Application Note

Transfection of E15 Primary Mouse Cortical Neurons with NeuroFECT™ Transfection Reagent*

***Data courtesy of Edoardo "Dado" Marcora, Ph.D., Senior Research Fellow,**
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Introduction

Primary mouse neuronal cells are used as a model for understanding the molecular mechanisms underlying local activation of transcription factors at synapses. This transfection experiment was part of a larger study of how transcription factors are transported from the synapse to the nucleus upon stimulation of glutamate receptors.

Materials & Methods

Cortices dissected from mice at embryonic day 15 were triturated and plated onto 12 mm diameter poly-D-lysine-coated glass coverslips in 24-well plates at a density of approximately 25,000 neurons/well in Neurobasal™/B27 medium (Invitrogen), with 25 µM glutamate.

The dissociated neurons were maintained in their original plating medium for 14 days in vitro without additional feeding. Then, the neurons were transfected with a vector containing mCherry fluorescent protein using the NeuroFECT Transfection Reagent as per manufacturer's recommendations with the following procedural modifications. The original plating medium was replaced with NeuroFECT-DNA complexes in fresh medium for 30-60 min maximum. Cultures were washed 2x with fresh medium, and then maintained in a 1:1 mixture of fresh medium and original plating medium for 24-48 hours. These modifications helped to reduce toxicity resulting from the standard transfection protocol.

Results & Discussion

The cells were visualized at low magnification for expression of the mCherry fluorescent protein.

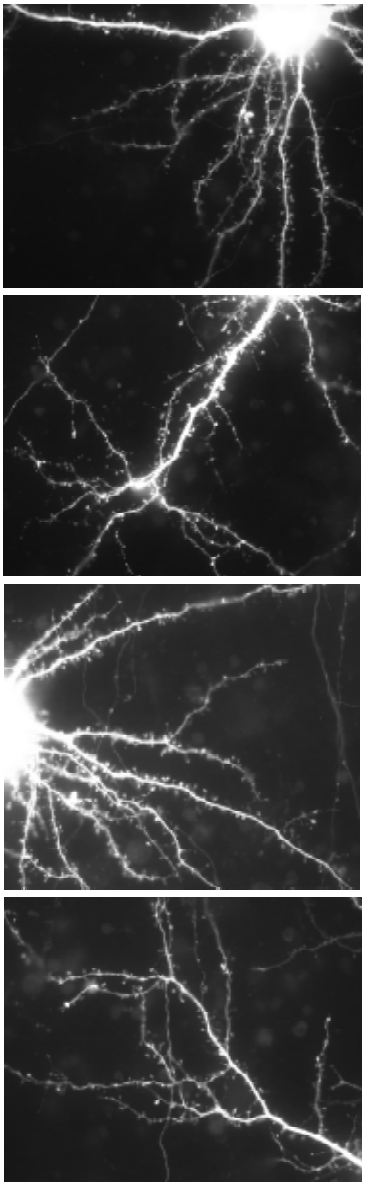
This procedure results in approximately 20-30 neurons transfected per well of a 24-well plate.

The images below show how healthy the dendritic spines look on the neurons following transfection.

Primary neurons are especially difficult to transfect. Many transfection reagents either do not deliver DNA efficiently to these cells or result in cytotoxicity. The NeuroFECT Transfection Reagent was developed using E18 primary rat cortical and hippocampal cells. Optimizing the standard NeuroFECT protocol, E15 mouse cortical cells were effectively transfected while maintaining cell health, as judged by appearance of the dendritic spines.

NeuroFECT™ Transfection Reagent		
0.75 ml kit	75-300 reactions	T800750
5 x 0.75 ml kit	375 - 1,500 reactions	T805750
Bulk applications	Custom	Inquire

Figure 1: Primary E18 Mouse Cortical Neuron Dendrites Following Transfection



Primary E15 mouse cortical neurons were transfected using the NeuroFECT Transfection Reagent. A modified protocol resulted in effective transfection and minimal cytotoxicity as seen by the healthy dendritic spines