Metabolic Pathway Assembly Case Study: Lycopene biosynthetic pathway optimization

1. Background

Lycopene, a carotenoid phytochemical best known for its bright red color and anti-oxidant properties, has various biological functions and is widely used in pharmaceutical, food and cosmetic industries. Structurally, it consists of six isopentenyl diphosphate (IPP) and two dimethylallyl diphosphate (DMAPP) molecules. As shown in Fig.1, the precursors IPP and DMAPP can be converted to lycopene by co-expressing four exogenous genes in *E.coli* cells: isopentenyl-diphosphaste delta-isomerase (*idi*); geranylgeranyl disphosphate (GGPP) synthase (*crtE*); phytoene synthase (*crtB*); and phytoene desaturase (*crtI*).

Engineering this pathway to optimize lycopene yield can be achieved through metabolic engineering principles. To manipulate metabolite flow through a biosynthetic pathway, homologs or orthologs of each enzyme can be employed, in different combinations and with different regulatory elements driving their expression. Constructing modular assembly libraries for screening is time-consuming and laborious, which consequently calls for an approach to manipulating multiple genes simultaneously.

In this case study, we applied a new technology to perform an all-in-one reaction to assemble multiple variants of each part of the lycopene biosynthetic pathway in many unique combinations. The resulting high-diversity pooled library was then transformed into *E. coli* hosts and colonies were screened to identify transformants with enhanced lycopene yield. The recombinant genetic circuits that gave rise to the best yield improvements could then be identified through restriction analysis and/or sequencing.

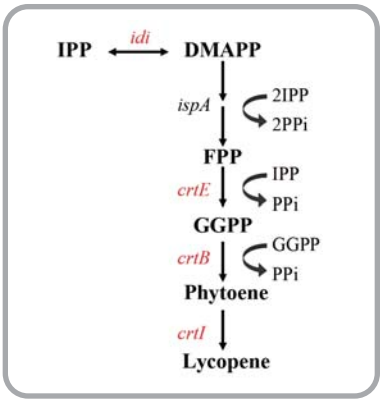

Figure 1. Lycopene biosynthetic pathway

2. Experimental design

Four homologs of *crtE*, *crtB*, and *crtI* from *Pantoea ananatis, Pantoea agglomerans, Pantoea vagans,* and *Rhodobacter sphaeroides* were cloned into a module plasmid with the same overhang for each gene. *idi* from *E.coli* K12 was cloned into the backbone plasmid, and fixed on the last position of the gene circuit. For each gene, 20 ribosome binding sites (RBS), including 10 reverse designed and 10 forward designed RBSs, were applied to balance the expression of *crtE*, *crtB*, and *crtI* genes.

2.1 Modular plasmid preparation

A total of 12 pHD plasmids were prepared (Fig.2) with nonsense mutation of *BsaI* site in genes applied.

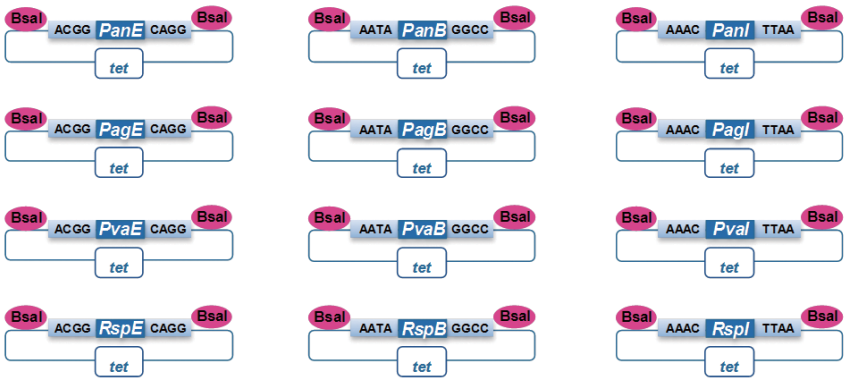

Figure 2. Sources of *crtE, crtB, crtI* genes. *Pan, Pag, Pva* and *Rsp* represent *Pantoea ananatis, Pantoea agglomerans, Pantoea vagans,* and *Rhodobacter sphaeroides,* respectively.

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2.2 RBS design

Each RBS for *crtE, crtB*, and *crtI* was synthesized as an oligo linker containing one of three versions of overhangs, depicted in Fig.3, in order to enable assembly of the first three genes in any order.

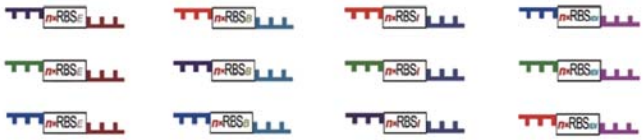

Figure 3. RBSs designed for *crtEBI* and *idi* genes. The overhangs are distinguished with different colors.

2.3 Assembly

An all in-one-reaction was performed to generate a modular metabolic pathway assembly construct library.

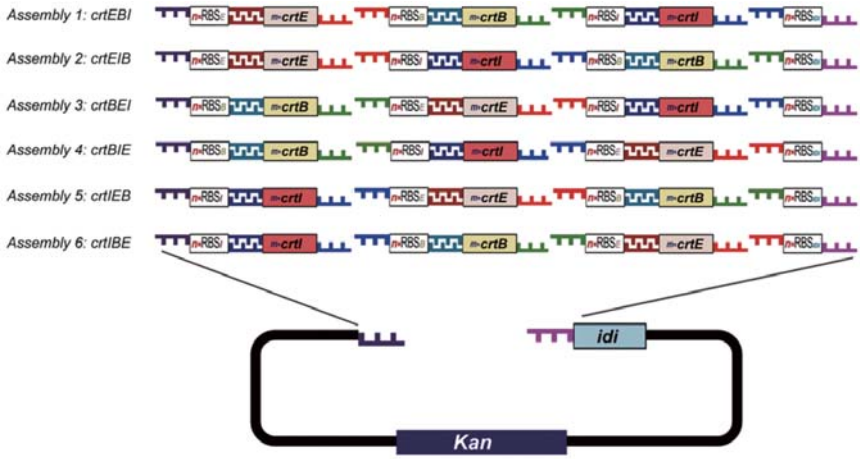

Figure 4. A high-diversity modular assembly construct library was generated via a single reaction.

3. Results

The plasmids mixture was transformed into *E.coli* PXIDF and the transformations were cultured for lycopene production. Lycopene was quantified by measuring OD472 absorption after extracting by ethanol-acetone (V4:1). 1080 red colonies were randomly picked for lycopene production measurement. A wide range of lycopene yield was observed (Fig.5).

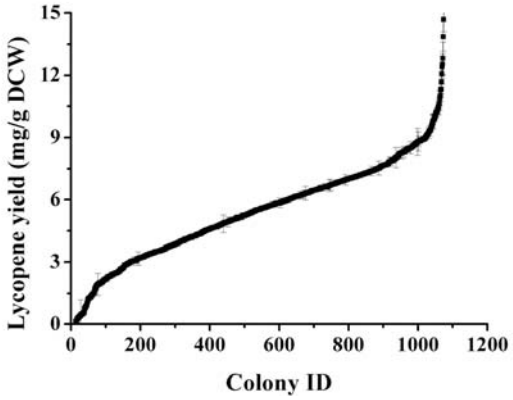

Figure 5. Lycopene yield after simultaneous optimization of RBS, gene order, and enzyme homologs using OLMA.

To learn more about GenScript's Pathway Assembly Services, please see www.genscript.com/metabolic-pathway-assembly.html

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