Synthetic biology strategies for the production of carotenoids in *Y. lipolytica*

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Yarrowia lipolytica is a well-known oleaginous yeast able to accumulate high amount of lipids. These lipids can be used as building blocks for the chemical and fuel industry and can replace petroleum. In *Y. lipolytica*, lipids are produced from Acetyl-CoA and are mainly stored in lipid bodies. This suggests that *Y. lipolytica* has a large pool of Acetyl-CoA and interestingly, this metabolite is the precursors of many other high value metabolites. For example, carotenoids are produced from Acetyl-CoA through the mevalonate pathway and they have a high market value. We thus decided to engineer Y. lipolytica to produce beta-carotene.

In order to do so, we first developed a Golden Gate library to allow us to quickly create expression cassettes of up to three transcriptional units using modular parts. In addition, this library allows us to make combinatorial assemblies, which are particularly useful with easy-to-screen molecules such as beta-carotene.

We used that tool to create a cassette for the heterologous expression of the genes required to produce beta-carotene. We additionally modified the mevalonate pathway in order to increase the precursors of the pathway. Then, we optimised the flux towards carotenoids using a combinatorial assembly method and the insertion of multiple copies of the expression cassettes. Finally, the best producer strain was grown on fed-batch in a 5 L bioreactor.

The generated strain was able to produce 6g/L of carotenoids one of the highest titers ever reported [1].

[1] Larroude et al. (2017) A synthetic biology approach to transform Yarrowia lipolytica into a competitive biotechnological producer of β-carotene. Biotechnol Bioeng 115(2) 464-472