

Video Article

# The use of SC1 (Pluripotin) to Support mESC Self-renewal in the Absence of LIF

Wen Xiong<sup>1</sup>, Yan Gao<sup>1</sup>, Xun Cheng<sup>1</sup>, Charles Martin<sup>1</sup>, Dongmei Wu<sup>1</sup>, Shuyuan Yao<sup>1</sup>, Min-Ju Kim<sup>2</sup>, Yang Liu<sup>1</sup>

<sup>1</sup>Research and Development, Stemgent

<sup>2</sup>Product Marketing, Stemgent

Correspondence to: Wen Xiong at [wen.xiong@stemgent.com](mailto:wen.xiong@stemgent.com)

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## Abstract

Mouse embryonic stem (ES) cells are conventionally cultured with Leukemia Inhibitory Factor (LIF) to maintain self-renewal.<sup>1</sup> However, LIF is expensive and activation of the LIF/JAK/STAT3 pathway is not absolutely required to maintain the self-renewal state.<sup>2</sup> The SC1 small molecule may be an economical alternative to LIF. SC1 functions through dual inhibition of Ras-GAP and ERK1.<sup>3</sup> Illustration of its mechanism of action makes it a useful tool to study the fundamental molecular mechanism of self-renewal. Here we demonstrate the procedure for culturing mouse ES cells in the presence of SC1 and show that they are able to maintain self-renewal in the absence of LIF. Cells cultured with SC1 showed similar morphology compared to cells maintained with LIF. Both exhibited typical mouse ES morphology after five passages. Expression of typical pluripotency markers (Oct4, Sox2, Nanog, and SSEA1) was observed after five passages in the presence of SC1. Furthermore, SC1 caused no overt toxicity on mouse ES cells.

## Video Link

The video component of this article can be found at <http://www.jove.com/video/1550/>

## Protocol

### 1. Preparation of MEF feeder plates

1. One day before culturing ES cells, thaw one vial of irradiated E14.5 CF-1 MEF feeder cells (see our procedure on how to thaw ES cells).
2. Plate the MEF feeder cells at a density of 10,000 cells/well in a 12 well plate. Incubate the cells overnight at 37°C and 5% CO<sub>2</sub>. MEF cell media: DMEM supplemented with 10% ES-PBS, 1X glutamine and 1X non-essential amino acids.

### 2. Maintenance of Self-Renewal of Mouse ES Cells

1. Detach the ES cells using trypsin. Seed the cells at a density of 50,000 cells/well on the previously prepared MEF feeder plates in growth media (Knockout MEM supplemented with 15% KSR, 4 mM L-glutamine, 0.1 mM non-essential amino acids and 1X BME) supplemented with SC1 at the desired concentration (we used 100 nM, 300 nM, and 1 μM). We also added a positive control well that was supplemented with 1000 μ/ml of LIF. Incubate the cells overnight at 37°C and 5% CO<sub>2</sub>.
2. Culture the cells at 37°C and 5% CO<sub>2</sub> for 3-4 days in each passage. Refresh media every 48 hours.
3. Passage cells 1:10 to 1:15 with trypsin to maintain a similar density as the initial seeding density. After 5 passages, the cells can be examined for the expression of typical pluripotency markers using immunocytochemistry.

### 3. Immunocytochemical Examination of Pluripotency Markers

1. Wash the cells gently three times with PBS.
2. Fix the cells with 500 μl of fixative for 20 minutes at room temperature.
3. Wash the cells gently three times with PBS.
4. Block non-specific binding with 500 μl blocking buffer for one hour at room temperature.
5. Incubate the cells with 250 μl of the specific primary antibody (we used Nanog, Sox2, SSEA1, and Oct4 at the following dilutions: 1:500, 1:2000, 1:500, and 1:500)
6. Wash the cells gently three times with PBS.
7. Incubate the cells with 250 μl of secondary antibody for two hours at room temperature, keeping away from light (we used donkey anti-mouse conjugated with Alexa Fluor® 555 at 1:2000 and donkey anti-rabbit conjugated with Alexa Fluor® 488 at 1:2000).
8. Wash the cells gently three times with PBS.

