Facile assembly and fluorescence-based screening method for heterologous expression of biosynthetic pathways in fungi.

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Abstract

Heterologous expression of multi-gene biosynthetic pathways in eukaryotic hosts is limited by highly regulated individual monocistrons. Dissimilar to prokaryotes, each eukaryotic gene is strictly controlled by its own regulatory elements, such as promoter and terminator. Consequently, parallel transcription can occur only when a group of genes is synchronously activated. A strategy to circumvent this limitation is the concerted expression of multiple genes as a polycistron. By exploiting the "stop-carry on" mechanism of picornaviruses, we have designed a sophisticated, yet easy-to-assemble vector system to heterologously express multiple genes under the control of a single promoter. For facile selection of correctly transformed colonies by basic fluorescence microscopy, our vector includes a split gene for a fluorescent reporter protein. This method was successfully applied to produce the psychotropic mushroom alkaloid psilocybin in high yields by

heterologous expression of the entire biosynthetic gene cluster in the mould *Aspergillus nidulans*.

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