Transfecting B35 Neuroblastoma Cells with Optogenetic Protein for Micropipette Excitation

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Research Question

The underlying objective of this research proposal is to generate a cell line of B35 cells that express humanized channelrhodopsin-2 (hChR2)-enhanced yellow fluorescent protein (EYFP) [1].

Overview

Subthreshold events are essential because they provide the foundation for neuroplasticity, adjusting the likelihood of reaching an action potential threshold. Current electrophysiological approaches lack the ability to assign functional activity to specific cells in a localized region of the brain. In combination with hChR2-EYFP and voltage sensitive dyes (VSDs), a fluorescence-guided micropipette electrode can be utilized to target specific neurons for higher resolution experiments. This work can have a direct impact on disabilities such as stroke, since understanding fundamental neuronal processes has the potential to translate into improved prognosis for those suffering from this devastating disability. The overall purpose of this study is to show how VSDs may be used to visualize subthreshold events in localized regions of the brain.

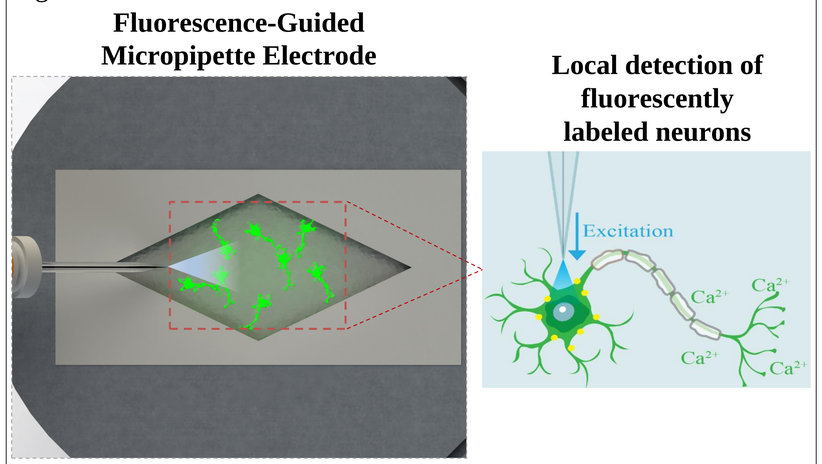

Fig 1: The tip of the micropipette electrode detects fluorescence as it approaches the transfected neuron [2].

Experimental Design

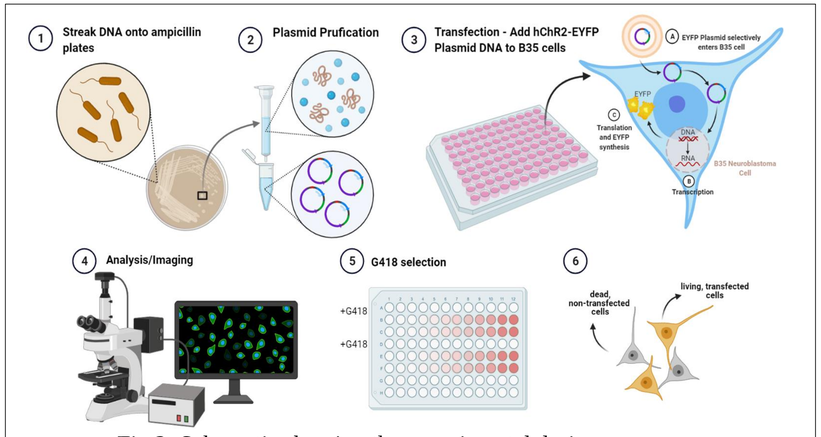

Fig 2: Schematic showing the experimental design process.

Results

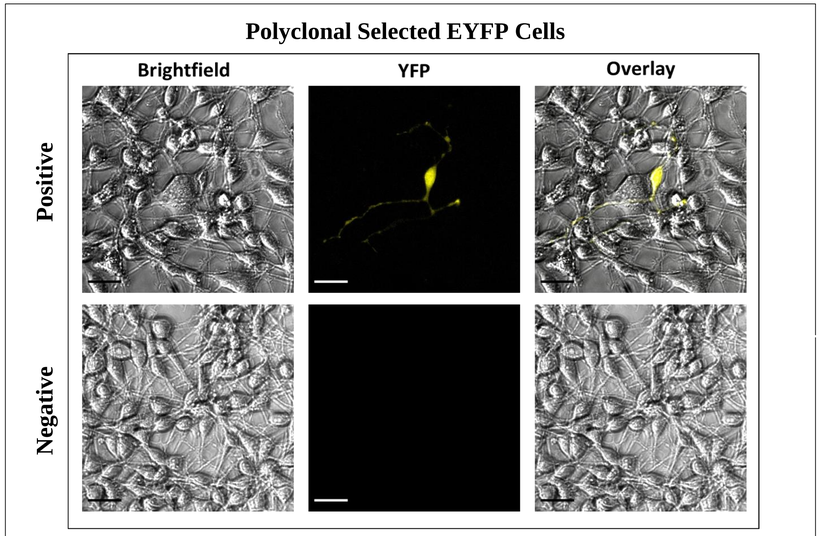

Fig 3: Fluorescence microscopy images of transfected polyclonal B35 neuroblastoma cells. Scale bar is 25 microns. Thresholds of images were done in ImageJ with the same values.

Conclusion

This research demonstrates the ability to successfully transfect B35 neuroblastoma cells with EYFP and hChR2. In this research, I purified plasmid DNA, transfected B35 cells with Lipofectamine and the bacterial plasmid, and stably transfected EYFP and hChR2-expressing polyclonal lines, as pictured in Figure 3.

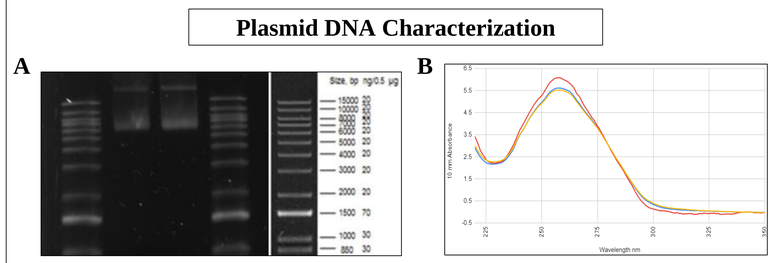

Fig 4: A) The gel electrophoresis verifies that the plasmid is present, since it reached between 6000-7000 base pairs. B) Nanodrop data was used to determine the quantity and quality of the plasmid.

Future Work

The stable cell line of B35 cells expressing hChR2-EYFP will be used to demonstrate the application of VSDs for visualizing subthreshold events utilizing our custom automated fluorescence-guided micropipette setup.

Acknowledgements

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References

[1] Multimodal fast optical interrogation of neural circuitry. Zhang F, Wang LP, Brauner M, Liewald JF, Kay K, Watzke N, Wood PG, Bamberg E, Nagel G, Gottschalk A, Deisseroth K. *Nature. 2007 Apr 5. 446(7136):633-9.* 10.1038/nature05744 PubMed 17410168
[2] C. Miranda, M.R. Howell, J.F. Lusk, E. Marschall, J. Eshima, T.Anderson, and B.S. Smith, "Automated microscope-independent fluorescence-guided micropipette," Biomed. Opt. Express 12, 4689-4699 (2021).

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