An Analysis of the Regulatory Effects of Inducible Promoters on the Gene Expression of Mammalian Cells

Kristen Cox, Thu Phan. [1] University of Minnesota, Department of Chemical Engineering

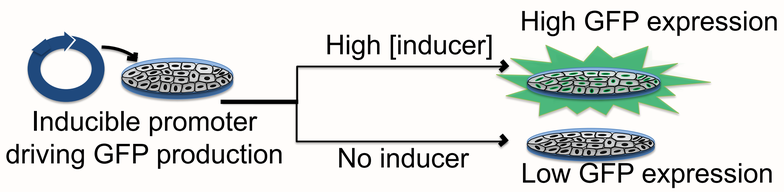
Introduction

Overarching Project

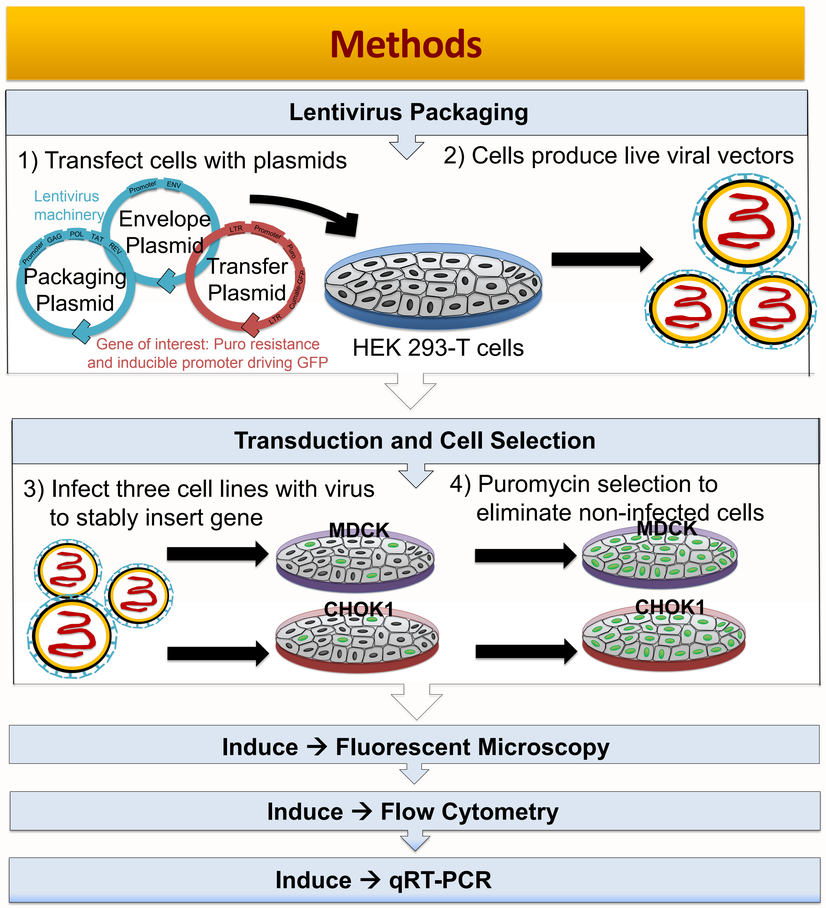
- The influenza A virus (IAV) is a highly mutatable and contagious virus that kills 250,000-500,000 people each year. The influenza vaccine is typically developed from seed virus amplified within chicken eggs, an intensive and expensive 6 to 12 month process.
- A potential alternative is an engineered system in which influenza genes are inserted into a mammalian cell via interchangeable cassettes, and the cell itself produces the virus necessary for a vaccine. To prevent to cell from producing too much virus too quickly and killing itself, production could be controlled by an inducible promoter system.

