Title:	A Genetic Screen for the Isolation of Mutants with Increased Flux in the Isoprenoid Pathway of Yeast
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Abstract:	The yeast Saccharomyces cerevisiae is one of the preferred hosts for the production of terpenoids through metabolic engineering. A genetic screen to identify novel mutants that can increase the flux in the isoprenoid pathway has been lacking. We present here the method that has led to the development of a carotenoid based visual screen by exploiting the carotenogenic genes from the red yeast Rhodosporidium toruloides, an organism known to have high levels of carotenoids. We also discuss the methods to use this screen for the identification of mutants that can lead to higher isoprenoid flux. The carotenoid based screen was developed in S. cerevisiae using phytoene synthase RtPSY1 and a hyperactive mutant of the enzyme phytoene dehydrogenase, RtCRTI(A393T) from Rhodosporidium toruloides. As validation of the genetic screen is critical at all stages, we describe the method to validate the screen using a known flux increasing gene, a truncated HMG1 (tHMG1). To demonstrate how this screen can be exploited to isolate mutants, we described how targeted mutagenesis of candidate gene, SPT15 a TATA binding protein involved in the global transcription machinery can be carried out to yield novel mutants with increased metabolic flux. Since it is also important to ensure that the isolated mutants are enhancing general isoprenoid flux, we describe how this can be established using an alternate isoprenoid, α-farnesene.
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