ENGINEERING CAROTENOID BIOSYNTHESIS IN *SACCHAROMYCES CEREVISIAE*

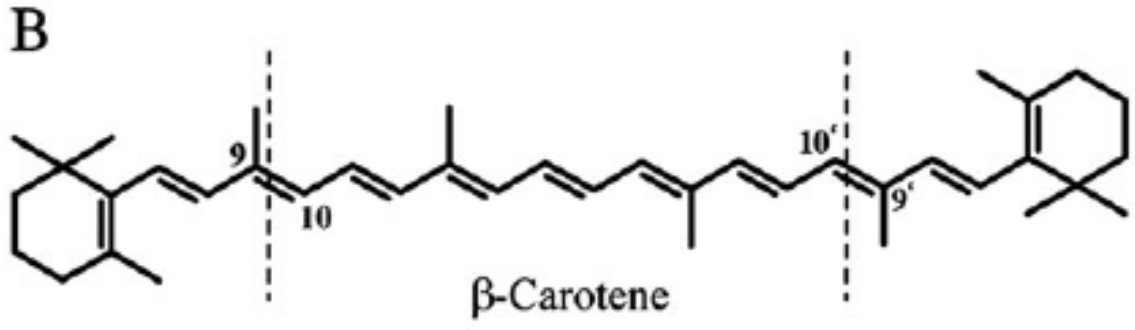
WashU IGEM Team 2011

Our Vision

- Vitamin A deficiency causes blindness in over 250,000 children annually
- Create a transgenic strain of *Saccharomyces cerevisiae* that produces β-carotene, the precursor to vitamin A
- When this GMO yeast is added to bread or other baked goods, it produces β-carotene in addition to its normal byproducts

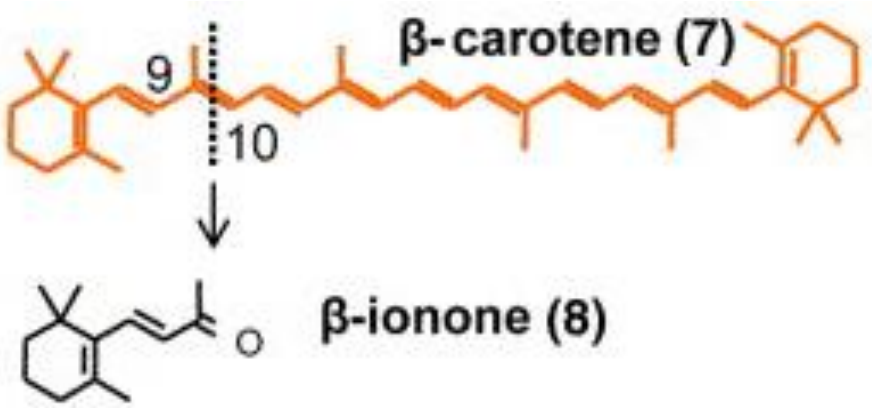
β-carotene

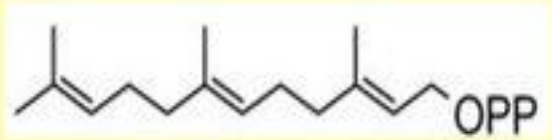
- Strongly-colored red-orange pigment
- Non-polar
- In humans, enzymes cleave β-carotene into Vitamin A
- Degrades in light and heat to form β-ionone



