Bachelor's thesis
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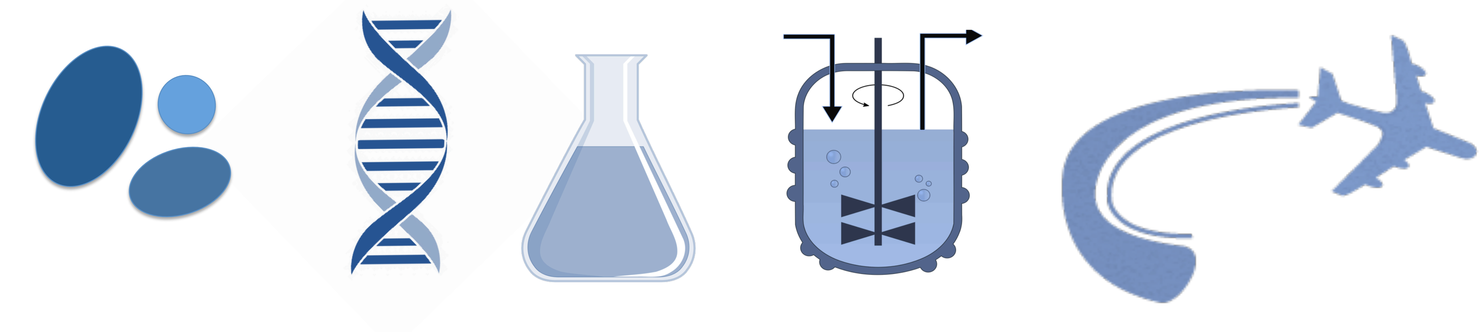
FARNESANE

as a biojet fuel production in *Saccharomyces cerevisiae*

Metabolic engineering and synthetic biology



Introduction| In the last years, we have been listening in different media and from different governments that petroleum will run out in a few years. Recently, several studies are trying to develop new approaches for the creation of different alternative biofuels. One of these biofuels is **farnesane**, a sesquiterpenoid. **Farnesane** is classified as a renewable diesel and can be used as a **Bio-Jet fuel** for jet engines, like planes and rockets. One production possibility is metabolic engineering in the mevalonate pathway of *Saccharomyces cerevisiae* and an expression plasmid introduction in order to produce farnesane.

OBJECTIVES !

- Learn how to modify *Saccharomyces cerevisiae* metabolic pathway and which strain is the best for the invention.
- Deal with molecular techniques and their applications.
- Learn how to design an expression plasmid.
- Evaluate new biofuel sources.

Disruption construct example

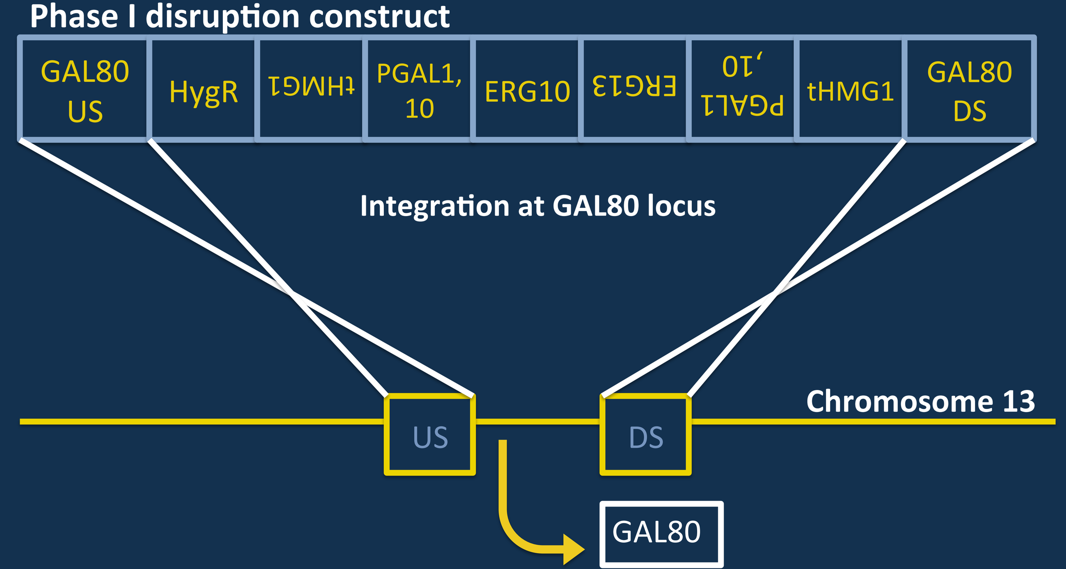


Figure 1. Disruption construct cassette with promoters and homologous recombination in GAL80 locus example

Disruption cassete triple function:

1. **Overexpressed mevalonate genes** by homologous recombination.
   - ◎ Selection genes used: **hygA, natR, kanA (resistance)** and **URA3 (without it, no growth).**
   - ◎ **Promoters: pGAL1↔pGAL10, pGAL4 (!), pGAL7.**
2. **Knock-out genes** by homologous recombination.
   - ◎ **STE5** and **IME1.**
3. **Promoter substitution** for expression repression by homologous recombination.
   - ◎ **ERG9 promoter** is substituted by a CTR3 promoter under Copper control.

