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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

Eroglu Lab Postnatal Astrocyte Labeling by Electroporation (PALE) general protocol

Materials

Supplies needed:

- Electrodes
- Needles (pulled glass, as in electrophysiological experiments)
- DNA constructs (add small amount of Fast Green Dye for visualization)
- Mouth pipet
- Small ruler
- Marker
- P20 pipet and some tips
- Parafilm
- Transfer pipet
- Extra glove
- PBS in 15 ml tube
- Ice bucket



Set up

- 1 In the procedure room, wipe down your bench space with trifectant.
- 2 Place snuggle safe heating pad in microwave for 5:30 – 6:00 minutes.
- 3 Go get animals and bring them to procedure room.
- 4 Also get small spring scissors (for cutting toes) and small regular scissors (for cutting needle).
- 5 Take 2-3 pups from the cage and begin anesthetizing on ice.
- 6 Cut extra glove, place on top of ice and make a groove into the ice.
- 7 Lay mice on glove in the groove and fold glove over mice.
- 8 Pile ice on top of glove.
- 9 In ~5 minutes mice will be ready. Look for purple color and no movement.
- 10 While waiting for pups to be ready, continue with set-up.
- 11 Set up electroporator and check settings.
Mode: LV
Voltage: 100V
Pulse length: 50 ms
pulses: 5
Interval: 950 ms
Polarity: Unipolar



- 12 Measure and mark needles using ruler. Every 3 mm is 1 μ l.
- 13 Use scissors and make a diagonal cut to remove the bendy top of the needle.
- 14 Pipet ~8 μ l of DNA onto parafilm.
- 15 Using mouth pipet, pull up ~3-4 μ l into needle.

Electroporation

- 16 Once mouse is ready, place on bench top.
- 17 Using mouth pipet, inject 1 μ l of DNA into lateral ventricle.
- 18 Use transfer pipet to wet the electrode with PBS.
- 19 Positive electrode has blue peg on it. Orient electrode so that the positive probe is on top of where DNA was injected (we are aiming for the visual cortex). The negative probe will be 180 degrees opposite, near the side of the jaw on the opposite side.
- 20 Cut toe to identify mouse if needed.
- 21 Record mouse ID and conditions.
- 22 Place mouse on snuggle safe to recover.
- 23 Mouse can go back to mom once it has regained pink color and is breathing and moving around.



- 24 Note: For CD1 mice, you can start anesthetizing the next 2-3 pups when you remove the first pups for electroporation. For other strains of mice, wait until your first 2 pups are ready to go back to mom before starting with more pups.