iPS cell induction from human T lymphocytes Keisuke Okita

<day -1>

Prepare 6 well plate coated with MEF feeder cells. (3 X 10^5 cell/well)

<day 0>

1. Add 2 mL of T cell stimulation medium to each well 6 well plates seeded with MEF feeder cells and store at 37°C, 5% CO_2.
2. Use Ficoll-paque Plus to purify mononuclear cells:
   a. To 10 mL of anti-coagulated blood (EDTA), add 10 ml of PBS.
   b. In two 15 mL tubes, add 5 mL Ficoll-Paque and the gently add 10 mL of blood+PBS.
   c. Spin tubes at 400 X g for 30 mins. at 18°C. Use slow acceleration and no brake.
   d. Remove the plasma for the top fraction without disrupting the mononuclear cells at the interface.
   e. Transfer the cells at interface to a 15 mL tube and add 12 mL of PBS.
   f. Spin at 200 X g for 10 mins. at 18°C (no brake).
   g. Resuspend cells in 3 mL of X-vivo 10 medium and count cells.
   h. Prepare aliquots of 3 x 10^6 cells in 1.5 mL tube.
   g. Spin at 200 X g for 10 mins. at 18°C (no brake). During spin, prepare nucleofection solution.
3. Nucleofection:

Amaxa Tcell Solution	81.8 µL
Supplement	18.2 µL
Plasmid mix (and GFP control)	3 µL

   a. Aspirate supernatant completely by hand using a pipette.
   b. Add nuclefection solution and suspend cells, be careful not to create any bubbles.
   c. Perform nucleofection using pre-stored program V-024.
4. Plating, 10^6 to 10^4 cells per well:
   a. Immediately following nucleofection, add 800 µL of X-vivo 10 to the electroporation cuvette, and harvest the cells. Metal ions in the nucleofection solution are toxic to cells!
   b. Plate the cells to incubated 6-well plate ranging from 90 µL (3 X 10^5 cells) to 9 µL (3 X 10^4 cells).

<day 2, 4, and 6>

Add additional 1.5 mL of primate ES cell medium per well.

<day 8>

Replace medium with 1.5 mL of primate ES cell medium per well. Most T cells are floating. Confirm that they are proliferating. If not, don't change medium for 2 more days.

* Medium replacement is performed every 2 days.

<day 20 to 30>

Pick colonies of about 2 mm diameter.

Reagents

Ficoll-paque Plus (GE Healthcare, 17-1440-02)

Dynabeads® Human T-Activator CD3/CD28 (Invitrogen, 111.31D)

X-vivo 10 (Lonza, 04-380Q)

Human IL-2, recombinant (PeproTech, 200-02)

T cell stimulation medium in 1 well

Washed Dynabeads	5 µL
X-vivo 10	2 mL
IL-2	30 U/mL

* Preparation : <100 wells

Add Dynabeads (**5** X **number of wells**) µL into 1 mL of X-vivo 10 in 1.5 mL tube.

Vortex 5 secs. then quick spin.

Place to magnetic rack for 1 min.

Remove supernatant and resuspend in (**2** X **number of wells**) mL X-vivo 10 medium.

Add 30 U/mL IL-2.

Primate ES Cell Medium (ReproCELL, RCHEMD001)

* Add bFGF (4ng/mL) before using.

Amaxa Human T Cell Nucleofector Kit (Lonza, VAPA-1002)

Nucleofector 2b (Lonza, AAB-1001)

Plasmid (Addgene, http://www.addgene.org/Shinya_Yamanaka)

Use following plasmid mixtures. Set 1 shows high efficiency. In set 2, we omitted WPRE sequence and replaced shRNA against p53 with dominant negative form of mouse p53, which exist in set 1.

Plasmid set 1	pCXLE-hOCT3/4-shp53-F	0.83 µg
	pCXLE-hSK	0.83 µg
	pCXLE-hUL	0.83 µg
	pCXWB-EBNA1	0.5 µg
Plasmid set 2	pCE-hOCT3/4	0.63 µg
	pCE-hSK	0.63 µg
	pCE-hUL	0.63 µg
	pCE-mp53DD	0.63 µg
	pCXB-EBNA1	0.5 µg

Optional

If you want to make iPS cells without genomic rearrangement, use following medium instead of T cell stimulation medium. But the induction efficiency is very low (1-10 colonies from 1 X 10^6 cells)

α-MEM containing 10% FCS and 10 ng/mL of cytokines (IL-3, IL-6, G-CSF, and GM-CSF)

Acknowledgement

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Reference

1. Okita, K., et al. An Efficient Non-viral Method to Generate Integration-Free Human iPS Cells from Cord Blood and Peripheral Blood Cells. *Stem Cells*. 2012 Nov 29

2. Okita, K., et al. A more efficient method to generate integration-free human iPS cells. *Nat Methods*. 8, 409-12 (2011).