°C 30 seconds

        65°C 45 seconds

        72°C 45 seconds

    c. Final extension at 72°C for 10 minutes.

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

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.

## Mouse iPS Cells Derived from MEF cells

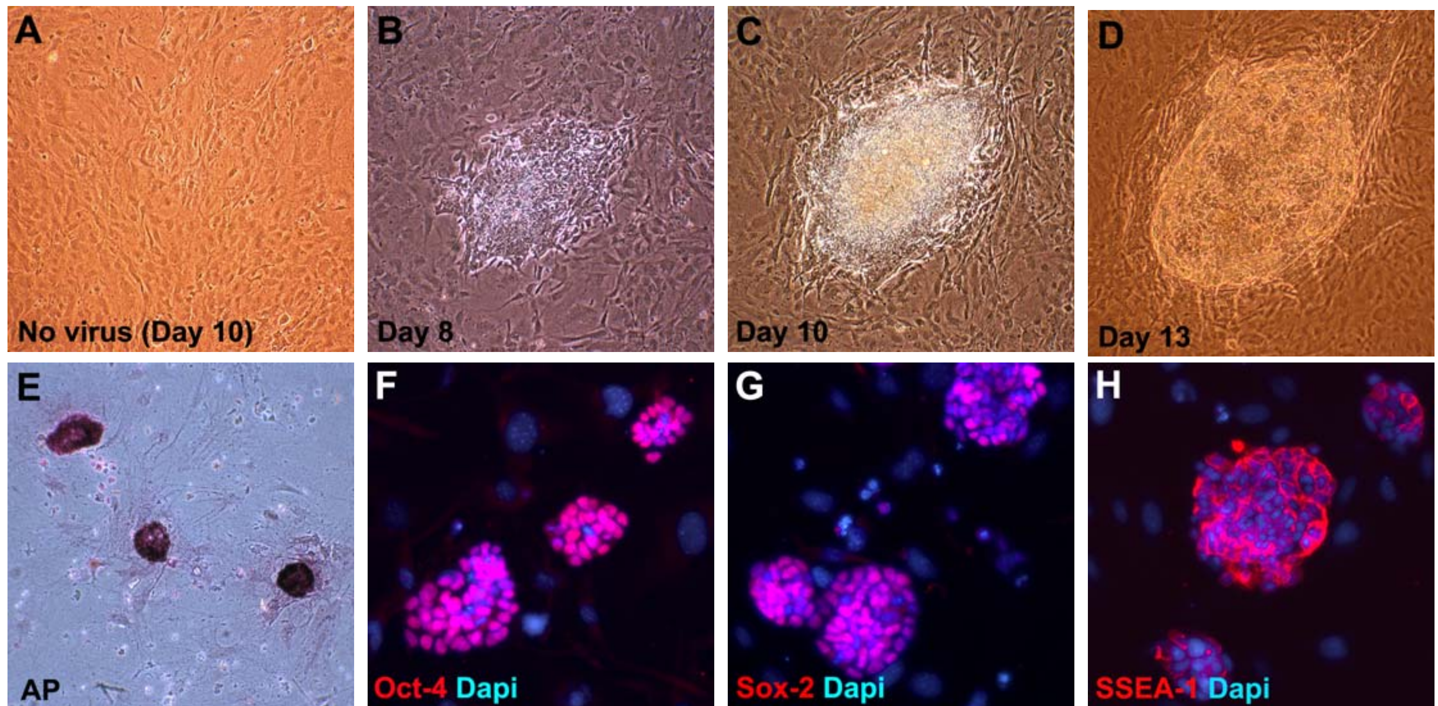


**Figure 2.** Mouse iPS cells derived from MEFs (passage 3) co-infected with the TetO-STEMCCA-LoxP (OKSM) and rtTA lentiviruses have cell morphology and staining characteristics of mouse ES cells. Lentiviral infection was performed with an MOI of 20 and 5 µg/mL Polybrene reagent. After 10-12 days, non-infected MEFs remained in a monolayer culture with no ES cell-like colonies observed (**A**) while infected MEFs formed multilayered, tightly packed cells with defined borders (**B-D**). Four factor derived mouse iPS cells exhibited 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).

