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 or dry ice (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 chilled 1x DPBS (15mL for a 15cm plate)
c. Aspirate media
d. Add enough chilled 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 **before** 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 15mL conical tube
j. Wash plate once with 5mL of chilled 1x DPBS and add to the same 15mL tube
k. Centrifuge the 15mL conical tube at 250 x g for 5 minutes at 4°C
l. Aspirate and discard supernatant
m. Resuspend in the desired amount of chilled 1x DPBS for flash freezing
    - Typically, 10×10^6 cells per mL
n. Transfer desired amount into 1.5mL Eppendorf Safe-Lock Tubes (or equivalent)
    - Typically, 1mL of 10×10^6 cells per mL
o. Spin down at 250 x g for 5 minutes at 4°C
p. Aspirate the supernatant and freeze by submerging the lobind-tubes completely in liquid nitrogen or on dry ice
q. After frozen (at least 30 seconds), remove from the liquid nitrogen (or dry ice) and store at -80°C