β-ionone

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

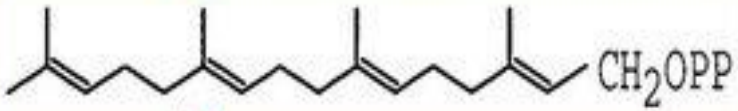




Farnesyl diphospahte

CrtE

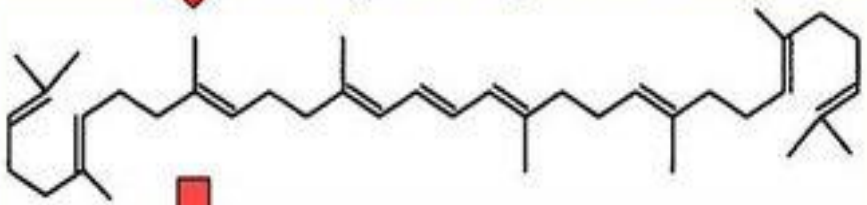
Geranylgeranyl diphosphate synthase



Geranylgeranyl diphospahte

CrtB

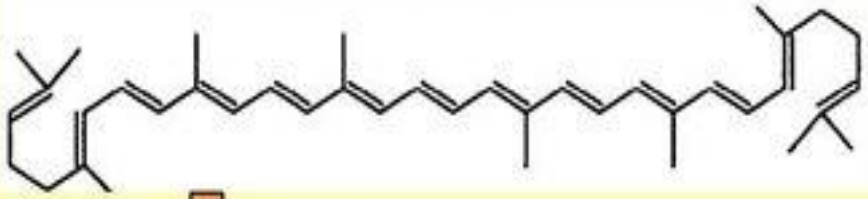
Phytoene synthase



Phytoene

CrtI

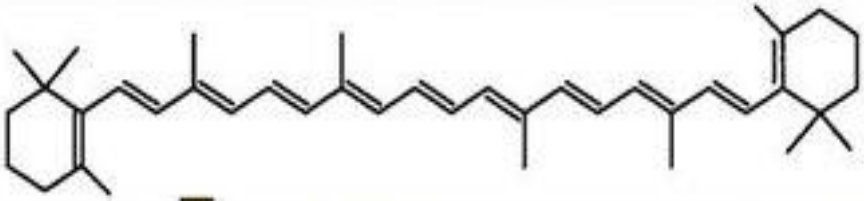
phytoene desaturase



Lycopene

CrtY

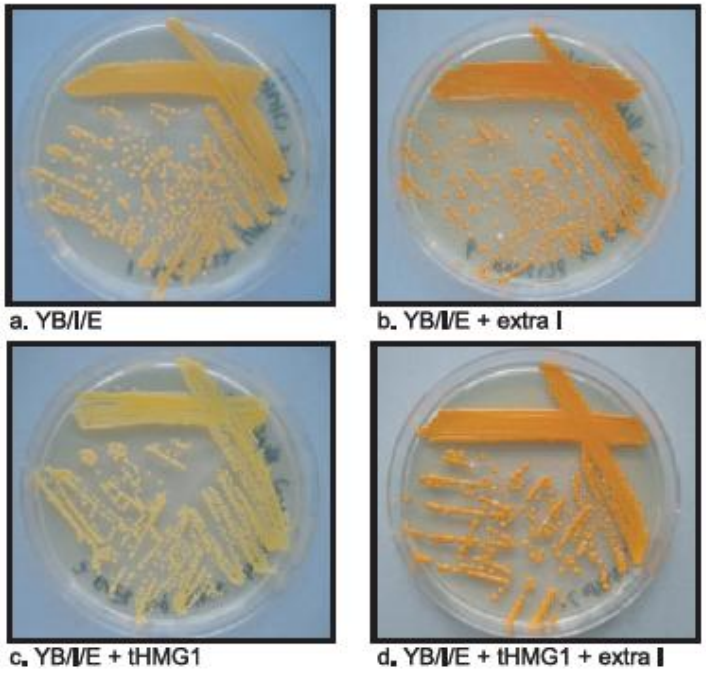
Lycopene β-cyclase

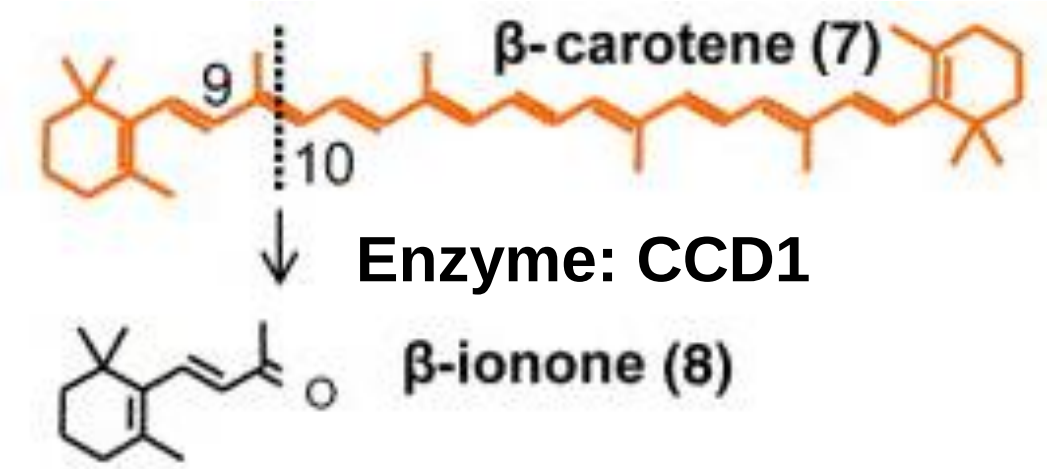


B- carotene

Carotenoids in Yeast

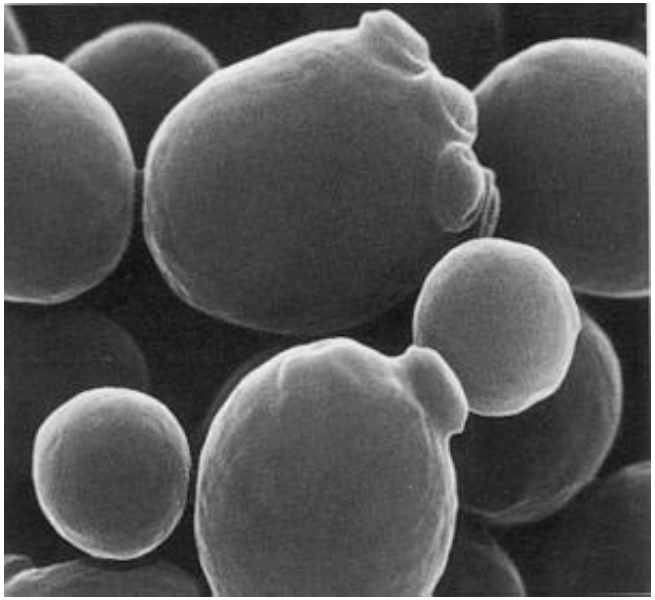
- Clone three enzymes into yeast in order to produce β-carotene
- Once producing β-carotene, a fourth gene will be added to cleave β-carotene into β-ionone.



