Development of a novel medium for culturing naïve iPS cells

Shunsuke Yoshida[1], Brad Hamilton[2], Robert Annand[2], Roseann Vardaro[2], Naoki Nishishita[3] * and Mitsuru Inamura[1] *

[1] ReproCELL Inc., Japan, KDX Shin-Yokohama 381 Bldg. 9F. 3-8-11, Shin-Yokohama, Kohoku-ku, Yokohama, Kanagawa 222-0033, Japan
[2] Stemgent Inc., 51 Moulton Street, Cambridge, MA 02139 USA
[3] National Institute of Biomedical Innovation, Health and Nutrition (NIBIOHN)., 7-6-8, Saito-Asagi, Ibaraki-shi, Osaka 567-0085, Japan

Email: [1] mitsuru.inamura@reprocell.com and [3] nnishishita@nibiohn.go.jp.

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Introduction

Human embryonic stem cells (ES cells) and induced pluripotent stem cells (iPS cells) differ from mouse stem cells in important areas such as pluripotency and growth characteristics. Recent papers have reported the existence of human naïve stem cells that are equivalent to mouse ES/iPS cells. We have developed a new culture medium (ReproNaïve™ base medium and ReproNaïve supplement) for converting conventional human iPS cells (primed stem cells) into naïve human iPS cells without genetic modification. After conventional primed human iPS cells are cultured 1-3 passages in ReproNaïve medium on neomycin-resistant SL10 feeder cells (ReproCELL Inc., RCHEF001), the cell colonies are converted to a dome-shape with packed round cell morphology typical of naïve cells. The average doubling time was significantly reduced from around 26 hours for primed human iPS cells down to approximately 20 hours for these converted cells. Nuclear localization of transcription factor TFE3 was enhanced in these converted cells, indicating these cells are in a naïve state. This medium requires no genetic modification, and provides researchers with higher growth rates than with previous medium. Moreover, this culturing system with neomycin-resistant SL10 feeder-cells can be useful for introducing genetically modified genes and single cell cloning by neomycin selection. This system can support basic research into the underlying mechanisms of naïve state conversion. Conversion and maintenance of naïve stem cells in ReproNaïve Medium will enhance stem cell research as well as have applications for disease modeling, studying mechanisms of stem cell biology and regenerative medicine.

Materials and Methods

Medium
- ReproNaïve base medium and ReproNaïve supplement (ReproCELL, Cat. No. RCHEMD008)
- Leukemia inhibitory factor, LIF (Stemgent, Cat. Nos. 03-0016, 03-0016-100)

CELL line
- 201B7 cells (Kyoto University, NHDF reprogrammed by 4 factors: OCT3/4, SOX2, Klf4, c-Myc)
- 253G1 cells (Kyoto University, NHDF reprogrammed by 3 factors: OCT3/4, SOX2, Klf4)
- SL10 (ReproCELL, Cat. No. RCHEFC001)

Immunocytochemistry
- Anti-SOX17 (R&D Systems, Cat. No. AF1924)
- Anti-SMA (Dako, Cat. No. M0851)
- Anti-Nestin (Millipore, Cat. No. AB5922)
- ES cell Characterization kit (Millipore, Cat. No. SCR001)
- Anti-Nanog (ReproCELL, Cat. No. RCAB0004PF)
- Anti-OCT3/4 (Santa Cruz, Cat. No. sc-5279)
- Anti-TFE3 (Sigma, Cat. No. HPA023881)
- Anti-βIII-tubulin (Covance, Cat. No. MMS-435P, PRB-435P)
- Anti-Tyrosine hydroxylase (TH) (Abcam, Cat. No. ab75875)
- Alexa Flour 488 Anti-mouse IgG (Invitrogen, Cat. No. A11029)
- Alexa Flour 488 Anti-mouse IgM (Invitrogen, Cat. No. A21442)
- Alexa Flour 488 Anti-rabbit IgG (Invitroge, Cat. No. A11034)
- Alexa Flour 488 Anti-Goat IgG (Invitrogen, Cat. No. A11055)

TaqMan® Gene Expression Assays
- Kruppel-like factor 2 (lung, Assay ID: Hs00360439_g1)
- Kruppel-like factor 4 (gut, Assay ID: Hs00358836_m1)

Equipment
- CellVoyager™ CV1000 (Yokogawa Electric Corporation)
- Incubator for hypoxic culture (37℃, 5% CO_2 and 5% O_2)

ReproNaïve and Naïve State

A

	PRIMED STATE	NAÏVE STATE
Morphology	Flat shape	Domed shape
Doubling time	26 hours	14 hours
Pluripotency factors	OCT4, NANOG	OCT4, NANOG, KLF2, KLF4
XX status	XaXi	XaXa
Differentiation bias	Variable	None
Response to LIF/STAT3	None	Self-renewal
Response to FGF/ERK	Self-renewal	Differentiation

Gafni et al., Nature 504:282 (2013): Nichols et al., Cell Stem Cell 4:487 (2009)

FIGURE 1A: (A) Characteristics of naïve and primed state stem cells.

