



Minicircle DNA and mc-iPS Cells

Cat. #SC301A-1, SRMXXXPA-1

User Manual

ver. 2-111910

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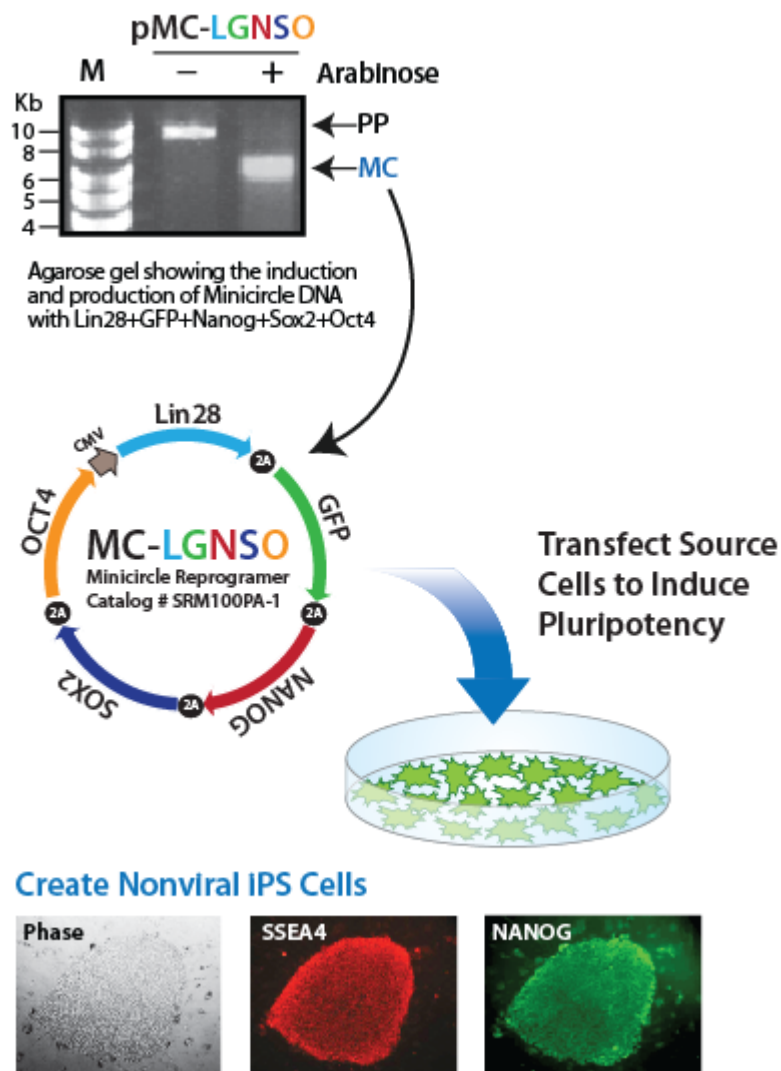
I. Introduction and Background

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.

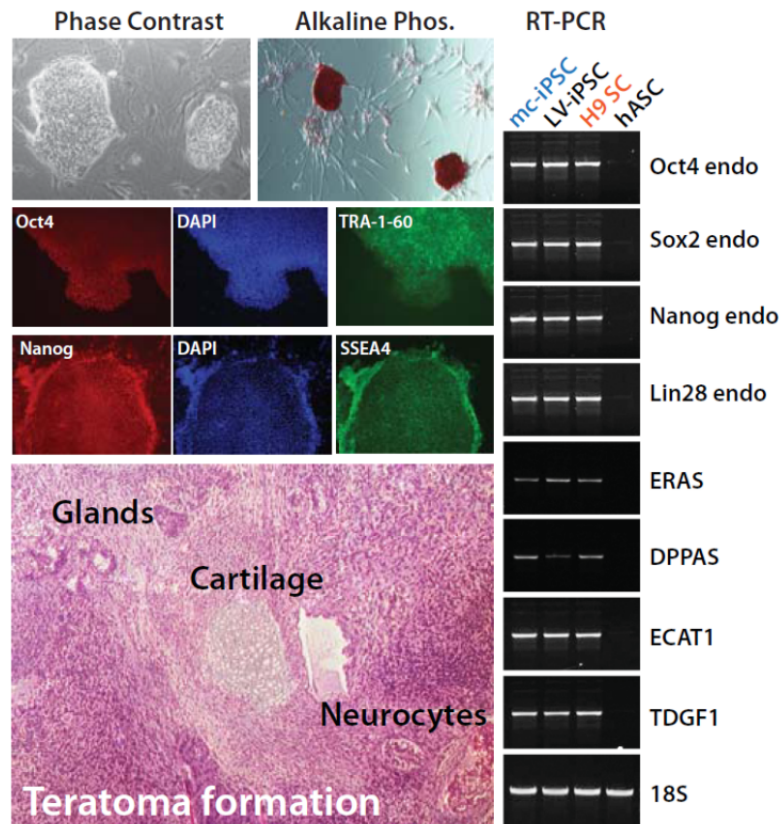
SBI's pre-made MC-LGNSO DNA features easy-to-transfect molecules that have an extended expression lifespan in mammalian cells to efficiently reprogram somatic cells to the pluripotent state. For dividing cells, expression of the minicircles lasts up to 14 days. For non-dividing cells, expression of the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