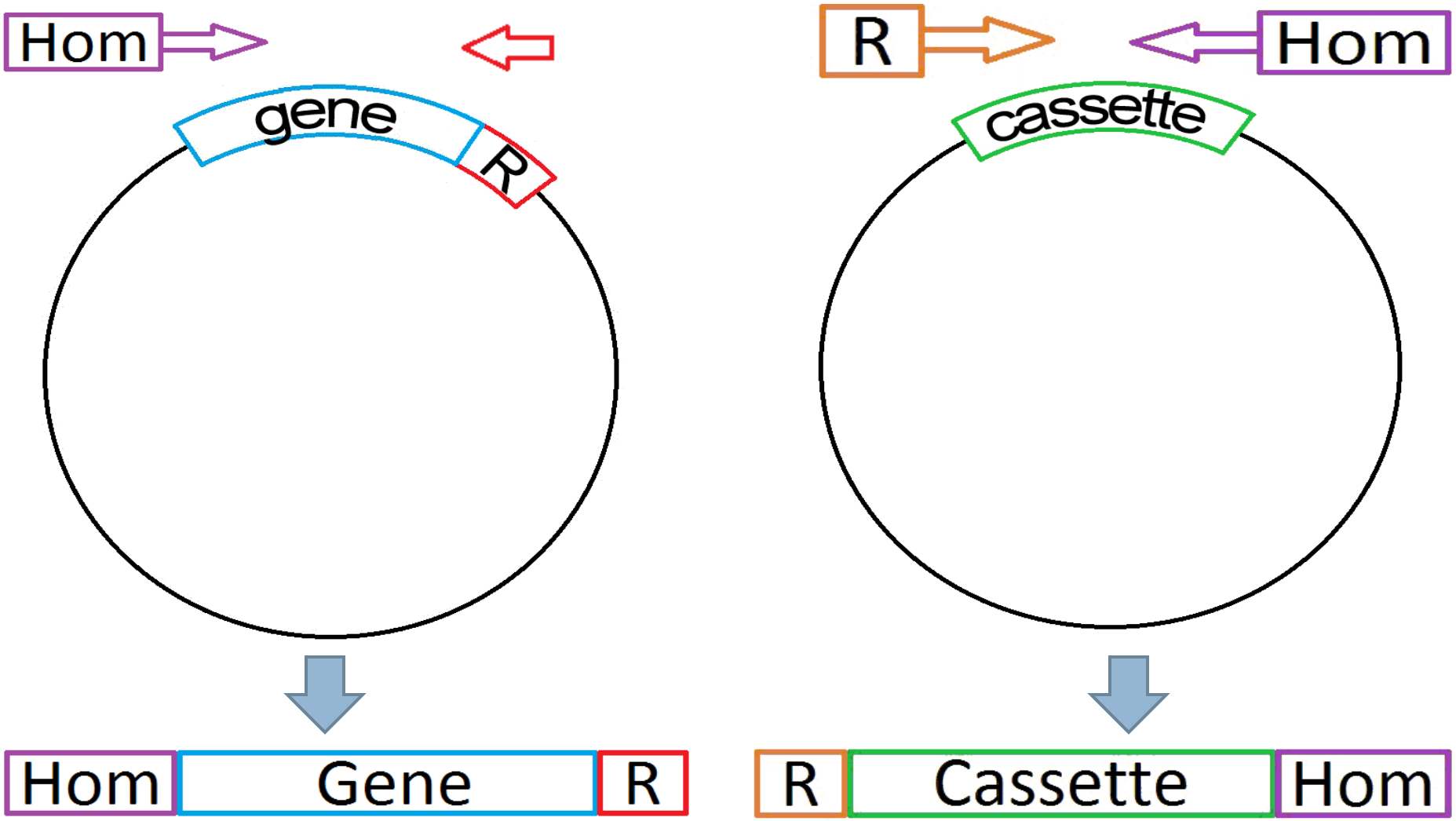


Why Yeast?

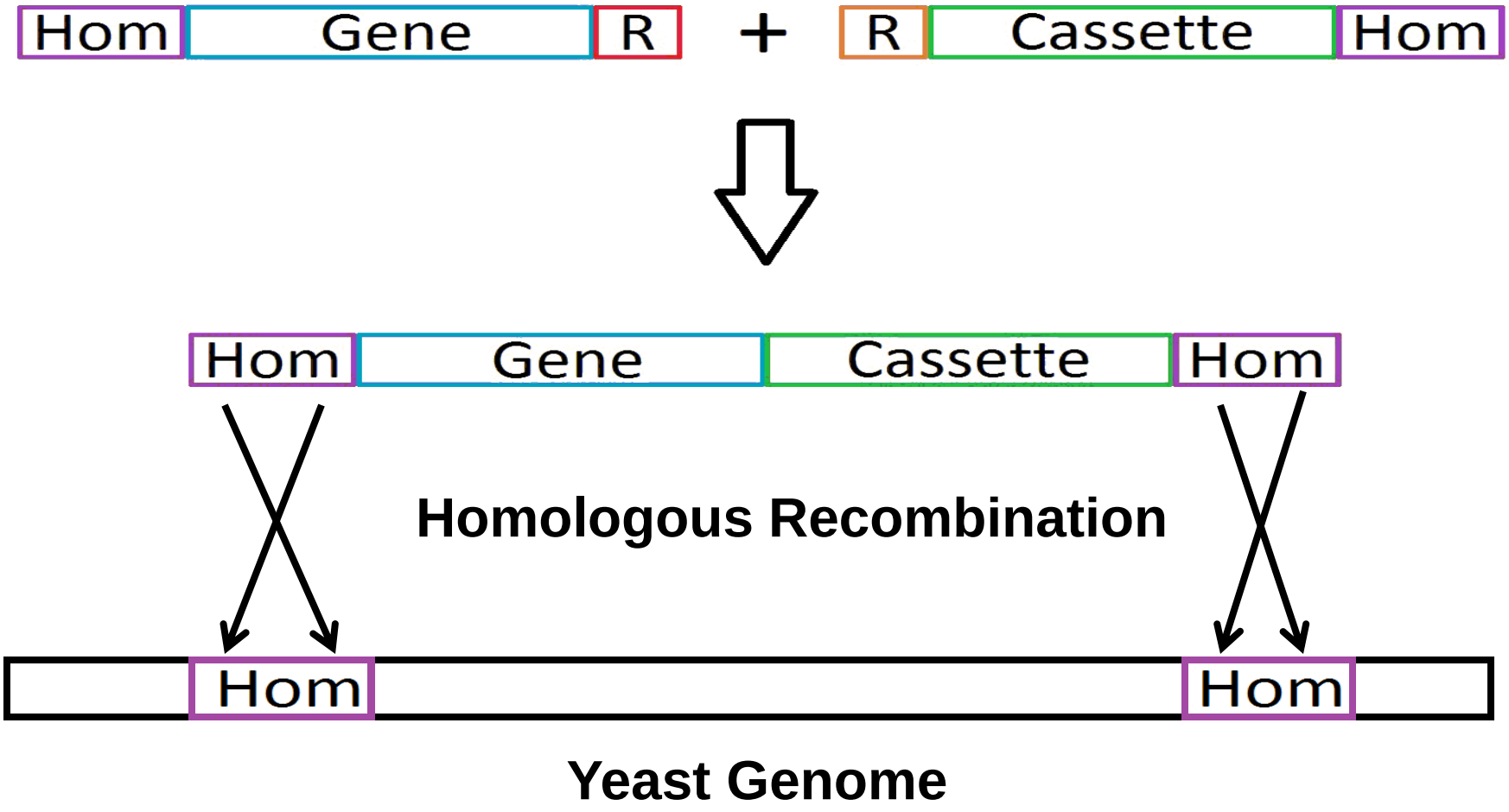
- Real-world applications
- No carotenoid precursor in *E. coli*
- Haploid or Diploid
  - Allows for successive transformations of multiple genes
- Well-studied organism



