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A MODULAR SYNTHETIC BIOLOGY TOOLKIT IN *STREPTOMYCES* FOR FLAVONOIDS BIOSYNTHESIS AND CELLULAR CHASSIS ENGINEERING

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A library of modular plasmids has been created for flavonoids biosynthesis gene clusters (BGCs) assembly through Modular Cloning (MoClo) and *Streptomyces* genome edition *via* CRISPR-Cas9 technology. For BGCs assembly, plasmids of different levels have been developed. Thus, Level 0 plasmids consist of a library of genetic parts (promoter, RBS, CDS, terminator), that can be assembled into Level 1 plasmids to create transcription units, and these ones into Level 2 plasmids to create BGCs, which can be delivered through genomic integration into four different specific sites of the *Streptomyces* chromosome. For genome edition, plasmids encoding the CRISPR-Cas9 machinery have been developed, enabling improved flavonoids production titers by chassis genetic engineering. By using this toolkit, a synthetic naringenin BGC (comprising four genes) has been integrated into ΦC31 site of *S. albus* chromosome and the transcription unit of a chimaera of flavone 3' hydroxylase-cytochrome P_{450} reductase into ΦBT1 site in order to achieve eriodictyol production. Improvement in eriodictyol titers was further accomplished by targeting *S. albus* MatABC endogenous system, in which malonate is imported by MatC, transformed to malonyl-CoA by MatB, and this converted to acetyl-CoA by MatA. Therefore, *matA* was knocked out by CRISPR-Cas9-mediated recombination, and malonate feeding experiments were conducted, leading to improved yields in eriodictyol.