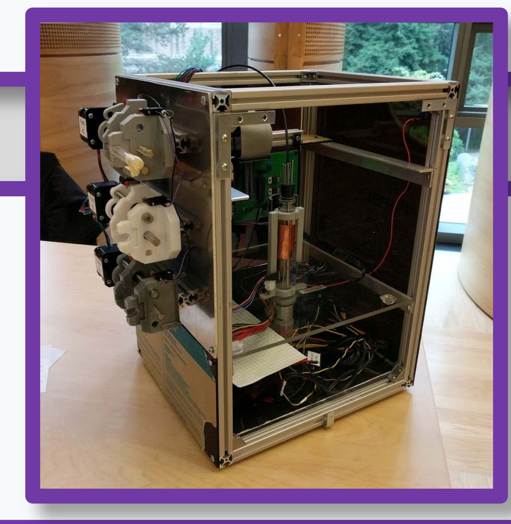
Viva la Violacein: a real-time metabolics tracker

Problem

Managing cultures + characterizing metabolic pathways are difficult and **time-consuming**. Constantly measuring and adjusting culture conditions in order to produce a desired metabolite is both tedious and **labor intensive**. Modern assays that accomplish this can also be prohibitively **expensive**.

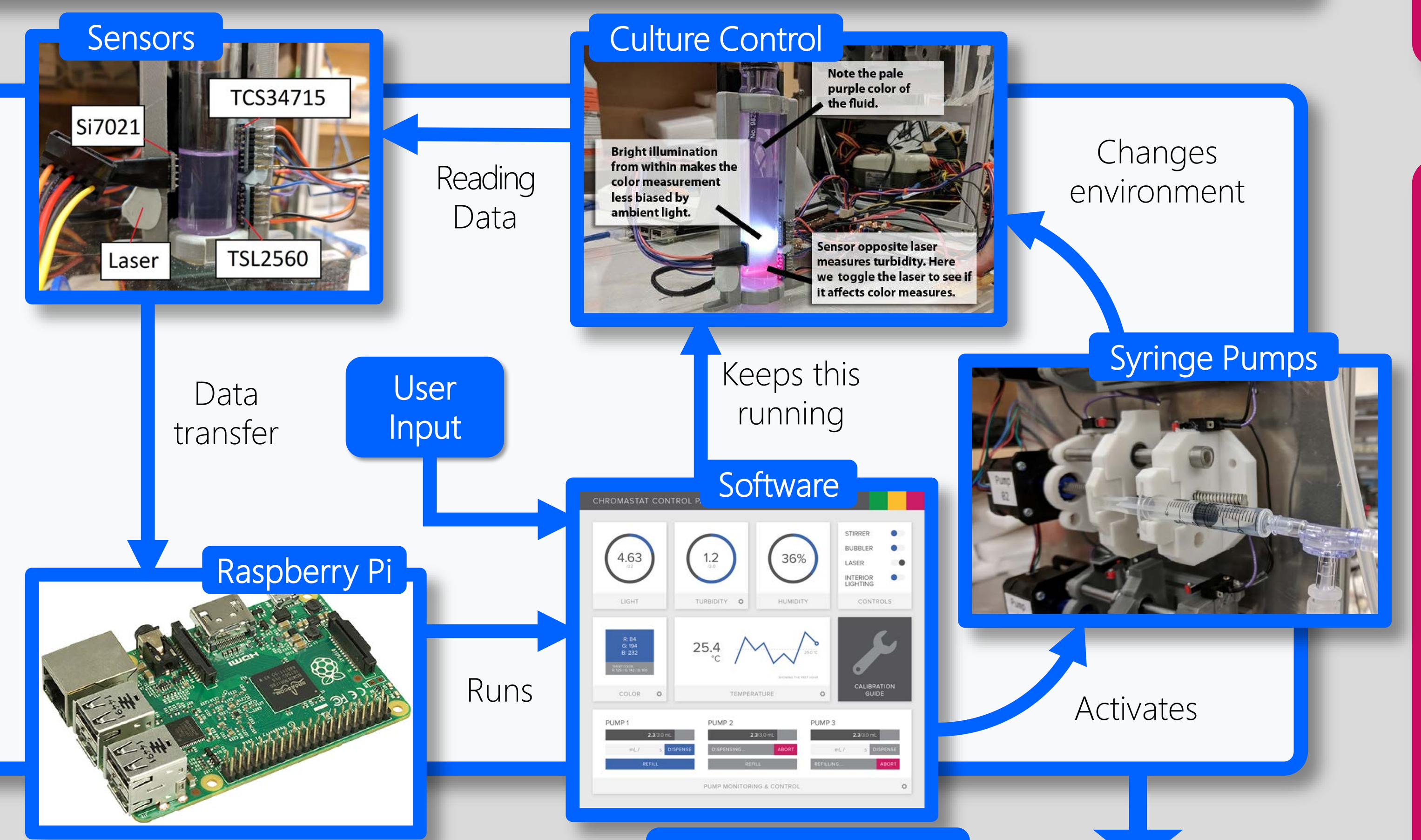


The Chromastat

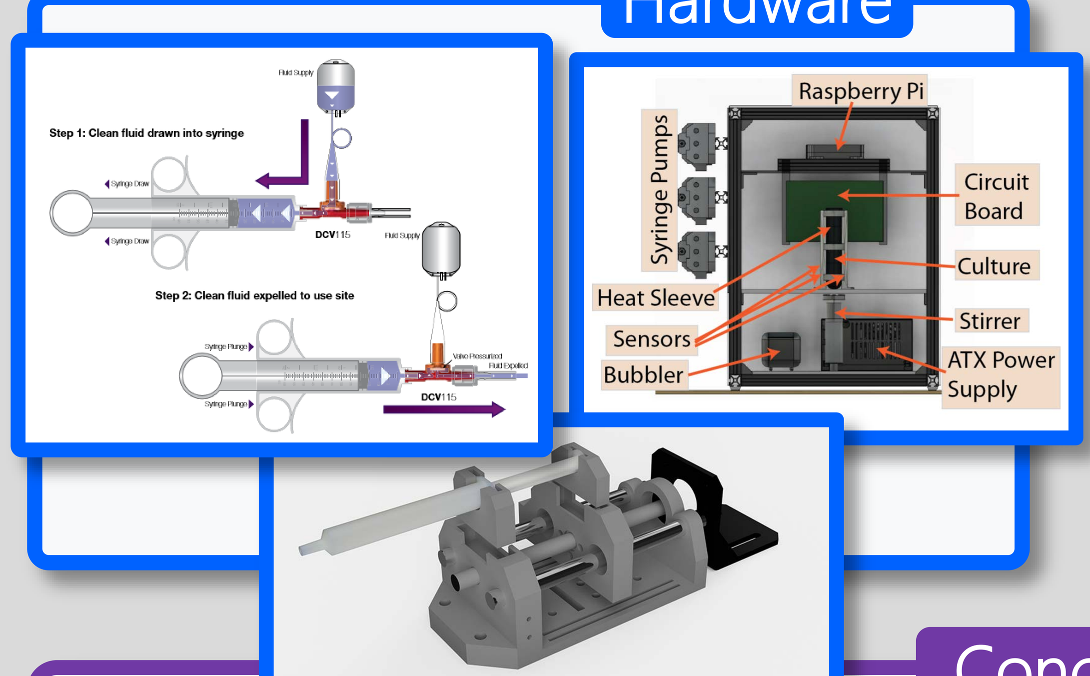
Our solution is the Chromastat: a proof of concept that utilizes a **closed-loop feedback control system** to **control + optimize production** of