Engineering Carotenoid Biosynthesis in *Saccharomyces cerevisiae*

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Washington University in St. Louis

International Genetically Engineered Machines Competition (IGEM) 2011

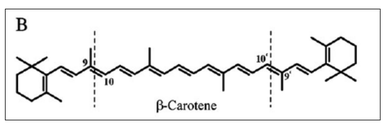
Abstract

Vitamin A deficiency causes blindness in over 250,000 children annually. The WashU iGEM team hopes to address this issue by creating a transgenic strain of *Saccharomyces cerevisiae* (baker's yeast) that produces beta-carotene, the precursor to vitamin A. The carotenoid biosynthesis pathway consists of four genes found naturally in the organism *Xanthophyllomyces dendrorhous*. Three of the four enzymes are required for beta-carotene production while a fourth enzyme cleaves beta-carotene to form beta-ionone, a rose-scented compound used in the fragrance industry.

Background

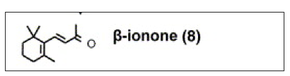
β-carotene

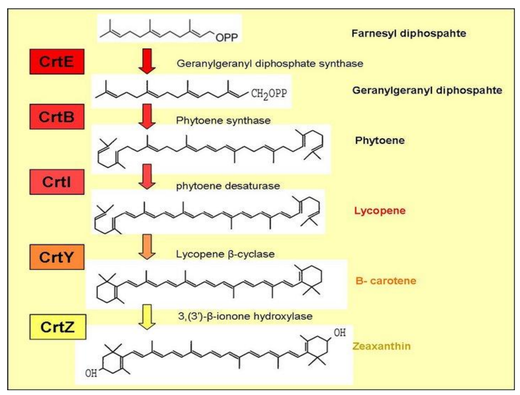
- Strongly-colored red-orange pigment
- Precursor to Vitamin A
- Non-polar
- β-carotene is cleaved in the body into Vitamin A
- Degrades in light and heat to form β-ionone



β-ionone

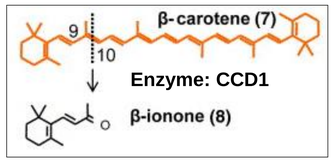
- Aroma Compound
- Characterized by a rose scent and is widely used by the perfume industry
- Currently produced via organic synthesis

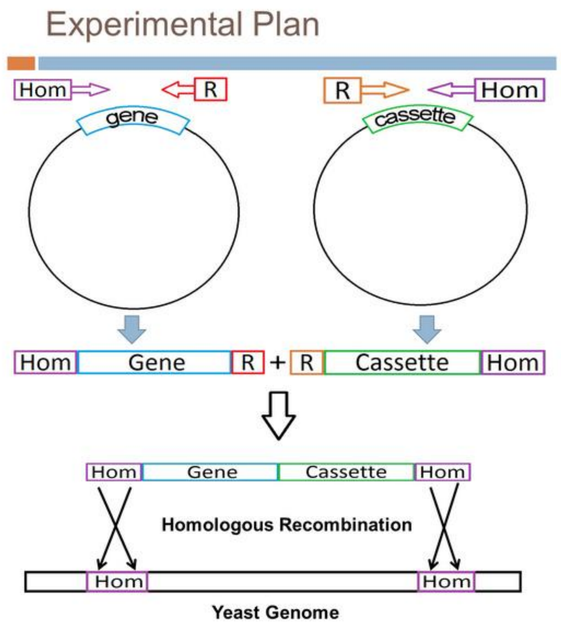




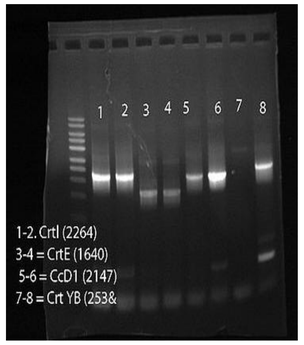
Objective

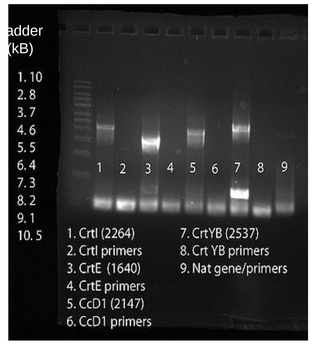
- Clone three enzymes (crtE, crtYB, and crtI) into yeast in order to produce β-carotene
- Once producing β-carotene, a fourth gene (CCD1) will be added to cleave β-carotene into β-ionone.



