Scenthase: Cellular Factories for the Production of Terpenes
Our Team After its First Year

- Founded by first-year students in December 2013
- First iGEM Team to compete in three Tracks in one year
- Small but passionate core team
The Problem: Terpenoid Production Today

• What do you get when you add:
  • Large quantities of deforested plant material
  • Known carcinogens and environmentally toxic chemicals
  • High economic demand for rare essential oils

• Answer: an impractical and expensive process for synthesizing and extracting rare essential oils and their active ingredients

(Hu and Corey 2002)
Where Our Project Comes In: A Better Way to Manufacture Terpenes

- *Escherichia coli* and *Saccharomyces cerevisiae*
  - Genetic model systems
  - Precursors already present

- Synthase genes
Meet the Terpenes

**Cadenine**
- Main essential oil: Cade oil
- Applications: Antifungal, bactericidal, and antioxidant
- Plant Species: *Gossypium hirsutum* (cotton)
- Synthase Gene: 8-cadene synthase (E.C. 4.2.3.13)

**Carene**
- Main essential oils: Rosemary and Cedar oil
- Applications: Insecticide, anti-inflammatory, and central nervous system depressant
- Plant Species: *Picea abies* (Norway spruce)
- Synthase Gene: carene synthase (E.C. 4.2.3.107)

**Humelene**
- Main essential oils: Hops oil
- Applications: Culinary spice, and anti-inflammatory
- Plant Species: *Zingiber zerumbet* (shampoo ginger)
- Synthase Gene: α-humulene synthase (E.C. 4.2.3.104)

**Sabinene**
- Main essential oils: Junior Coriander oil
- Applications: Spice, and antimicrobial
- Plant Species: *Salvia officinalis* (sage)
- Synthase Gene: (+)-sabinene synthase (E.C. 4.2.3.110)

**Myrcene**
- Main essential oils: Thyme and Hops oil
- Applications: Fragrance, analgesic, and anti-inflammatory
- Plant Species: *Perilla frutescens* (green shiso)
- Synthase Gene: myrcene synthase (E.C. 4.2.3.45)

**R-Linalool**
- Main essential oils: Lavender oil
- Applications: Fragrance, and insecticide
- Plant Species: *Mentha citrata* (lemon mint)
- Synthase Gene: (R)-linalool synthase (E.C. 4.2.3.26)

**S-Linalool**
- Main essential oils: Citrus and Coriander oil
- Applications: Fragrance, and insecticide
- Plant Species: *Arabidopsis thaliana* (thale cress)
- Synthase Gene: (S)-linalool synthase (E.C. 4.2.3.25)

**Zingiberene**
- Main essential oils: Ginger oil
- Applications: Flavoring and pesticide
- Plant Species: *Ocimum basilicum* (basil)
- Synthase Gene: α-zingiberene synthase (E.C. 4.2.3.65)

**Santalene**
- Main essential oils: Sandalwood oil
- Applications: Fragrance, antiviral, and tumor-suppressant
- Plant Species: *Santalum album* (sandalwood tree)
- Synthase Gene: α-santalene synthase (E.C. 4.2.3.82)
Novel Approaches

- Don’t synthesize: Grow your own genes
- Go where no sequencer has gone before
- Full spectrum of fragrance
- Assembly lines in the lab
- Why settle with one copy when you can have two?

(Scherens and Goffeau 2004)
Team Structure: An Experiment in SynBio Education

- Nine projects, nine times the opportunities for engagement
- 122 members, 60 trained in lab, 4 experiments in 4 weeks
- Collaboration with Ravenwood High School iGEM
Getting Genes From Their Source

• Greenhouse
• Genomic Extractions
• Synthase PCR Isolation
Success, But With a Catch

(Chang et al 2007)
Another Way for Extracted Genes

- RNA Extraction from Plants
- RNA $\rightarrow$ cDNA
- cDNA $\rightarrow$ Synthase

(Hossain and Levy 2014)

(Integrated DNA Technologies)
From Genes to Biobricks to Production

- Biobrick standard
  - Mutagenesis
  - Prefix and Suffix
  - pSB1C3

- Cloning into our vector

- Expression in E. coli and yeast

- GCMS confirmation, plus strep-tag and inducible promoter.
pVU14006: The Amenities
Building the Vector

- PCR Extract Gene Cassettes from existing plasmids
- Combine into pUC19 MCS
- Mutagenize RFC10 sites
- Ligate synthase gene

Diagnostic digest produces bands of exactly the correct size (from left to right, 2700, 2700 and 950, 2700 and 1700), confirming that pVU14004 was successfully constructed.
<table>
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<th>Terpene</th>
<th>Plant Genomic DNA Successfully Extracted</th>
<th>Synthase Gene Successfully PCR Isolated</th>
<th>Plant RNA Successfully Extracted</th>
<th>Synthase gene Successfully Reverse Transcribed</th>
<th>Terpene Successfully Produced in E. coli or yeast</th>
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Results Summary

pVU14006

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Questions and Answers