ENCODE4 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 300 x g for 3 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. Divide the cells between two wells of a 6 well plate for duplicate samples.
9. Add approximately 12 drops of transfection cell mixture into the duplicate wells using a transfer pipette.
10. **Day after transfection**: Spin down the cells at 300 x g for 3 min. at room temperature. Resuspend the cells in 3mL complete media and place back into the 6 well plate.
11. **Two days after transfection**: Spin down the cells again at 300 x g for 3 min at room temp. and place in 6 well plates with 3mL of complete medium per well. Add Geneticin G418 to a concentration of 50µg/mL to begin light selection.
12. **Five days after transfection**: Replace the media with full selection media (500µg/mL G418) and place back into 6 well plate.
13. Continue to replace selection media every two days (usually M-W-F).
14. **Nineteen days after transfection**:
    a. Collect 1.5ml of cells and isolate genomic DNA with the Qiagen DNEasy kit. Prepare PCR validation reactions.
    b. Place the remaining cells in a T-25 flask and bring volume up to 5mL with fresh media.
    c. Continue feeding cells.
15. Once cells are 1M cells/ml
    a. Collect 10M cells and isolate protein for Western Blot

b. Place the remaining cells in a T-75 flask and bring volume up to 25mL with fresh media.

16. Ensure negative control has completely died prior to this point.
17. Once cells have reached log growth phase, doubling approximately every day:
    a. Create aliquots of viable cells:
        i. Collect $7x10^6$ cells and centrifuge 500 x g for 5 min.
        ii. Resuspend the pellets in 7mL recovery cell culture medium with 5% DMSO and aliquot into 7 cryogenic vials.
        iii. Place tubes in “Mr. Frosty” Nalgene box with isopropanol and place at -80ºC.
        iv. Once frozen, the cells can be placed in liquid nitrogen for storage.
    b. Continue to feed the remaining cells in the T-75 flask.
18. **Once cells have reached ~2M cells/ml collect 40M cells per replicate.**
    a. **Create crosslinked cells**:
        i. With the cells suspended in RPMI media, add formaldehyde to a final concentration of 1%.
        ii. Incubate cells for 10 min.
        iii. Add a 1:20 volume of 2.5M Glycine, and keep on ice.
        iv. Wash twice with cold PBS, centrifuged as above. Pellets can be snap frozen and stored at -80°C.
    b. Add fresh media to the remaining cells and continue feeding.
19. Once 6 replicates of frozen crosslinked-cells have been collected, discard the flask.