MCF-7 Cell Culture and 4-hydroxytamoxifen treatment
(Farnham lab protocol)

Cells: MCF7/HAE2F1wt

MCF7 cells (ATCC # HTB-22) stably transfected with a plasmid containing human E2F1 fused at the N-terminus to an HA tag and the estrogen receptor ligand binding domain.

DMEM Media

500 ml DMEM High Glucose 1X Phenol-Red Media, GIBCO/Invitrogen, Cat no: 11960-044
50 ml (to 10%) FBS
5 ml Pen Strep
5 ml L-Glutamine
Filter sterilize

Thawing Cells:

1. Take one vial out from LN2 tank or -150° C freezer and thaw vial immediately in 37° C water bath. Keep O ring above the water surface to prevent contamination. Thaw content with slight shake until only small ice is left in vial. It usually takes 1 min. Spray vial with 70% ethanol all over and wipe its surface with clean tissue in the hood.
2. Open the vial and transfer the content to a 15 ml Falcon tube already containing 5 ml of fresh medium.
3. Spin down at 1000 rpm or 200g for 3-5 mins at 4° C. Aspirate supernatant.
4. Resuspend cells in fresh medium and transfer to 150mm x 25mm tissue culture dish.
5. Check the cells under microscope.
6. Cells are cultured in CO2 incubator and medium is changed about every 3 days.
7. It usually takes 3 days or more for cells to recovery from freezing. After cell culture reaches 80-85% confluence, subculture is conducted. Subculture ratio is about 1:3 or 1:4.

Passaging Cells:

1. Observe cells to see how confluent they are, whether the cells are alive, whether the cells are contaminated, and whether the cells have the correct morphology. After cell culture reaches 80-85% confluence, subculture is conducted.
2. Remove media from dish.
3. Wash 1x with 10 ml of PBS
4. Add 5 ml of Trypsin and trypsinize for 3 min at 37C. Whack hard – you should see the cells coming down. (Important: never over-trypsinize the cells, so work quickly)
5. Add 5 ml of media and use it to rinse the dish to detach the cells off (4-5 times).

   (The serum in the media will neutralize the trypsin)

6. Spin down at 1000rpm for 3-5 min at room temperature. Aspirate supernatant.
7. Add 15 ml of media to 15ml tube containing cell pellet, and pipette up and down to mix.
8. Add 15 ml of media to each new 150mm x 25mm tissue culture dish (we split 1 dish into 3-4 dishes)
9. Add 5mls of cell culture-containing media to each tissue culture dish. Make sure the media covers the entire area of the dish. Put the cells into 37C with 5% CO2.

4-Hydroxytamoxifen treatment

When cells reach 50-65% confluency, add 4-hydroxytamoxifen (Sigma H7904) into each plate to a final concentration of 600 nM, incubate for 30 min, and harvest for ChIP assays.