AliveLIGHT™: Developing a Natural Light Generating Machine

Cate Barton, Anya Gemos, Meredith Hoole, Jackson Lundberg, Trey Marcantonio, Kenneth Moore, Amy Murrant-Johnson, Naama Perliger, and Ian Wood

BioBuilder Club, Tyngsborough High School, Tyngsborough, Massachusetts, USA

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Candles and light bulbs are commonly purchased forms of illumination, even though these lighting options can consume a considerable amount of energy and pose potential health risks, including the release of toxic heavy metals, susceptibility to contact burns, and an increased risk of fire. Our product seeks to replace these more hazardous and energy-consuming options with a self-sustaining biological desk lamp that lights up at night and recharges during the day, is equipped with a gas release valve, and is enclosed in shatter-proof glass. This allows for a more sustainable and eco-friendly method for natural luminescence. By transforming the well-characterized bread yeast *Saccharomyces cerevisiae* to include a copper-inducible promoter that regulates the expression of firefly luciferase, the goal of engineering a sustainable, biological light source will be accomplished. This system will be constructed through plasmid design, assembly, and integration, followed by an evaluation of luminescence, niche engineering, and system optimization. Here, work towards plasmid assembly will be shown. This phase will require the amplification of the copper-inducible promoter and luciferase-containing vector, Gibson assembly of PCR products, and transformation of yeast. In preparation for this phase, a proof of concept transformation was conducted, resulting in the successful expression of tryptophan in red, orange, yellow, and white color variants of the yeast.

Keywords: *Yeast, light, luciferase, self-sustaining, eco-friendly, Chlamydononas reinhardtii*

Mentors: Rebekah Ravgiala and Michael Sheets
Direct correspondence to rebekah.ravgiala@tyngsboroughps.org

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Background

The purpose of AliveLIGHT™ is to reduce the hazards that other light sources create including, but not limited to, the emission of toxic heavy metals, susceptibility to contact burns, and an increased risk of fire (Ahrens 2019). AliveLIGHT™ is a biological system that will act as a replacement for other luminescent technology, reducing the emission of greenhouse gases into the environment and thus slowing the process of global climate change.

In order to construct this system, *Saccharomyces cerevisiae* will first be engineered to express luciferase for the production of light. In order to create a sustainable system, the re-programmed yeast will be cultured with *Chlamydononas reinhardtii* to induce an artificially engineered niche. *C. reinhardtii* is a protist that produces ammonia in the presence of potassium nitrite, which is supplied in the growth media. The reprogrammed yeast will consume the ammonia generated, which will serve as a nitrogen source for the production of essential amino acids. Conversely, the carbon dioxide produced by the yeast will provide the carbon source for the photosynthetic *C. reinhardtii*. In this way, a fabricated symbiotic relationship will be established. To sustain this relationship in the long term, glucose will be required to promote cellular respiration in the yeast.

To regulate the expression of luciferase, a copper-inducible promoter was chosen for both its accessibility and practicality. Gene regulation is important in developing a genetically engineered system because it allows for greater consumer control over the final system. By adding copper (II) sulfate to this system, luciferase production is induced, while in its absence, luciferase production is repressed. The CUP1 promoter works to activate downstream transcription by binding copper I ions produced from the reduction of copper (II) sulfate.

Although copper is a trace element, it is vitally important to biological systems. It is a component of nearly every tissue in the human body, plays a role in immunity and nerve function, assists with cellular respiration as a required cofactor for mitochondrial, cytosolic, and vesicular oxygen-processing enzymes, and protects against oxidative stress (Pufahl et al. 1997; Yasokawa et al. 2008). Copper ions are essential for life, but even in small concentrations they can be highly toxic. Therefore, copper toxicity testing in *S. cerevisiae* and *C. reinhardtii* must first be conducted to determine the optimum concentration of copper (II) sulfate tolerated by the system.

