eCLIP: Preparation and UV crosslinking of suspension cells

Required materials:

1. UV crosslinker with 254-nm wavelength UV bulbs *(*UVP CL-1000 Ultraviolet Crosslinker or equivalent)
2. Liquid nitrogen (sufficient to submerge tubes in appropriate container)
3. 1x DPBS (*Corning cat# 21-031-CV* or equivalent)
4. Trypan blue stain (*ThermoFisher Scientific, cat# 15250-061* or other equivalent live cell counting assay)
5. Standard cell counting system (hemocytometer or automated cell counter)

Cell viability validation (prior to crosslinking):

a. Use Trypan blue stain (*ThermoFisher Scientific, cat# 15250-061* or other equivalent live cell counting assay to assay cell viability)
b. Cell viability should be > 95% to ensure intact RNA

Preparation of suspension cells:

a. Pool all cells per biosample (if multiple plates)
b. Transfer cells with media to 50mL conical tube(s)
c. Centrifuge at 200 x g for 5 minutes at room temperature
d. Aspirate spent media

Wash cells:

a. Resuspend the pellet(s) in 25mL of 1x DPBS at room temperature
b. Count cell concentration (either with automated cell counter or hemocytometer)
c. Spin down remaining sample in 50mL conical tube(s) at 200 x g for 5 minutes at room temperature
d. Aspirate supernatant
e. Resuspend cells to no more than 20×10^6 cells per mL

UV crosslinking:

f. Aliquot at most 60×10^6 cells (re-suspended in 1x DPBS) in at least 3mL total volume to a standard 10cm tissue culture grade plate.
    - **Note**: Ensure the cells are evenly dispersed and the plate is fully covered (3mL should be sufficient volume).
g. Place the tissue culture plate on leveled ice or a cooling block pre-chilled to 4°C
h. Place the above (plate plus ice or cooling block) into the UV cross-linker.
    - Notes: **Ensure the plate is leveled**
    - **Remove tissue culture plate lid** for cross-linking
i. Cross-link at 254-nm UV with an energy setting of 400 mJoules/cm^2
    - **Note**: this is a setting of **4000** on many cross-linkers which display values in 0.1 mJoules/cm^2
j. After crosslinking is completed, transfer cells to a 50mL conical tube
k. Wash plate once with 7mL of 1x DPBS and add to the same 50mL tube

l. Count cell concentration (either with automated cell counter or hemocytometer)
    - **Note**: ensure cells are re-suspended well before counting
m. Centrifuge the 50mL conical tube at 200 x g for 5 minutes at room temperature.
n. Aspirate and discard supernatant.
o. Resuspend in the desired amount for flash freezing
    - Typically 20×10^6 cells per mL.
p. Transfer desired amount into 1.5mL Eppendorf Safe-Lock Tubes (or equivalent)
    - Typically 1mL of 20×10^6 cells per mL.
q. Spin down at 200 x g for 5 minutes at room temperature.
r. Aspirate the supernatant and freeze by submerging the epi-tubes completely in liquid nitrogen.
s. After frozen (at least 30 seconds), remove from the liquid nitrogen and store at -80°C.