IGSB CRISPR transfection of K562 cells Nucleofector Kit V (Lonza VACA-1003)

1. Using 1 million cells per transfection, Spin down desired amount of cells at 500 x g for 5 min.
2. Resuspend cells in 82µl of Nucleofector Solution and 18µl of Supplement per transfection.
3. Label tubes for each transfection and add 100µl K562 cell suspension into each tube.
4. Add 1µg of guide DNA and 10 µg of donor DNA to each tube and mix by flicking tube.
5. Add transfection mixture into labeled Nucleofector Electroporation Cuvettes.
6. Electroporate K562 cells using the appropriate Nucleofector Program (T-016) for Nucleofector I Device.
7. After electroporation add 0.5mL of complete RPMI media into electroporation cuvette.
8. Add entire volume of transfection cell mixture into the 12 well plate using transfer pipette.
9. **Day after transfection**: Spin down the cells at 500 x g for 5 min. at room temperature. Resuspend the cells in 2mL complete media and place back into the 12 well plate.
10. **Two days after transfection**: Spin down the cells again at 500 x g for 5 min at room temp. and place in 6 well plates with 4mL of complete medium per well. Add Geneticin G418 to a concentration of 50µg/mL to begin light selection.
11. **Five days after transfection**: Replace the media with full selection media (500µg/mL G418) and place back into 6 well plate.
12. Continue to replace selection media every two days (usually M-W-F).
13. **Nineteen days after transfection**: expand the cells to a T-25 flask and bring volume up to 10mL.
14. When negative control has completely died (~2.3 to 3 weeks), expand cells to a T-75 flask.
15. Once cells have reached log growth phase, doubling approximately every day. Collect $7x10^6$ cells and centrifuge 500 x g for 5 min.
16. Resuspend the pellets in 7mL recovery cell culture medium with 5% DMSO and aliquot into 7 cryogenic vials.
17. Place tubes in “Mr. Frosty” Nalgene box with isopropanol and place at -80ºC. Once frozen, the cells can be placed in liquid nitrogen for storage.
18. **Crosslinked Cells**: With the cells suspended in RPMI media, add formaldehyde to a final concentration of 1%. Incubate cells for 10 min.
19. Add a 1:20 volume of 2.5M Glycine, and keep on ice.
20. Wash twice with cold PBS, centrifuged as above. Pellets can be snap frozen and stored at -80°C.

IF protocol

1. Place 200µL of cells in a 1.5mL tube.
2. Centrifuge at 2000 rpm and wash twice with 500µL of PBS with 4% formaldehyde.
3. Incubate for 20 min at RT.
4. Wash twice with 500µL of PBS with 0.1% BSA.
5. Centrifuge and replace wash buffer with 500µL Blocking Buffer.
6. Incubate for 45 min at RT.
7. 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.
8. Incubate overnight at 4ºC with rotation.
9. Centrifuge and wash twice with 500 uL Wash buffer.
10. Resuspend in 500µL Dilution buffer with 1µL of Alexa488. Incubate for 1 hr, rotating in the dark.
11. Centrifuge and wash twice with 500µL Wash buffer.
12. Leaving ~30µL of Wash buffer behind add two drops Prolong mounting media with DAPI (Life Tech. P36935) and transfer to a slide.
13. Place coverslip and 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.