Systems Level

On a systems level, we plan to use *C. reinhardtii* to establish a mutualistic relationship with the genetically modified *S. cerevisiae*. This mutualistic relationship relies on the exchange of molecules to prevent the AliveLIGHT™ unit (Figure 1) from filling up with carbon dioxide while continuing to support yeast growth. *C. reinhardtii* produces ammonia that the yeast uses to generate essential amino acids while also consuming the carbon dioxide produced by the yeast during cellular respiration. In order to ensure the health and sustainability of the living unit, a master mix of glucose (to feed the yeast), potassium nitrite (to promote ammonia production by *C. reinhardtii*), and copper (II) sulfate (the copper source) is needed to sustain the transcription of luciferase.

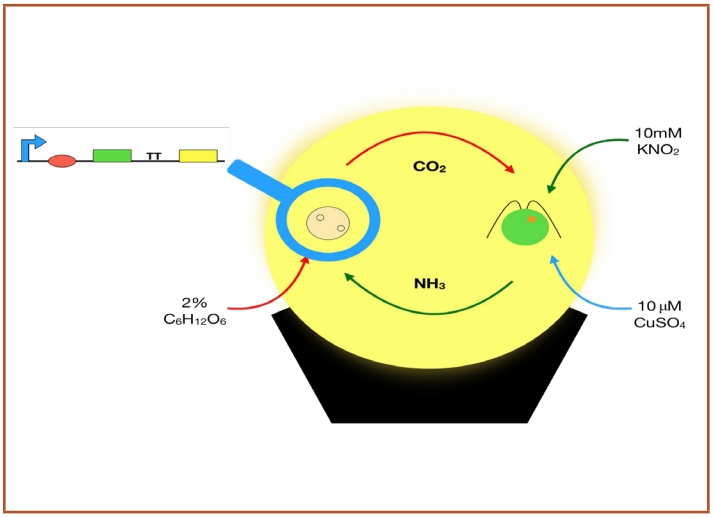

Figure 1. *Systems level representation.*

Device Level

On a device level, Cu2+ (from CuSO4) is first reduced into Cu+ before Cu+ is transported across the cellular membrane through transport proteins. Intracellularly, Cu+ promotes the transcription of Fluc, the open reading frame that codes for luciferase expression, by binding to the CUP1 promoter. When luciferase complexes with the substrate D-luciferin in the presence of ATP and oxygen, adenosine monophosphate, carbon dioxide, inorganic phosphate, oxyluciferin, and light are formed (Branchini, 2013). This is depicted in Figure 2.

Parts Level

CUP1 is a copper-inducible promoter that enables the activation of downstream transcription when Cu2+ is present in the growth media. At the parts level, a strong ribosome binding site is necessary for high copy translation of luciferase to increase the intensity of light production. This involves an RNA sequence found in mRNA to which ribosomes can bind and initiate translation. Downstream of the ribosome binding site is the coding sequence for firefly luciferase. The coding sequence will be transcribed into

