**Optimization of Neon Electroporation for TSCs**

**Prepare TS cells for Neon Transfection**

1. Remove media from the cells, wash with PBS, trypsinize for 3 minutes.

2. Quench cells with standard DMEM media. Take an aliquot for counting.

3. Centrifuge cells at 500g for 5 minutes.

4. Resuspend in a small amount of PBS-aliquot ~ 3 million cells into microcentrifuge tubes.

5. Centrifuge 500g for 5 minutes at RT.

6. Remove the PBS wash and resuspend in Resuspension Buffer R (other vial in cold PBS) at a final density of 1.0 x 10^7 cells/ml. (At this point, do not store for more than 15-30 minutes)

7. Prepare 24 well plate the day before with MEFs. Change media right before transfection (500ul of culture media without antibiotics, including no PenStrep) and preincubate plates about 30 minutes before transfection.

**Electroporation Protocol- 24 well optimization, using 10ul kit**

1. Ensure that 1ug of DNA is added/10ul reaction (for 3 million cells, resuspend in 270ul of buffer R + 30ul of GFP plasmid (starting concentration of 1ug/ul) --> 100,000 cells/10ul)

2. Set up Neon tube with 3ml Electrolytic Buffer (buffer E for 10 ul) at Neon pipette station.

3. Use preprogrammed 24 well optimization protocol.

4. Push pipette down to second stop to grab the tip, release and then press down again to completely load tip.

5. Push pipette to first stop to load liquid, ensure that there are no air bubbles. Place in machine and push start.

6. After electroporation, transfer the 10ul to the appropriate well. Swirl plate frequently.

7. Change optimization protocol each time. Use each tip 2x, and change buffer E every 10x.

 Note: Watch machine each time to ensure no sparks.