An RBS library in cyanobacteria to boost biocatalytic activity

Julia Jodlbauer, Florian Rudroff, Marko D. Mihovilovic

Institute of Applied Synthetic Chemistry, TU Wien, Getreidemarkt 9/163, 1060 Vienna / Austria

E-mail: julia.jodlbauer@tuwien.ac.at

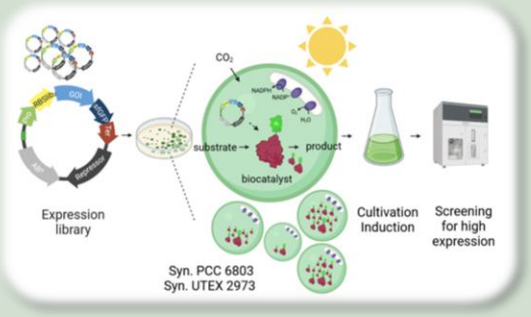
Introduction

Cyanobacteria are promising candidates for microbial biocatalysts. With our interest in redox-enzymes, their photoautotrophic metabolism equips them with unique advantages [1].
During photosynthesis

(i) they produce great amounts of high-energy cofactor NADPH, which can be used directly to drive enzymatic reactions [2]

(ii) oxygen is produced as by-product, which can feed oxygen-driven reactions [3].

Despite these great advantages, there are still remaining challenges. On the one hand, obtaining sufficient expression levels of recombinantly expressed genes within cyanobacteria is still challenging. On the other hand, high throughput screenings are limited by their special cultivation conditions.



General aim

Based on these major limitations, we developed a strategy, which aims to tackle both bottlenecks.

A ribosome binding site (RBS) library

- To test multiple genetic constructs for improved expression

A GOI-sfGFP fusion protein

- To enable high throughput screening of diverse genetic constructs

With this, we aim to improve the performance of biocatalytic enzymes in the cyanobacterial strains Syn. PCC 6803 and Syn.UTEX 2973.

As enzymes, we investigate

- an alcohol dehydrogenase **(RR-ADH)** [4]
- a ketoreductase **(LfSDR1M50)** [5]
- an enoate reductase **(YqjM)** [6],
- and a Baeyer-Villiger monooxygenase **($CHMO_{Acineto}$ S7)**

Library design – Cloning strategy

Library design:

- RBS library: 20 RBSs from 5 different studies
- RiboJ: genetic insulator (promises more reliable expression)
- Promoter: Ptrc10 (IPTG inducible)
- sfGFP: superfolder green fluorescent protein

Cloning strategy:

- Based and compatible with the CyanoGate cloning kit [7]

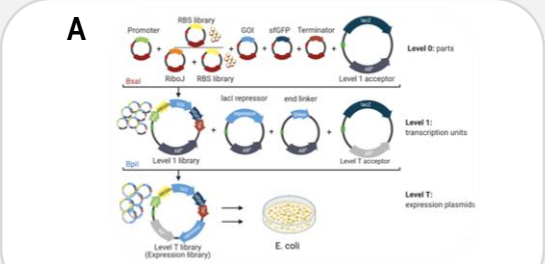


Figure A: Cloning strategy based on CyanoGate Kit. MoClo assembly of individual genetic parts (including RBS library) in Level 1 cloning with BsaI. Assembly of transcription cassettes (GOI- and lacI transcription cassette) via BpiI in Level T cloning.

Check 1: An approach to clone them all..?!