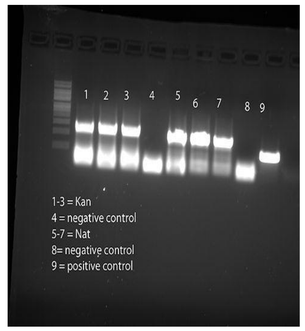


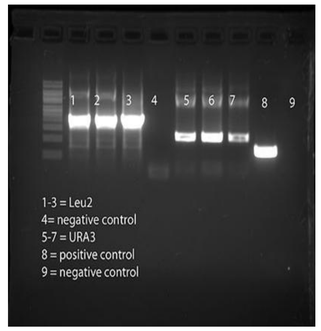
Results and Data



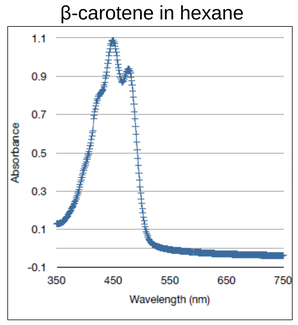


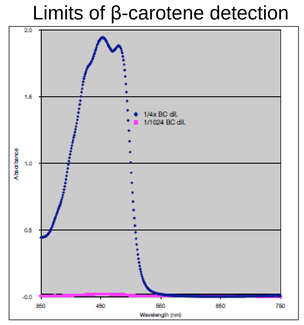
PCR amplification of Synthesized Genes: CrtI, CrtE, CCD1, and CrtYB

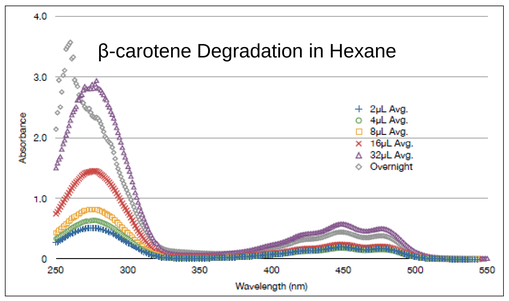


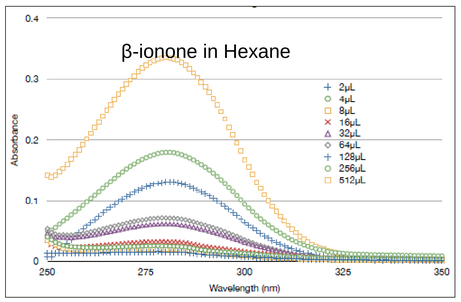


PCR amplification of KanMX4, NatMX4, LEU2, and URA3 Cassettes









Summary

Team WashU succeeded in creating expression constructs for each of the four genes in the carotenoid biosynthesis pathway. Subsequently, we have submitted our original biobricked constructs in the "DNA Planning" stage in the Registry of Standard Biological Parts. Despite the fact that we have all of our DNA sequences complete and synthesized, we ran out of time before we could transform our yeast strains or submit our DNA constructs to the Registry of Standard Biological Parts. However, should next year's WashU IGEM team decide to continue this project, the biobricks we submitted would undoubtedly be completed for use by future IGEM teams.

Acknowledgements

Advisors

Joseph Jez, Barak Cohen, Larry Page, and Bert Berla

Corporate Support

Sigma-Aldrich
Monsanto
BioMerieux
Lesaffre

Washington University in St. Louis

Departments of Biology and Engineering
Office of Undergraduate Research
Chancellor Mark Wrighton

Synthetic Biology based on standard parts