UROP Project

- Inducible promoter systems can be inserted in cells and used to turn genes on and off via addition of an inducer, like a light switch. Yet the regulatory possibilities of such systems have not been formally characterized in many mammalian cell lines.
- The cumate-inducible system will be inserted into two cell lines, CHO-K1 and MDCK, and induced at a range of concentrations. We hypothesize that some combination of inducible promoter and mammalian cell line will provide tight and regulatable control of gene expression.



Methods



Results: CHOK1

The cells were induced with 0, 25, and 150 ug/mL cumate, and imaged at 10x, 24 hours later.

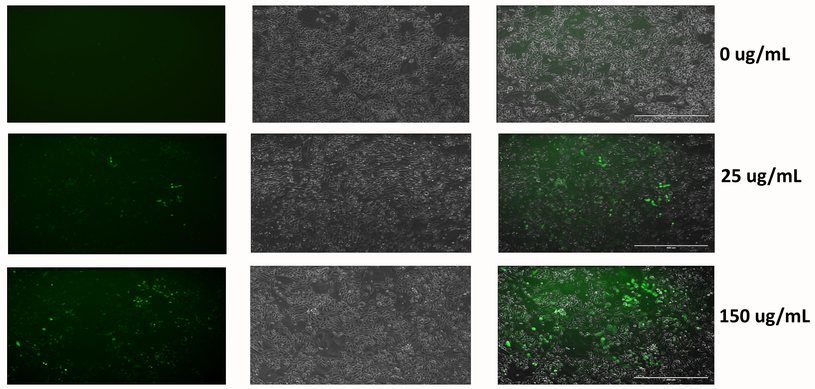
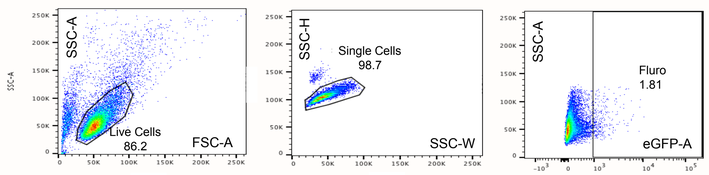


Figure 1 (a-i): Fluorescent, brightfield, and overlay images of CHOK1 engineered cell line induced with 0, 25, and 125 ug/mL cumate.



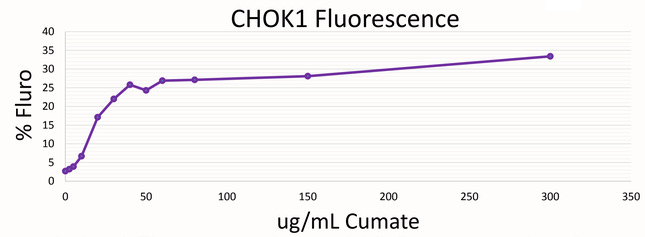


Figure 3: (a-c) Flow sort gating and **(d)** fluorescent data for twelve induction concentrations

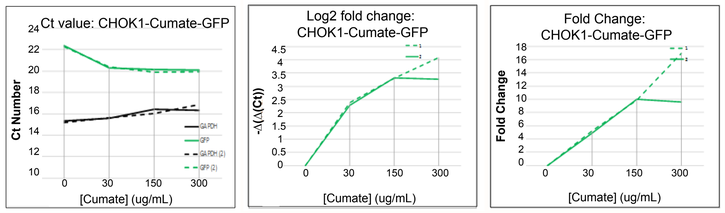


Figure 5: qRT-PCR results. **(a)** Ct number, **(b)** delta delta Ct value, and **(c)** fold change for CHOK1 induced with 0, 30, 150, and 300 ug/mL cumate.

Results: MDCK

The cells were induced with 0, 25, and 150 ug/mL cumate, and imaged at 10x, 24 hours later.

