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CAS9-BASED PLASMIDS FOR ENGINEERING SYNECHOCYSTIS SP. PCC 6803 CYANOBACTERIA

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These plasmids were developed by Washington University researchers as tools for genetically engineering *Synechocystis* sp. PCC 6803 (S6803), a model cyanobacterium used commonly in research and biotechnology applications. Because S6803 is polyploid, genetic modification of this strain typically requires multiple rounds of culturing with increasing concentrations of antibiotics to select for mutants that have the desired mutation on all copies of the chromosome. Used together, these plasmids utilize the Cas9 endonuclease to reduce the number of required culturing rounds to one, enabling one‐step, segregation‐free engineering. First, the pYX170 (p6803‐cas9) plasmid is used to integrate Cas9 into the genome. Specifically, this suicide plasmid contains a pBR322 replication origin and the Cas9 insertion cassette, which includes the cas9 gene, kanamycin and tetracyline resistance genes, an sgRNA targeting the neutral site slr0168 in the S6803 genome, and RecJ recombination arms. Next, the pYX226 (pCCM1‐FbFP) shuttle vector, which was engineered from the native pCC5.2 plasmid, is used to introduce a desired heterologous gene sequence into S6803 for expression. This plasmid contains the flavin mononucleotide (FMN)-binding fluorescent protein (FbFP) reporter gene (to be replaced by another desired gene), a spectinomycin resistance gene, and the pBR322 replication origin (allowing plasmid construction in *Escherichia coli*). These tools allow researchers to quickly introduce heterologous genes into S6803, with the heterologous DNA remaining stable for at least 30 days without maintenance via selective pressure.

Publication

Developing a Cas9-based tool to engineer native plasmids in Synechocystis sp. PCC 6803