Minicircle DNA and mc-iPS Cells
Cat. #SC301A-1, SRMXXXPA-1

User Manual

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ver. 2-111910

Contents

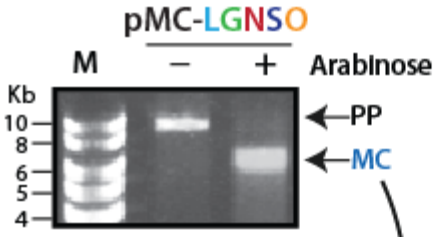
I. Introduction and Background

A. The Minicircle Technology

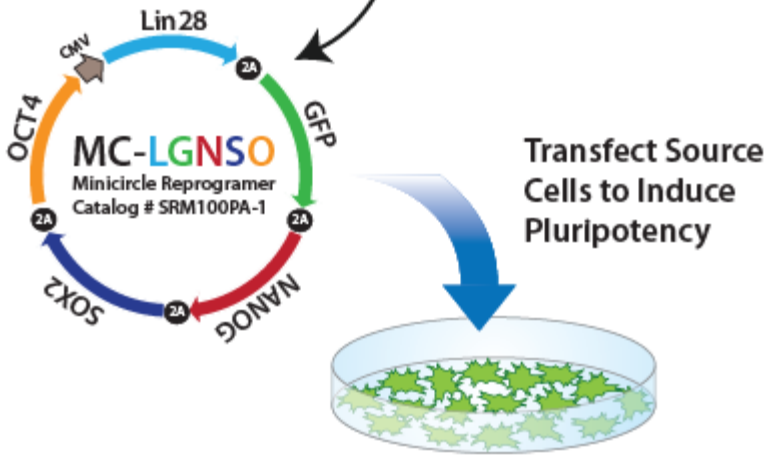
Minicircles (MC) are circular non-viral DNA elements that are generated by an intramolecular (cis-) recombination from a parental plasmid (PP) mediated by ΦC31 integrase. The full-size MC-DNA construct is grown in a special host *E. coli* bacterial strain. This strain harbors an Arabinose-inducible system to express the ΦC31 integrase and the I-SceI endonuclease simultaneously. The ΦC31 integrase produces the MC-DNA molecules as well as PP-DNA from the full-size MC-DNA construct.

The PP-DNA contains several engineered I-SceI restriction sites that ultimately lead to the destruction of the PP-DNA but not the MC-DNA. The difference between MC and standard plasmid vectors is that the MC no longer contains the bacterial origin of replication or the antibiotic resistance markers. Sequences within the bacterial plasmid backbone contain signals for methylation and transgene silencing. Thus delivering only the minicircles to cells lengthens the expression of the transgene over traditional transient transfections of plasmids.

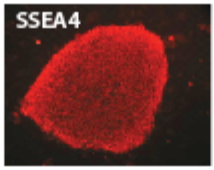
SBI's pre-made MC-LGNSO DNA features easy-to-transfect molecules that have an extended expression lifespan in mammalian cells to efficiently reprogram somatic cells to the pluripotent state. For dividing cells, expression of the minicircles lasts up to 14 days. For non-dividing cells, expression of the minicircles drops slightly after the first week, but then can continue expressing the transgenes for months.

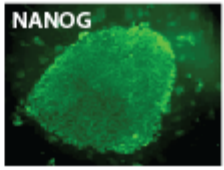


Agarose gel showing the induction and production of Minicircle DNA with Lin28+GFP+Nanog+Sox2+Oct4