9. Add DAPI (final concentration 1  $\mu\text{g/ml}$ ) to the last wash and incubate 5 minutes to visualize nuclei.
10. Analyze the cells under a fluorescent microscope.

## 4. Representative Results:

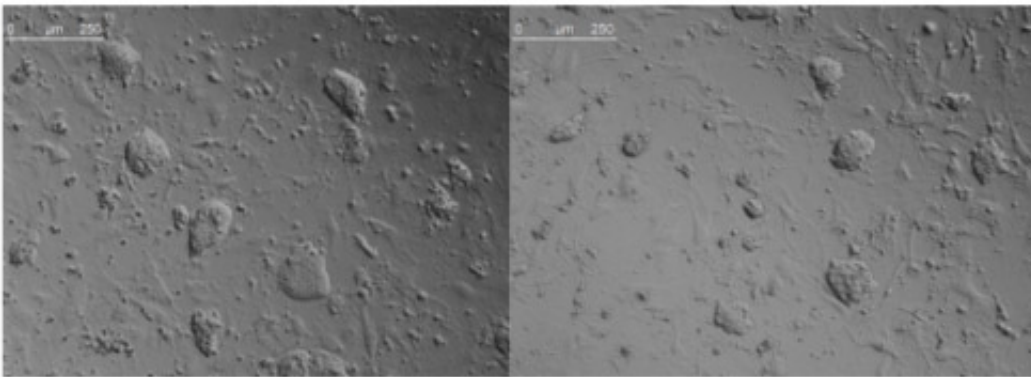
### 1. Morphology results:

Mouse ES cells (ESC R1) were grown on MEF feeder cells in the presence of either LIF or SC1 to sustain self-renewal at an undifferentiated state. After the second passage, differentiation could be observed in cells without LIF or SC1 (negative control) and after 5 passages essentially no undifferentiated colonies were observed. LIF (positive control) treated colonies remained in an undifferentiated state throughout the 5 cell passages. Cells treated with SC1 at concentrations of 300 nM or 100 nM also remained undifferentiated after 5 passages, however, the cells treated with 1  $\mu\text{M}$  SC1 experienced cell death after the fourth passage. (fig 2 of app note) Table 1 summarizes the morphological observations.

### 2. Expression of Pluripotency Markers

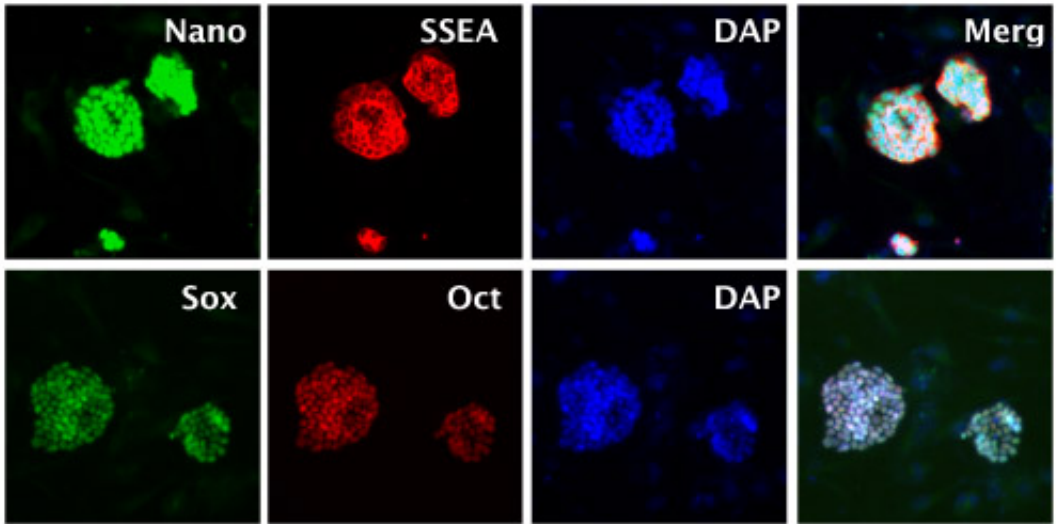
Mouse ES cells maintained for 5 passages were fixed and examined by immunocytochemistry for SSEA1, Oct4, Nanog and Sox2. Marker expression was similar to cells maintained in LIF. (fig 3 and 4 of app note)