Carried out techniques

Modified mevalonate pathway

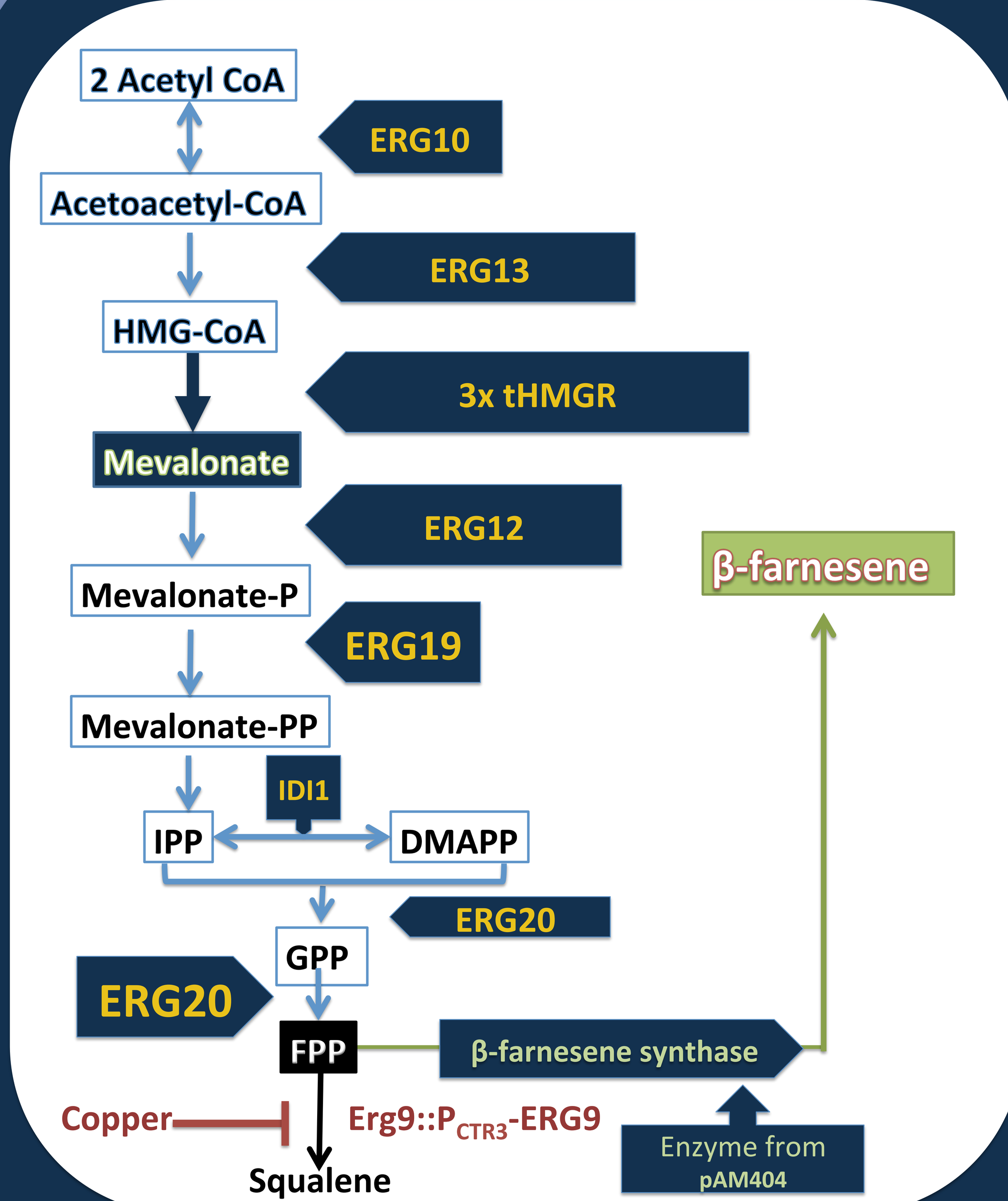


Figure 2. Metabolic pathway showing which genes have been modified to synthesize ß-farnesene