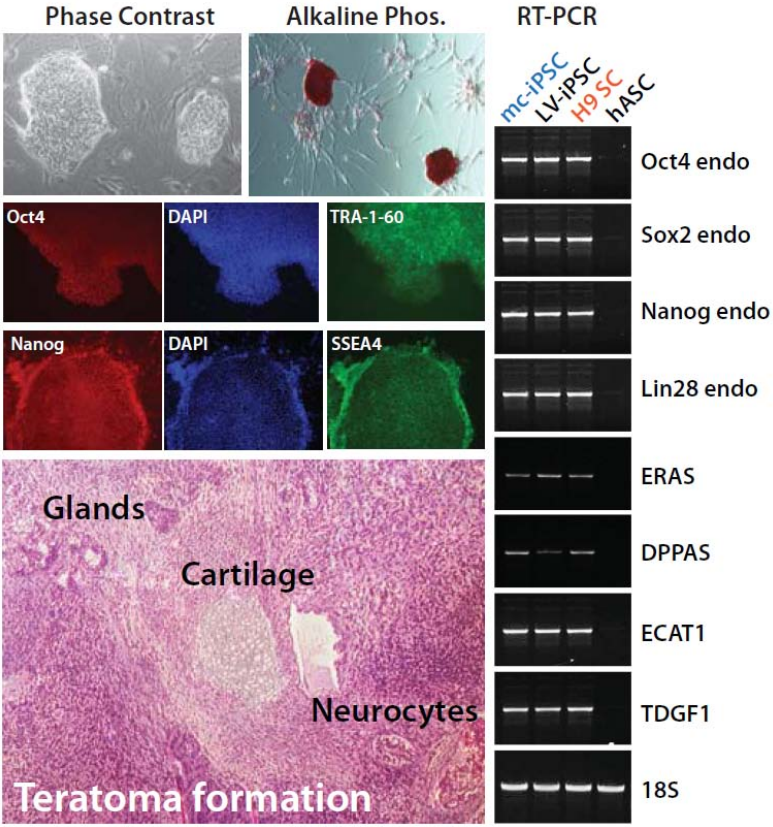
Create Nonviral iPS Cells



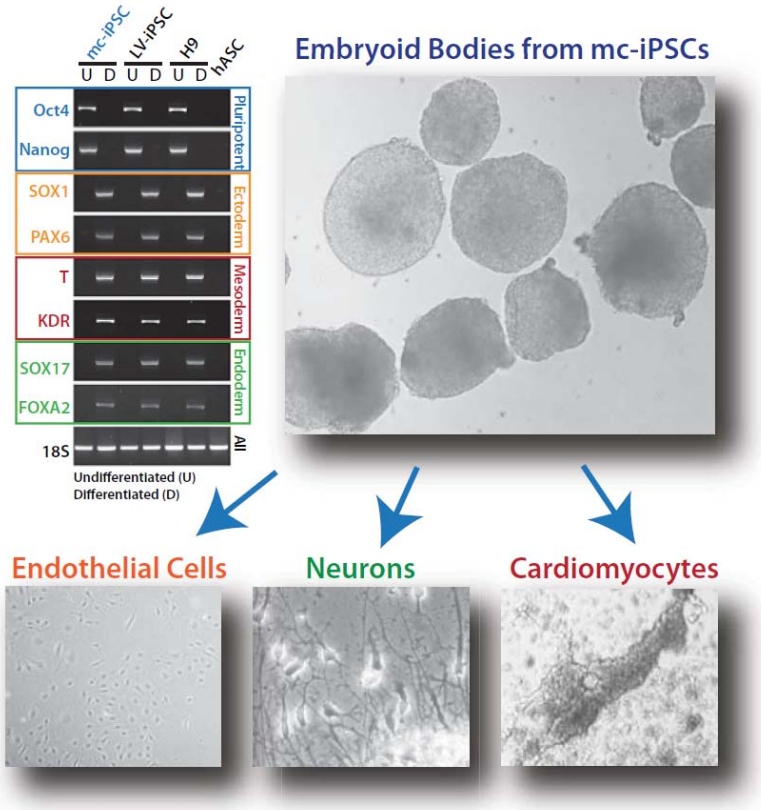


B. Minicircle derived iPS cell line

In addition to the pre-made, ready-to-transfect 4-in-1 minicircle reprogramming DNA, SBI also offers the Human mc-iPS Cell line highlighted in **Nature Methods**, A nonviral minicircle vector for deriving human iPS cells. Jia F, et al., 2010 Mar;7(3):197-9. The mc-iPS cell line was derived from adult human adipose stem cells (hASCs) and is certified positive for pluripotency protein marker immunostaining and by gene expression.



The mc-iPSCs also demonstrate multiple lineage potential.



