Cost Comparison of Calcium Phosphate Transfection and Lipofectamine Transfection for Production of PCDH19 Protein in HeLa Cells

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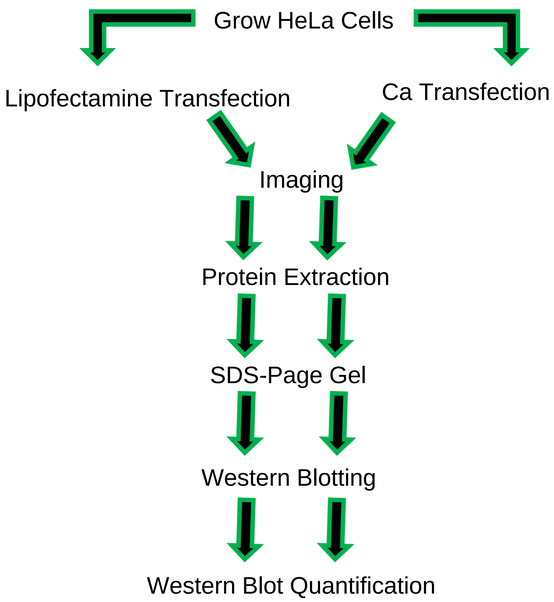
Introduction

Protocadherin-19 (PCDH-19) is a specific gene located within the brain of most organisms. This gene, if mutated would cause epilepsy which often coincides with various degrees of intellectual disabilities. In addition, PCDH-19 is known for its contribution to the cranial nerve pathways along with other sensory structures and motor nuclei. During study of the gene, the zebrafish (Danio rerio) is the best model to discover its behavior. Most of the research conducted regarding this gene utilize a technique similar technique to situ hybridization, which is labeling and localizing DNA, RNA, and molecules from their associated tissue or cell (Liu, et al. 2015). Ultimately, by discovering how and why this gene behaves as it does within the brain, we can further our study into neurology. In order to study PCDH-19, we must first determine the best strategy scientifically and costly. The goal of this research is to determine which transfection is most cost efficient.

Methodology



References

- Liu, Q., Bhattarai, S., Wang, N., & Sochacka-Marlowe, A. (2015). Differential expression of protocadherin-19, protocadherin-17, and cadherin-6 in adult zebrafish brain. The Journal of comparative neurology, 523(9), 1419–1442. https://doi.org/10.1002/cne.23746

