**TS Cell Transfection and selection of stable colonies with Neon**

**Day 0:**

1) Thaw and plate DR4 MEFs (1 vial--> two gelatinized 10 cm plates)

**Day 1**:

1) About 30 minutes before transfection, change media on MEFs to TSM+GF, no antibiotics (including no PenStrep).

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

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

4) Centrifuge cells at 500g for 5 minutes.

5) Resuspend in a small amount of PBS-aliquot ~ 2 million cells each into 4 microcentrifuge tubes.

6) Centrifuge 500g for 5 minutes at RT.

7) Remove the PBS wash and resuspend in Resuspension Buffer R:

 - 95ul buffer R per tube, for each add 10ul of DNA at 1ug/ul

 - 5 extra ul of the buffer to each tube to makes it easier to pipette without bubbles.

8) Set up Neon tube with 3ml Electrolytic Buffer (buffer E2 for 100 ul) at Neon pipette station.

9) Use program 18 for TSCs

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

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

12) After electroporation, transfer the 100ul to the appropriate plate. Swirl.

13) Change tip and buffer each time because different DNA in each sample.

Note: Watch machine each time to ensure no sparks.

**Day 2:** Change media on 10 cm plates- no PenStrep and no neomycin.

**Day 3:** Start neomycin selection (200ug/ml), no PenStrep

**Day 4-10:** Change media everyday.

**Day 11 (9th day of selection):** Pick colonies.

Transfer colonies onto DR4 MEFs- 1 vial DR4 MEFs onto 2 24 well plates (gelatinized)

**Day 12-?:**

Change media everyday, continue with neomycin, no Pen/Strep.

As colonies are ready, transfer to 6 well plates with normal MEFs, normal media with PenStrep, no neomycin.