In biotechnology, as with any manufacturing process, the overall goal is to maximize output while minimizing inputs. As a group, the VCU iGEM team sought to implement and improve the efficiency of isoprenoid production, in the cyanobacterium *Synechococcus elongatus* PCC7942. These organisms grow almost exclusively on water, carbon dioxide and light, eliminating most expenses from inputs. As well, since these organisms utilize a circadian rhythm, we postulated that we could induce increased production and fitness, by gating production to the ‘dark’ cycle of the circadian rhythm. Our target molecule belongs to an extremely diverse class of molecules known as terpenes, terpenoids or isoprenoids, which are produced by enzymes known as terpene synthases. Some of these terpenoids are extremely valuable, such as Taxol® (cancer treatment), artemisinin (malarial treatment), and Famesol (fuel). We decided to attempt to produce Nerolidol, an isoprenoid that has been identified as a component in traditional delivery of drugs, fragrances and flavorings, and as a pesticide of Aedes aegypti larvae – the mosquito responsible for the spread of Dengue fever. To carry out this project we decided to divide it into several sub-projects. The first project was to perform the molecular biology needed to assemble and express our gene of interest. The second part of the project focused on a bioinformatics approach to identify terpene synthases and relative promoter strengths. The final part of our project was to investigate the circadian rhythmic properties in *Synechococcus elongatus* to allow gating of terpene production to the dark cycles. This sub-project involved constructing a small library of circadian-responsive promoters, and characterizing them using a dynamic reporter.

We designed our cassette sequence in 60 base pair, single-stranded oligonucleotides with 20 base pair overlaps. With these oligos we attempted several different assembly methods including polymerase chain assembly (PCA) and Gibson isothermal assembly. The PCA protocol consisted of two variants; a one-step assembly and a two-step assembly. In the two-step assembly the oligos are assembled in equimolarity, and then there is a subsequent PCR reaction using primers that land on the outside of the insert. We found that the two-step assembly protocol had the highest titers of correct assembly and the production of the fewest side-products.

To investigate the circadian behaviors of *Synechococcus* promoters we utilized the native *psbAI* promoter, which has been well documented to become expressed solely in the dark cycle of the circadian rhythm. Much of the control is from the use of a alternative -10-hexameric sequence. We designed two promoters from the *psbAI* promoter, one with the endogenous -10 sequence and one with the -10 mutated to the consensus sequence. Each of these promoters were assembled up stream of a superfolding GFP variant, known as turboGFP (or tgFP). These two constructs were also made with variants containing a ssrA-degradation tag, to assess the effect of increased degradation.

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