Materials:

- Neon Transfection Devices and kits (Invitrogen; 100 µL sets)
- Human fibroblasts
- HDF medium without pen/strep
- HDF medium
- Episomal plasmids (pCXLE-hOsP, -hSK, -hUL, and –GFP)
- Feeder cells (mitomycin-c treated-SNL cells)
- hESC medium (KSR based)
- Trypsin / EDTA (Gibco)
- Dulbecco’s Phosphate Buffered Saline (DPBS)
- Gelatin-coated 6 well plates and 10 cm dishes

HDF (Human dermal fibroblast) Media:

- High glucose DMEM media
- Sodium pyruvate
- Non-essential amino acids
- 10% Hyclone FBS (Characterized)
- Pen/Strep

Note: pCXLE-hOsP= Oct3/4 & p53 shRNA
   pCXLE- hSK= Sox2 & Klf4
   pCXLE-hUL= L-Myc & lin28
   pCXLE-GFP= GFP only

Protocols:

Day -1 (or before): Culture HDFs:

1) Seed human fibroblasts at 1 x 10^6 (or 5 x 10^6) cells onto a gelatin-coated 10 cm dish.

Day 0: Electroporation of Episomal Plasmids to HDFs:

1) Prepare the Neon Transfection Devices and kits.
2) Prepare the gelatin-coated 6-well plates and warm the culture medium (w/o P/S) up in the plates.
3) Detach the HDFs using trypsin/EDTA and count the cell number.
4) Resuspend the cells in DPBS.
5) Take 3 x 10^5 cells into one 1.5 ml tube. (The cell number can be modified to 1.5 – 6 x 10^5 cells/tube.)
6) Spin down the cells. During the spin, mix plasmids (1 µg each) in 100 µL of Solution R and add 3 mL of solution E2 to each microporation tube.
7) Aspirate the supernatant and add the mixed Solution R to the cell suspension.
8) Set the cell suspension solution to the electroporation device.
9) Electroporate the cell suspension. The conditions are 1,650 Volts, 10 ms width, and 3 pulses.
10) Immediately after electroporation, the cell suspension solution is poured into warmed FP medium in 6-well plates.
11) Culture the electroporated cells in 37 °C, 5% CO₂ incubator.
Day 1, 3, 5: Change the FP Medium:

If the GFP plasmid is used to monitor the transfection efficiency, the GFP expression can be seen during these days.

1) Change medium to the normal FP medium (with P/S).
2) In day 5, feeder cells should be prepared.

Day 6: Replating the Transfected Cells (Replating Day can be changed):

1) The transfected HDFs are detached by trypsin/EDTA.
2) Seed the transfected HDFs onto feeder cells at 1.5 x 10^5 cells / 10 cm dish. (The cell numbers can be changed from 5 x 10^4 to 3 x 10^5 cells / 10 cm dish.)

Day 7: Change the hES Medium:

1) Change the medium to hES medium. (Change the medium every other day.)

Day 20 – 30 : Picking up the iPS-like colonies:

1) Pick up the iPS-like colonies and culture in 24-well plate with feeder cells.

Figure

A typical image of the transfected HDFs electroporated with pCXLE-GFP. (24 hours after electroporation.) ~50% of cells are GFP positive and a little bit of cell death can be seen.