eCLIP: Preparation and UV crosslinking of adherent 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

Wash cells:

a. Aspirate spent media
b. Wash the plate gently with 1x DPBS at room temperature (15mL for a 15cm plate)
c. Aspirate media
d. Add enough 1x DPBS to just cover the plate (5mL for a 15cm plate)
   *** Note:** If all plates are at equal cell density, one plate can be sacrificed for counting – this plate would be dissociated (with trypsin, accutase, or equivalent) and cell number (per plate) counted at this stage. **This is recommended for cell types that require chemical dissociation** enzymes to dissociate and be properly counted, as this is not recommended post-crosslinking

UV crosslinking:

e. Place the tissue culture plate on leveled ice or a cooling block pre-chilled to 4°C
f. 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
g. 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
h. While keeping the cells on ice, use a cell scraper (*Corning cat# CLS3010-10EA*) to scrape the plate
i. Transfer the cells to a 50mL conical tube
j. Wash plate once with 10mL of 1x DPBS and add to the same 50mL tube
k. Gently resuspend until the sample is homogeneous

l. Count cell concentration (either with automated cell counter or hemocytometer)
    - **Note**: ensure cells are re-suspended well before counting
    - **Note**: for cells that do not easily dissociate into single cells, a separate plate of cells can be counted instead (see step d *note above)
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