a desired product. This would **reduce the amount of time and effort needed to maintain cultures** through automated, real-time metabolite analysis.

Closed-loop controller

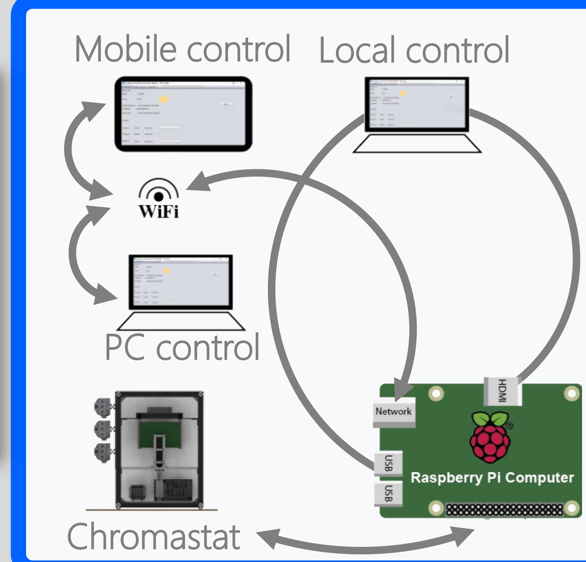
We **implemented** control theory through a feedback loop which uses a sensor to measure output, compare it to a user-defined value, and then change the input to get the output closer to the defined value. In our system, the inputs are **inducers**, and the output is the **color of the solution**. The Chromastat adds in more of the inducer to achieve expression of the desired color, using a proportional control algorithm.



