Creating a ready-to-use sigma factor toolbox for pathway control

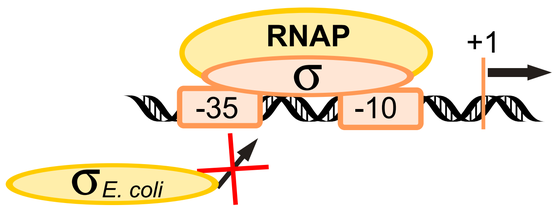
Maarten Van Brempt[1], Indra Bervoets[2], Jo Maertens[1], Daniel Charlier[2], Marjan De Mey[1]

[1] MEMO, Metabolic Engineering Group, Laboratory of Industrial Biotechnology and Biocatalysis, Ghent University
[2] MICR, Research group of Microbiology, Free University of Brussels (VUB)
Maarten.vanbrempt@ugent.be

Introduction

The field of synthetic biology is supplying biological engineers with tools to address the increasingly complex optimization questions handled. In this respect, the ability to tune heterologous gene expression independently from the native machinery is of prime importance. To this end, we aim to develop **orthogonal** non-native **Sigma factor**-promoter library combinations.

σ-factors associate with the RNAP core to form the RNAP holoenzyme, which results in an increased specificity for promoters, initiating transcription at the correct sites.

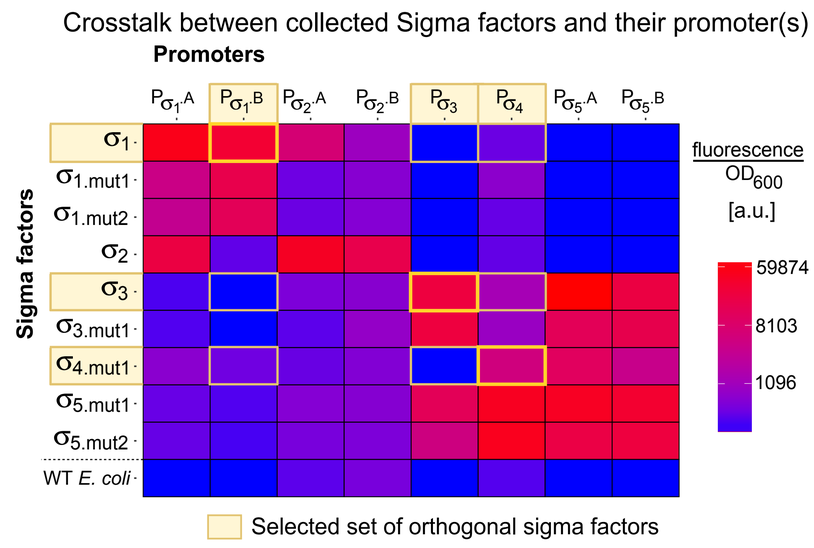


A sigma factor and corresponding promoter displaying orthogonality towards *Escherichia coli*

Set of orthogonal Sigma factors (σ)

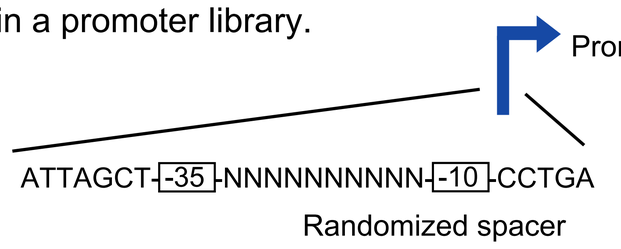
We searched *Bacillus* sp. for various sigma factor-promoter combinations. With a reporter gene under control of the promoters, undesired crosstalk could be detected by expressing all (non-)corresponding promoter -Sigma factor combinations in *E. coli*. (see map)

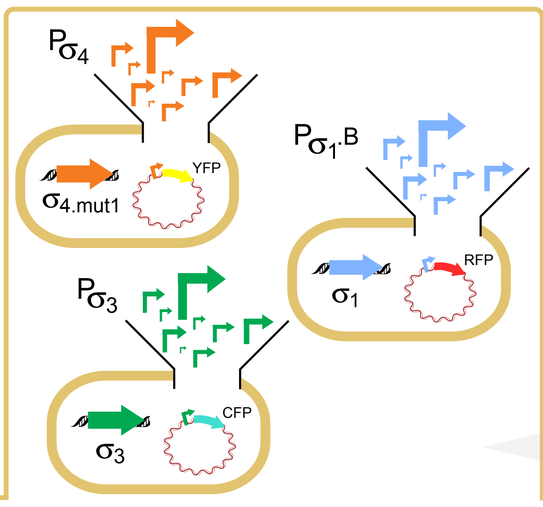
Based on these results, we selected 3 sigma factor-promoter combinations.



Promoter libraries construction

Promoters are characterized by a conserved -35 and -10 box. The spacer in between was randomized ('N'-nucleotides) to vary the affinity of the sigma factor for the promoter, which results in a promoter library.