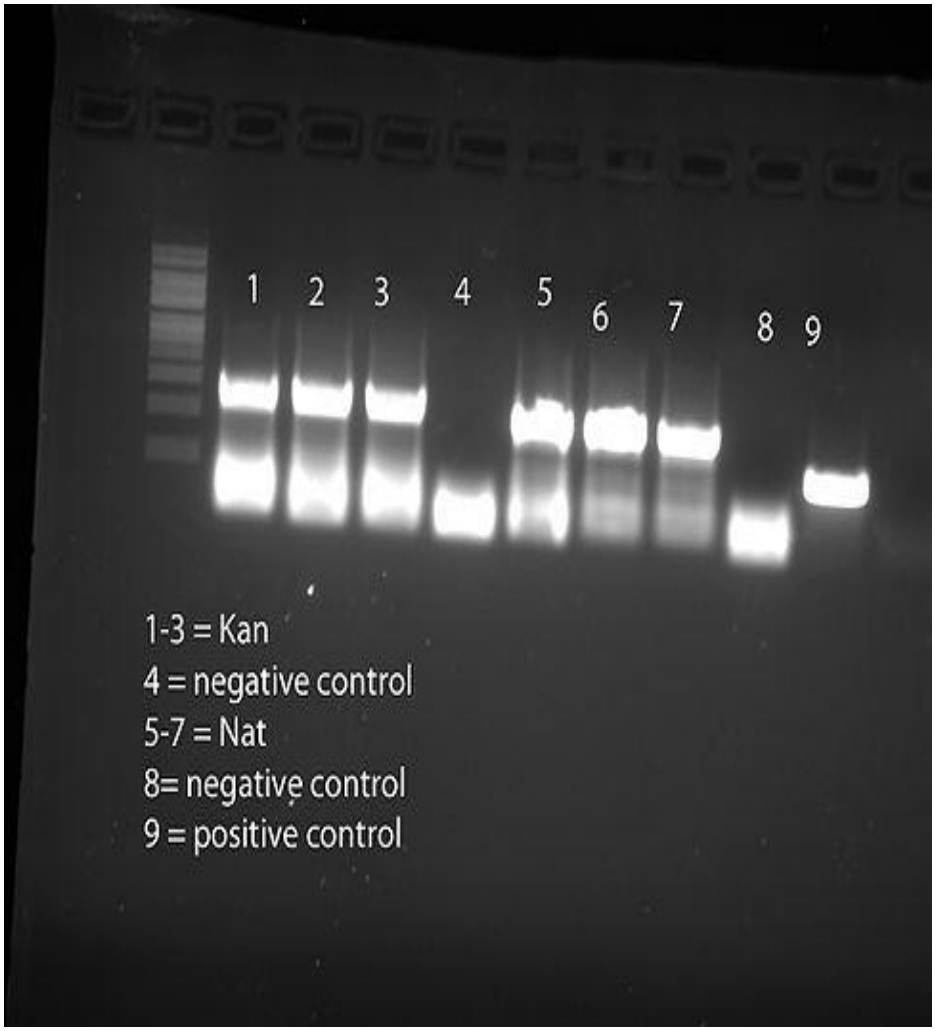
Experimental Plan – Part 1

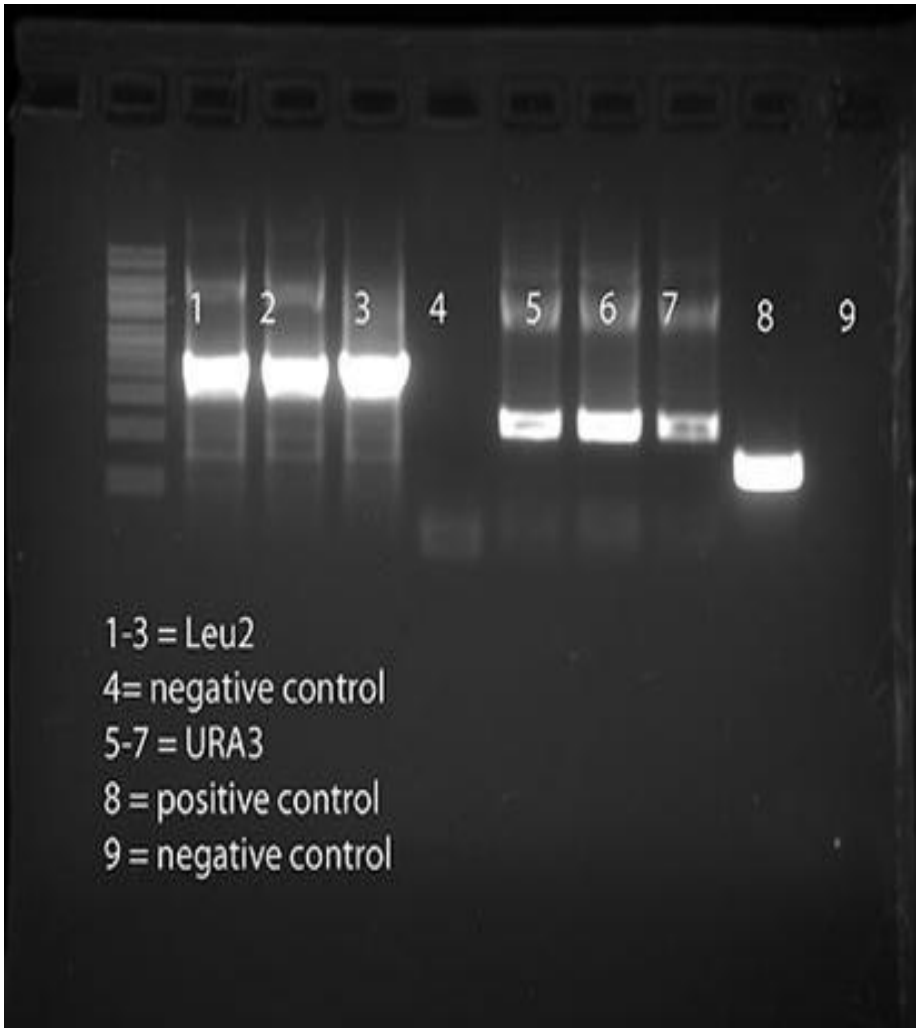


Experimental Plan – Part 2