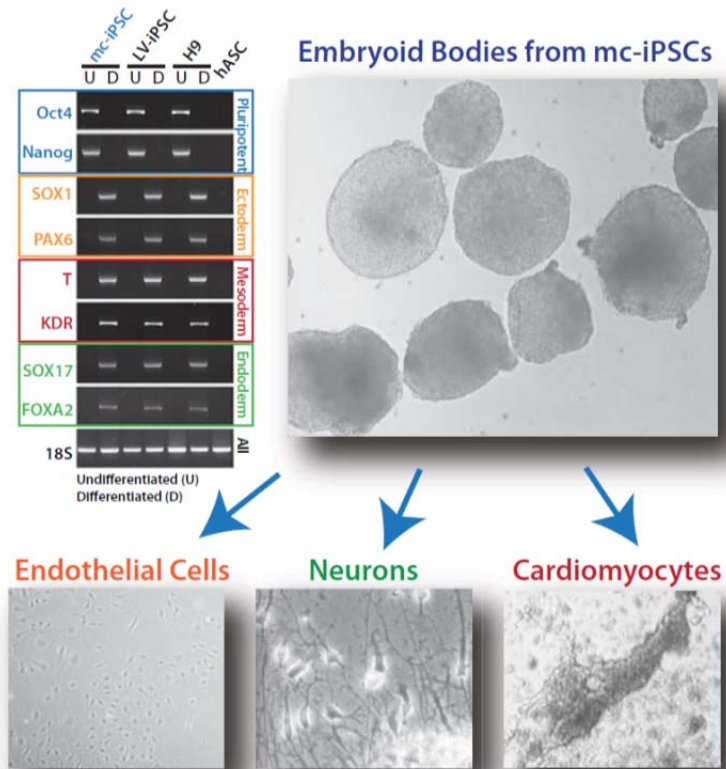
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B. Minicircle derived iPS cell line

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. 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.

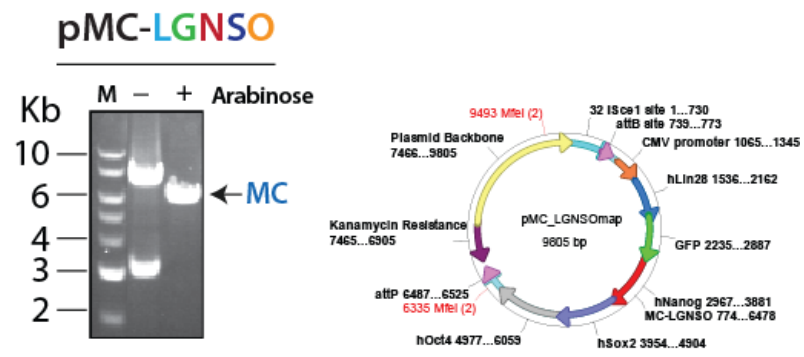
Materials

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.
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.

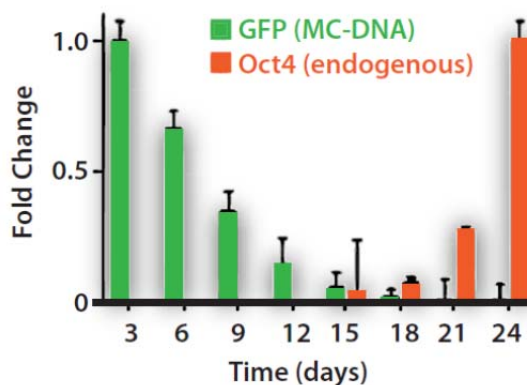


B. Transfection of Minicircle DNA for reprogramming

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.

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 in Feeder-Free Media

Materials

- **mTeSR-1 basal media and supplements (Cat. no. 05850, StemCell Technologies)**
- **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.
- **Matrigel (Cat. no. 354277, BD Biosciences)**
- **Accutase (Cat. no. SCR005, Millipore)**
- **ROCK Inhibitor Y-27632 (Cat. no. Y0503, Sigma)**

Preparation of Feeder-free medium

1. Thaw mTeSR1 5X Supplement (Cat. 05850, STEMCELL Technologies) at room temperature or overnight at 4°C.
2. Add the 100 mL of thawed 5X Supplement to 400 mL Basal Medium for a total volume of 500 mL aseptically. Mix well. Filter through a 0.2 µm, low-protein binding filter, if desired.
3. Aliquot into appropriate amount according to usage and store the aliquots at 4°C.

