Nucleofection Protocol for Human iPSCs

1. Add fresh E8 + Ri to the wells at least 1 hour before trypsinization and coat new 96-well plates with Matrigel.
2. Prepare separate microcentrifuge tubes with 3 ug of each TALEN/pX335 and 6ug of donor DNA. *Note: Increased total DNA volume (>10 uL) may increase cell death so it is better work with concentrated DNA preps
3. Transfer conditioned media into 50 mL conical.
4. Wash cells with PBS w/o Ca2+ and Mg2+ (2 mL/6-well) and add Accutase (0.5 mL/6-well).
5. Incubate at 37°C for 2-3 min until complete detachment of cells.
6. Add 2.5 mL PBS w/o Ca2+ and Mg2+ to each well and pool cells into a 50 mL conical tube.
7. Count an aliquot the cell suspension (10 uL cell suspension + 10 uL Trypan Blue (2X)) using IN Cell hemacytometer or the Countess to determine cell density.
8. Transfer total number of cells needed (2 million cells per transfection) to 15 mL conical.
9. Spin at 500 rpm for 2 minutes.
10. Aspirate supernatant and resuspend cell pellet in 100 ul human Stem Cells Solution I per transfection.
11. Transfer 100 ul cell/Solution I suspension into tube with premixed TALEN/pX335+donor oligo DNA, pipette up and down to mix, and transfer to cuvette without creating air bubbles.
12. Load cuvette into Nucleofector™ 2b Device and execute program A-23.
13. Take cuvette out of holder and add 500 uL of conditioned media.
14. Aspirate Matrigel from coated 96-well plates.
15. For control transfections with unrelated TALENs, transfer 75 ul cell suspension to a new tube. Add 1.25mL conditioned media and plate 100uL/well onto 12 Matrigel-coated wells of a 96-well plate.
16. For mutagenesis conditions, transfer 300 ul of cell suspension to a new tube. Add 5 mL conditioned media and plate 100 ul/well onto 48 Matrigel-coated wells of a 96-well plate.