MDA-MB-231 Cell Culture Protocol

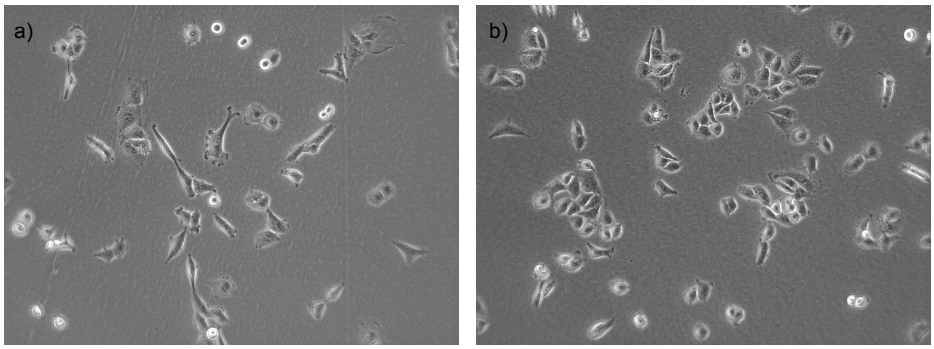
For MDA-MB-231(pMIG-GFP) and MDA-MB-231(pMIG-GATA3-GFP) cells

MDA-MB-231 cells (ATCC # HTB-26) were stably transduced with a pMIG retrovirus containing either GFP only or human GATA3-IRES-GFP. The retroviral construct used to make these cells can be found on Addgene (Plasmid 21629 pMIG-GATA3; http://www.addgene.org/pgvec1?f=c&cmd=findpl&identifier=21629).

Culture Medium
450 ml DMEM High Glucose H-21 Media
50 ml (to 10%) FBS
5 ml Pen Strep

Thawing Cells:
1. Take one vial (of approximately 1 x 10^6 cells) out from LN2 tank and thaw vial immediately in 37ºC 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 10 ml of fresh medium.
3. Spin down at 1200 rpm for 3 min at 4ºC. Aspirate supernatant.
4. Resuspend cells in fresh medium and transfer to 10cm tissue culture dish.
5. Cells are cultured at 37°C in 5% CO2 and medium is changed about every 3 days.
6. It usually takes about 2 days for cells to recovery from freezing. After cell culture reaches 80-85% confluence, subculture is conducted. Subculture ratio is about 1:10.

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 (see below). After cell culture reaches 80-85% confluence, subculture is conducted.



a) MDA-MB-231 pMIG cells b) MDA-MB-231 GATA3-GFP cells

2. Remove media from dish.
3. Wash 1x with 10 ml of Dulbecco's Phosphate Buffered Saline (without Ca++ or Mg++)
4. Add 1 ml of 0.05% Trypsin and trypsinize for 3-5 min at 37°C. Gently tap the side of the dish – you should see the cells coming down. (Important: it is easy to over-trypsinize the cells, so work quickly)

5. Add 8 ml of media (the serum in the media will neutralize the trypsin) and use it to rinse the dish by pipetting up and down 4-5 times to detach the cells off, using the same 8 ml of media.
6. Spin down at 1200rpm for 3 min at room temperature. Aspirate supernatant.
7. Add fresh 8 ml of media to 15ml tube containing cell pellet, and pipette up and down to mix. Depending on the desired concentration of your culture, split an appropriate volume of cells. For example, for a 1:10 split, take 800 uL of cells, and add to a new 10cm tissue culture dish with 8 ml of fresh media.
8. Make sure the media covers the entire area of the dish. Put the cells into 37°C with 5% CO_2.
9. When cells reach 75-80% confluency, harvest for ChIP assays. For a 1:10 split, this will take approximately 3 days.