



Minicircle DNA and mc-iPS Cells

Cat. # SC301A-1, SRMXXXPA-1

User Manual

Store DNA at -20°C on receipt

Store cells at -80 C on receipt

A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the Licensing and Warranty Statement contained in this user manual.

(ver. 1-072210)

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I. Introduction and Background

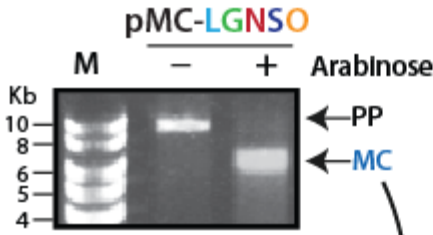
A. The Minicircle Technology

Minicircles (MC) are circular non-viral DNA elements that are generated by an intramolecular (cis-) recombination from a parental plasmid (PP) mediated by Φ C31 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 Φ C31 integrase and the I-SceI endonuclease simultaneously. The Φ C31 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.

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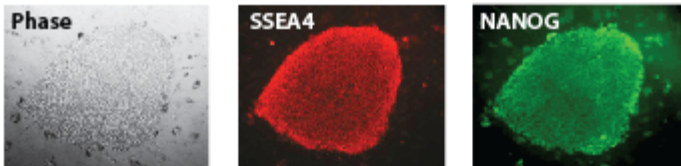
Agarose gel showing the induction and production of Minicircle DNA with Lin28+GFP+Nanog+Sox2+Oct4



Transfect Source Cells to Induce Pluripotency

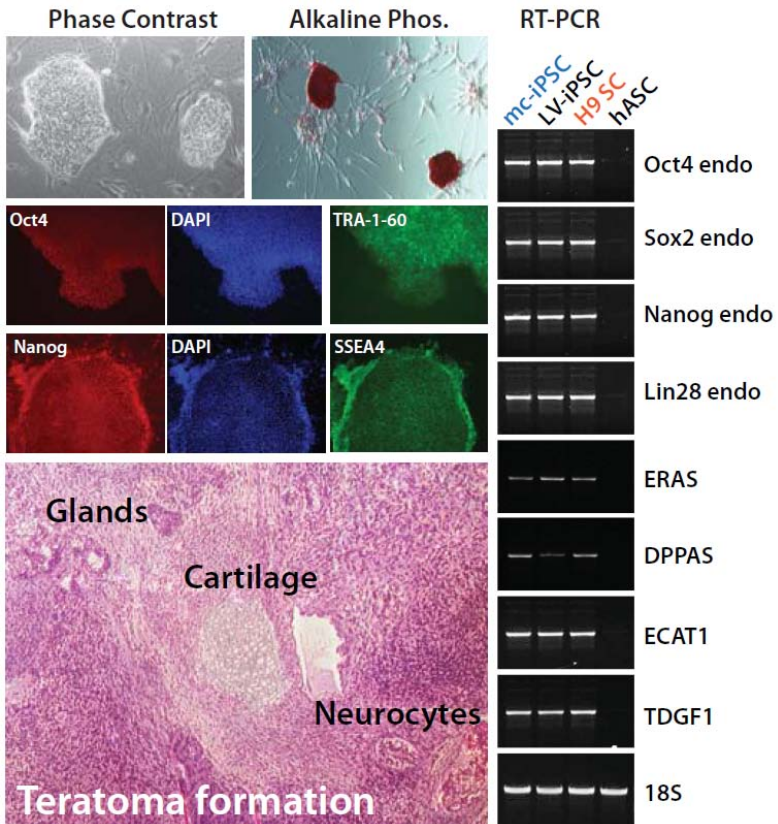


Create Nonviral IPS Cells



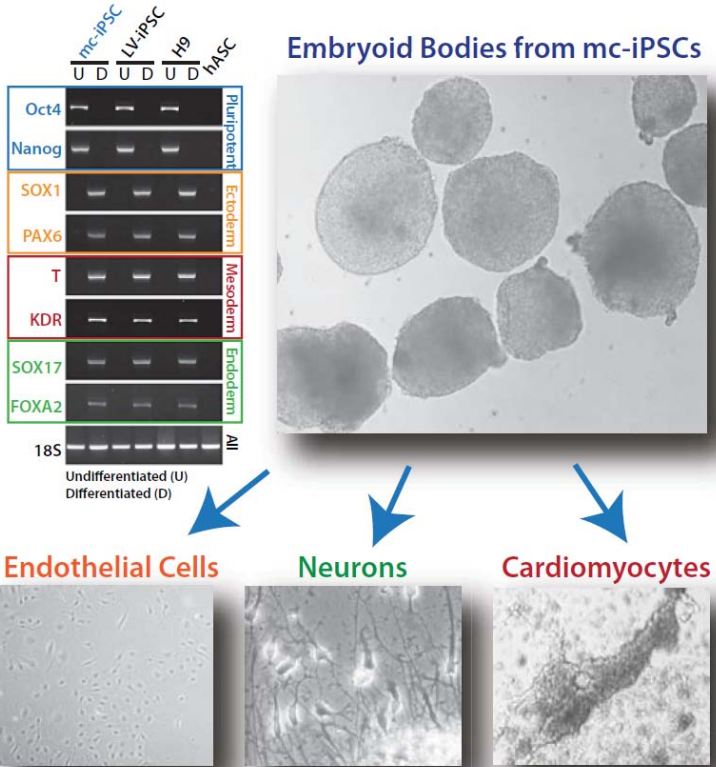
B. Minicircle derived iPS cell line

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.