Coating Plates with Matrigel

Matrigel (Cat. 354277, BD) should be aliquoted and stored at -80°C for long term use.

1. Thaw matrigel on ice until liquid. Dilute matrigel 1:50 to 1:100 with pre-chilled KO DMEM/F12.
2. Immediately use the diluted matrigel solution to coat tissue culture-treated plates. For a 6-well plate, use 0.8 mL of diluted matrigel solution per well, and swirl the plate to spread the matrigel solution evenly across the surface.
3. Let the coated plate stand for 1 h at 37°C or overnight at 4°C. If plate has been stored at 4°C, allow the plate to incubate at 37°C for 30 minutes before removing the matrigel solution.

Thawing Cryopreserved hiPSCs

1. Quickly thaw the hiPSCs in a 37°C waterbath by gently shaking the cryovial continuously until half thawed. Remove the cryovial from the waterbath and spray with 70% ethanol to sterilize.

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2. Transfer the contents of the cryovial to a 15 mL conical tube. Add 5 mL warm mTeSR1 dropwise to the tube, gently mixing as the medium is added.
3. Centrifuge cells at $200 \times g$ for 5 minutes at room temperature.
4. While centrifuging, remove the matrigel solution from a coated tissue culture 6-well plate. Add 1 mL of warm mTeSR1 containing 10 μ M ROCK inhibitor to one well of 6-well plate.
5. After centrifugation, aspirate the medium from 15 mL tube. Gently resuspend the cell pellet in 1 mL mTeSR1 with 10 μ M ROCK inhibitor, taking care to maintain the cells as small aggregates.
6. Transfer the medium containing the cell aggregates to the matrigel coated 6-well plate.
7. Place the plate into the 37°C incubator and move the plate in quick side to side, forward to back motions to evenly distribute the clumps within the wells. Culture the cells at 37°C, with 5% CO₂ and 95% humidity.
8. Change medium daily. Check for undifferentiated colonies that are ready to passage approximately 7-10 days after thawing.

Passaging hiPSCs Grown under Feeder-free condition

1. Look under microscope to identify regions of differentiation. Mark the differentiated colonies using lens marker on the bottom of the plate.
2. Remove regions of differentiation by scraping with a pipette tip or by aspiration.
3. Aspirate medium from the hiPSC culture and rinse with DPBS (2 mL/well).
4. Add 0.5 mL per well of accutase (Cat. SCR005, Millipore, diluted 1:1 with DPBS before use). Let it stand at room temperature for 1-2 minutes.
5. Remove accutase, and gently rinse each well 2 - 3 times with 2 mL of DMEM/F-12 per well to dilute away remaining enzymes.
6. Add 2 mL/well mTeSR1 and scrape colonies off with a cell scraper.
7. Transfer the detached cell aggregates to a 15 mL conical tube and rinse the well with an additional 2 mL mTeSR1 to collect any remaining aggregates. Add the rinse to the 15 mL tube.
8. Centrifuge the 15 mL tube containing the aggregates at $200 \times g$ for 5 minutes at room temperature.
9. Aspirate the supernatant. Resuspend pellet in mTeSR1 containing 10 μ M ROCK inhibitor by gently pipetting and ensure that cells are maintained as aggregates.
10. Plate the hiPSC aggregates with mTeSR1 onto a new plate coated with matrigel. (Remove matrigel solution before plating). *If the colonies are at an optimal density, the cells can be split every 5 - 7 days using 1:6 to 1:10 splits.*
12. Place the plate into the 37°C incubator and move the plate in quick side to side, forward to back motions to evenly distribute the clumps within the wells. Culture the cells at 37°C, with 5% CO₂ and 95% humidity.
13. Change medium daily.

Cryopreserving hiPSCs

1. Prepare freezing medium which contains 90%FBS, 10%DMSO and 10 μ M ROCK inhibitor.
2. Perform step 1-8 from **Passaging hiPSCs Grown under Feeder-free condition**
3. Gently aspirate the supernatant taking care to keep the cell pellet intact.
4. Gently resuspend the pellet in freezing medium, taking care to leave the clumps larger than would normally be done for passaging.
5. Transfer 1 mL of clumps in freezing medium into each labeled cryovial.
6. Place vials into an isopropanol freezing container and place the container at -80°C to -150°C overnight.
7. Transfer to a liquid nitrogen tank the next day.

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>

For additional information or technical assistance, please call or email us at:

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