A combinatorial approach to synthetic transcription factor-promoter combinations for yeast strain engineering

Despite the need for inducible promoters in strain development efforts, the majority of engineering in Saccharomyces cerevisiae continues to rely on a few constitutively active or inducible promoters. Building on advances that use the modular nature of both transcription factors and promoter regions, we have built a library of hybrid promoters that are regulated by a synthetic transcription factor. The hybrid promoters consist of native S. cerevisiae promoters, in which the operator regions have been replaced with sequences that are recognized by the bacterial LexA DNA binding protein. Correspondingly, the synthetic transcription factor (TF) consists of the DNA binding domain of the LexA protein, fused with the human estrogen binding domain and the viral activator domain, VP16. The resulting system with a bacterial DNA binding domain avoids the transcription of native S. cerevisiae genes, and the hybrid promoters can be induced using estradiol, a compound with no detectable impact on S. cerevisiae physiology. Using combinations of one, two or three operator sequence repeats and a set of native S. cerevisiae promoters, we obtained a series of hybrid promoters that can be induced to different levels, using the same synthetic TF and a given estradiol. This set of promoters, in combination with our synthetic TF, has the potential to regulate numerous genes or pathways simultaneously, to multiple desired levels, in a single strain.
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