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

Reagents:

- 5-Bromouridine (Aldrich 850187)
- DRB (5,6-Dichlorobenzimidazole 1-β-D-ribofuranoside) (Sigma-Aldrich D1916)
- TRIzol (Invitrogen 15596026)
- PBS

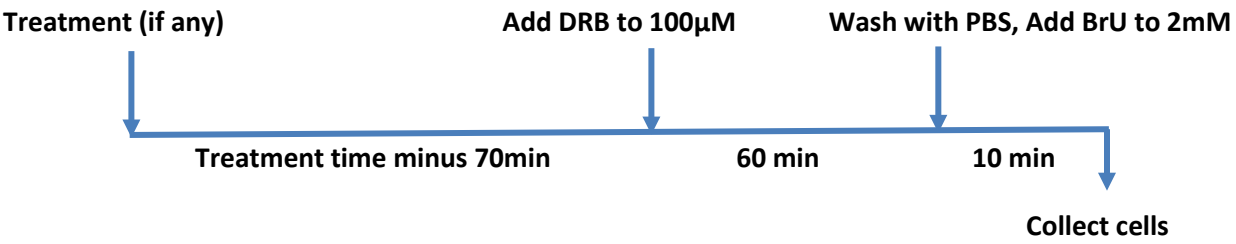
Before starting:

- Make a fresh stock solution of 50mM 5-Bromouridine (BrU) in PBS (protect from light). Store at 4°C for up to 6 weeks.
- Make a fresh stock solution of 50mM DRB.
- **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.



DRB treatment/Bromouridine(BrU)-labeling:

1. Remove 9.6ml of media from each plate of cells to a sterile tube and keep warm.
2. Add DRB to remaining media in plate for final concentration of 100µM. Incubate at 37˚C for 60 minutes.
3. Add 400µl of BrU stock (final concentration is 2mM) to the saved 9.6ml media.
4. Aspirate DRB media, wash cells with PBS, remove PBS and add reserved Bru-containing media.
5. Incubate at 37°C for 10 minutes
6. 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].
7. Store the samples at -80°C.