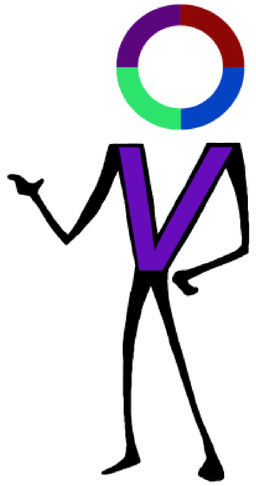
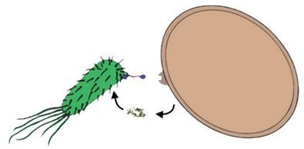
Synthetic Unity

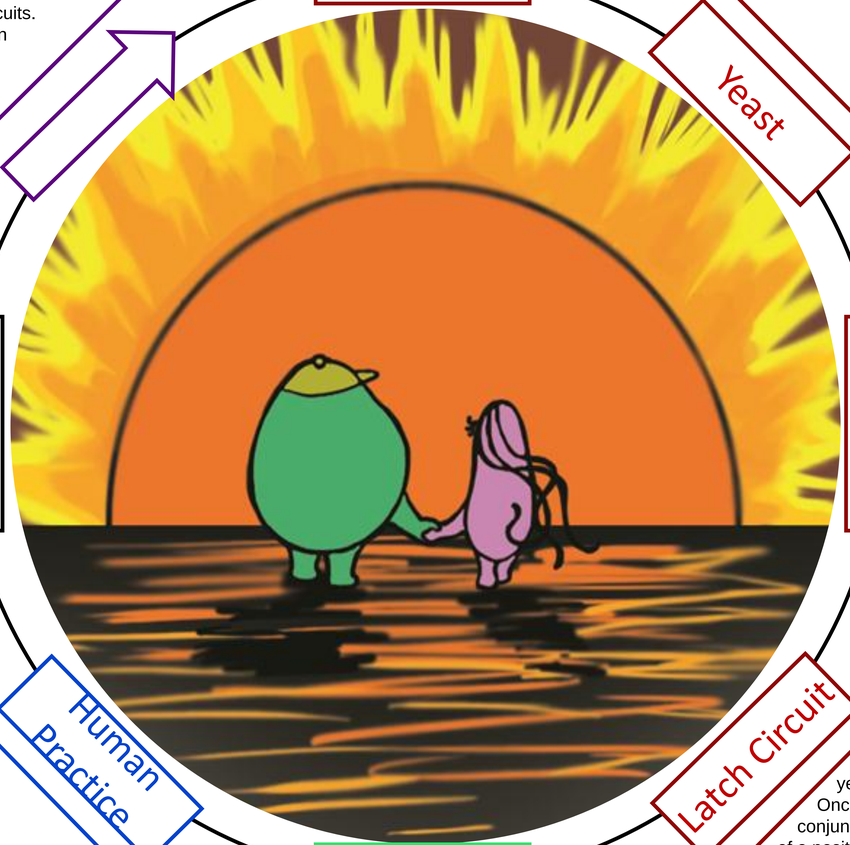
Abstract



Our project explores synthetic obligate mutualism as a means to produce novel, multi-species chassis as foundations for innovative synthetic biological applications. These applications could exploit unique biochemical potentials emerging within chimeric systems. Here we explore the pairing of *Saccharomyces cerevisiae* and *Escherichia coli* by constructing genetic circuits for reciprocal induction of essential histidine biosynthetic genes. Since one species relies on the other for induction of their own essential gene, mutualism is obligatory. Another key component of our efforts addresses the need for effective interactive models to facilitate teaching and understanding of synthetic biology concepts. Victor the Vector is an electromechanical device, which combines circuits and software to not only model components of our project, but to facilitate understanding of gene regulation and the synthesis of genetic circuits. *In toto*, Team RHIT strives to stimulate innovation and education in synthetic biology.

Our wet lab work was designed to induce an endosymbiotic event between *S. cerevisiae* and *E. coli*, where these organisms engage in a scenario in which each organism requires something produced by the other organism for survival. To achieve this goal, a construct was designed for each organism. Both constructs have two qualities: the ability to produce a product that will activate gene expression in the other organism, and the ability to make the other organism dependent on the activation of this gene. To achieve this goal, lactate and mating-induced gene systems and histidine biosynthesis were implemented as a way to select for cells properly expressing the system.