Hardware



Software

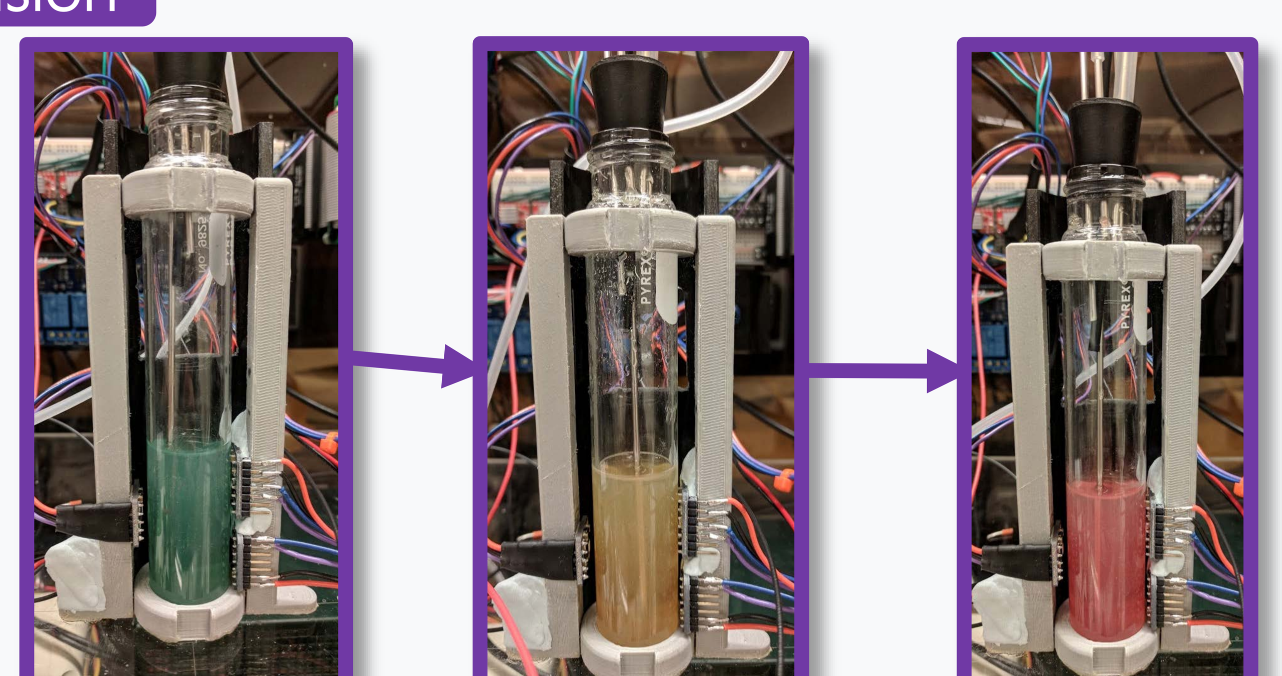


Our software is both **simple to use** and **easy to modify**. Our adherence to Java standard programming patterns and documentation practices means that our code base will be familiar and understandable to anyone with Java experience.

Conclusion

Our project can measure metabolites in the violacein pathway using an RGB sensor and autonomously regulate gene expression by varying inducer input. This proof of concept is modular; any part of this can be switched with another part or combined with additional parts so that it is customizable for both research and industry.

