REPROGRAMMING OF FIBROBLASTS INTO iPS CELLS USING SENDAI VIRUS (CYTOTUNE^{TM})

In BSCC we are using the CytoTune^{TM} reprogramming vectors that do not integrate into the host genome. CytoTune^{TM} -iPS Reprogramming System uses vectors based on replication in competent Sendai virus (SeV) to safely deliver and express reprogramming factors (OCT4, SOX2, KLF4, and c-MYC) necessary for reprogramming somatic cells. CyotTune^{TM} is commercially available from Invitrogen LifeTechnologies. Complete manual for iPSC induction and other supporting documents can be found from LifeTechnologies web pages.

Safety:
This product must be used under Biosafety Level 2 (BL-2) containment with biological safety cabinet and laminar flow hood, and with appropriate personal safety equipment to prevent mucosal exposure/splash.

Materials and media needed:

- 15ml conical centrifuge tube
- Centrifuge
- Incubator (+37^{0}C, 5%CO_2)
- Water bath +37^{0}C
- Sterile serological pipettes (5ml, 10ml)
- 6-well tissue culture-treated plates
- Inverted microscope
- Sterile cell culture hood (bio safety level 2)
- Sterile cell culture hood (bio safety level 2) equipped with stereo microscope
- 21-gauge needle
- Feather disposable scalpels
- TrypLE Select cell dissociation reagent
- CytoTune Sendai Reprogramming Vectors
- Human fibroblast cells
- Human foreskin fibroblasts (HFF, ATCC line CRL-2429)
- Mytomycin inactivated MEFs (see SOP reagents and media preparation)
- MEF media (see SOP reagents and media preparation)
- hESC media (see SOP reagents and media preparation)

Procedure:
We have used the following protocol for successful reprogramming of human fibroblasts from many different donors (complete protocol can be obtained from LifeTechnologies web pages). As a positive control we are using human foreskin fibroblasts (HFF, ATCC line CRL-2429).

1. Plate human fibroblast cells into two wells of a 6-well plate at the appropriate density to achieve $5x10^5$ cells per well on the day of transduction (Day 0).
NOTE: You can try to do inductions with less fibroblasts in a smaller plates. That will reduce the amount of Sendai virus needed. You will need to adjust the induction conditions accordingly.
2. Remove one set of CytoTune™ Sendai tubes from the –80°C storage. Thaw each tube one at a time by first immersing the bottom of the tube in a 37°C water bath for 5–10 seconds, and then removing the tube from the water bath and allowing it to thaw at room temperature. Once thawed, briefly centrifuge the tube and place it immediately on ice.
3. Add the indicated volumes of each of the four CytoTune™ Sendai tubes ($3x10^6$ CIU each; see the CoA for the appropriate volume) to 2 mL of fibroblast medium, pre-warmed to 37°C.
4. Aspirate the fibroblast medium from the cells, and add one half of the solution prepared in Step 3 to each of the two wells. Place the cells in a 37°C, 5% CO2 incubator and incubate overnight.
5. 24 hours after transduction, replace the medium with fresh fibroblast medium.
6. Culture the cells for 6 more days, changing the spent medium with fresh fibroblast medium every other day.
7. One to two days before passaging the transduced fibroblasts onto MEF feeder-cells, prepare 35-mm MEF culture dishes.
8. Seven days after transduction, fibroblast cells are ready to be harvested and plated on MEF culture dishes. To remove the cells from the 6-well plate, use 0.5 mL of TrypLE™ Select reagent and incubate at room temperature. When the cells have rounded up (1–3 minutes later), add 2 mL of fibroblast medium into each well, and collect the cells in a 15-mL conical centrifuge tube.
9. Centrifuge the cells at 500rmp for 5 minutes, aspirate the medium, and re-suspend the cells in an appropriate amount of fibroblast medium.
10. Count the cells and seed the MEF culture dishes with $2x10^4$–$1x\ 10^5$ cells per 35-mm dish and incubate at 37°C, 5% CO2 incubator overnight.
11. 24 hours later, change the medium to hESC medium.
12. Observe the plates every other day under a microscope for the emergence of cell clumps indicative of transformed cells.
13. Three to four weeks after transduction, colonies should have grown to an appropriate size for transfer. The day before transferring the colonies, prepare mitomycin inactivated MEF 12- or 24-well plates.
14. Manually pick colonies using scalpel or a needle and transfer them onto prepared MEF plates.
15. Propagate the cells as other human pluripotent stem cells using mechanical splitting for first three passages.
16. Further passages as other human pluripotent stem cells.