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# Contributions

## Cyanobacteria in the Registry

We created new parts for future iGEM teams choosing to work with cyanobacteria. This model organism is ideal for studying photosynthesis due to its well characterized metabolic pathways and natural competency that readily allows transformation. Cyanobacteria are relatively low-cost and easy to maintain, as well as extremely safe experimental organisms as highlighted on our Safety page. Overall, these features make cyanobacteria an excellent choice as a model or chassis, especially in synthetic biology.

The iGEM Registry currently has few parts specific for cyanobacteria, contrasting with the well characterized *Escherichia coli* and *Saccharomyces cerevisiae*. This lack of available constructs for cyanobacteria limits future photosynthetic-focused iGEM work, as these heterotrophic organism parts are usually not as effective or applicable when applied to cyanobacteria. This shortage requires major expansion, reorganization, and characterization of parts and tools to expand future iGEM opportunities and photosynthetic research.

As part of our project, we created three overexpression plasmids and one deletion plasmid, detailed below. Within these plasmids, consisting of several composite parts, we added numerous basic parts involving regulatory and expression functions within cyanobacteria. All our registered parts are detailed below in contextual descriptions, with technical details being on the linked registry pages.

## Parts Registered

### Overexpression Plasmids

Our overexpression plasmids were created via Gibson Assembly, of which we registered all relevant parts. These plasmids were all used within *Synechococcus elongatus* PCC 7942 with intended alterations to the Calvin-Bensen-Bassham (CBB) cycle.

[BBa\_K4047000](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047000) and [BBa\_K4047001](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047001) are primers for amplifying the transaldolase (*tal*) gene ([BBa\_K4047034](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047034)) from wild type *S. elongatus* PCC 7942. The primer contains EcoRI and BamHI restriction enzyme sites for confirmation testing. Transaldolase participates in the CBB cycle by catalyzing the forward and reversion conversion of sedoheptulose-1,7-bisphosphate and glyceraldehyde-3-phosphate into erythrose-4-phosphate and fructose-1,6-bisphosphate. In a similar fashion, [BBa\_K4047002](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047002) and [BBa\_K4047003](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047003) are primers for amplification of the fructose-1,6-bisphosphatase (*fbp*) gene ([BBa\_K4047035](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047035)). They also contained the EcoRI and BamHI restriction enzyme sites. Fructose-1,6-bisphosphatase catalyzes the hydrolysis of a phosphate group from fructose-1,6-bisphosphate. This process, part of the CBB cycle, is also vital to many other central metabolic pathways.

Additional primers were designed so that both tal and fbp, once amplified, could be inserted in a third overexpression plasmid ([BBa\_K4047004](http://bba_k4047004) and [BBa\_K4047005](http://bba_k4047005)).

All our overexpression plasmids used pAM2991 as the expression vector ([BBa\_K4047024](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047024)). This vector was created by Susan Golden in 2005 specifically for *S. elongatus* PCC 7942, and is publically available on [addgene.org](https://www.addgene.org/40248/). Its multiple components include an origin of replication ([BBa\_K4047029](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047029)), a trc promoter ([BBa\_K4047032](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047032)), an rrnB T1 terminator ([BBa\_K4047025](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047025)), an rrnB T2 terminator ([BBa\_K4047026](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047026)), a base of mobility region ([BBa\_K4047028](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047028)), a lac repressor ([BBa\_K4047031](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047031)), a lacIq promoter ([BBa\_K4047030](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047030)), a lac operator ([BBa\_K4047033](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047033)), and spectinomycin/streptomycin resistance ([BBa\_K4047027](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047027)). Sequences homologous to the neutral site I region allow for these components along with our target genes to be inserted into the genome of *S. elongatus* PCC 7942.

Our first plasmid, called pGEM-tal ([BBa\_K4047036](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047036)), aimed to induce transaldolase overexpression by inserting an additional copy of the encoding gene, *tal* (Figure 1).

Our second plasmid, named pGEM-fbp ([BBa\_K4047037](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047037)), induced fructose-1,6-bisphosphate overexpression by inserting an additional copy of the encoding *fbp* gene (Figure 2).

