Direct Combinatorial Pathway Optimization

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Introduction

Combinatorial engineering approaches are becoming increasingly popular, yet they are hindered by the lack of specialized techniques for both efficient introduction of sequence variability and assembly of numerous DNA parts, required for the construction of lengthy multigene pathways. As a solution, we present the Direct Combinatorial Pathway Optimization workflow which combines the strenghts of Single Strand Assembly (SSA) methods1 and Golden Gate Assembly (GGA)2.

Acknowledgments

The lycopene biosynthetic pathway was chosen as proof-of-principle. This pathway of 3 enzymes (CrtE, I, B) leading up to the red carotene lycopene, which is used as food colorant and antioxidant.

To allow for sufficient precursor supply, a Trc-MEP (dxs-idi-spfD) and a T7-MEP overexpressed strain were used3.

Consistencies between the individual libraries could not be observed, yet huge differences were observed between individual transformants.

The 5 best ranked strains for every expression host-plasmid combination (right). The best lycopene producer produces up to 448 mg lycopene/g CDW, twice as much in comparison with Rad et al. (198 mg lycopene / g CDW)6.

References


Lycopene test case

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References