Easy-MISE toolkit: a novel combination of synthetic biology tools towards Plant Natural Products production in *Saccharomyces cerevisiae*

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Abstract:
Plant Natural Products (PNPs) exhibit a wide range of applications as bioactive molecules in pharmaceutical, food and agriculture sectors. Despite that, they rarely reach the market since extraction from natural sources lead to the formation of mixtures of compounds and total chemical synthesis has low yields. Biotechnological production through the exploitation of microbial cell factories stands out as a possible solution. To facilitate the fast and effective engineering of novel cell factories expressing complex plant biosynthetic pathways, the application of synthetic biology principles of standardization, modularity and reusability is needed.
The goal of this project is the design, the construction and the test of a standardized and versatile system for the expression of complex PNPs heterologous biosynthetic pathways in *Saccharomyces cerevisiae*. We designed and built a novel combination of synthetic biology tools, named Easy-MISE toolkit (Easy-Modular Integrative Scaffold-ready Expression toolkit). This framework allows the building of double expression unit cassettes in a modular fashion, thanks to a novel Golden Gate Assembly framework with a more than 60 parts library. The synthetic constructs are designed to be easily integrated in *S. cerevisiae* genome by using a CRISPR/Cas9 marker-free technology. Furthermore, the toolkit allows the standardized colocalization of the desired enzymes on a scaffold protein, a well-established strategy to enhance heterologous productions.
The toolkit effectiveness has then been verified by its application to the reconstruction and the study of glucobrassicin biosynthetic pathway in yeast. The product is an indolyl-glucosinolate with cancer chemoprevention properties, naturally produced by Brassicaceae plants. We exploited the flexibility of the toolkit to build a panel of strains expressing different combinations of plant enzymes and scaffolding strategies in order to find which one maximizes glucobrassicin production. To verify and quantify the production, an LC-MS analysis of cultures supernatants is currently ongoing.