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In vivo BioID protein purification

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We use this protocol and it's working

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Disclaimer

Abstract

In vivo BioID protein purification

1 **Animal Preparation** - Breed genotype-matched animals (wild-type C57BL6 or LRRK2 G2019Ski/ki) to produce single-genotype litters. - For each genotype (WT or G2019S), prepare 6 pups for injection.

2 **AAV Injection** - Inject 1 µL of AAVs carrying Astro-Ezrin-BioID (PHP.eB.GfaABC1D-Ezrin WT-BioID2-HA) or Astro-CYTO-BioID (PHP.eB.GfaABC1D-BioID2-HA) bilaterally into the cortex of P0-P2 mouse pups using a Hamilton syringe. - Monitor pups until they recover on a heating pad.

3 **Biotin Injection** - At P18, P19, and P20, subcutaneously inject biotin at 24 mg/kg to increase biotinylation efficiency.

4 **Tissue Collection** - At P21, remove the cerebral cortices and store at -80°C. - Pool 2 genotype-matched cortices at the time of protein isolation, yielding 3 independent replicates per BioID construct.

5 **Protein Purification** - Lyse each cortex in a buffer containing: - 50 mM Tris/HCl, pH 7.5 - 150 mM NaCl - 1 mM EDTA - Protease inhibitor mixture (Roche) - Phosphatase inhibitor mixture (PhosSTOP, Roche) - Add an equal volume of buffer containing: - 50 mM Tris/HCl, pH 7.5 - 150 mM NaCl - 1 mM EDTA - 0.4% SDS - 2% TritonX-100 - 2% deoxycholate - Protease inhibitor mixture - Phosphatase inhibitor mixture

6 **Sonication and Centrifugation** - Sonicate samples. - Centrifuge at 15,000 g for 10 minutes. - Ultracentrifuge the supernatant at 100,000 g for 30 minutes at 4°C.

7 **Sample Preparation for Protein Binding** - Add SDS detergent to the samples and heat at 45°C for 45 minutes. - Cool on ice.

8 **Protein Binding** - Incubate each sample with High-Capacity Streptavidin Agarose beads (ThermoFisher) at 4°C overnight.

9 **Bead Washing** - Wash beads serially:

10 Twice with a solution containing 2% SDS.

11 Twice with a buffer containing 1% TritonX-100, 1% deoxycholate, and 25 mM LiCl.

12 Twice with 1 M NaCl.

13 Five times with 50 mM ammonium bicarbonate.

14 **Protein Elution** - Elute biotinylated proteins attached to the agarose beads in a buffer containing: - 125 mM Tris/HCl, pH 6.8 - 4% SDS - 0.2% β-mercaptoethanol - 20% glycerol - 3 mM biotin - Heat at 60°C for 15 minutes.

15 **Downstream Analysis** - Subject the 12 total samples (3 per genotype per construct) to LC-MS/MS and downstream analysis.