Finally, our third overexpression plasmid, pGEM-tal+fbp ([BBa\_K4047038](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047038)), combined the encoding elements of the above overexpression plasmids, aiming to insert both *tal* and *fbp* genes to overexpress both genes (Figure 3).

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### Deletion Plasmid

Our deletion plasmid, named pGEM-SBPase ([BBa\_K4047039](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047039)), was designed to delete sedoheptulose-1,7-bisphosphate (*glpX*) from the *S. elongatus* PCC 7942 genome through homologous recombination with upstream ([BBa\_K4047022](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047022)) and downstream ([BBa\_K4047023](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047023)) sequences (Figure 4). Two sets of primers ([BBa\_K4047009](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047009) and [BBa\_K40470010](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047010), [BBa\_K4047013](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047013) and [BBa\_K4047014](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047014)) were used to amplify 800 base pair upstream and downstream fragments of *glpX* from the *S. elongatus* genome. We designed additional primers ([BBa\_K4047007](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047007) and [Bba\_K4047008](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047008)) to amplify the backbone of pXWK3-glgC. This backbone is well-established as a vector within the Wang Microbiology Lab at Miami University, used in a [previous publication](https://academic.oup.com/plphys/article/182/1/507/6116245). The backbone ([BBa\_K4047018](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047018)) contains an origin of replication and a gentamicin resistance gene ([BBa\_K4047016](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047016)), promoter ([BBa\_K4047015](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047015)), and terminator ([BBa\_K4047017](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047017)). In addition, we used primers ([BBa\_K4047011](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047011) and [BBa\_K4047012](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047012)) to amplify a kanamycin resistance cassette from pXWK3-glgC ([BBa\_K4047019](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047019), [BBa\_K4047020](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047020), [BBa\_K4047021](http://parts.igem.org/wiki/index.php?title=Part:BBa_K4047021)).

### Part compatibility

Our parts have a variety of compatibility detailed in Table 1. The vast majority of our parts are compatible with all RFC standards. All our plasmids, due to possessing various restriction sites, are not RFC compatible. Several of our components, despite having limited RFC compatibility, may be extremely useful for future cyanobacteria teams due to their vast array of functions, suitability for the widely adaptable and accessible pAM2991 vector, and handling of the central metabolic genes *tal* and *fbp*.

Table 1: Complete list of registered parts and their compatibilities within the iGEM registry.

| **Parts** | **Compatability** |
| --- | --- |
| BBa\_K4047000, BBa\_K4047001, BBa\_K4047002, BBa\_K4047003 | **RFC[1000]** |
| BBa\_K4047004, BBa\_K4047005, BBa\_K4047007, BBa\_K4047008, BBa\_K4047009, BBa\_K4047010, BBa\_K4047012, BBa\_K4047013, BBa\_K4047014, BBa\_K4047016, BBa\_K4047017, BBa\_K4047019, BBa\_K4047020, BBa\_K4047025, BBa\_K4047026, BBa\_K4047028, BBa\_K4047029, BBa\_K4047030, BBa\_K4047031, BBa\_K4047032, BBa\_K4047033 | **RFC{10][12][21][23][25][1000]** |
| BBa\_K4047011, BBa\_K4047021 | **RFC[21][1000]** |
| BBa\_K4047015 | **RFC{10][12][21][23][25]** |
| BBa\_K4047018, BBa\_K4047022, BBa\_K4047024, BBa\_K4047035, BBa\_K4047036, BBa\_K4047037, BBa\_K4047038, BBa\_K4047039 |  |
| BBa\_K4047023, BBa\_K4047027 | **RFC{10][12][21][23][1000]** |
| BBa\_K4047034 | **RFC[10][21][23]** |

Figure captions

Figure 1: Annotated SnapGene viewer of pGEM-tal.

Figure 2: Annotated SnapGene viewer of pGEM-fbp.

Figure 3: Annotated SnapGene viewer of pGEM-tal+fbp.

Figure 4: Annotated SnapGene viewer of pGEM-SBPase.

## Wiki Development

Content