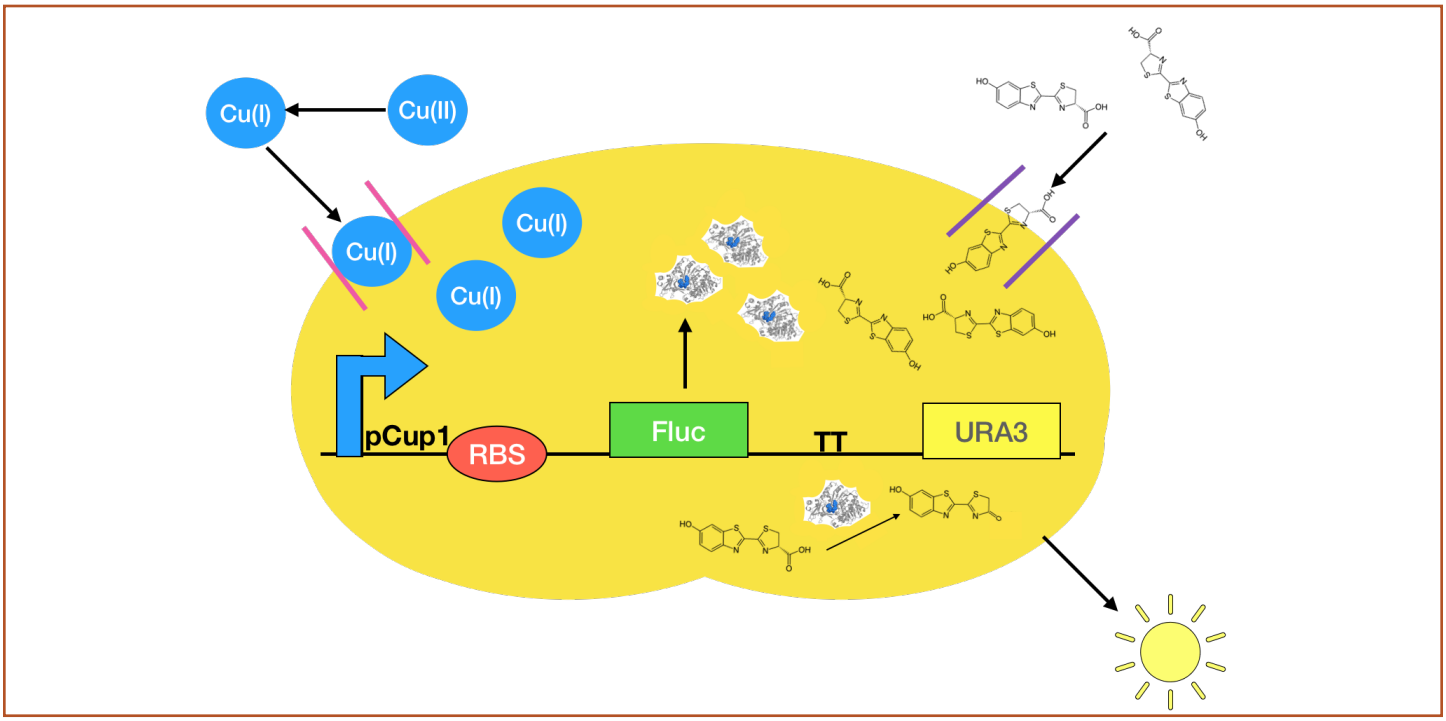

Figure 2. *Device level description.*

mRNA and ultimately translated into a functional protein for the expression of light. Since luciferase is typically imported into peroxisomes for enzyme substrate interaction, the light produced is not expected to be intense (Leskinen et al. 2003). Modification of luciferase with a serine, lysine, and leucine at the C-terminus of the protein, which would inhibit transport into peroxisomes for optimal light emission, will be considered. In order to stop transcription, a terminator is required, which is a genetic part usually occurring at the end of the gene or operon. The terminators present in this circuit include T7, a sequence with a bacteriophage origin that allows for efficient transcriptional termination, and RrnG, a bacterial terminator. Generally, BioBricksTM parts would be used, but we are starting with a pre-assembled, firefly luciferase yeast vector donated by Oxford Genetics (catalog number OG545) that we will edit by replacing the existing promoter with the CUP1 promoter (donated by Dr. Natalie Kuldell). The final genetic construct is depicted in Figure 3.

Safety

There are safety protocols that our potential customers need to be aware of since our product will contain potassium nitrite and copper sulfate in the master mix, which are potentially hazardous (Ware 2017). On a device level, the assembled product will include a kill switch designed so that the engineered biotechnology can survive only in the shatterproof glass chamber. This will decrease the likelihood that the engineered yeast will make its way into the environment. In this way, if any components of the mixture are accidentally discharged from the chamber, the kill switch will activate a sequence of biochemical reactions leading to the eradication of the biohazard. The exact components involved have not yet been determined for the kill switch. In comparison to other light sources, this biological light source lacks the hazard of an open flame, the negative biological impacts involving the disposal of hazardous items like light bulbs and batteries, and the potential hazard of heat production in the home.