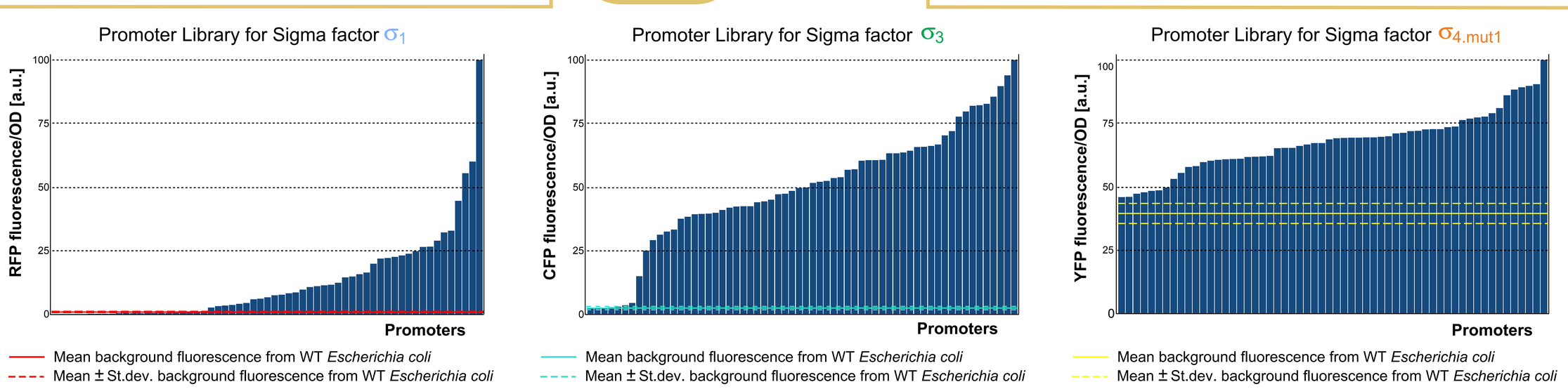


Selection of orthogonal libraries

1. By randomizing the spacer base pairs, loss of orthogonality can occur.
   First, colonies from the created library were randomly picked and screened on MTP scale for orthogonality.

2. Second, the orthogonal promoters were selected and the resulting libraries were characterized using three complementary fluorescent proteins (FPs).

Orthogonal promoter libraries



Issue(s)	Only relatively low promoter activities in library (± 3 times lower than original promoter)	Low and high expression regions under-represented	Only very low promoter activities in library (± 2x background) Very high WT *E. coli* fluorescence
Solution	Less stringent orthogonality criterion for screening Higher throughput screening method	Higher throughput screening method	Less stringent orthogonality criterion for screening Higher throughput screening method Change fluorescent protein reporter

Strategy 2.0

Design

Next to the 'fully-randomized spacer' libraries (scenario ①), also different partially-randomized libraries are created (scenario ②) for each sigma factor.

Evaluation

The different libraries are evaluated with Flow Cytometry (FC).
In case of ②, the library preserving the largest diversity with respect to ① is selected for further steps.

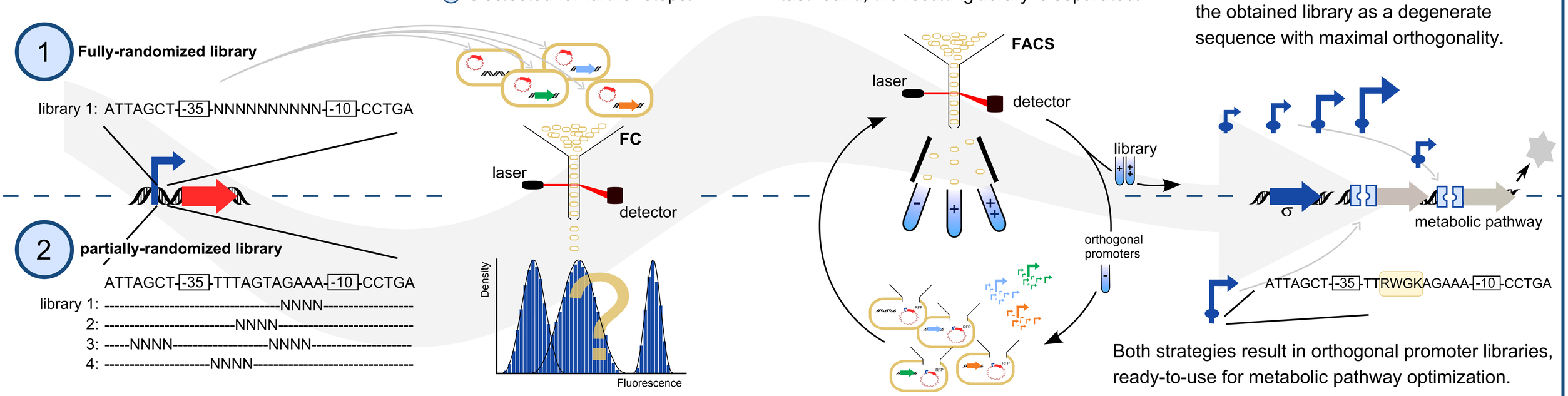
Selection

FACS is used in consecutive rounds, where in each round the promoter library is screened versus another Sigma factor, selecting all orthogonal promoters. In the last round, the resulting library is separated.

Result

① Following scenario 1, for each Sigma factor a defined set of fully characterized, orthogonal promoters will be obtained.

② Following scenario 2, the goal is to define the obtained library as a degenerate sequence with maximal orthogonality.



Both strategies result in orthogonal promoter libraries, ready-to-use for metabolic pathway optimization.

Acknowledgements

The authors wish to thank the Institute for the Promotion of Innovation through Science and Technology in Flanders (AIO-Vlaanderen) for financial support in the framework of the Ph.D grant of M. Van Brempt.
This research was also supported by project G.0321.13N (CondEx).