B

FIGURE 1B: Human iPS cells cultured in ReproNaïve medium showed naïve state colony morphology.

Derivation and Colony Growth

A

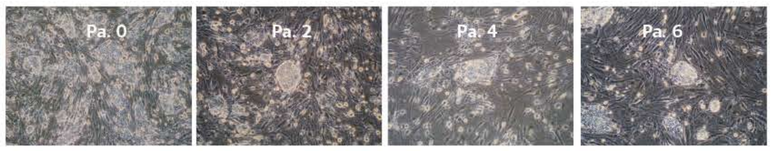


FIGURE 2A: iPS cells were converted to the naïve state by culturing in ReproNaïve medium for six passages. Cell count decreased for about 4 passages before recovering in passages 5 and 6.

B

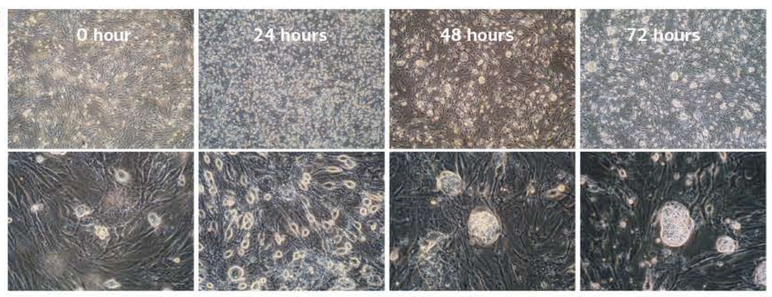
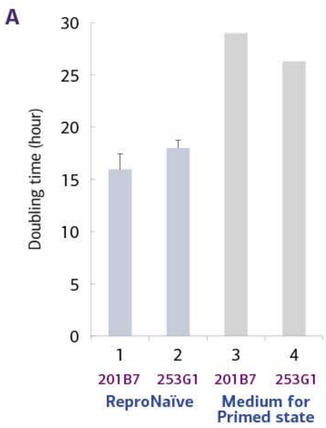


FIGURE 2B: Naïve stem cells were seeded as individual cells and cultured in ReproNaïve medium for 3 days to form dome-shaped colonies

Doubling Time and Growth Curve

A



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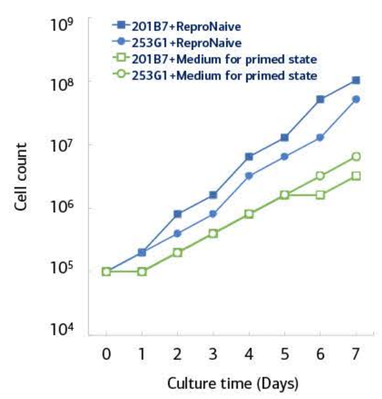

FIGURE 3A: Cell doubling time between passages 5 and 8 was estimated from the average cell counts between day 3 and 4 in culture for the 4 passages. The average doubling time was significantly reduced to less than 20 hours .

FIGURE 3B: Growth curve of iPS cells in culture.

Characterization of Naïve iPS Cells Cultured with ReproNaïve Medium

A

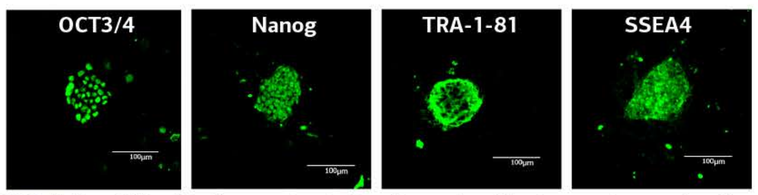

FIGURE 4A: ReproNaïve. iPS cells were cultured in ReproNaïve medium (A) and in primed state medium (B).

