High-throughput gene overexpression and knockdown in primary neurons by plasmid and siRNA electroporation

UNIVERSITY OF Miami Neuroscience Program

MILLER SCHOOL OF MEDICINE UNIVERSITY OF MIAMI

William Buchser, Yan Shi, Jose Pardinas, John Bixby, Vance Lemmon

Miami Project to Cure Paralysis

Introduction

To elucidate the contributions of multiple molecular effectors on a cellular phenotype, it has proven valuable to determine the impact of gene expression perturbations. We have developed high-throughput assays to either "overexpress" or "knockdown" gene targets in central nervous system neurons.

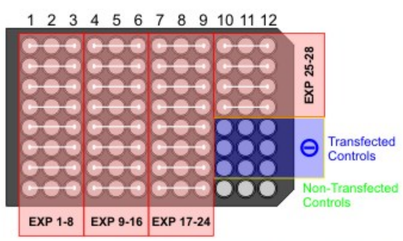
- Introduction of mammalian-expression vectors has been validated using eGFP in 96-well plate electroporations.
- Protein expression levels were reduced using siRNA oligonucleotides from various suppliers.
- Electroporations of L1CAM siRNAs were effective at knocking down their protein target. Protein levels were detected using immunofluorescence.
- The effects of these treatments on protein levels and axon outgrowth were scored via 96-well plate fluorescent microscopy using automated imaging with a neuron identification algorithm.
- Neurite and branches of identified neurons were measured and analyzed.
- We will expand our target set to include candidate axon growth-promoting genes. This will further our understanding of the underlying mechanisms in axonal regeneration and, in particular, of spinal cord regeneration after injury.

High Content Overexpression

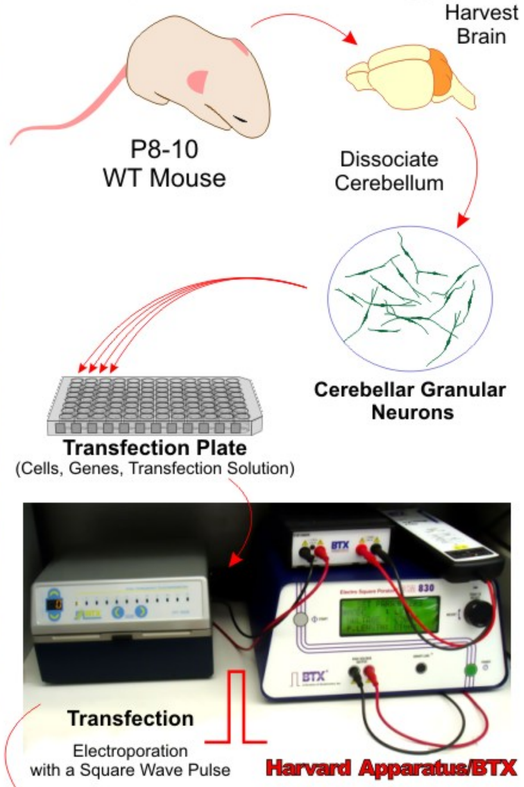
Overview of 1 Experiment

1 96 Well Plate:
1 Mouse
4 Million Neurons
30+ Different Transfections
3 Replicates/Condition
20% Transfection Efficiency
50% Viability

Example Layout:



Techniques & Technology

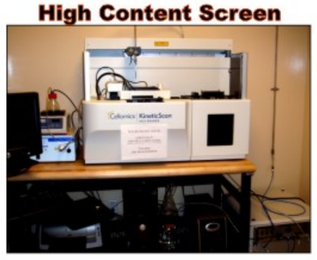


After electroporation, cells are plated onto laminin-coated 96-well plates.

Fix & Stain

A Qiagen BioRobot 3000 performs fixation and immunohistochemistry.

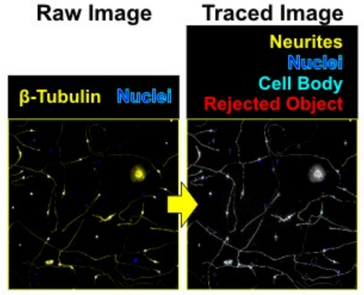
High Content Screen



Imaging

A Cellomics KineticScan Reader automatically images 5 fields from each well.

Raw Image **Traced Image**

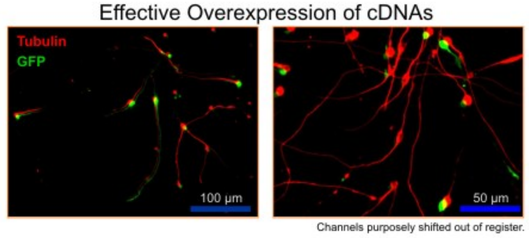


Automated Tracing

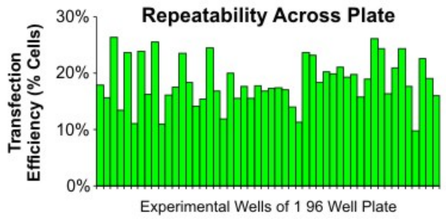
Cellomics software automatically identifies neurons and traces neurites. Both "cell-based" and "well-based" data are reported.

High Content Screen Results

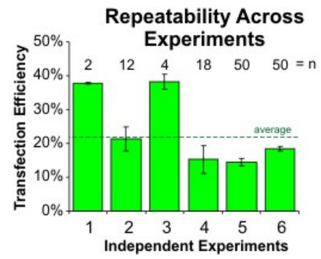
Effective Overexpression of cDNAs



Channels purposely shifted out of register.



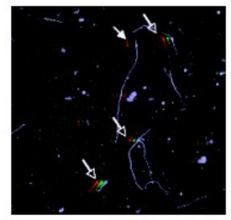
Both across plates and across independent experiments, we have found that our transfection technique yields expression of GFP in ~20% of neurons.



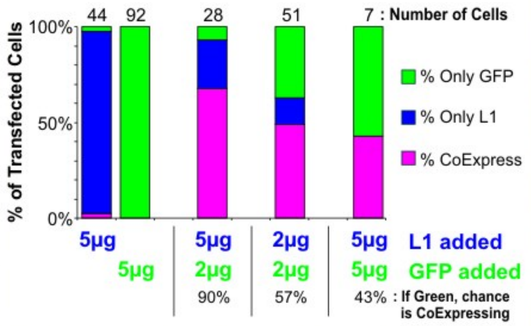
Co-Transfection & Co-Expression

Optimization of two-gene transfections

L1CAM
GFP
Tubulin



Black Arrows: Neurons Co-Expressing L1& GFP
White Arrow: A neuron expressing L1 alone

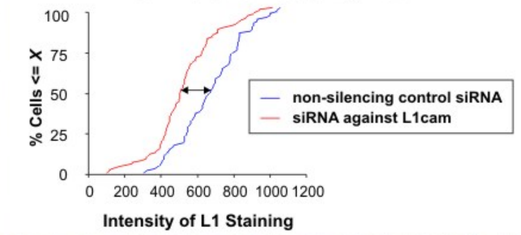


siRNA Knockdown

Using Electroporation:

~ 10% of neurons exhibit significantly reduced protein expression 48 hours after transfection (48 hours is used so that neurites are short enough to trace reliably)

Cummulative Probability Plot



Conclusions

1. 96 well electroporation can be used to express cDNAs in cerebellar granule cells for studies on neurite extension.
2. Co-transfection with eGFP allows identification of transfected neurons
3. 96 well electroporation can be used with siRNAs to knockdown protein expression in cerebellar granule cells but protein half-lives can influence the effectiveness of this approach in short-term (2 DIV) assays.

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