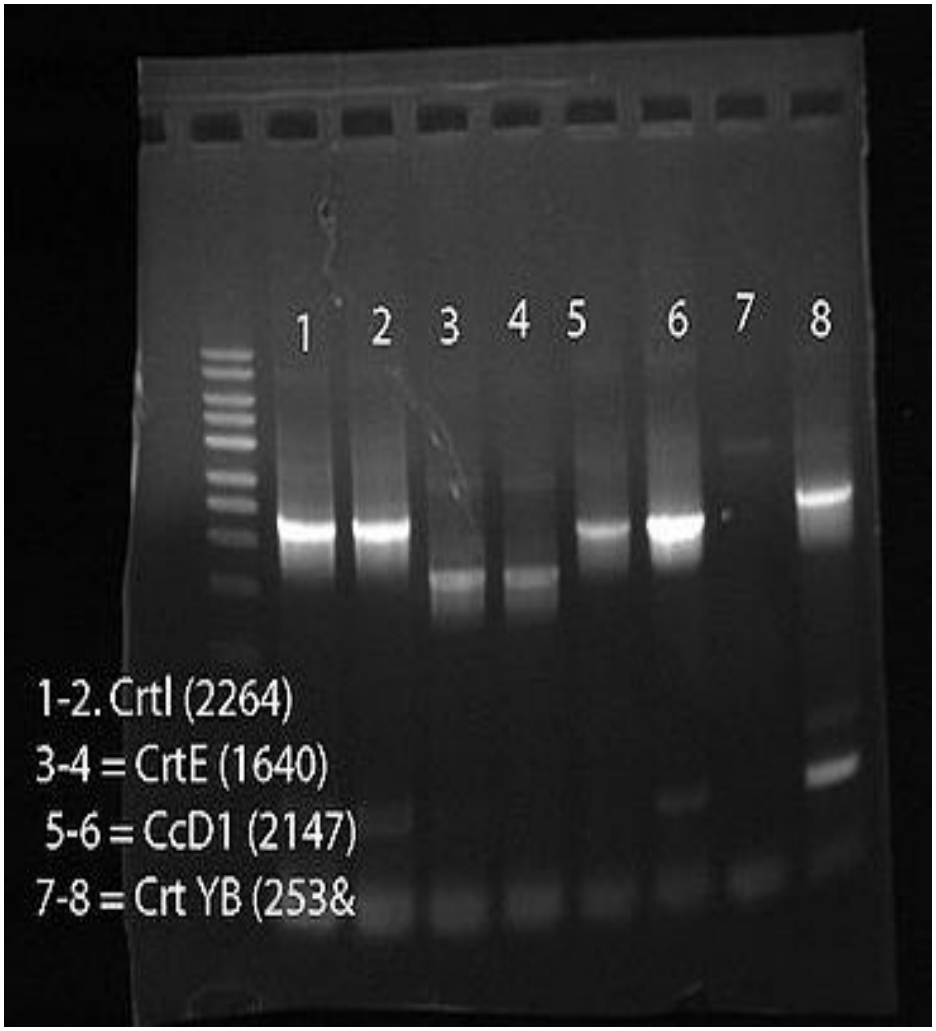


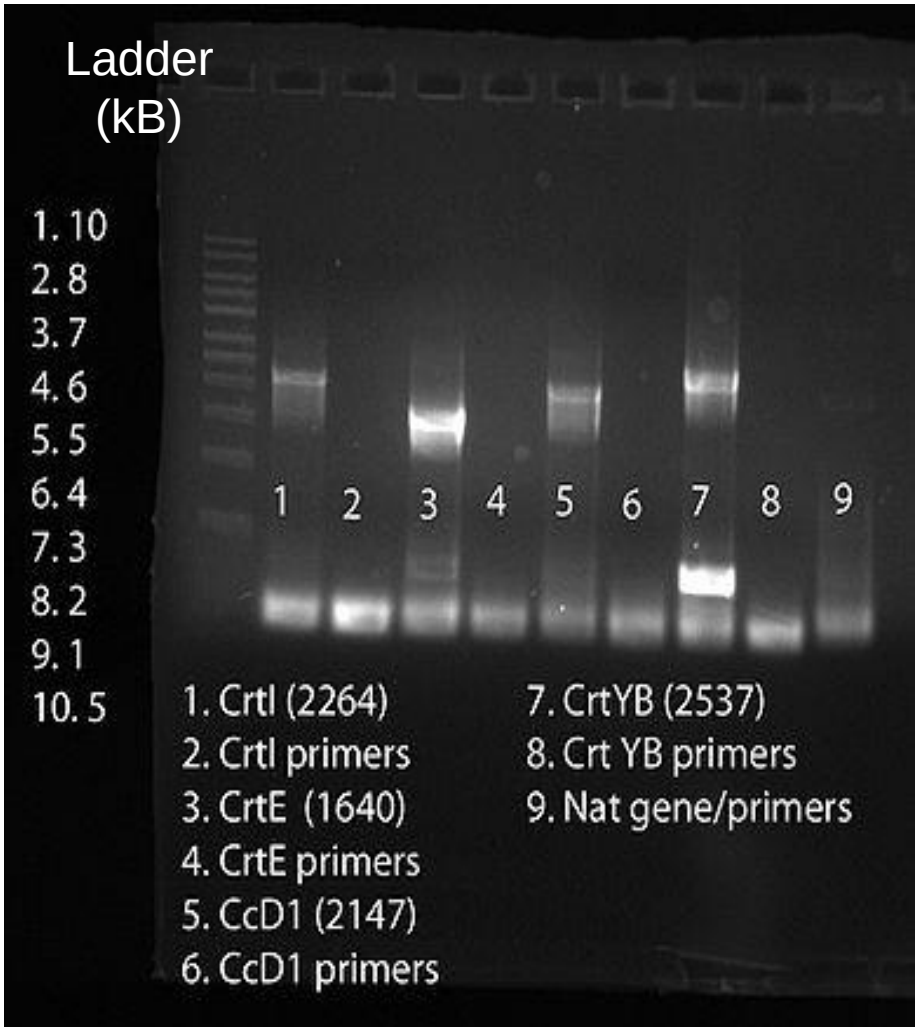
PCR amplification of KanMX4, NatMX4, LEU2, and