B

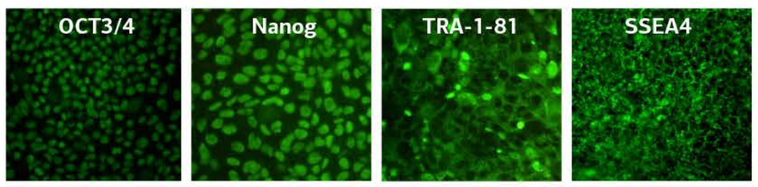

FIGURE 4B: Medium for primed state. Staining for pluripotency markers showed both media retained pluripotent state.

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bioserve Stemgent reinnervate

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Expression and Localization of Naïve State Markers

A

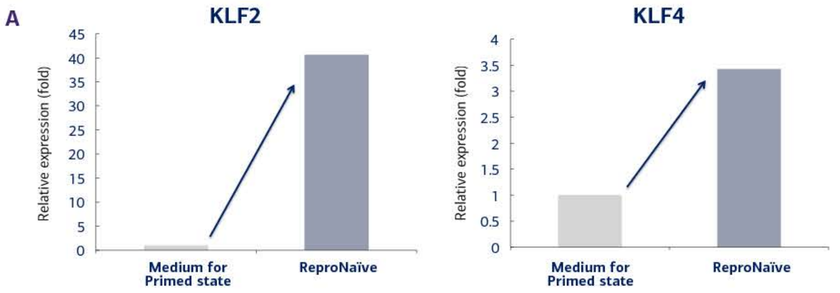

FIGURE 5A: qPCR was used to estimate the relative expression level of KLF2 and KLF4 in cells cultured in ReproNaïve or primed state medium.

B

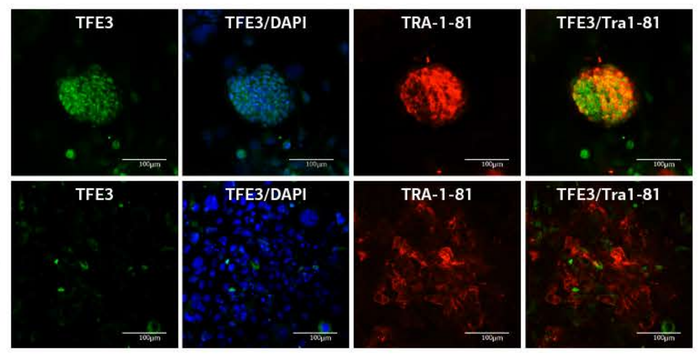

FIGURE 5B. Expression and localization of naïve state markers TFE3 and TRA-1-81 was determined by ICC. Nuclear localization of transcription factor TFE3 was enhanced in cells cultured in ReproNaive, indicating these cells are in a naïve state.

Differentiation Capacity of Naïve State Cells Cultured in ReproNaïve Medium

A

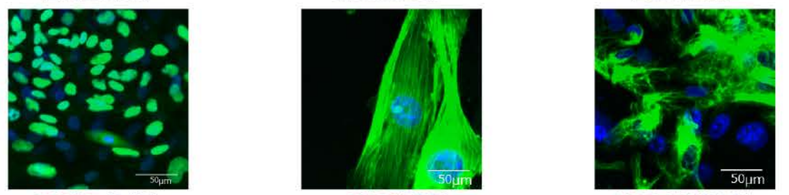

FIGURE 6A: To form embryoid bodies, naïve stem cells were cultured in ReproNaïve medium for one week on a low-attachment round bottom plate, and then transferred to gelatin-coated cell culture plates for one week culture with ReproNaïve + 10% FBS. Cells were stained with markers for all three germ layers and analyzed by ICC.

B

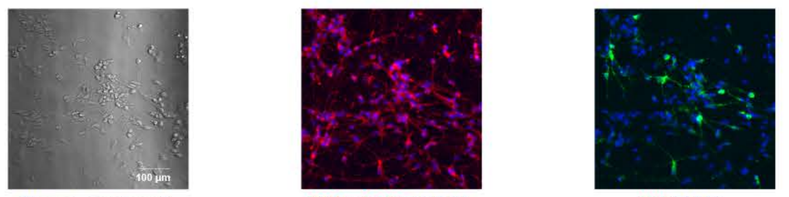

FIGURE 6B: Naïve stem cells cultured in ReproNaïve medium were differentiated into neural cells. Cells were stained for neural markers and analyzed by ICC.

Summary

- **ReproNaïve Medium** induces formation of human naïve iPS cells from established primed human iPS cells.
- Genetic modification is **not necessary** to transform primed human iPS cells to a naïve state.
- This culturing system with **neomycin-resistant SL10 feeder-cells** can be useful for introducing genetically modified genes and single cell cloning by neomycin selection.

Genetic Engineering