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Cas9-Assisted Direct Cloning and Refactoring of a Silent Biosynthetic Gene Cluster

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Abstract : Natural products produced from marine bacteria serve as an immense reservoir for anti-infective drugs and therapeutic agents. Nowadays, heterologous expression of gene clusters of interests has been widely adopted as an effective strategy for natural product discovery. Briefly, the heterologous expression flowchart would be: biosynthetic gene cluster identification, pathway construction and expression, and product detection. However, gene cluster capture using traditional Transformation-associated recombination (TAR) protocol is low-efficient (0.5% positive colony rate). To make things worse, most of these putative new natural products are only predicted by bioinformatics analysis such as antiSMASH, and their corresponding natural products biosynthetic pathways are either not expressed or expressed at very low levels under laboratory conditions. Those setbacks have inspired us to focus on seeking new technologies to efficiently edit and refractor of biosynthetic gene clusters. Recently, two cutting-edge techniques have attracted our attention - the CRISPR-Cas9 and Gibson Assembly. By now, we have tried to pretreat Brevibacillus laterosporus strain genomic DNA with CRISPR-Cas9 nucleases that specifically generated breaks near the gene cluster of interest. This trial resulted in an increase in the efficiency of gene cluster capture (9%). Moreover, using Gibson Assembly by adding/deleting certain operon and tailoring enzymes regardless of end compatibility, the silent construct (~80kb) has been successfully refactored into an active one, yielded a series of analogs expected. With the appearances of the novel molecular tools, we are confident to believe that development of a high throughput mature pipeline for DNA assembly, transformation, product isolation and identification would no longer be a daydream for marine natural product discovery.

Keywords : biosynthesis, CRISPR-Cas9, DNA assembly, refactor, TAR cloning
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