Project Title

Identifying Two Populations of Neurons in the Developing Cerebellum

Objectives/Goals
In the past, genes for fluorescence have been utilized for tagging viruses. The insertion of these genes allowed for viral strains to be more immediately visible and facilitated additional analysis of the effects of the virus. The purpose of our experiment is to further the use of fluorescent genes in this capacity. We aimed to create separate lentivirus each containing a unique fluorescence and tested to see if the two could be independently distinguished in one specimen.

Methods/Materials
Plasmids were injected with their corresponding restriction enzymes. Genes for the chosen fluorescence were inserted into the gaps created by the enzymes. The plasmids were turned into virus from their constructs and were then humanely injected into mouse pups' developing cerebellums. The cerebellums were sectioned, stained, and imaged.

Results
Based on the imaging results, it appears that the experiment was successful. In addition to the standard calbindin staining of Purkinje neurons, other neurons seem to have been stained as well. Both the green fluorescent proteins (GFP) and the cyan fluorescent proteins (CFP) appear to have successfully infected cells. Most infected cells appear to exhibit both blue and green staining; however, there are a substantial number of cells with only one visible color, either green or blue.

Conclusions/Discussion
The results indicate that it is certainly possible to stain neurons with different fluorescent proteins in the same specimen. While the overwhelming presence of dually infected cells is peculiar, the fact that numerous cells are stained with only one GFP or only CFP seems to suggest that the outcome, while not what was theoretically expected, did successfully demonstrate the potential for multi-colored staining. A multi-colored approach to studying the impact of the cerebellum certainly will open new doors in research in the field of molecular biology. Through the use of multiple fluorescent proteins, researchers can analyze the effects of multiple viruses when targeting different cells or the same cell in a single specimen.

Summary Statement
This project sought to demonstrate that cells infected with different virus can be distinguished by using fluorescent proteins.

Help Received
Used lab equipment and conducted experiments at Stanford University under the supervision and leadership of Dr. Ashvin Sangoram