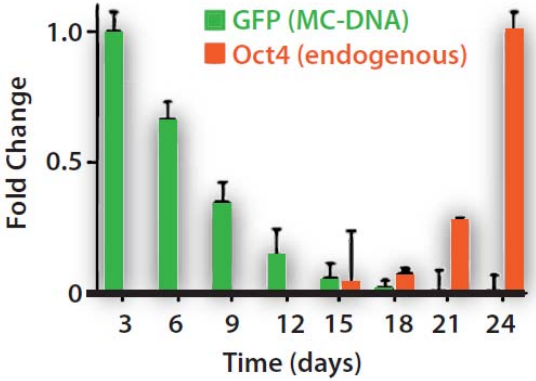
II. Protocols

A. Transfection of Minicircle DNA for reprogramming

The following protocol has been optimized for human adipocyte-derived stem cells according to the method described in Jia et. al. Other source cells may require transfection optimization for efficient reprogramming. In general, reprogramming requires approximately 5µg per transfection per well in 6 well plate three times.

1. Use Nucleofector Kit R (Amaxa) and program U-023 according to the manufacturer's instructions.
2. Plate transfected cells in 10 cm dishes and culture in DMEM/F12 medium (Invitrogen) supplemented with 10% FBS.
3. GFP-positive cells can be sorted by flow cytometry 3 days after transfection. The sorted cells should then seeded on gelatin-coated 6-well plates at ~0.5×10^5 cells per well.
4. Switch cells to human ESC culture medium 1 day after seeding. Refresh culture medium every 2–3 days.
5. On days 4 and 6, transfect the cells again with minicircle DNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions.
6. Colonies with morphologies similar to hESC colonies are clearly visible by day 18 after the initial transfection.
7. At day 26–28 after transfection, GFP-negative mc-iPSC colonies can be individually picked for further expansion and analysis.

The GFP signal should decrease over time correlating with the disappearance of the minicricle DNA. A simultaneous increase of the endogenous pluripotency marker expression should also be observed.



B.Growing mc-iPS cells

Materials

Human ESC medium

DMEM/F12 containing 20% knockout serum replacement, 2 mM glutamine, 1 x 10^{-4} M nonessential amino acids, 1 x 10^{-4} M 2-mercaptoethanol, 10 ng/ml bFGF, and 50 U and 50 µg /ml penicillin and streptomycin.

MEF medium

DMEM containing 10% FBS, 2 mM glutamine, 1x 10^{-4} M nonessential amino acids, and 50 U and 50 µg /ml penicillin and streptomycin.

ROCK Inhibitor Y-27632 (Sigma)

NOTE: This protocol is for growing mc-iPS cells on MEF feeder cells. These should already be growing before you plate your mc-iPS cells.

Processing and culturing mc-iPS Cells

Upon receiving the vial of human iPS cells, quickly thaw the vial of frozen cells in a 37°C water bath. Remove the vial from the water bath as soon as the cells are half thawed, and sterilize by spraying the outside of the tube with 70% ethanol.

Add the cells to 10 ml of pre-warmed human ES medium in a 15-cm conical tube. Pellet the cells by centrifugation at 200 g for 5 min. While centrifuging, remove MEF medium from the 6-well plate with MEF feeder cells, wash the wells twice with 1 ml of DMEM/F12, and add 1ml of human ES medium.

Discard the supernatant of the tube containing human iPS cells. Resuspend the cells with 1 ml of fresh human ES medium supplemented with 10 µM ROCK inhibitor Y-27632 (final concentration), to decrease spontaneous differentiation and to assist in recovery after freeze/ thawing.

Plate the cells in one well of a 6-well plate with MEF feeder cells. Incubate the plate overnight at 37°C. Change the medium every day until the cells reach 80% confluency. If desired, Y-27632 can be added into the culture media for a few days after thawing.

Usually, it takes about one week to observe the iPSC colonies.

III. References

Fangjun Jia et al. A nonviral minicircle vector for deriving human iPS cells. Nature Methods 2010 Mar;7(3):197-9.

Elayne Chan et al. Live cell imaging distinguishes bona fide human iPS cells from partially reprogrammed cells. Nature Biotechnology 27 (11) 1033-1037 (November 2009).

Zhi-Ying Chen et al. Improved production and purification of minicircle DNA vector free of plasmid bacterial sequences and capable of persistent transgene expression in vivo. Human Gene Therapy 16 (1) 126-131 (January 2005).

Zhi-Ying Chen et al. Minicircle DNA Vectors Devoid of Bacterial DNA Result in Persistent and High-Level Transgene Expression in Vivo. Molecular Therapy 8 (3) 495-500 (September 2003).

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