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

Reagents:

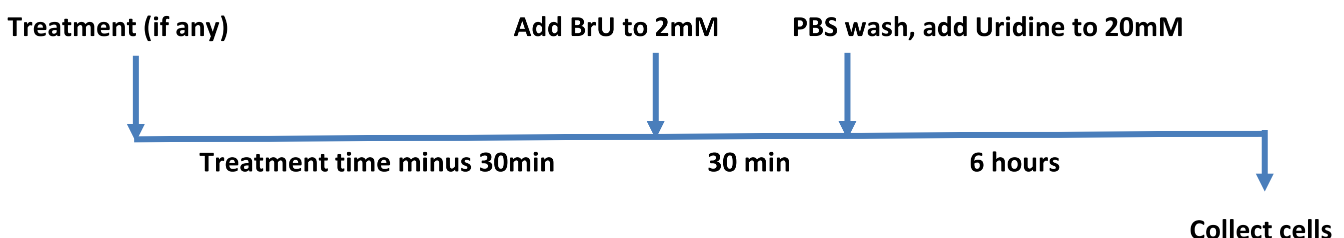
- 5-Bromouridine (Aldrich 850187)
- Uridine (Sigma U3750)
- TRIzol (Invitrogen 15596026)
- PBS

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 1M Uridine in PBS for chase (for doing stability analysis).
- **Use conditioned media 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.



Labeling cells with Bromouridine (BrU):

1. Remove 9.6ml of media from each plate of cells to a sterile tube and add 400µl BrU for a final concentration of 2mM. **Save remaining media from plate to use for the uridine chase (keep warm).**
2. Add back BrU-containing media to plate and incubate at 37˚C for 30 minutes.
3. For the chase, after the 30min incubation, rinse plate once with PBS, then add back saved media containing 20mM uridine and incubate for 6 hours.
4. 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].
5. Store the samples at -80C.