Flp-In BioID Streptavidin sepharose chromatin optizided
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Affinity capture of biotinylated proteins:

1. Grow 2x15cm plates of Flp-in BirA*-X stably transfected cells to 75% confluence.
2. Treat cells with 1ug/uL of tetracycline (2ul of 10mg/mL stock per 15cm plate) + 50uM biotin (50uL of 400X [20uM] stock per 15cm plate).
3. Wash each plate 1X in 10mL of cold PBS.
4. Add 1ml of cold PBS per plate and scrape the cells into 2ml eppendorf. Two plates for two replicates.
5. Pellet cells at 500g for 5min at 4°C. Remove supernatant and freeze pellets at -80 or on dry ice.
6. Thaw pellet in 1.5mL of RIPA lysis Buffer.
7. Sonicate sample for 30 secs (10 secs ON, 2 secs OFF) at amplitude 0.35 using the Gingras lab sonicator.
8. Add 1uL (250units) of benzonase to each sample.
9. Centrifuge for 20 minutes at 12,000g at 4°C.
10. Collect supernatant in new eppendorf. *You may save and freeze pellet as insoluble fraction.*
11. Wash streptavidin-sepharose beads 2 times in 500ul of lysis buffer (60 ul of slurry per sample). Spin on a table-top centrifuged for a few seconds to pellets beads at each wash step.
12. Add lysate to the now washed beads.
13. Incubate at 4°C with rotation for 3hours.
14. Spin on a table-top centrifuged for a few seconds. *You may save supernatant as unbound.*
15. Transfer beads in 1mL of Lysis Buffer (no protease inhibitors) to a new 1.5mL eppendorf.
16. Spin on a table-top centrifuged for a few seconds and discard supernatant.
17. Wash once more with 1mL RIPA buffer.
18. Wash 3x with 1mL of 50mM ABC (add NH_4OH to ABC to get pH to 8.0).
19. Resuspend beads in 100uL of 50mM ABC and add 1ug of trypsin (resuspend trypsin in Tris-HCl, pH 8,0 [10uL from a 200ul stock containing 20ug tube]).
20. Incubate overnight at 37°C with rotation.
21. In the morning add another 1ug of trypsin and incubate for a further 2-4hours.
22. Pellet the beads 2min at 1000g. Transfer supernatant (peptides) to new tube.
23. Rinse the beads 2x in 100uL of mass spec water and pool with collected supernatant.
24. Add formic acid to the solution to a final concentration of 2% to end digestion (30 uL of 50% stock).

25. Spin sample 10min at 10000g and transfer supernatant to new tube (be sure not to collect any beads [leave 5-15uL in bottom of tube to prevent aspirating any beads].
26. Speedvac sample to dryness.
27. Resuspend in 13uL of 5% formic acid for MS. Centrifuge at 12000g for 5 min. Transfer 6uL to new tube to shoot 5uL (Save leftover sample for another technical replicate.Speedvac down other 5ul of sample and save).

Preparation of RIPA lysis buffer:

For 500mL (stock solution):

- 5mL of NP-40
- 5mL of 10% SDS stock
- 25mL of 1M Tris-HCl pH7.4
- 15mL of 5M NaCl
- 2.5g of Sodium Deoxycholate
- 1mL of 0.5M EDTA
- Water to 500mL

Add fresh to 50mL aliquot for daily use:

- 500µL of 100mM PMSF
- 50µL of 1M DTT
- 100µL of SIGMA protease inhibitor

Preparation of FLAG lysis buffer:

For 500mL (stock solution):

- 25mL of 1M Hepes-NaOH pH 8.0
- 20mL of 2.5M KCl
- 2mL of 0.5M EDTA
- 5mL of 10% NP-40
- 50mL of glycerol

ddH$_2$O to 500mL

Biotin Stock solution (20mM, 400x)

100mg Biotin (BioBasic BB0078)
2.04mL of 30% NH4OH (Sigma 221228)
18mL of 1N HCl.
Store at 4°C.

50mM ammonium bicarbonate (ABC), pH 8.5 (50mL)

200mg in 50mL Mass spec H$_2$O