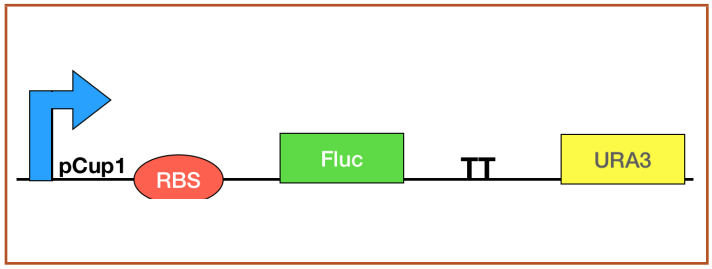

Figure 3. *In this parts level description of firefly luciferase, the pCUP1 promoter will be turned on in the presence of copper and will allow for downstream expression of the open reading frame. An auxotrophic marker is also included as a way to select yeast colonies that have successfully taken up the engineered plasmid when grown in uracil deficient environments.*

Discussion

The purpose of the AliveLIGHT™ system is to produce a safe, long-lasting light source using genetically modified yeast. Before working towards the goal of a functioning AliveLIGHT™ system, a consumer analysis survey was administered using a Google Form. The total number of

respondents participating in this survey was 107, ranging in age from 13 to over 55 years old. In response to the question "Would you still be interested in this product if you knew that it contained genetically modified yeast?", 46.7% of respondents answered "yes", 15.9% answered "no", while 37.4% answered "maybe." This suggests that while there is still some uncertainty about purchasing genetically modified products in the marketplace, many are receptive to the idea. This further suggests that there will be a space, and possible demand, for a product of this nature in the existing market if one remains mindful of the implications related to knowledge about, and acceptance of, genetically modified products.

As with any new project, there are potential, unanticipated limitations. The first limitation that might be problematic is the brightness of luciferase. Compared to existing light sources, the intensity of biologically produced light may not be competitive. In order to characterize the intensity of light produced by AliveLIGHT™, the biological system must be tested with the use of a luminometer. The results will be analyzed and strategies for optimizing the light intensity will be determined. In addition to light intensity, the rate at which the system requires nutrient replenishment yields yet another limitation. The objective of this final system is to offer a biological light source that is self-sustaining. In its present form, the system requires nutrient supplementation in the form of a complete master mix composed of copper sulfate, potassium nitrite and glucose.

Lastly, since copper is a known biological toxin at high concentrations, it is necessary to test the tolerance of the yeast (Yasokawa et al. 2008). Copper sulfate is a required component in the master mix because it regulates the production of luciferase by inducing the CUP1 promoter. Currently, copper toxicity tests are being conducted to determine the tolerance range of the yeast. Preliminary results at concentrations of 10 mM did not suppress yeast growth on yeast peptone dextrose (YPD) plates. This is likely due to the high concentration of yeast cells plated. Future testing will employ the use of serial dilutions. Both 1:10 and 1:100 dilutions will be plated and subsequently incubated at 30°C. Colonies will be counted and plate coverage will be analyzed using ImageJ, an imaging processing program. The experimental results will confirm the copper sulfate tolerance range in yeast. Since the final assembly of AliveLIGHT™ involves the establishment of a mutualistic relationship between yeast and a photosynthetic protist, similar experiments will be conducted with *C. reinhardtii* during artificial niche engineering.

To move forward in the construction of AliveLIGHT™, the following phases will be executed: 1. Plasmid design, 2. plasmid assembly, 3. plasmid integration, 4. evaluation of luminescence, 5. niche engineering, and 6. system optimization. Currently, the project is entering the assembly phase. This will be accomplished through DNA amplification by polymerase chain reaction followed by Gibson assembly and will culminate with the introduction of this final genetic construct into a *S. cerevisiae* chassis by way of transformation. After engineering an artificial niche utilizing both *C. reinhardtii* and *S. cerevisiae*, a more sustainable and eco-friendly form of luminescence in the form of AliveLIGHT™ will be ready for market.

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