

ELECTROPORATION OF HYPOCAMPAL NEURONS IN A 96 PLATE WITH THE AMAXA NUCLEOFECTOR KIT (Amaxa biosystems)

Per transfection:

- dissect and dissociate 2 hippocampi (post-natal) with trypsin.
- Spin-down dissociated neurons (900 rpm; 4 min) and resuspend in 100 μ l of nucleofector. Once neurons are in the nucleofector solution work quickly (do not leave them in that solution for more than 15 min.)
- Add DNA (0.5 to 1 μ g, DNA should be super clean) mix gently with the plastic pipettes provided.
- Transfer solution into a cuvette (with the plastic pipette) and electroporate using the G-13 program. (they also recommend the 0-03 program but I have never tested it)
- Immediately after transfection, add 500 μ l of NM (neuron medium) and transfer to a eppendorf tube containing 700 μ l NM (1200 μ l NM total)
- Distribute 50 μ l in 24 wells of a 96-well plate. Allow neurons to adhere for 30 min. (10 min. at RT and 20 min in the incubator at 37C)
- After 30 min add 150 μ l NM.
- After 2hrs, one may want to remove entirely the solution and add 200 μ l fresh NM.

Remark: the amount of cells plated per 96-well is twice that used normally (I typically plate out 2 hippocampi in 48 wells, or 4 hippocampi (2 pups) per 96-well plate. The reason for that is that one loses cells during the centrifugation and (or) poration steps.

The amount of cells and DNA added to the 100 μ l may be varied. I did not try many other conditions.