URA3 Cassettes





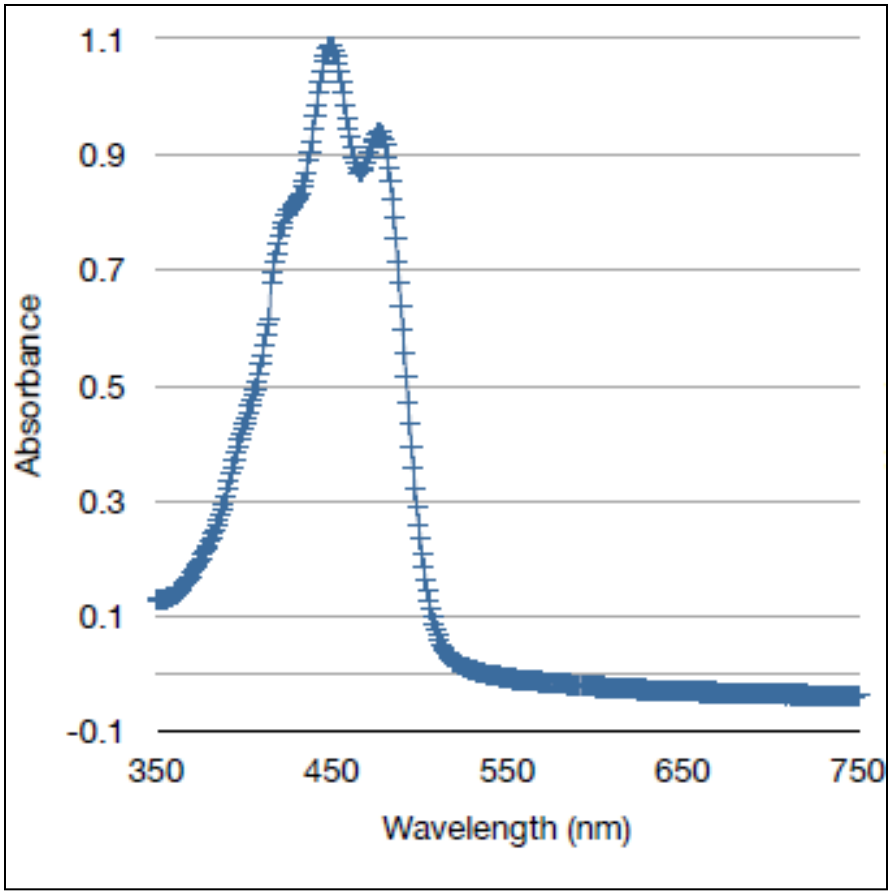
PCR amplification of Synthesized Genes: CrtI, CrtE, CCD1, and CrtYB



