Transfection of MCF7 Cells

This protocol uses the Lipofectamine 3000 kit (Invitrogen #L3000015).

1. Each transfection requires 200,000 cells. Add cells in a volume of 2-3mL of complete DMEM media to 6-well plate. Include a well for a negative control. Incubate overnight.
2. The following day, exchange the complete DMEM media and let grow overnight.
3. On day three, in a 1.5mL tube combine 1µg of BAC DNA, 250µL pre-warmed Opti-mem media (Gibco #31985088) and 2µL P3000 reagent. Mix and let stand.
4. In a second 1.5mL tubes, combine 7.5µL Lipofectamine 3000 and 125µL Opti-mem and mix with slow pipetting.
5. Add the lipofectamine solution to the DNA mixture tube. Mix, and let stand for 5 minutes.
6. Combine with cells and incubate the plate overnight.
7. **Day after transfection**: Replace media with an equal volume of complete DMEM growth media.
8. **Two days after transfection**: Culture the wells in complete DMEM media supplemented with 500µg/mL Geneticin G418 to begin selection.
9. Replace selection media every two days (usually M,W,F)
10. **When 30-50% confluent**: Trypsinize the cells by adding 0.05% Trypsin-EDTA solution (Gibco #25300-054) using ~30-40% of the original culture volume for 3 to 5 min. Inactivate by adding an equal volume of DMEM. Plate 50µL onto two coverslips for IF. Transfer remaining cells to T-25 flask. Increase the volume to 10mL with complete media.
11. When the T-25 flask is confluent, trypsinize as before and place cells in a T-75 flask with fresh media.
12. Once cells are doubling approximately every day. Collect aliquots of ~$7x10^6$ cells.
13. **Frozen Cells**: Cells can be frozen in 1mL DMEM with 5%DMSO by placing tubes in “Mr. Frosty” Nalgene box with isopropanol and place at -80ºC. Once frozen, the cells can be placed in liquid nitrogen for storage.
14. **Crosslinked Cells**: With the cells suspended in DMEM, add formaldehyde to a final concentration of 1%. Incubate cells for 10 min.
15. Add a 1:20 volume of 2.5M Glycine, and keep on ice.
16. Wash twice with cold PBS, centrifuged as above. Pellets can be snap frozen and stored at -80°C.

MCF7 IF Protocol

1. Place 50µL of cells on a cover slip with 0.5mL of DMEM and incubate overnight.
2. The following day, replace change media and incubate again overnight.
3. Wash the coverslips twice with 500µL of PBS with 4% formaldehyde.
4. Incubate for 20 min at RT.
5. Wash twice with 500µL of PBS with 0.1% BSA.
6. Replace wash buffer with 500µL Blocking Buffer.
7. Incubate for 45 min at RT.
8. Remove blocking buffer and add 500µL Dilution Buffer with a 1:1000 dilution of Ab290 (Abcam). Stock can be stored at 4ºC for several months or 5 times.
9. Incubate overnight at 4ºC.
10. Wash twice with 500µL Wash buffer.
11. Resuspend in 500µL Dilution buffer with 1µL of anti-rabbit Alexa Flur488. Incubate for 1 hour in the dark.
12. Centrifuge and wash twice with 500µL Wash buffer.
13. Add 1 drop of Prolong mounting media with DAPI (Life Tech. P36935) and place coverslip on a slide. Allow to dry at RT in the dark for several hours. Store at 4ºC.

Blocking Buffer:

PBS with 0.3% Triton X-100, 10% Goat serum.

Wash Buffer:

PBS with 0.1% BSA.

Dilution Buffer:

PBS with 1% BSA, 1% goat serum, 0.3% Triton X-100.