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The mc-iPSCs also demonstrate multiple lineage potential.



II. Protocols

A. Minicircle Production

The following protocol requires that you use both the pMC-LNSO parental construct in combination with the ZYCY10P3S2T *E. coli* strain. This strain has been specifically engineered to express the Φ C31 integrase and I-SceI endonuclease upon arabinose induction.

Materials

Component	Cat. #	Source
LB Agar Kanamycin 50 plates, 20/pk	L1025	Teknova
20% Arabinose solution 50ml	A2010	Teknova
Kanamycin Solution, 50mg/mL (25 mL)	K2125	Teknova
Terrific Broth Complete 500 ml	T7055	Teknova
LB 1L	L8000	Teknova
PureLink HiPure Plasmid Filter Purification Kit	K2100- 14	Invitrogen

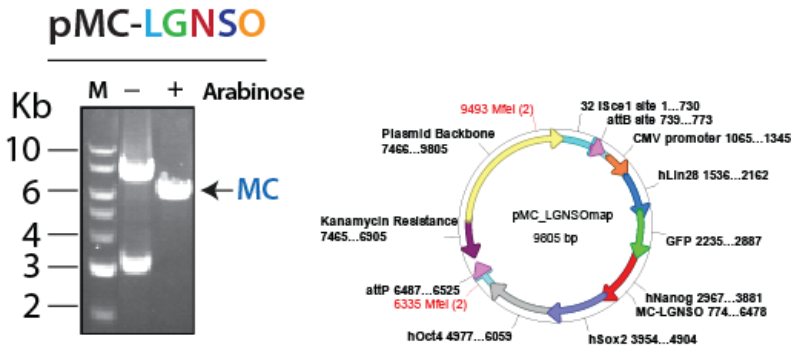
Procedure

1. On day 1 in the morning, grow cells from a plasmid-transformed colony in 5 ml LB containing 50 μ g/ml kanamycin at 37°C, with shaking at 250 rpm.
2. On day 1 in the evening, grow an overnight culture by combining 100 μ l of the culture in step 1 per 400 ml of fresh TB (Terrific Broth) containing 50 μ g/ml kanamycin in a 2 liter flask and culture at 37°C, with shaking at 250 rpm. For scaling up the protocol, keep the ratio of flask size to O/N culture volume at 5:1 (vol:vol).
3. On day 2 in the morning, the OD600 of the overnight culture will be between 4 and 5.

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4. Prepare a Minicircle Induction Mix comprising 400ml LB, 16 ml 1N NaOH and 400 μ l 20% L-arabinose.
5. Combine the Minicircle Induction Mix with the overnight culture, and incubate at **30°C** with shaking at 250 rpm for 5 hours or longer.
6. Pellet the bacteria, and use a PureLink HiPure Plasmid Filter Purification kit (Invitrogen, Cat #K2100-14) to isolate minicircle DNA according to manufacturer's protocol.

The gel image below shows uninduced (minus arabinose) and the LGNO minicircle preps digested with MfeI. The parental construct generates 2 bands of 6.7kb and 3.2kb. The Minicircle (MC) DNA produces a single band about 6kb.



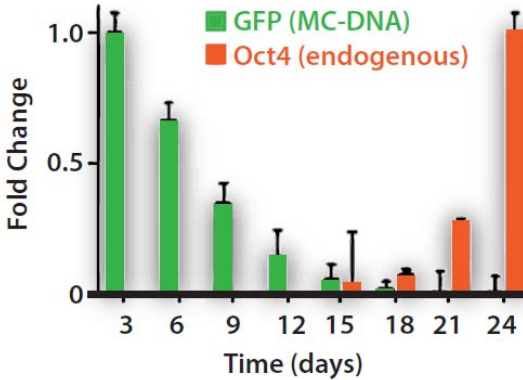
B. Transfection of Minicircle DNA for reprogramming

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 $\sim 0.5 \times 10^5$ cells per well.
4. Switch cells to hESC 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.

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



C. Growing mc-iPS cells

Materials

- **Human ES 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 mg/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 mg/ml penicillin and streptomycin.

ROCK Inhibitor Y-27632 (Sigma)

NOTE: mc-iPS cells must be grown 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 μ M 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).

IV. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

<http://www.systembio.com>

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Use of the mc-iPS cell line and mc-LGNSO DNA (*i.e.*, the “Product”) is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

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The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

**** This Product shall be used by the purchaser for internal research purposes only and distribution is strictly prohibited without written permission by System Biosciences.**

Limited Warranty

SBI warrants that the Product meets the specifications described in the accompanying Product Analysis Certificate. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

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