## Phase Comparison Between LIF and SC1 Maintained R1 Cells

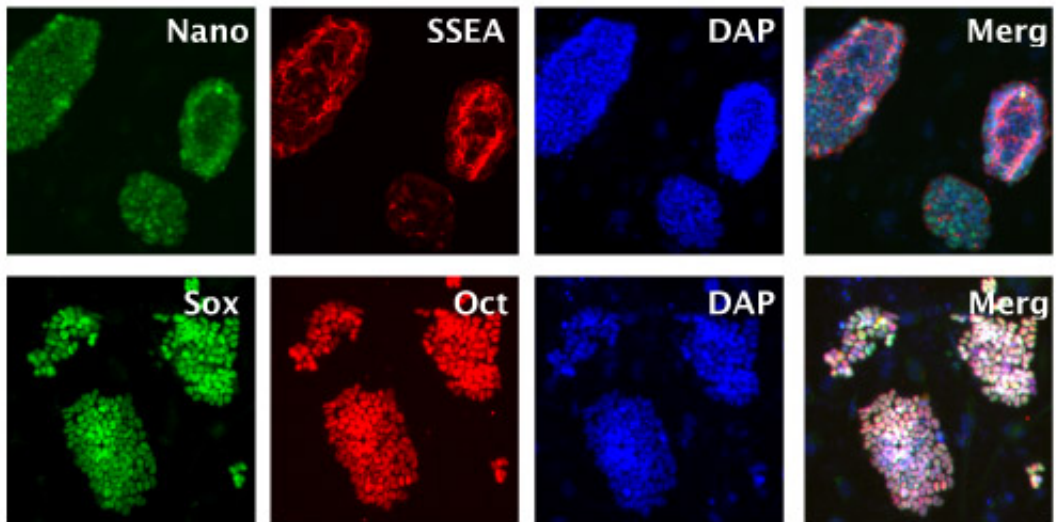


**Figure 1:** Morphology comparison of cells maintained in LIF or SC1. No difference in morphology was observed.

Positive control with LIF  
10X Images



ICC of R1 cells maintained with SC1  
10X Images



**Figure 2 and Figure 3:** Nanog, SSEA1, Sox2, and Oct4 staining of ES cells maintained with SC1 or LIF (fig 4 of app note). Cells maintained in SC1 show similar pluripotency marker expression to cells maintained in LIF.

**Table 1**

	Negative Control	LIF Positive Control	SC1 1uM	SC1 300 nM	SC1 100 nM
<b>1st Passage</b>	Normal ES cell morphology	Normal ES cell morphology	Normal ES cell morphology	Normal ES cell morphology	Normal ES cell morphology
<b>2nd Passage</b>	Some differentiated morphology	Normal ES cell morphology	Normal ES cell morphology	Normal ES cell morphology	Normal ES cell morphology
<b>3rd Passage</b>	Majority of cells differentiated	Normal ES cell morphology	Normal ES cell morphology	Normal ES cell morphology	Normal ES cell morphology
<b>4th Passage</b>	No ESC colonies	Normal ES cell morphology	Cell death observed	Normal ES cell morphology	Normal ES cell morphology
<b>5th Passage</b>	differentiated	Normal ES cell morphology	Cell death observed	Normal ES cell morphology	Normal ES cell morphology

## Discussion

The small molecule SC1 can be used to maintain self-renewal with an undifferentiated state in mouse ES cells. Before using on an untested cell line, however, it should be titrated to determine the optimal concentration. For example, we tested three concentrations on the mouse ES cell line ESC R1. 100 nM and 300 nM concentrations were able to sustain mouse ES cells, however, a 1  $\mu$ M concentration was toxic.

SC1 can also be used under feeder-free conditions.

## Disclosures

The authors of this article are employed by Stemgent that produces reagents and instruments used in this article.

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