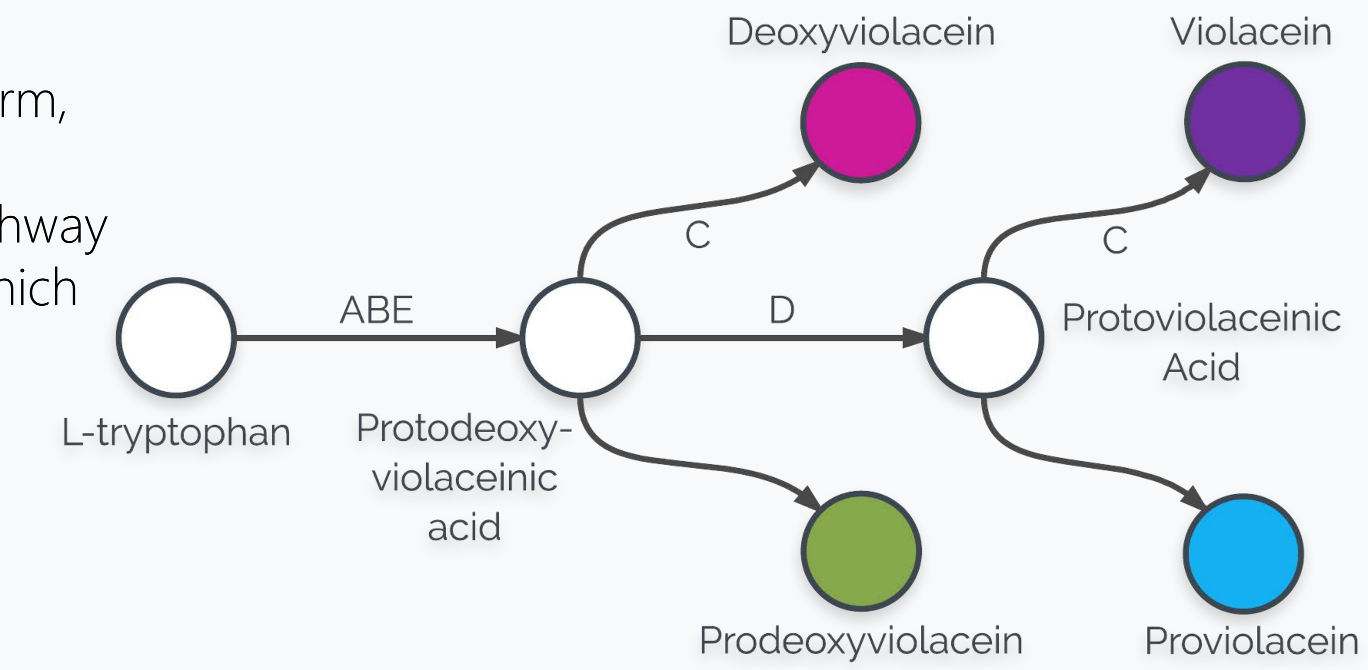
The applications of this are truly boundless.



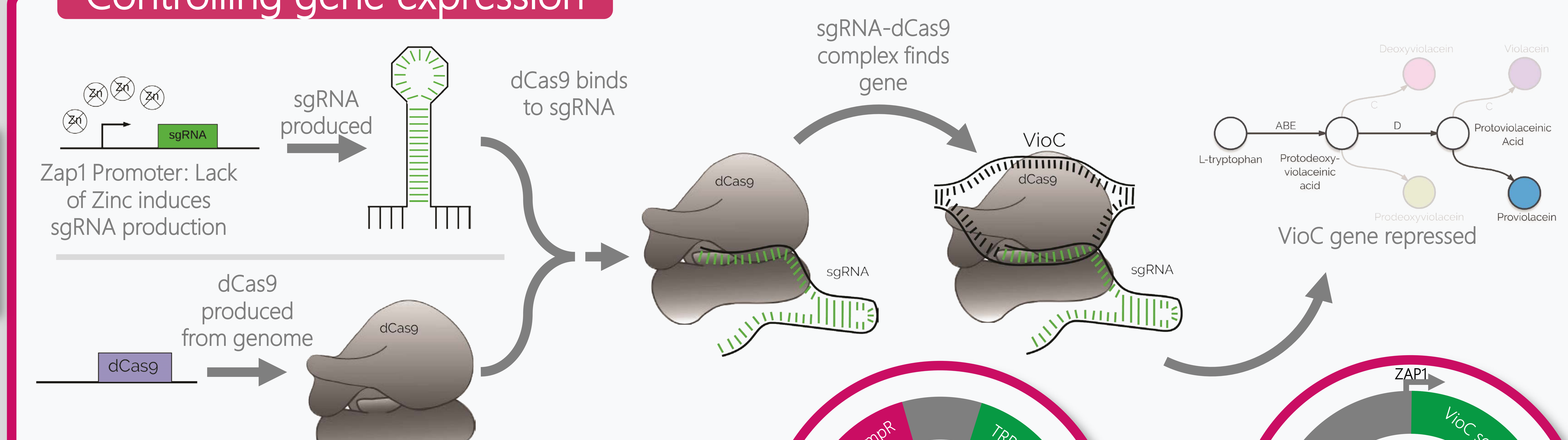
Employing a violacein pathway

In order to realize our goal of creating an autonomous yeast culture management platform, we are employing the violacein pathway to **visualize metabolic processes** in yeast. The pathway consists of five genes: **VioA, VioB**, and **VioE** (which are constitutively expressed), **VioC** and **VioD**.