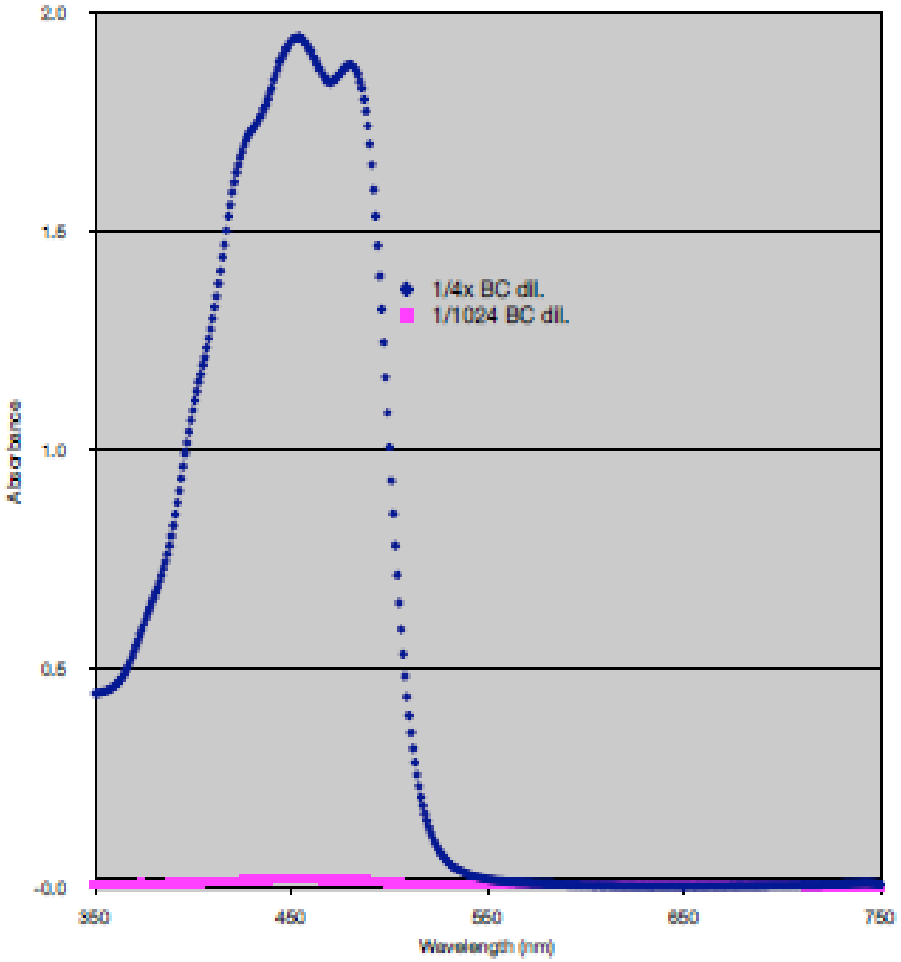


β-carotene Assay

β-carotene in hexane

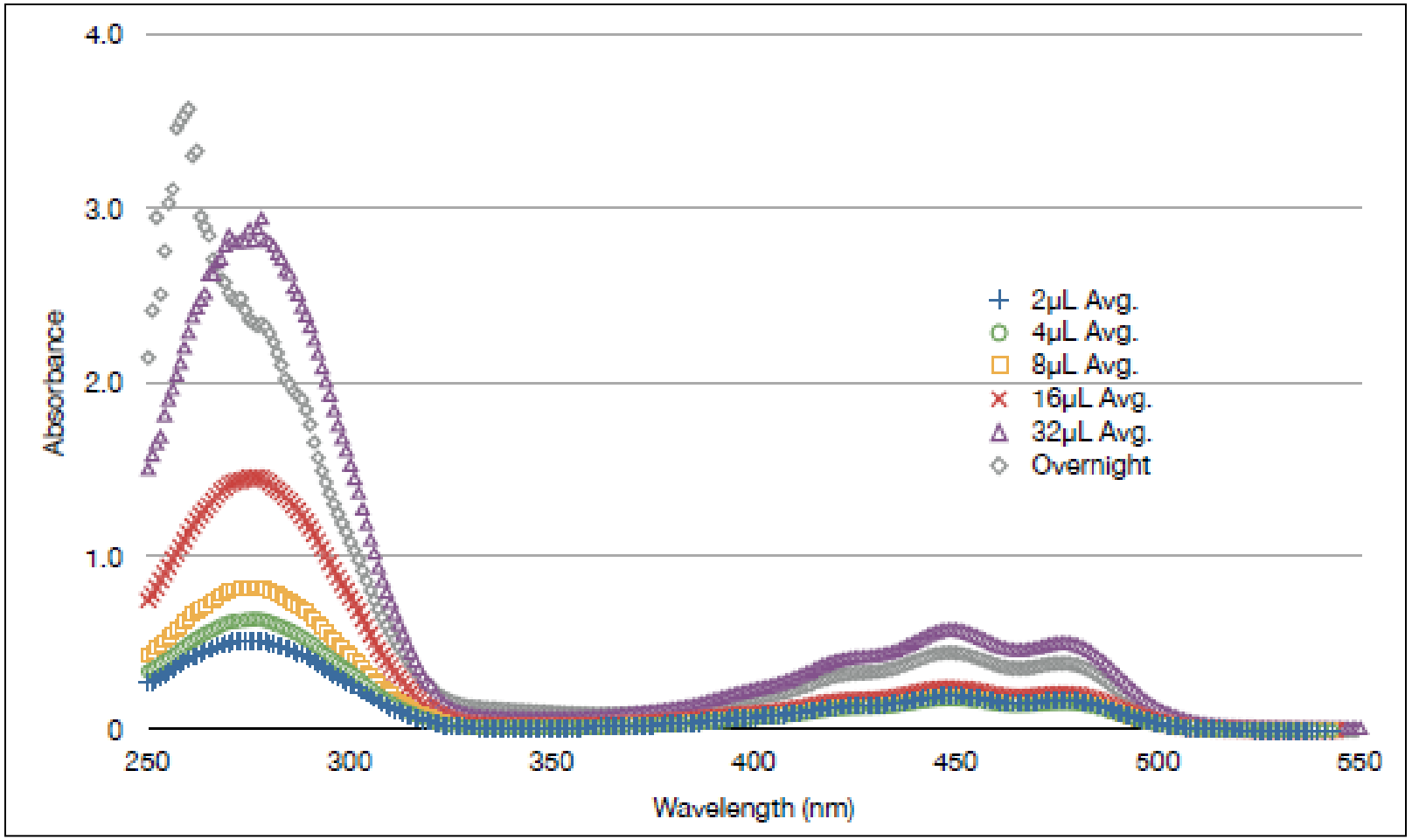


Limits of β-carotene detection



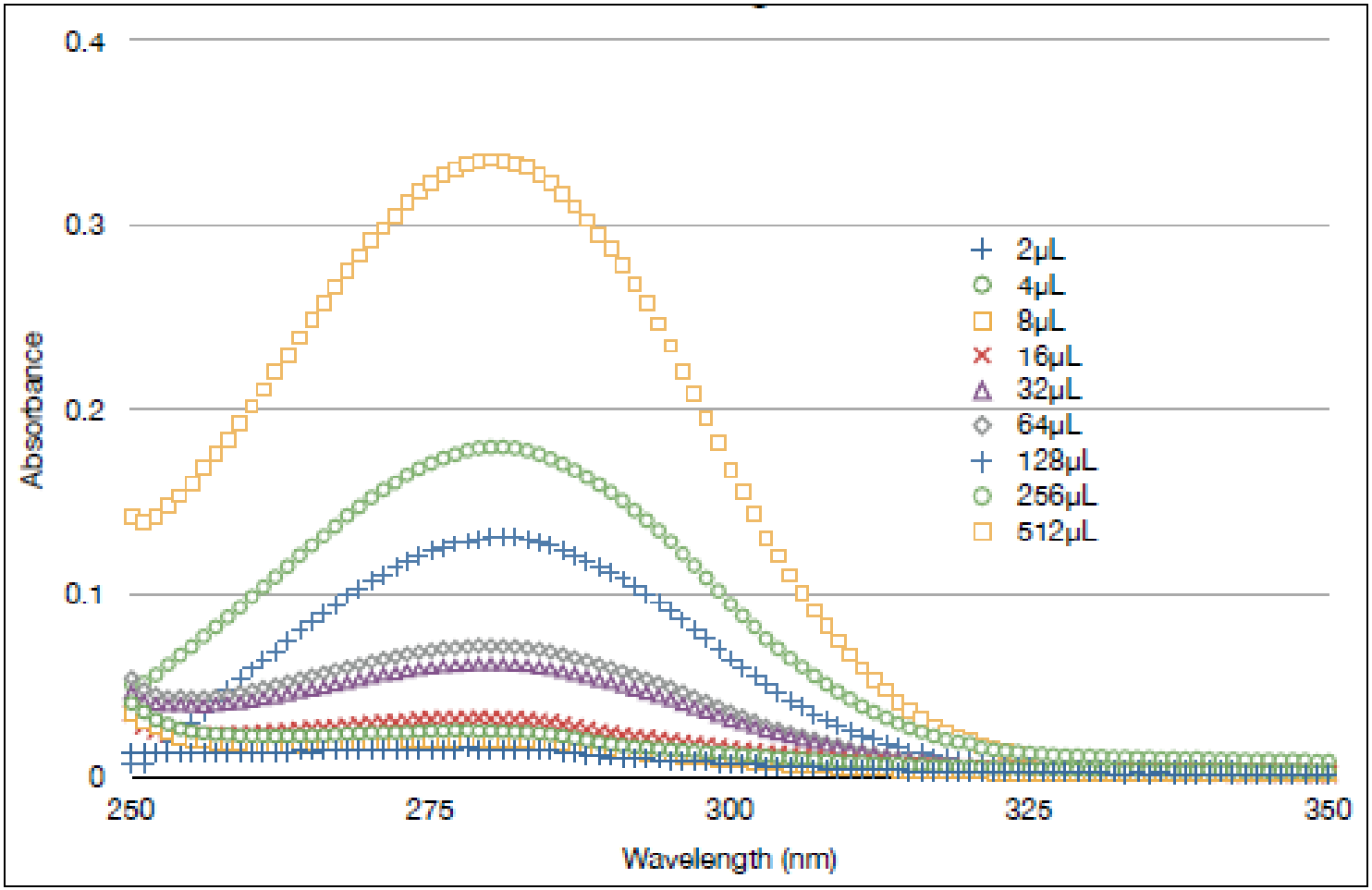
β-carotene Degradation

β-carotene in Hexane



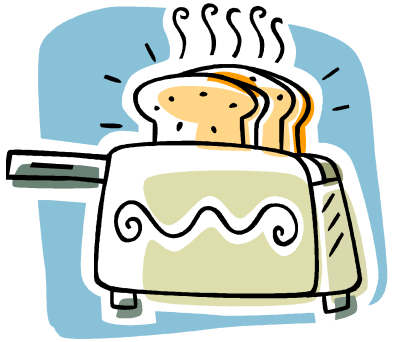
β-ionone Assay

β-ionone in Hexane



Future Plans

- Transform yeast with our synthesized constructs.
- Perform quantitative assays determining efficiency of the beta-carotene and beta-ionone pathways.
- Industrial applications:
  - Vitamin A Bread
  - Vitamin A Beer?
  - Beta-Ionone biosynthesis



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  - Office of Undergraduate Research
  - Career Center
  - Chancellor Mark Wrighton

References

- Susanne Baldermann, Masaya Kato, Miwako Kurosawa, Yoshiko Kurobayashi, Akira Fujita, Peter Fleischmann and Naoharu Watanabe. **Functional characterization of a carotenoid cleavage dioxygenase 1 and its relation to the carotenoid accumulation and volatile emission during the floral development of Osmanthus fragrans Lour**. Journal of Experimental Botany, Vol. 61, No. 11, pp. 2967–2977, 2010
- Rene Verwaal, Jing Wang, Jean-Paul Meijnen, Hans Visser, Gerhard Sandmann, Johan A. van den Berg, and Albert J. J. van Ooyen. **High-Level Production of Beta-Carotene in *Saccharomyces cerevisiae* by Successive Transformation with Carotenogenic Genes from *Xanthophyllomyces dendrorhous.*** APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2007, p. 4342–4350
- **http://parts.mit.edu/igem07/index.php/Edinburgh/Yoghurt/Wet_Lab**

Questions?