Cell Immunoflourescence
NDJ V.1

1. Plate cells as needed in p60 or p100.
2. Transfect or manipulate per the experimental design
3. Sterile cover slips in 6-well plate by exposing to UV in cell culture hood. Flip coverslips over with tweezers. Keep coverslips on side. Expose for a 30 seconds on each side.
4. Store 6-well plate, with cover slips inside, in the hood.
5. Remove cells from p60 or p100. Plate onto coverslips in 6well plate. $2X10^5$ cells in 2ml volume in each well. Otherwise, a 90% coverslip can be split into 40 wells without counting.

6. Set up tubs with Chen boats to fix and wash slides. Place slides all in Chen boats facing same way and keep track of direction and order. Wash 10% formaldehyde, PBS, H20, -20°C acetone, H20, PBS.

    a. Fix cells 10 minutes 3.7% formaldehyde

    b. Wash 5 minutes in PBS

    c. Dip Chen boat in 10x H20 (use double distilled H20)

    d. Incubate 2 minutes in -20°C acetone.

    e. Wash 5 minutes in PBS. Leave in PBS until right before adding antibodies.

7. **Stain cells 60 minutes with primary antibodies**. Use 40ul per coverslip. Carefully lift the coversliop out of the Chen boat in PBS. Set cell-side up on Kimwipe. Lift vertically to let PBS off. Place coverslip in clean dish and carefully place 40ul of diluted antibody (standard dilution in 1:100). Place dish in 37°C antibody.

8. **Wash the coverslips 3x in PBS**

9. **Stain cells with secondary antibodies in dark. Cover with aluminum foil in 37°C and place in incubator** (standard dilution 1:200).

10. **Wash the coverslips 3x in PBS.**

11. **Place slides at -20°C until ready to visualize.**