iPS cell induction from human non-T, B cells from peripheral blood

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<day 0>

1. Culture medium preparation:
   a. Add 2 mL of the following culture medium containing cytokines to a well 6 well plate.
      StemSpan H3000
      100 ng/mL IL-6
      300 ng/mL SCF
      300 ng/mL TPO
      300 ng/mL Flt3 ligand
      10 ng/mL IL-3
   b. Add 2 mL of PBS in the remaining wells of the plate and store the plate 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 H3000 medium and count cells.
   h. Prepare aliquots of 3 x 10^6 cells in 1.5 mL tube.
   i. Spin at 200 X g for 10 mins. at 18°C (no brake).
   j. Aspirate supernatant.
3. Plating:
   a. Resuspend the cells in the medium prepared in 1.
   b. Store the plate at 37°C, 5% CO_2 for around 6 days. Medium change is not needed.

<day 5>

1. Prepare 6 well plate coated with MEF feeder cells. (3 X 10^5 cell/well)
2. iPSCs can be established in non-feeder condition, but the efficiency is low.
   a. Add 2 ml of RetroNectin solution (20 μg/ml) to a well 6 well plate .
   b. Store at RT for 2 hr.
   c. Wash the plate once with PBS before cell seeding.

<day 6>

1. Culture medium preparation:
   a. Prepare the following culture medium.

StemSpan H3000
100 ng/mL IL-6
300 ng/mL SCF
300 ng/mL TPO
300 ng/mL Flt3 ligand
10 ng/mL IL-3

2. Harvest the cultured cells:

a. Resuspend the cells in the medium and count cells. Number of live cells is usually around 1 x 10^6.

b. Harvest the floating cells into 15 ml tube.

c. Spin at 200 X g for 10 mins. at 18°C (no brake). During spin, prepare nucleofection solution.

3. Nucleofection:

Amaxa CD34 Solution	81.8 µL
Supplement	18.2 µL
Plasmid mix	3 µL (3 µg)

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 U-008.

4. Plating, 10^6 to 10^4 cells per well:

a. Immediately following nucleofection, add 800 µL of H3000 to the electroporation cuvette, and harvest the cells. Metal ions in the nucleofection solution are toxic to cells!

b. Plate the cells to MEF feeder or RetroNectin coated 6-well plate ranging from 5 X 10^5 cells to 1 X 10^4 cells per well with the medium prepared above.

<day 8, 10, and 12>

Add additional 1.5 mL of TeSR2 medium per well.

<day 14 - >

Replace medium with 1.5 mL of TeSR2 per well.

* Medium replacement is performed every 2 days.

<day 25 to 35>

Pick colonies of about 2 mm diameter.

Reagents

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

StemSpan H3000 (StemCell Technologies, 09800)

IL-6 (PeproTech, AF-200-06B)
SCF (PeproTech, AF-300-07B)
TPO (PeproTech, AF-300-18B)

Flt3 ligand (PeproTech, AF-300-19B)
IL-3 (PeproTech, AF-200-03B)

Amaxa Human CD34+ cell Nucleofector Kit (Lonza, VPA-1003)

Nucleofector 2b (Lonza, AAB-1001)

RetroNectin (Takara, T100A)

TeSR2 (StemCell Technologies, ST-05860)

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

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).

3. Mack, AA., et al. Generation of Induced Pluripotent Stem Cells from CD34+ Cells across Blood Drawn from Multiple Donors with Non-Integrating Episomal Vectors. *PLoS One*. 6, e27956 (2011).