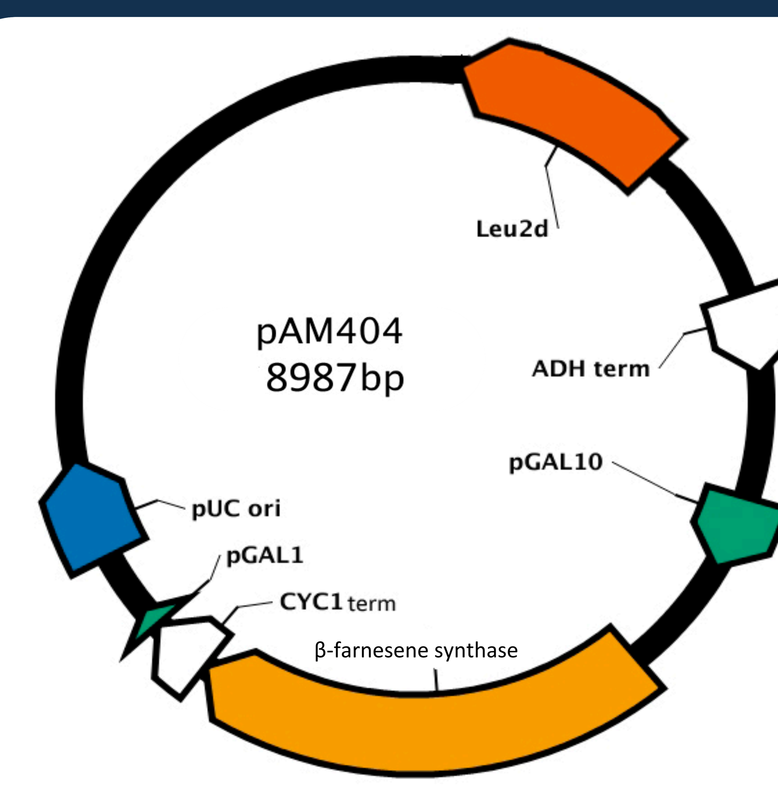


Figure 3. Overview of pAM404 and genes that makes up of it

Transformed plasmid

Inactivated genes by homologous recombination.

STE5. Mating – pheromone response gene. Without it, the possibility of sexual reproduction is 6 times less possible than with it (WT).

IME1. Meiosis transcriptional regulator

Diploid and an immutable strain.