- **An RBS library is prepared for each investigated enzyme (with and without RiboJ)**
- **To evaluate the performance of the RBS library approach, we evaluated the cloning efficiency and the distribution of the individual RBSs.**
- **The cloning strategy is very efficient: 99.5% (748/752 transformants)**
- **The individual RBSs are represented in nearly all (RiboJ)-RBSlib:GOIs**

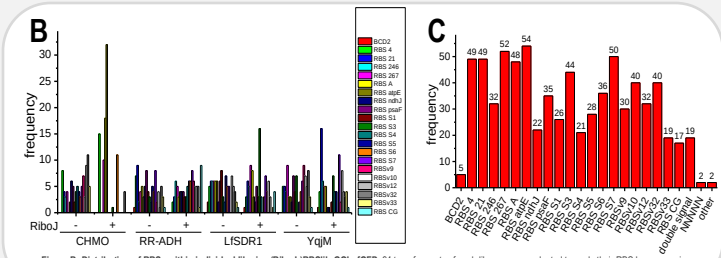
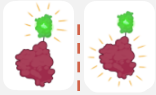


Figure B: Distribution of RBSs within individual libaries (RiboJ-)RBSlib:GOI-sfGFP. 94 transformants of each library were evaluated towards their RBS by sequencing.
Figure C: Frequency of individual RBSs within all 752 transformants. In average, each RBS should occur 36 times. Except BCD2, all RBS were represented well.

Check 2: Fluorescence of GOI-sfGFP



- **All GOI-sfGFP fusion proteins show fluorescence.**
- **A broad spectrum of different expression levels was achieved in *E. coli* - Screening of spectrum must be repeated for (RiboJ-)RBSlib:RR-ADH-sfGFP**

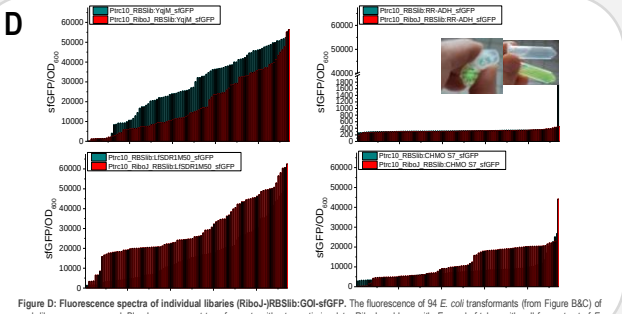


Figure D: Fluorescence spectra of individual libaries (RiboJ-)RBSlib:GOI-sfGFP. The fluorescence of 94 *E. coli* transformants (from Figure B&C) of each library was measured. Blue bars represent transformants without genetic insulator RiboJ, red bars with. Eppendorf tubes with cell free extract of *E. coli* pPMQAK1-Ptrc10_BCD2:RR-ADH-sfGFP (yellow) and *E. coli* wildtype (white) show the activity of sfGFP within RR-ADH-sfGFP fusion protein

Check 3: Activity of GOI-sfGFP

- **RR-ADH-,YqjM- & CHMO S7-sfGFP are active and show comparable activity to respective enzyme without sfGFP**
- **LfSDR1M50-sfGFP is not active – reclone with different linker design**

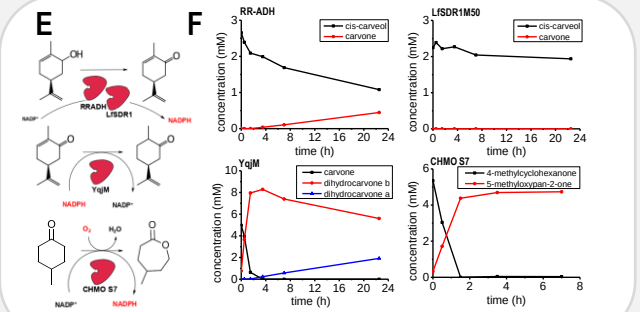
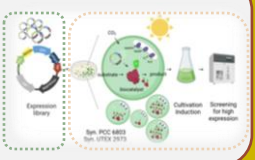


Figure E: Schematic biocatalytic reactions. Figure F: Biotransformations of GOI-sfGFP to confirm activity. Biotransformation of RR-ADH-, LfSDR1M50-, YqjM- & CHMO S7-sfGFP (from left to right, from top to bottom).

Conclusion – Next Steps

- **The RBS library proofs efficient in *E. coli***
- **The next step is to transfer and establish all experiments in Syn. PCC 6803 and Syn. UTEX 2973**



References

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Schematic illustrations were created with BioRender.com.