Results: Fluorescent Images

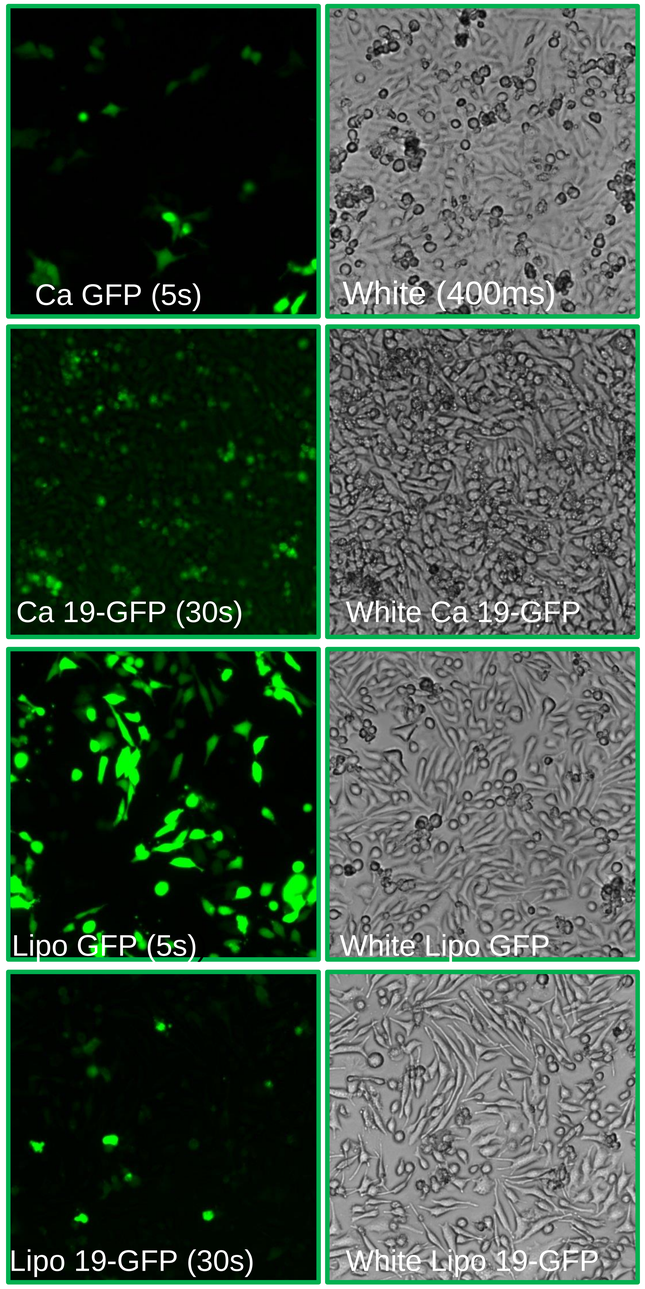


Figure 1: Expression of protein using Ca+ phosphate and lipofectamine techniques.

Results: Western Blot Analysis

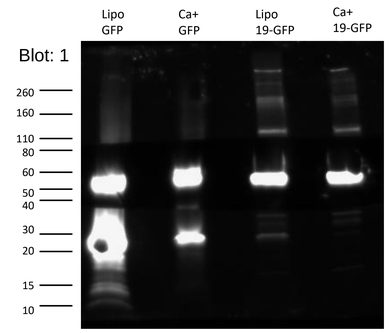
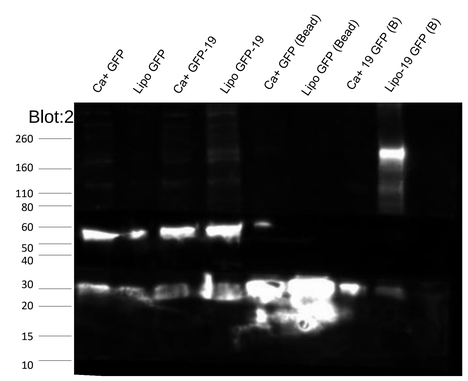


Table 1:	Lipo-GFP	Ca+ GFP	Lipo-GFP-19	Ca+ GFP-19
ratio (GFP/loading control)	0.5497	0.180452	0.015626	0.006102
ratio (PCDH-19/loading control)	0.152335	0.142651	0.32757	0.194692



- Protein GFP- three Ca+ phosphate plates would be needed to produce the same amount of proteins as lipofectamine.
- PCDH-19 GFP- 1.7 plates are necessary of Ca+ phosphate to produce the same amount of proteins as lipofectamine.

Cost Analysis

Table 1: Ca Phosphate
Total Cost: $1.90 per reaction

Reagent	Cost	Amount Needed	Final Cost
DMEM Media	$27.20 per 500ml	4ml	$1.01 per reaction
2X HBS	$10.80 per 100ml	200µL	$0.02 per reaction
FBS	$146 per 100ml	0.4ml	$0.58 per reaction
2M CaCl2	$17.29 per 100ml	24.4µL	$.01 per reaction
DNA	$82.96 per 300 reactions	4µg	$0.28 per reaction
Water	$53.50 per liter (5,000 reactions)	200µL	$0.01 per reaction

Table 2: Lipofectamine
Total Cost: $9.17

Reagent	Cost	Volume Needed	Final Cost
DMEM Media	$27.20 per 500ml	4ml	$1.01 per reaction
FBS	$146 per 100ml	0.4ml	$0.58 per reaction
Opti-MEM I Media	$22.84 per 100ml	580 µL	$0.13 per reaction
Lipofectamine 2000	$215 per 0.3ml	10µL	$7.17 per reaction
DNA	$82.96 per 300 reactions	4 µg	$0.28 per reaction

Conclusion

- Lipo-transfection nearly costs five times as much compared to Ca+ phosphate.
- We only get three times as much GFP protein from lipo-transfection compared to Ca+ phosphate.
- PCDH-19 has contradicting results; additional research must be done.
- Overall, we can conclude that the GFP construct for Ca+ phosphate is more cost effective than lipofectamine. On the contrary, the PCDH-19 construct needs more experimentation.