Generation of stable TETRIS cell lines and luciferase assay.

1. Seed 4x105 E14 ESCs in a 6 well plate
2. Next day transfect:
   1. 125ul Optimem + 7.5ul Lipofectamine 3K
   2. 125ul Optimem + (0.5ug TETRIS cargo, 0.5ug rtTA, 1ug transposase) +7.5ul P3000
   3. Mix (a) and (b)
   4. Incubate 5 mins at RT
   5. Add dropwise to 6 well, gentle swirl
3. Next day change media (start selection?)
4. Next day start selection on Puro [2ug/ml] and G418 [200ug/ml]
5. Select for 6 to 12 days. Will need to split every 2-3 days.
6. Split for luc. assay and freeze a vial.
7. Luc assay (Bright-Glo™ Luciferase Assay System, Promega E2620):
   1. 2 day assay:
      1. 1x105 cells per well of 24 well plate in triplicate or more.
      2. Need cells for “no dox” and “(+) dox”.
      3. 0.5ml total per well. Add 0.25ml of media (no dox and (+ 2x) dox) to each well before adding cells. Then R/S cells @ 4x105 per ml and add 0.25 ml to each well.
      4. 48 hours after plating, do luc. assay.
   2. **3 day assay:**
      1. 5x104 cells per well of 24 well plate in triplicate or more.
      2. Need cells for “no dox” and “(+) dox”.
      3. R/S cells @ 1x105 per ml and add 0.5 ml to each well.
      4. 24 hours later, change media on all cells, adding 1x dox to correct samples.
      5. 72 hours after plating, do luc. assay.
   3. Luc assay:
      1. Make 100 uL of cell lysis solution per well. Dilute passive lysis buffer (5x) with water
      2. Aspirate medium off wells
      3. 1 mL PBS wash, aspirate
      4. Add 100 uL cell lysis solution into each well
      5. Shake plate for 20 min @ RT
      6. Add 10 uL of lysate into each well (96W flat bottom med binding cat # 655075 greiner-bio-one)
      7. Bring bright glo reagent (-80C) and measure activity using Pherastar
      8. Pherastar program:
      9. Manage protocols -> edit -> layout -> empty to erase -> sample (highlight cells) -> plate out -> remove lid & insert 96W plate -> start measurement -> measure one plate -> plateout -> add 25 uL of bright glo to each well -> start measurement -> OK -> measure one plate -> plate in -> exit
   4. Protein concentration via Bradford
      1. Prepare Bradford reagent (5X, dilute with water)
      2. Add 150uL of solution per well in a 96W flat bottom plate
      3. Make BSA standards in eppendorf tubes
      4. Use the 20mg/mL BSA stock (-20°C)
         1. 0.8 mg/mL 4uL 20 BSA + 96uL water
         2. 0.4 mg/mL 50uL of 0.8 BSA + 50uL water
         3. 0.2 mg/mL 50uL of 0.4 BSA + 50uL water
         4. 0.1 mg/mL 50uL of 0.2 BSA + 50uL water
         5. Add 3uL of standard (0.0, 0.1, 0.2, 0.4, 0.8) Do duplicate series
         6. Add 3uL of each sample into each well
         7. Wait 5 minutes, then analyze for protein concentration using SPF program