5.1 Genome Editing for therapy, biotechnological & food use

O.232. New genome-editing tools for *Ashbya gossypii*

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Ashbya gossypii is a splendid riboflavin biofactory that suppresses nearly half of the worldwide demand for vitamin B2. The availability of its genome sequence allowed a better understanding of this fungus' metabolism and the development of targeted genome editing tools. Such progresses allowed increasing the vitamin B2 natural production levels, and to expand the potential of this fungus towards the production of nucleosides, folic acid, single cell oil and fragrances [1,2]**.**

Now, with a broadening range of potential genetic targets, modular and flexible pathway assembly tools are more convenient than ever. There is also an urgent need for a larger set of well characterized promoters for metabolic flux optimization towards improved production of target products. As such, this work aimed at developing a modular, flexible and fast pathway assembly platform for targeted chromosomal integration in *A. gossypii* that offers a wide range of expression strengths (promoters).

The developed pathway assembly platform is in an early-stage, designed to easily allow fast cloning and exchange of selection markers, promoters, genes and terminators. As endogenous promoters provide the required regulatory elements for gene expression, several transcriptomic datasets were scanned for medium to high strength promoters. A set of uni- and bi-directional promoters was selected, and each promoter cloned into promoter-probes for characterization in terms of expression strength and regulation, a task that is under way using multiple reporter systems. Preliminary results revealed that the bi-directional promoter *AgCCW12*/*HOG1*p drives moderate gene expression through the *HOG1*p side and strong expression through the *CCW12*p side. The datasets were also used for transcription factor binding motif mining, in order to find important regulation elements that confer strong gene expression values. Five transcription factor binding motifs were identified as strong targets for promoter engineering. This information can hereafter enable the rational design of synthetic/semi-synthetic minimal promoters.

With the current genetic engineering turnaround time to genetically modify *A. gossypii* being a limiting factor to establish this fungus as a state-of-the-art microbial factory, this pathway

assembly platform has great relevance. With a growing number of promoters characterized, it will become increasingly easier to genetically channel the *A. gossypii* metabolism towards the production of value-added chemicals.

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