Triple expression system for efficient and controlled production of the enzymatic cascade for flavonoid rhamnosylation

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The traditional concept of biotransformations, conducted by unmodified whole-cell biocatalysts, is diminishing in significance in favour of genetically engineered microorganisms, recombinant enzymes, or enzymatic cascades. Artificially designed biocatalysts are engineered to be more efficient, robust, and easy-to-implement. Thus, the biocatalyst design is gaining well-deserved attention as a vital step of process optimisation. Here, we present a characterisation of a set of bacterial plasmids tailored for recombinant expression of enzymes of interest in the broadly used *Escherichia coli* or *Pseudomonas putida.*

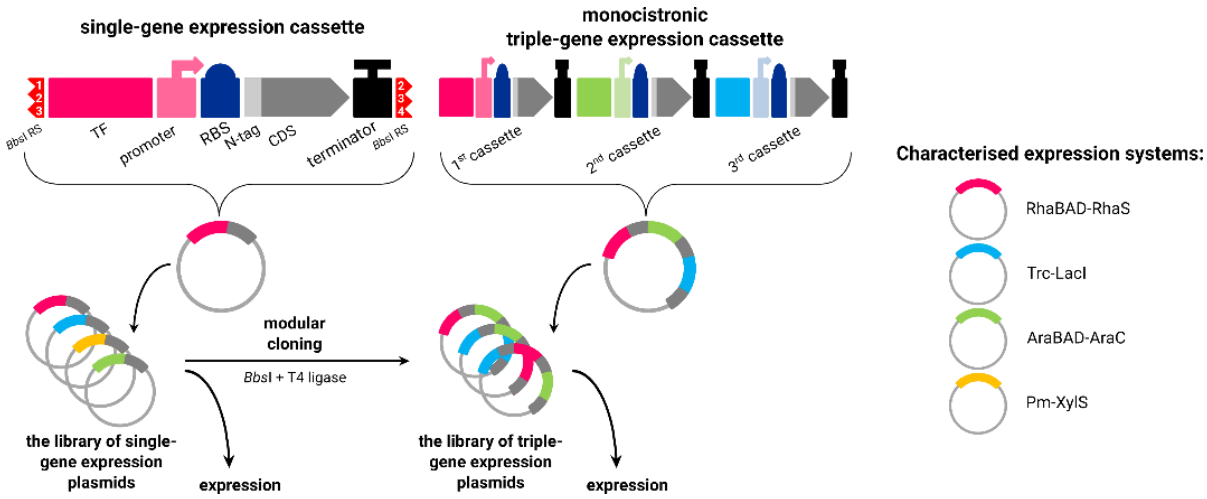

Figure 1. The framework of the expression cassettes design. Abbreviation: TF – transcriptional factor; RS – restriction site; RBS – ribosome binding site; CDS – coding sequence.

The plasmids set consists of four different bacterial expression cassettes (Fig. 1), which can be freely combined in up to three-gene expression plasmid using Golden Standard MoClo assembly [1]. Due to the independent induction of each cassette, it is possible to produce recombinant enzymes in desirable proportions, to get optimal cascade activity. The expression of triple-enzyme cascade consisting of sucrose synthase, UDP-rhamnose synthase and flavonol-7-*O*-rhamnosyltransferase was used as an example of designed plasmids utilisation.

References

[1]. B. Blázquez, D. S. León, J. Torres-Bacete, Á. Gómez-Luengo, R. Kniewel, I. Martínez, et al., *Nucleic Acids Research*, 51 (2023), e98; doi.org/10.1093/nar/gkad758

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