Signal Peptides for extracellular protein transport in *Synechocystis* sp. PCC6803

Introduction

Production of proteins with clinical or industrial value can be performed by bacterial cell cultures. Once synthesized, however, problems may arise in recovering these recombinant proteins in substantial yields. Protein recovery oftentimes requires the cells to be lysed in order to recover the protein of interest, thus limiting the amount of product to a one-time yield of the total biomass that can be maintained at a given time. Production can be optimized if multiple yields can be collected over time from a living cell culture.[1] Ideally, this can be done by transporting the protein product out of the cell and collecting it from the growth medium.

Background

Cyanobacteria are defined by their ability to photosynthesize and can be engineered to synthesize substances of biotechnological interest. Thus cyanobacteria provide the opportunity for autotrophic protein production. For example, the Gram-positive bacterium *Bacillus subtilis* has been used to produce high quality industrial enzymes and a few eukaryotic proteins via secretion into the growth medium.[2] Alternatively, *Synechocystis* sp PCC6803 is a gram-negative bacterium that has undergone continuous study. The genome of *Synechocystis* has been completely sequenced[3], making it a model organism for the exploration of secretion in gram-negative bacteria.[4]

The major obstacle for secretion of proteins in gram-negative bacteria is the additional outer membrane with a cell wall made of the peptidoglycan murein cell. As a Gram-negative bacterium, *Synechocystis* sp. PCC6803 cells possess a functional periplasm between its inner membrane and outer membrane[5]. Proteins destined for extracellular secretion must be transported through both of these membranes and the periplasmic space.

Proteins are known to be secreted via a number of different pathways.[6] The simplest way to have PCC 6803 act as a factory for protein production is to take advantage of naturally existing protein secretion pathways in the organism.

Seven distinct proteins have been found to be secreted by *Synechocystis* sp. PCC6803 into its culture medium under normal growth conditions[7]. Analysis of the amino-terminal sequences of these proteins allowed Sergeyenko and Los to identify the proteins' signal polypeptides. Additional studies have produced recombinant cyanobacterial strains that use PCC6803 signal peptides to secrete a foreign reporter protein, lichenase, into the culture medium[8]. (See Figure 1)

Fig. 1 Oligonucleotides and corresponding signa l peptides experimentally proven to result in protein secretion in *Synechocystis* PCC 6803. Courtesy of Seryenko and Los

Objective

To create BioBricks encoding signal peptides that can be combined with a protein coding sequence in order to express the protein of interest extracellularly. The ability of the signal peptide to export a protein will be tested by combining it in a BioBrick device with the BioBrick part for Green Fluorescent Protein (GFP) and a BioBrick part of the nitrate-inducible *nirA* promoter.

Materials/Methods

All BioBrick precursors and parts will be amplified in *E.coli* before being assembled into a single plasmid and introduced into *Synechocystis* sp. PCC6803 via transformation or conjugation.

Step 1. Synthesize signal peptide oligonucleotides in BioBrick format

The following signal peptides will be synthesized artificially with the appropriate BioBrick restriction enzyme prefix and suffix:

Table 1. Signal peptide BioBricks to be synthesized

Gene	Nucleotide Sequence 5' → 3', (BioBrick restriction site sequences in bold)
pilA	***GAATTCATCTAGA***ATGGCTAGTAATTTTAAATTCAAACTCCTCTCTCAACTCTC CAAAAAACGGGCAGAGGTA***ACTAGTACTGCAG***
slr2016	***GAATTCATCTAGA***ATGGCAGCAAAACAACTATGGAAAATTTTCAATCCTAACC GATGAAGGGTGGAA***ACTAGTACTGCAG***

The nucleotides coding for the following putative signal sequences will also be artificially synthesized in BioBrick format for testing: *Sll1694, Sll10044, Slr0841.*

Step 2. Isolate and purify nirA promoter in BioBrick format

The 380bp *nirA* promoter will be isolated from the *Synechocystis* sp. PCC6803 genome and converted to BioBrick format by PCR[9].

Table 2. *Synechocystis* sp. PCC6803 *nirA* promoter sequence and proposed forward and reverse primer sequences

Type	Nucleotide Sequence 5' → 3' (BioBrick restriction site sequences in italics)
nirA promoter	CTAAATGCGTAAACTGCATATGCCTTCGCTGAGTGTAATTTACGTTACAAATTTTA ACGAAACGGGAACCCTATATTGATCTCTAC
Forward (primer)	***GAATTCATCTAGA***TGTAGGTACCCAACCTCAGAATGCTGC
Reverse (primer)	CAACGGTACCAGCCAGATAACAGTAGAGAT ***ACTAGTACTGCAG***

Step 3. Contstruct devices and ligate into BioBrick vector

The synthesized signal peptides and *nirA* promoter BioBricks will be combined with four existing BioBricks to create two (or more) nitrate-regulated protein secretion devices according to the scheme in figure 2. The resulting devices will be placed in a *Synechocystis* compatible BioBrick vector derived from the RSF1010 derived plasmid pRL1383a.

Table 3. Existing BioBrick parts required for proposed device

Part Number	Part Number
B0034	Ribosomal Binding Site (RBS)
E0040	GFP
B0010	Transcription Terminator
B0012	Transcription Terminator

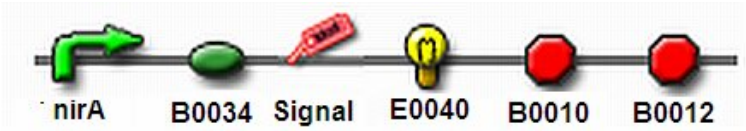

Fig. 2 Proposed nitrate-regulated protein secretion device for Syn. PCC6803.

In the proposed devices, the signal peptides will be situated so they are in-frame with the GFP. The translated polypeptide should consist of a signal polypeptide leader sequence attached to a fluorescent protein.

Step 4. Test for Protein Secretion

The BioBrick vector can be inserted into *Synechocystis* sp. PCC6803 by transformation, conjugation, or electroporation.

Plated *Synechocystis* sp. PCC6803 colonies successfully transformed would exhibit a glowing halo of secreted GFP. Transformed *Synechocystis* sp. PCC6803 grown in liquid media would result in fluorescent culture media. The efficacy of the signal peptides in transporting GFP into the extracellular media can be measured using a spectrofluorometer.

Sources Cited

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