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Postnatal astrocyte labeling by electroporation (PALE)

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We use this protocol and it's working

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Abstract

Postnatal astrocyte labeling by electroporation (PALE)

1 **Animal Preparation**

1.1 - Sedate late P0/early P1 mice by hypothermia until anesthetized.

2 **Plasmid Preparation**

2.1 - Prepare plasmid DNA mixed with Fast Green Dye for visualization.

3 **Injection Procedure**

3.1 - Inject 1 µl of plasmid DNA mixture into the lateral ventricle of one hemisphere using a pulled glass pipette (Drummond).

4 **shRNA Knockdown Experiments in Wild-Type CD1 Mice**

4.1 - Prepare 1 µl of DNA containing 1 µg of pGLAST-PBase and 1 µg of pPB-shRNA-mCherryCAAX for injection.

5 **Astrocyte Labeling in WT and LRRK2 G2019Ski/ki Mice**

5.1 - Prepare 1 µl of DNA containing 1 µg of pGLAST-PBase and 1 µg of pPB-mCherry-CAAX for injection per mouse.

6 **PALE-Mediated Overexpression of Phospho-mimetic Ezrin in shRNA Knockdown Experiments**

6.1 - Prepare 1 µl of DNA mixture containing 0.5 µg pGLAST-PBase, 0.5 µg pPB-shRNA-mCherryCAAX, and 1 µg pZac2.1-GfaABC1D-Ezrin T567D-BioID2-HA.

7 **Phospho-Dead Ezrin Overexpression in WT and LRRK2 G2019Ski/ki Mice**

7.1 - Prepare 1 µl of DNA mixture containing 0.5 µg pGLAST-PBase, 0.5 µg pZac2.1-gfaABC1D-mCherry-CAAX, and 1 µg pZac2.1-GfaABC1D-Ezrin T567A-BioID2-HA.

8 **Electrode Placement and Electroporation**

8.1 - Orient electrodes with the positive terminal above the frontal cortex and the negative terminal below the chin of the pups.

8.2 - Apply 5 discrete 50 ms pulses of 100 V spaced 950 ms apart.

9 **Recovery**

9.1 - Recover pups on a heating pad and return them to their home cage.

9.2 - Monitor pups until collection at P21.

10 **Animal Monitoring and Sample Collection**

10.1 - Monitor all animals for health status until collection at P21.

11 **Brain Section Examination**

11.1 - Examine brain sections for the presence of electroporated cells before subsequent staining procedures.

12 Notes:

12.1 - Ensure all procedures are performed in compliance with institutional guidelines for animal care and use.

12.2 - Maintain sterile conditions during plasmid preparation and injection procedures.

12.3 - Optimize electroporation parameters for consistent and reproducible results.