CRISPR Transfection of MCF7 Cells Nucleofector Kit V (Lonza VACA-1003)

1. Each transfection requires ~1 million cells. Wash cells once with PBS using a third of the original culture volume. 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.
2. Transfer cells to Falcon tubes and pellet cells at 500 x g for 5 min.
3. Resuspend cells in 82µl of Nucleofector Solution and 18µl of supplement per transfection.
4. Label tubes for each transfection and add 100µl MCF7 cell suspension into each tube.
5. Add 1µg of guide DNA and 10µg of donor DNA to each tube and mix by flicking tube.
6. Add transfection mixture to labeled Nucleofector Electroporation Cuvettes. Electroporate MCF7 cells using the appropriate Nucleofector Program (E-014) for Nucleofector I Device.
7. After electroporation add 0.5mL of complete DMEM media into electroporation cuvette.
8. Add entire volume of transfection cell mixture into the 6 well plate using transfer pipette with 1.5 to 2mL of pre-warmed DMEM.
9. **Day after transfection**: Replace media with an equal volume of complete DMEM growth media.
10. **Two days after transfection**: Culture the wells in complete DMEM media supplemented with 500µg/mL Geneticin G418 to begin selection.
11. Replace selection media every two days (usually M,W,F)
12. **When 30-50% confluent**: Trypsinize as previously described. Plate 50µL onto two coverslips for IF. Transfer remaining cells to T-25 flask. Increase the volume to 10mL with complete media.
13. When the T-25 flask is confluent, trypsinize as before and place cells in a T-75 flask with fresh media.
14. Once cells are doubling approximately every day. Collect aliquots of ~$7x10^6$ cells.
15. **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.
16. **Crosslinked Cells**: With the cells suspended in DMEM, add formaldehyde to a final concentration of 1%. Incubate cells for 10 min.
17. Add a 1:20 volume of 2.5M Glycine, and keep on ice.
18. Wash twice with cold PBS, centrifuged as above. Pellets can be snap frozen and stored at -80°C.

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.