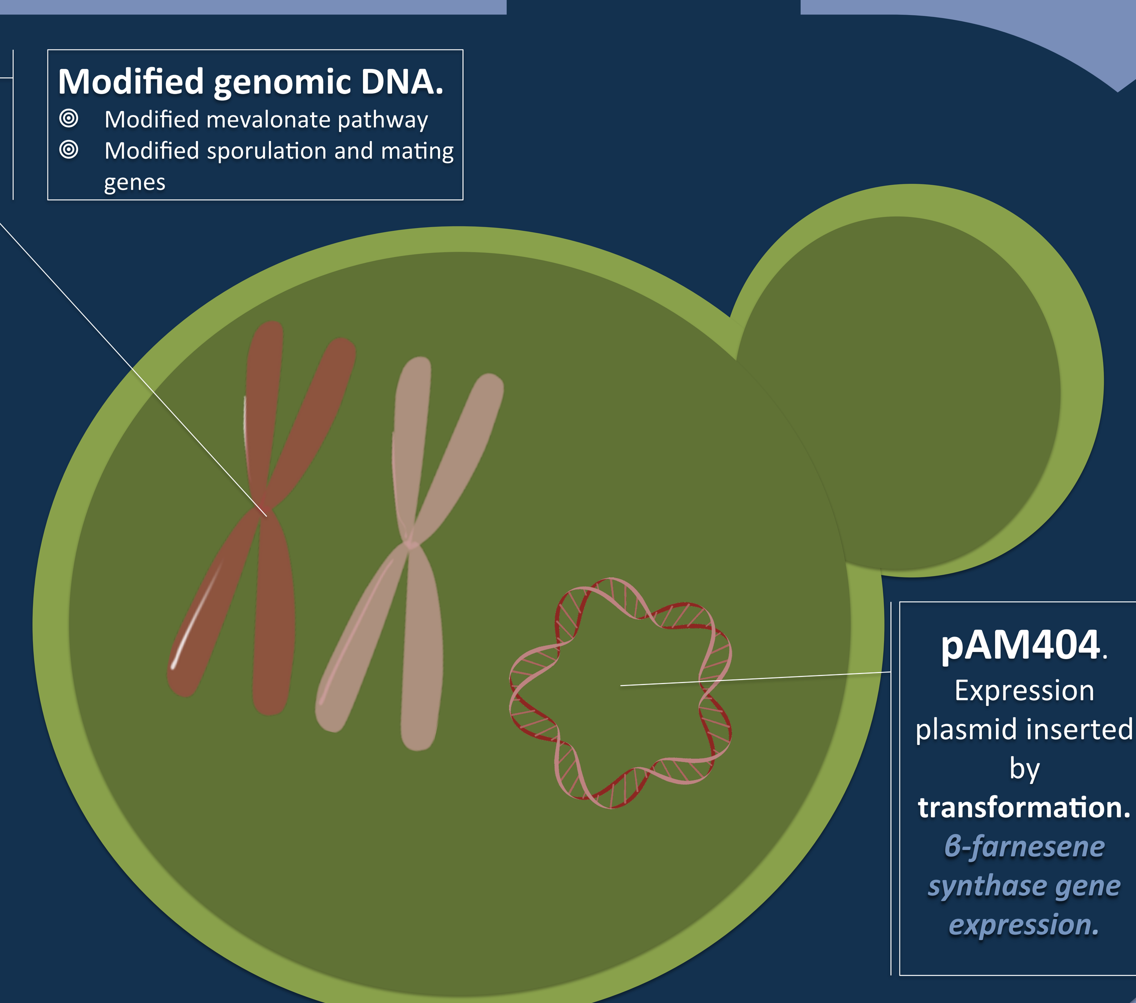


Figure 4. Schematic figure of a Saccharomyces cerevisiae strain and what includes on it

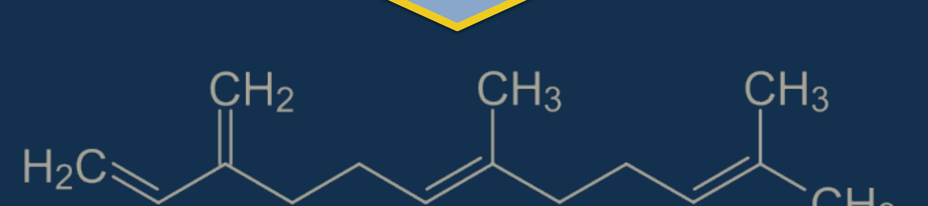
Modified yeast used | Y1979

Codon optimized for *Saccharomyces cerevisiae.*	Under a very strong **pGAL10** promoter
Synthetically generated **β-farnesene synthase** gene from ***Artemisia annua.***	
Inserted into a pAM178, yielding **pAM404,** using restriction enzymes.	**Leu2** gene as a selectable marker

Techniques used

fermentation

Sugarcane as a substrate

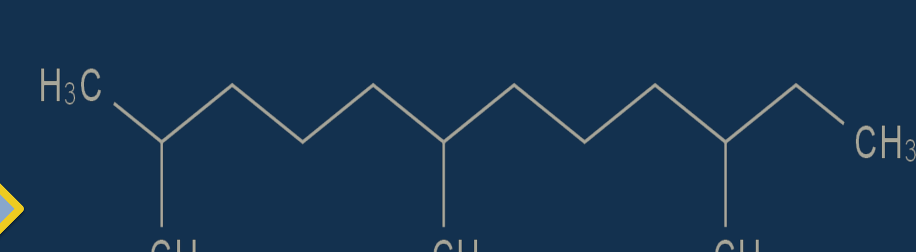


β-Farnesene **Biofene®**

10% Pd/C, H2, 25 ºC

hydrogenation

Farnesane



AMYRIS

Company and application

Conclusions

- ◎ Part of the information taken in this project comes from patents. That is why there is a lack of information in different points.
- ◎ There are different strategies for modifying *Saccharomyces cerevisiae.* The use of disruption cassettes involving more than one gene is innovative and different from what other research group have tested.
- ◎ Farnesane production shows that yeasts, and in this case *Saccharomyces cerevisiae,* are a good platform for sesquiterpenoids production.
- ◎ Farnesane can be one of the future biojet fuels available against the imminent lack of oil and petrol.

[1] Mcphee, D. J., & Toomer, P. E. D., Stephen Renninger, N. (2010). Oakland, California, (US). Patent. Fuel compositions comprising farnesane and farnesane derivatives and method of making and using same. Patent No.: US 7846222 B2.

[2] Ubersax, J.A., Emervylle, (2010), California, (US). Methods for generating a genetically modified microbe. Patent. Pub. No.: US 2010/0304490.

[3] Nielsen J, Larsson C, van Maris A, Pronk J. Metabolic engineering of yeast for production of fuels and chemicals. Curr Opin Biotechnol [Internet]. Elsevier Ltd; 2013;24(3):398–404.

[4] Asadollahi MA, Maury J, Schalk M, Clark A, Nielsen J. 2010. Enhancement of farnesyl diphosphate pool as direct precursor of sesquiterpenes through metabolic engineering of the mevalonate pathway in *Saccharomyces cerevisiae. Biotechnol Bioeng* 106:86-96.

Main references