NBCC Proteomics Protocol 3
Version – January 4, 2019
Adapted from Protocols in the Gingras lab

Induction of Flp-IN T-REx Pools and Cell Collection

Cell culturing:

1. Culture stable Flp-IN T-REx cells (see Note 1) in 15 cm dishes (1 dish per sample, see Note 2) so they are 70-75% confluent on day of induction.

Transgene induction and biotin labeling:

2. Replace media with media containing 1 µg/ml doxycycline and 50 µM biotin (see Notes 3 and 4). Incubate for 24 hours.

Cell harvest:

3. Wash each plate with 2 x 10 ml ice-cold PBS. Keep plates cold and harvest a manageable number of plates to minimize harvesting time.
4. Using a cell scraper, scrape the cells in 1 ml of PBS and transfer to a pre-weighed 2 ml microfuge tube (See Note 5).
5. Using an additional 500 µl of PBS, collect the residual cells and pool and add to the microfuge tube.
6. Centrifuge the cells at 500x g for 5 minutes to collect the cells. Carefully remove all supernatant by aspiration. Weigh cell pellets and record on tube (see Note 6).
7. Flash freeze the tubes on dry ice and place at -80^{o}C.

For streptavidin enrichment and preparation of samples for mass spectrometry, proceed to NBCC Protocol PR5.

Key Reagents

This protocol has been optimized using these suggested reagents.

Reagent	Company	Catalog Number
Bir*-FLAG and BioID2-FLAG parent vectors	Gingras Lab	Available upon request
Biotin	BioBasic	58-85-5
Doxycycline	Sigma-Aldrich	D9891
Flp-IN T-REx HEK293 cells	ThermoFisher	R78007
Tetracycline	Bioshop	TET701.25

Notes

[1]This protocol is designed to work with BirA*-FLAG and BioID2-FLAG-tagged constructs that have been stably transfected into a Flp-IN T-REx cell line. This protocol has been optimized for HEK293 stable cell pools but other Flp-IN T-REx cell lines may be used. More notes on the BioID method are discussed in Hesketh *et al.*, Methods Mol Biol. 2017;1550:115-136.

[2]Experiments should be performed with a minimum of 2 biological replicates. The following controls are recommended: 1) untransduced control that will identify endogenously modified proteins and background binding to streptavidin beads; 2) BirA* expressed alone or fused to a fluorescent protein (e.g. EGFP) to identify non-specific biotinylation; and 3) if the protein is nuclear, a BirA-EGFP-NLS to reduce compartment specific background.

[3]Prepare 10 mg/mL doxycycline stock and add 2 µL per 20 mL media. Precipitation of stock may occur with long-term storage but should not affect your experiments. Prepare aliquots to avoid freeze-thawing and warm the solution to room temperature prior to use. Tetracycline can be substituted for doxycycline at the same concentration.

[4]The recommended range for biotin labelling is 4 to 24 hours. The addition of biotin should be timed to end at the 24 hour transgene induction point. Biotin stock solution (for 20 mL of 20 mM which is a 400× stock): add 2 mL of 30% NH_4OH to 100 mg of biotin and place on ice. Slowly add 5 mL of 1 N HCl, wait 5 min, and repeat for a total of 18 mL added. Store at 4°C protected from light. Extreme care must be taken in preparing biotin solutions, as it can easily precipitate from the solution if the pH is raised too rapidly or if it is not kept adequately chilled during preparation (this may be reversed by the re-addition of a small amount of ammonium hydroxide). In our experience, the biotin stock solution is very stable and can be used for approximately 6 months to a year without any change in potency when prepared and stored appropriately.

[5]Autoclaving tips and tubes may result in a residue being deposited on to plasticware, which may result in contamination during MS analysis. As sterility is not essential for the protocols (other than during cell culture), use tips and tubes directly as received from the manufacturer. Keep all plasticware and reagents protected from dust and other environmental contaminants as much as possible and use gloves for all preparation steps.

[6]Pellet weight should be a minimum of 0.1 to 0.15 g (after wash is fully aspirated) to ensure sufficient starting material. In HEK293 cells, this translates to 3 to 5 mg total protein in the lysate. As tube weights vary, it is recommended to weigh the tube prior to adding cells to get a more accurate weight.