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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 μ l 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 μ l of DNA containing 1 μ g of pGLAST-PBase and 1 μ g of pPB-shRNA-mCherryCAAX for injection.
- 5 ****Astrocyte Labeling in WT and LRRK2 G2019Ski/ki Mice****
 - 5.1 - Prepare 1 μ l of DNA containing 1 μ g of pGLAST-PBase and 1 μ g of pPB-mCherry-CAAX for injection per mouse.
- 6 ****PALE-Mediated Overexpression of Phospho-mimetic Ezrin in shRNA Knockdown Experiments****
 - 6.1 - Prepare 1 μ l of DNA mixture containing 0.5 μ g pGLAST-PBase, 0.5 μ g pPB-shRNA-mCherryCAAX, and 1 μ g 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.