Biotechnology
Experimental Research
Application Notes

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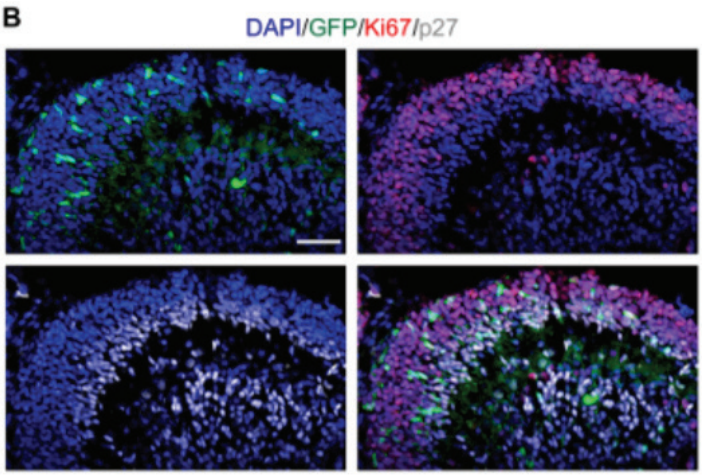
CRISPR-mediated Loss of Function Analysis in Cerebellar Granule Cells Using In Utero Electroporation-based Gene Transfer

Brain deformities are conditions in which the brain is not fully formed in pregnancy and is associated with several neurological and developmental problems. The malformations are often caused by genetic mutations and deciphering the key genes involved in the normal brain development can help identify the potential factors contributing to brain diseases and tumor formation. To help identify the somatic mutations in the cerebellum, the researchers utilized genetically engineered mouse models and developed a novel approach by combining electroporation-based gene delivery with the CRISPR/Cas9 technology.

Briefly, a single plasmid carrying a guide RNA (sgRNA) together with Cre was introduced into the cerebellar stem/ progenitor cells of Rosa26-CAG-LSL-Cas9-P2A-EGFP (conditional knock-in) mice using in utero electroporation. This approach enabled successful labeling of the granule neuron precursor cells and allowed to track their progeny (daughter cells) during post-natal brain development.



The GFP expressing cells in the outer granular cell layer can be observed proliferating as marked by Ki67. Immunostaining of GFP, Ki67, and p27 on the P7 cerebella from a Rosa26-CAG-LSLCas9-P2A-EGFP mouse were subjected to electroporation at E13.5 with pU6-sgRNA-Cbh-Cre plasmid constructs expressing control sgRNA. The section is counterstained with DAPI (blue).

ECM® 830 & GEMINI™ Electroporation Protocol

Cell Line: Cerebellar Granule Cell, CRISPR – sgRNA plasmid
Application: In Utero Electroporation E13.5
Electrode: 5 mm diameter Platinum Tweezertrode Item# 45-0489

Preparation:
Prepare the guide RNA (sgRNA) and the cre recombinase plasmids to a final concentration of at least 1 µg/µL for each plasmid and color the plasmid-solution with fast green (final concentration of 0.05%). Prepare 1% fast green stock solution with endotoxin-free distilled water and filter through a 0.22 µm filter to remove dye particles from the solution. Prepare a glass capillary with a micropipette puller (inside diameter: 0.6-0.8 mm) and aspirate ~ 15 µl colored plasmid-solution per mouse (~ 12 embryos) and immediately proceed with the surgery. Timed pregnant mice 13 day post-coitum are anesthetized using isofluorane and the uterine horn from the abdominal mouse cavity is exposed. Approximately 1 µl of the colored plasmid-solution is injected into the fourth ventricle by penetrating through the dorsal hindbrain. Apply electric square pulses with platinum tweezertrodes.

Square Wave Electroporation Settings:

Set Voltage:	32 V
Set Pulse Length:	50 ms
Number of Pulses:	5
Pulse Interval:	950 ms

Electroporation Procedure:
Sample Volume: ~15 µl plasmid-solution per mouse (~ 12 embryos)

Electroporation Procedure: Place the electrodes laterally with the negative pole covering the ear and the positive pole positioned at the cerebellar primordium over the uterine wall

Pulse: Press Go icon or Start button to activate the Automatic Charge and Pulse Sequence

Post Treatment: Carefully place uterine horns back into the abdominal cavity and close the peritoneum and skin separately with a simple continuous suture, monitor the animal during waking phase

Reference:
Feng, W., Herbst, L., Lichter, P., Pfister, S. M., Liu, H. K., Kawauchi, D. CRISPR-mediated Loss of Function Analysis in Cerebellar Granule Cells Using In Utero Electroporation-based Gene Transfer. J. Vis. Exp. (136), e57311, doi:10.3791/57311 (2018).