

## **ECLIP: PREPARATION AND UV CROSSLINKING OF TISSUE**

### **Required materials:**

1. UV crosslinker with 254-nm wavelength UV-C bulbs (*UVP CL-1000 Ultraviolet Crosslinker or equivalent*)
2. Liquid nitrogen (LN<sub>2</sub>) (sufficient to submerge materials in appropriate container)
3. Small container that's dry ice compatible (that can fit into crosslinker)
4. Tissue culture plates (5cm recommended)
5. CryoGrinder (*Ops Diagnostic cat. #CG 08-01, set includes mortar and pestles*)
6. Mortar and pestles
7. Razor blades (*Genesee Scientific cat. #38-100*)
8. Tweezers
9. Tongs
10. Analytical Balance
11. Tubes (1.5mL *Eppendorf cat. #022431021* or 5mL *Eppendorf cat. #0030119401* depending on tissue size)
12. Liquid Nitrogen suitable gloves

### **Prepare materials:**

- a. Weigh and record mass of each empty Eppendorf sample tube using analytical balance
- b. Place tubes on dry ice to chill
- c. Chill mortar, pestles and tweezers in LN<sub>2</sub> for 5 minutes
- d. Place several razor blades in an empty tissue culture plate, then place on dry ice to chill
- e. Label tissue plates with sample names, then place on dry ice to chill

### **Prepare tissue:**

- **Preparation note:** It is imperative that tissue remains frozen during the entirety of the crosslinking procedure.
- a. Obtain frozen tissue from -80°C and place on dry ice
  - b. Carefully remove mortar from LN<sub>2</sub> using tongs
  - c. Transfer frozen tissue into mortar
  - d. Grind tissue until consistency is a very fine powder. Tissue that is not grinded to the appropriate consistency will not lyse properly in solution (can re-submerge pestle to cool during grinding step; ensure hands are protected from extreme temperatures while gripping mortar)
  - e. Carefully transfer tissue powder to pre-chilled tissue culture plate. Spread evenly on plate

### **UV crosslinking:**



- f. Place the tissue culture plate on leveled dry ice
- g. Place the above (plate plus ice) into the UV cross-linker
  - Notes: **Ensure the plate is leveled**
  - **Remove tissue culture plate lid** for crosslinking
- h. Cross-link at 254-nm UV with an energy setting of 400 mJoules/cm<sup>2</sup>
  - **Note:** this is a setting of **4000** on many cross-linkers which display values in 0.1 mJoules/cm<sup>2</sup>
- i. Remove plate while still on dry ice and mix tissue powder using pre-chilled tweezers
- j. Repeat step h for a total of 2 crosslinking rounds
- k. Remove plate from crosslinker
- l. While keeping the tissue on dry ice, use a pre-chilled razor blade to scrape and scoop the tissue from the plate
- m. Transfer the tissue powder to a pre-chilled Eppendorf tube
- n. Store tube on dry ice
- o. Repeat step a-n for each sample
- p. After all samples have been successfully prepared and crosslinked, carefully weigh each tube containing tissue powder without allowing the tissue to melt
- q. Record new mass
- r. Determine weight of tissue (New weight – empty tube weight), record weight on tube
- s. Freeze samples at -80°C or continue to cell lysis