MICORES
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UMICH Bru-seq Lab

Preparing samples for BruUV-seq (150mm plate - adherent cells):

Reagents:

- 5-Bromouridine (Aldrich 850187)
- TRIzol (Invitrogen 15596026)
- PBS

Equipment:

- UV-C light source (e.g., portable germicidal UV lamp)
- Radiometer to measure UV-C output (e.g., UXV radiometer from UVP, Inc. Upland CA)

Before starting:

- Make a fresh stock solution of 50mM 5-Bromouridine (BrU) in PBS (protect from light). Store at 4°C for up to 6 weeks.
- **Use <u>conditioned media</u> for everything, including treatments.**

Notes:

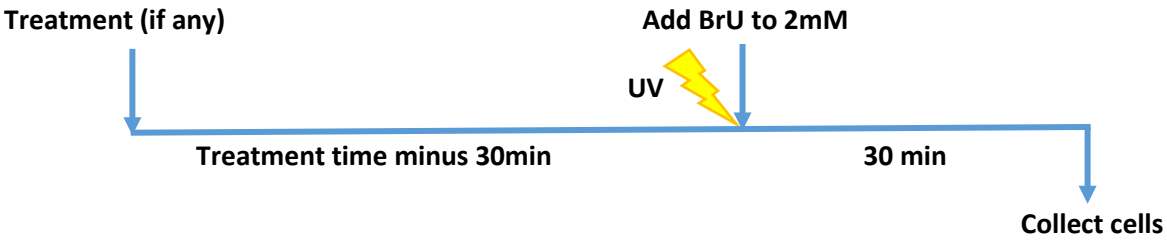
This protocol is optimized for one ~80-90% confluent 150mm plate per sample, which works well for most cell lines. This could be scaled down or up, depending on the cell type. The protocol can also be modified to be used with suspension cells.

Minimum cell number required per sample is 5 million, but more should be used if possible. We have had success starting with less cells, but prefer to use less only when absolutely necessary. Bru-RNA is only ~1% of total RNA so a large amount of starting material is needed to get sufficient RNA for library preparation.

Preparing UV source:

Safety precaution: Wearing appropriate PPE is recommended to prevent UV exposure to skin, face, and eyes. Exposing only a small region of a large lamp (by covering the rest) can also mitigate the UV light-related hazards.

1. Warm up UV lamp for at least 10 min.
2. Measure UV output with UVX radiometer.
    1. Position lamp relative to irradiation surface where radiometer reads approximately 150 in the 200µW/cm^2 sensitivity range.
    2. This radiometer reading should be taken at the position where cells will be placed.
3. Calculate time to desired dose.
   At 150, the output is 1.5 J/m^2/sec and thus a 66 sec exposure gives a dose of 100 J/m^2.



Labeling cells with Bromouridine (BrU):

1. Remove 9.6ml of media from each plate of cells to a sterile tube and add 400µl BrU to a final concentration of 2mM. Set aside.
2. Aspirate any remaining media from plate, and wash plate once with PBS, remove PBS.
3. Remove lid to plate and irradiate with 100 J/m^2.
4. Immediately following irradiation, add back saved BrU-containing media to plate and incubate at 37˚C for 30 minutes.
5. To collect cells, aspirate media then immediately add 3ml Trizol to the plate to lyse cells. Scrape plate and transfer lysate to an appropriate tube. [We use 14ml round bottom Falcon tubes, but any polypropylene tube is fine].
6. Store the samples at -80C.