

[https://colab.ws/virginia.edu/access/content/group/f85bed6c-45d2-4b18-b868-6a2351586804/2/Ch21\\_Schwartz\\_M\\_Rubisco\\_-\\_Spinacia\\_oleracea\\_-\\_Ch21\\_Schwartz\\_M\\_Rubisco\\_-\\_Spinacia\\_oleracea\\_Rubisco.html](https://colab.ws/virginia.edu/access/content/group/f85bed6c-45d2-4b18-b868-6a2351586804/2/Ch21_Schwartz_M_Rubisco_-_Spinacia_oleracea_-_Ch21_Schwartz_M_Rubisco_-_Spinacia_oleracea_Rubisco.html)

# Using Directed Evolution to Improve Rubisco Function

Shloke Srivastava and Dr. Jake Stout

Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada



## Background

Global food demands, human populations and CO<sub>2</sub> emissions are rising.

Rubisco is a key enzyme in the process of photosynthesis. Improving it could help improve plant functionality and efficiency, aiding the fight against the growing issues.

## Methods

Error-prone PCR (epPCR)

- Inserts mutations randomly into enzyme.

Transformation into *Ralstonia eutropha*

- Optimal strain of bacteria developed for Rubisco.

Screening the library

- Oxygen consumption graphs to screen for improved enzymes for the next round of epPCR.

## Objective

To use directed evolution to evolve the Rubisco enzyme to have some level of improvement by random mutagenesis using error-prone PCR

## Predictions

If Rubisco were to be improved, it could be for many reasons including:

- Higher enzyme efficiency
- Increased carbon sequestration
- Higher affinity for CO<sub>2</sub>
- Decreased O<sub>2</sub> utilization

## Conclusions

An improvement in the Rubisco enzyme would improve the process of photosynthesis. This could have many downstream effects including:

- Improving rate of plant growth
- Improving crop yield
- Increase in carbon sequestration

This research could also provide inspiration for continuing to apply directed evolution in plant enzymes, improving biofuel production, secondary plant product extraction and more.

## Acknowledgments

Thank you to Dr. Stout for being an excellent advisor, Dr. Waterman for being a dedicated professor and the 2020 class of BIOL3100 for their unwavering support.

## Expected Results

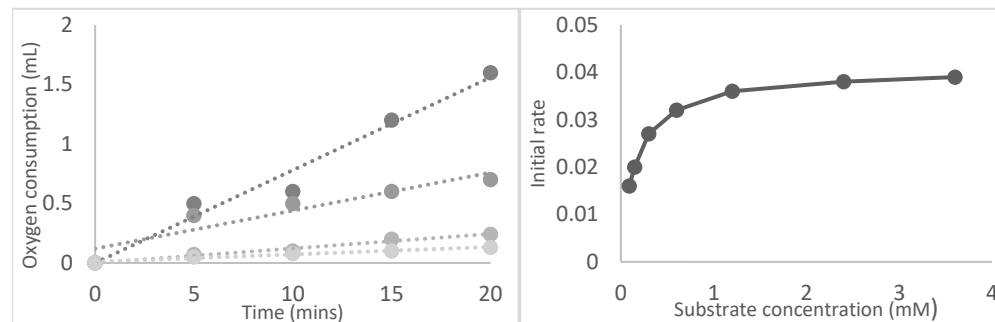


Figure 1. Hypothetical O<sub>2</sub> consumption graph screening mutated Rubisco enzymes to identify template for the next round of epPCR.

Figure 2. Hypothetical Michaelis-Menten curve to calculate enzyme kinetics values such as K<sub>M</sub> and K<sub>cat</sub> to determine level of improvement.