VioABE → prodeoxyviolacein
VioABE + VioC → deoxyviolacein
VioABE + VioD → proviolacein
VioABE + VioC + VioD → violacein

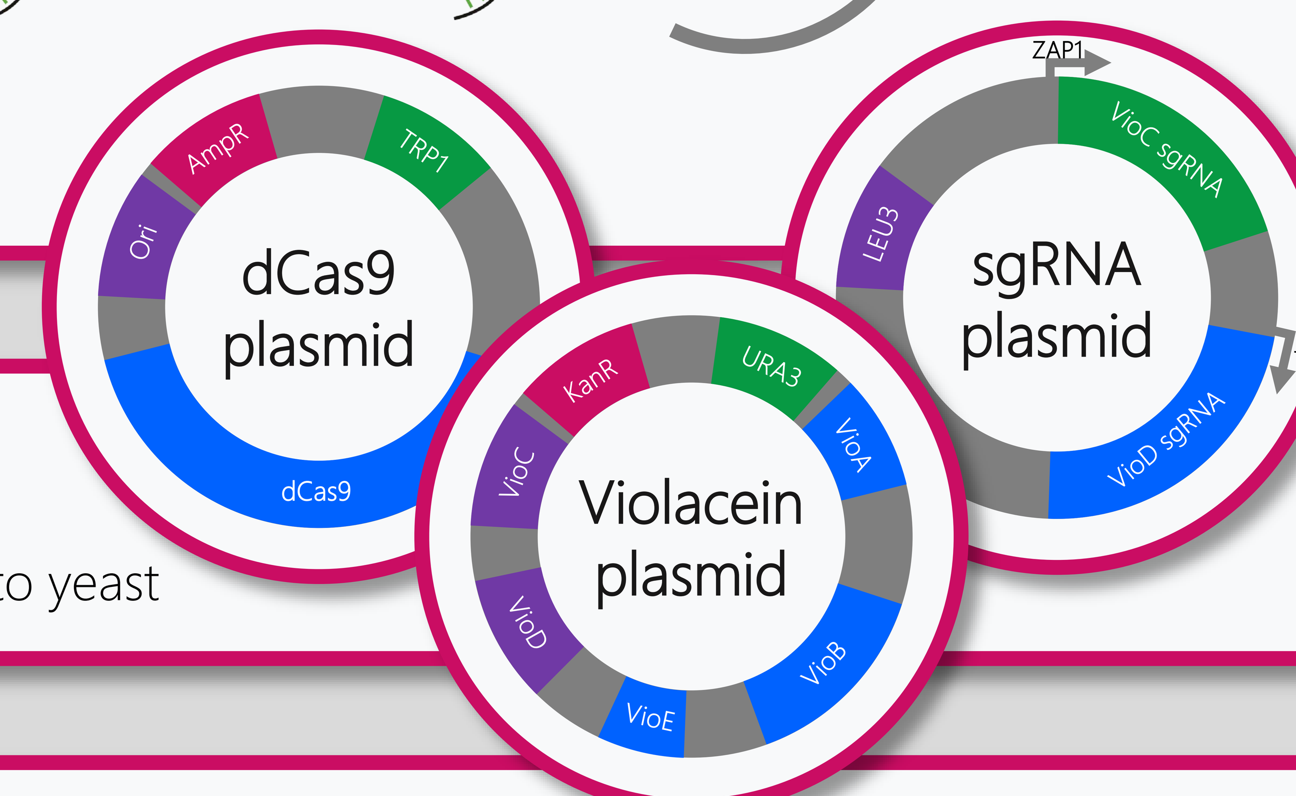


Controlling gene expression



Three Plasmid System

- dCas9 – **Integrated** into yeast genome
- Violacein – **Integrated** into yeast genome
- sgRNA + inducible promoters – **Transformed** into yeast



BioBricks

Yeast is used ubiquitously in synthetic biology, but the BioBricks Repository still has **very few** yeast BioBricks. Thus, we submitted **two fundamental BioBricks**: The ICL1 Yeast Promoter (BBa_K2391000) and the ZAP1 Yeast Promoter (BBa_K2391001).

Human Practices

We engaged multiple audiences to further the awareness and practice of synthetic biology. This included:

- Founding iTESLA, the **first** all high school student iGEM team based in Washington State
- Obtaining input from the general public and educated professionals about the safety, responsibility, and usability of our project to improve our plans
- Spreading scientific awareness to students from preschool to high school age

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Special thanks to: Dr. Eric Seibel, Dr. Herbert Sauro, Dr. Liangcai Gu, Dr. Karen Thickman, Kevin Li, Natalie Johnston, and Cameron Roots

Cress, B., Jones, A., Kim, D., Leitz, Q., Englaender, J., Collins, S., Linhardt, R., Koffas, M. (2016, May 9). Rapid generation of CRISPR/dCas9-regulated, orthogonally repressible hybrid T7-lac promoters for modular, tunable control of metabolic pathway fluxes in Escherichia coli. Nucleic Acids Research., 44(9), 4472-4485.

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