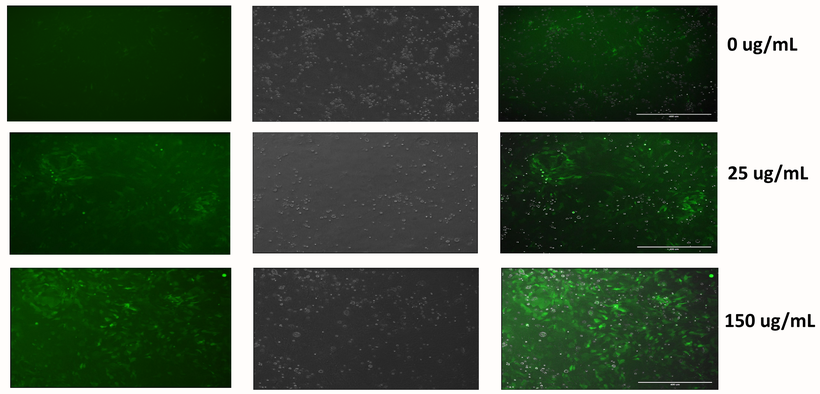
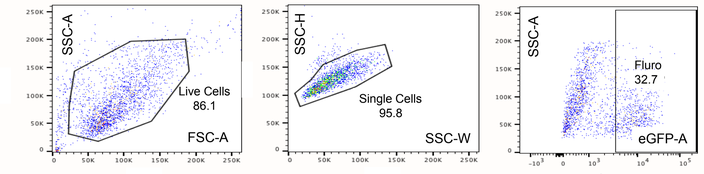


Figure 2 (a-i): Fluorescent, brightfield, and overlay images of MDCK engineered cell line induced with 0, 25, and 125 ug/mL cumate.



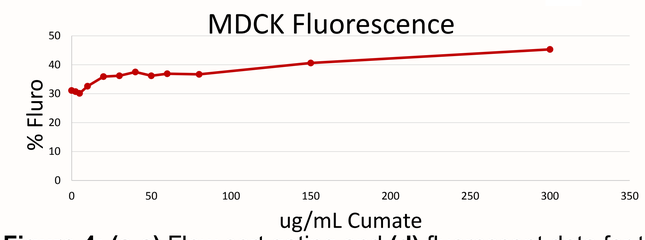


Figure 4: (a-c) Flow sort gating and **(d)** fluorescent data for twelve induction concentrations

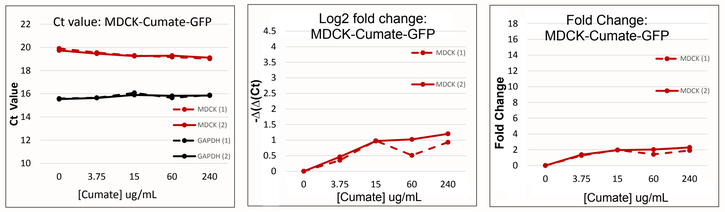


Figure 6: qRT-PCR results. **(a)** Ct number, **(b)** delta delta Ct value, and **(c)** fold change for MDCK induced with 0, 3.5, 15, 60, and 240 ug/mL cumate.

Conclusions and Future Work

- Two cell lines, CHOK1-Cumate-GFP and MDCK-Cumate-GFP, were successfully created.
- CHOK1 is a superior cell line over MDCK for the cumate-inducible system, providing tighter and more regulatable control of GFP production as shown by fluorescent imaging, flow cytometry data, and qRT-PCR
- Future work: Repeat experiment with HEK-293T cell line, as well as TetON inducible promoter. Test functionality of CHOK1-cumate engineered cell line for virus production.

Acknowledgements & Citations

1 - Matias G, Taylor R, Haguinet F, Schuck-Paim C, Lustig R, Shinde V. Estimates of mortality attributable to influenza and RSV in the United States during 1997–2009 by influenza type or subtype, age, cause of death, and risk status. Influenza and Other Respiratory Viruses. 2014;8(5):507-515
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4 - Tetracycline (Tet) Inducible Expression. Plasmid Resources, Addgene
5 - Cumate-Inducible Systems. System Biosciences