Wet Lab

Yeast

E. coli

Latch Circuit

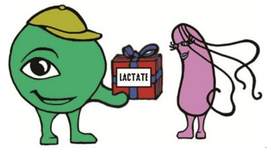
PLA Recycling

Human Practice

Attributions

To create yeast for this endosymbiotic event, we built a yeast plasmid that would require dependence on *E. coli* for survival, and allow for the production of something that the *E. coli* will need to survive. To do this, the gene coding for His3, an enzyme that is essential for survival, was hooked to the FUS1 promoter from the mating pheromone response pathway; this causes the yeast to be dependent on the activation of this promoter sequence, which happens when mating factor α produced by the *E. coli* binds to receptors on the surface of the yeast. In addition, a constitutive promoter was hooked to the gene coding for lactate dehydrogenase. This allows the yeast to produce the lactate that the *E. coli* will be dependent on for histidine biosynthesis, which is necessary for survival.

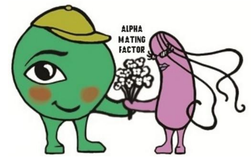
***S. cerevisiae* gives *E. coli* Lactate!**



Lactate

MF α

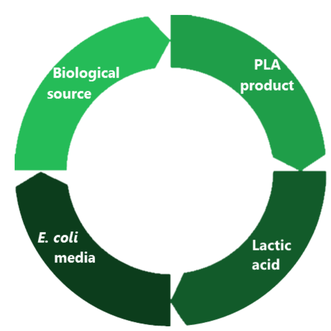
***E. coli* gives *S. cerevisiae* Alpha Mating Factor!**



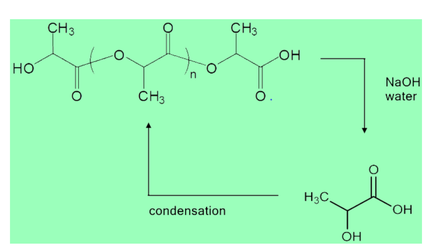
To create *E. coli* that allows for the survival of the yeast, we needed to express the mating factor α on the surface of the *E. coli*. To do this, the Ice Nucleation Protein-based surface display (Penn iGEM, 2012) and the gene for mating factor α were hooked to a constitutive promoter; this allows for the activation of the Fus1 promoter and histidine biosynthesis, and therefore survival, in the yeast. To make the *E. coli* dependent on the yeast for survival, a lactate-inducible promoter was hooked to the His3 gene, causing the *E. coli* to be dependent on the lactate provided by the yeast.

MF α

This “latch circuit” was designed to detect the expression of mating factor α by *E. coli* and produce a blue fluorescent heteroprotein in response via a positive feedback loop. The initial production of the heteroprotein is controlled by the binding of mating factor α to receptors on the yeast cell surface, which activates the mating pheromone response pathway and the Fus1 promoter. Once the protein is produced, the LexA binding domain binds to the LexA-regulatory element, and in conjunction with the VP64 activator domain, facilitates the further production of the heteroprotein in the form of a positive feedback loop.



A 3D printer was used to create parts made of polylactic acid (PLA) for Victor the Vector. PLA is a Biodegradable polyester developed from various biological sources, such as corn starch, and is used in several plastic products, including cups, packaging material, and various parts of medical implants; because PLA is produced from biological sources it can be broken down and recycled, thus it is a desirable alternative to petroleum-based plastic. Hydrolysis of the polymer will degrade it back into lactic acid. The scrap pieces of PLA from the 3D printing were converted back into lactate to use in the *E. coli* media.



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iGEM 2014

AIT Bioscience

Victor the Vector

Victor the Vector was created to aid in teaching high school students around the world about synthetic biology and the ethical debate surrounding this emerging field. Victor the Vector is a hands on, interactive device that allows students to build basic synthetic biology systems and watch instructional videos that demonstrate how synthetic biologists take different genetic parts and put them together in a novel way to create a new synthetic system. We hope that this interactive device will allow students to gain a better appreciation for and understanding of this emerging science.

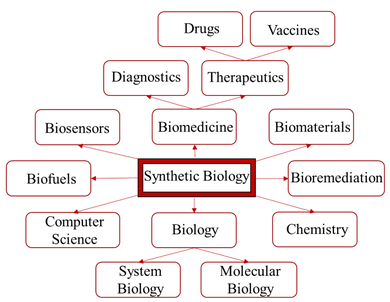
Potential Harms

Synthetic biology shows great promise, but there are potential harms associated with this emerging field.

Type of Harms	Definition	Example
Known	Potential physical harms that people realize are associated with synthetic biology	A synthetically engineered small pox virus is released into a population of people
Unknown	Potential physical harms that people can conceptualize, but lack the knowledge to fully understand	Not knowing how synthetically engineered bacteria will mutate
Unknown Unknown	Potential physical harms that given the human race’s current state of knowledge cannot be understood.	Humans cannot conceptualize these potential physical harms, so there are no examples.

Potential Benefits

Synthetic biology has many potential applications



For more information about the ethical debate surrounding synthetic biology please visit 2014.igem.org/Team:RHIT/Ethics or scan the QR code to the right.