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CRI-SPA mediated screening for metabolic engineering targets to improve small molecule production in *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae is a eukaryotic model organism and represents a commonly used cell factory chassis for the production of a wide range of small molecules, such as terpenes, non-ribosomal peptides, and polyketides. The expansive knowledge of *S. cerevisiae* genetics and metabolism has allowed for the development and use of genome-scale models and flux balance analysis to guide metabolic engineering efforts. However, even in a model organism such as *S. cerevisiae*, our understanding of gene regulation and cell metabolism remains incomplete, making it likely that potential targets for cell factory improvement are missed in such approaches. We have recently developed a mating-based method, CRI-SPA [1], which combines CRISPR-Cas9 induced gene editing with Selective Ploidy Ablation (SPA). This allows for high throughput transfer of a genetic feature of interest from a donor strain to a library of recipient strains. We have applied CRI-SPA to transfer several different biosynthetic pathways—betaxanthin (shikimate pathway-derived), bikaverin (polyketide), and aspulvinone E (non-ribosomal peptide-like)—to the genome-wide gene deletion library. All three biosynthetic pathways result in visible colour production when expressed in *S. cerevisiae*, allowing for visual-based screening for high and low producers. The approach generates a comprehensive dataset of the effect of each gene deletion on product formation and host fitness, which in turn can be used to devise superior metabolic engineering strategies for the production of these valuable small molecules in *S. cerevisiae*.

References

[1] Paul Cachera, Helén Olsson, Nucleic Acids Research, Volume 51, Page e91, (2023)