Human Fibroblast Reprogramming to make human iPS Cells (iPSC)

1. Plate $1 \times 10^5$ human dermal fibroblasts in fibroblast media (DMEM with 10% FBS) on a gelatin-coated 35mm plastic tissue culture dish. For fresh adult cells, passage 3-4 is best and reprogramming efficiency declines with each passage.

2. The next day, change to 1 mL of fibroblast media adding polybrene to the media (5 ug/mL).

3. Add EF1a-hSTEMCCA-loxP lentivirus (4 factor, excisable vector) at a multiplicity of infection (MOI)=0.1-1 (usually from 0.2 to 5 mcl of concentrated lentiviral supernatant, depending on the viral titer). Infect overnight (around 16 hours).

4. The next day (day 1) change to fresh fibroblast media without polybrene.

5. On day 2, change media to serum-free ‘iPSC media’ (see below).

6. On day 6 trypsinize the well (0.25% trypsin/EDTA) and pass at a 1:16 split by plating the cells onto one 10cm gelatin-coated culture dish (pre-seeded the day before with mitomycin C-inactivated mouse embryonic fibroblast (MEF) feeder cells). Discard the other half of cells (or keep to passage if more plates/colonies are needed). Refeed the cells every 2 days or so and observe occasionally to watch the reprogrammed cells changing morphology as early colonies form. Resist the urge to pick colonies early prior to day 30. If dense overgrowth and too many colonies are observed then repeat the reprogramming from step 1, but passage 1:40 onto MEFs on day 6 (by discarding 80% of the fibroblasts on day 6 and passing the remainder onto a 10cm plate of feeders).

7. On day 30, mechanically pick colonies of good morphology with a P200 pipette. An inverted microscope cleaned and placed in a sterile culture hood is recommended to visualize colonies for picking. (For 3 factor reprogramming, where indicated, GSK3 inhibitor (Bio) (EMD Biosciences, 361550; 10μM) can be added to the culture media on days 7-30 of reprogramming. Colonies take longer to emerge and are far fewer when reprogramming with the 3 factor hSTEMCCA-RedLight-loxP vector).

8. The picked colonies are placed in 1 well of a 24 or 96 well plate, pre-plate with inactivated MEF feeders on gelatin. The picked colony during picking should be gently mechanically broken up by pipetting but kept in chunks. Do not trypsinize.

9. After outgrowth of the picked colony, passage the well to a new 24 well plate and then expand as usual with passaging (see below).

Tissue culture maintenance of undifferentiated iPSC and iPSC media:

‘iPSC Media’ consists of Dulbecco’s Modified Eagle’s medium (DMEM) F12 (Sigma-Aldrich) with 20% KnockOut Serum Replacement (Invitrogen), 1 mM of non-animal L-glutamine (Sigma-Aldrich), 0.1 mM "- mercaptoethanol (Sigma-Aldrich), 1% non-essential amino acid solution (Invitrogen), and 10 ng/mL of FGF2 (Invitrogen). Culture dishes are coated with sterile gelatin (Millipore) before use. The cells were cultured on a feeder layer of mitomycin-C (Fisher) treated mouse embryonic fibroblasts (MEFs), and
were incubated at 37°C at 5% CO₂. iPSC lines are typically passaged approximately every five days at a one-to-three split ratio. Collagenase IV (Invitrogen) is used to loosen the cells from the dish before mechanically scraping to passage. As needed, the cells are occasionally ‘cleaned’ to maintain them in an undifferentiated state by scraping off differentiated cells with a glass pipette or alternatively by mechanical passage of individual colonies of undifferentiated cells.