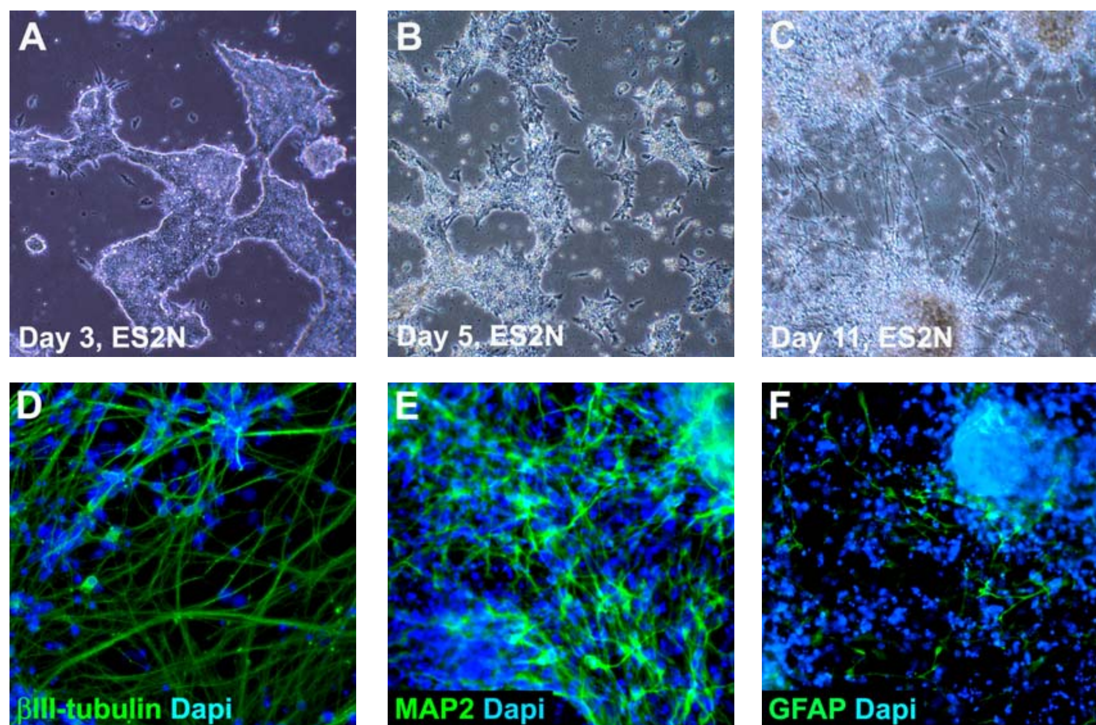


**Figure 3.** Directed differentiation of mouse iPS cells to mostly neuronal phenotype using serum-free ES2N media (Cat. No. SCM082). Mouse iPS cells were directly differentiated in ES2N media for 3 (**A**), 5 (**B**), and **11** (**C**) days, with media changes every other day. After 11 days of differentiation, majority of the cells expressed neuronal markers, βIII-tubulin (**D**, Cat. No. MAB1637) and MAP2 (**E**, Cat. No. MAB3418) with few GFAP-positive astrocytes (**F**, Cat. No. MAB3402) detected.

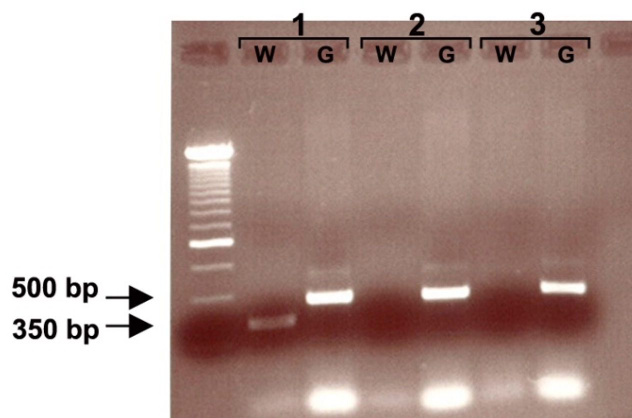


**Figure 4.** PCR analyses of mouse iPS clones that have undergone Cre recombinase excision. In this example, excision was demonstrated on mouse iPS clones generated from EF1α-STEMCCA-LoxP (OKSM) lentivirus. Presence of a 350 bp PCR product, corresponding to the WPRE transcript specific to the STEMCCA 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.

## References


1. Takahashi K and Yamanaka S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**: 633-676.
2. Okita ,*et al*. (2007) Generation of germline-competent induced pluripotent stem cells. *Nature* **448**: 313-317.
3. Wernig M, *et al*. (2007) In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* **448**: 318-324.
4. Takahashi K, *et al*. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factor. *Cell* **131**: 861-872.
5. Yu J, *et al.* (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science* **318**: 1917-1920.
6. Sommer CA, *et al*. (2010) Excision of reprogramming transgenes improves the differentiation potential of iPS cells generated with a single excisable vector. *Stem Cells* **28(1)**: 64-74.
7. Sommer CA, *et al.* (2009) iPS cell generation using a single lentiviral stem cell cassette. *Stem Cells* **27**: 543-549.
8. Hanna J, *et al.* (2009) Direct cell reprogramming is a stochastic process amenable to acceleration. *Nature* **462**: 595-601.
9. Stadtfeld M, *et al.* (2010) A reprogrammable mouse strain from gene-targeted embryonic stem cells. *Nature Methods* **7**: 53-55.
10. Carey B, *et al*. (2010) Single-gene transgenic mouse strains for reprogramming adult somatic cells. *Nature Methods* **7**: 56-59.
11. Carey B, *et al.* (2009) Reprogramming of murine and human somatic cells using a single polycistronic vector. *PNAS* **106**:157-162.
12. Wernig M, et al. (20008) A drug-inducible transgenic system for direct reprogramming of multiple somatic cell types. *Nat. Biotechnol.* **26(8)**: 916-924.
13. Markoulaki S., et al (2009) Transgenic mice with defined combinations of drug-inducible reprogramming factors. *Nat. Biotechnol.* **27(2)**: 169-171.
14. Staerk J., et al (2010) Reprogramming of human peripheral blood cells to induced pluripotent stem cells. *Cell Stem Cell* **7**: 20-24.

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