Abstract

Terpenoids or Isoprenoids have uses in pharmaceuticals, agrochemicals, fragrances, synthetic rubber, and fuels. Terpenoids can be produced by metabolic engineering by expressing heterologous terpene synthases (TPSs) in bacteria and yeasts. Since both substrates and products of TPSs are colorless, diverse in structure, and mostly volatile, there is a need for screening system to screen for mutants with higher catalytic activity. The broad goal of the project aims to develop a visual carotenoid-based genetic screen in yeast to identify heterologously expressed superior catalytic variants of TPSs (specifically diterpene synthases) depending on the variation in the color intensity of the colonies. In the present study, towards this goal, lycopene biosynthesis enzymes were attempted to be integrated into yeast genome using the CRISPR/Cas9 system in a markerless integration strategy. Also, since the visual carotenoid-based screen is functional only in a small window, a delicate balance of carotenoid production concomitant with the diterpene production is needed. This aim has been targeted by making cassettes for all the genes under different strength of promoters.