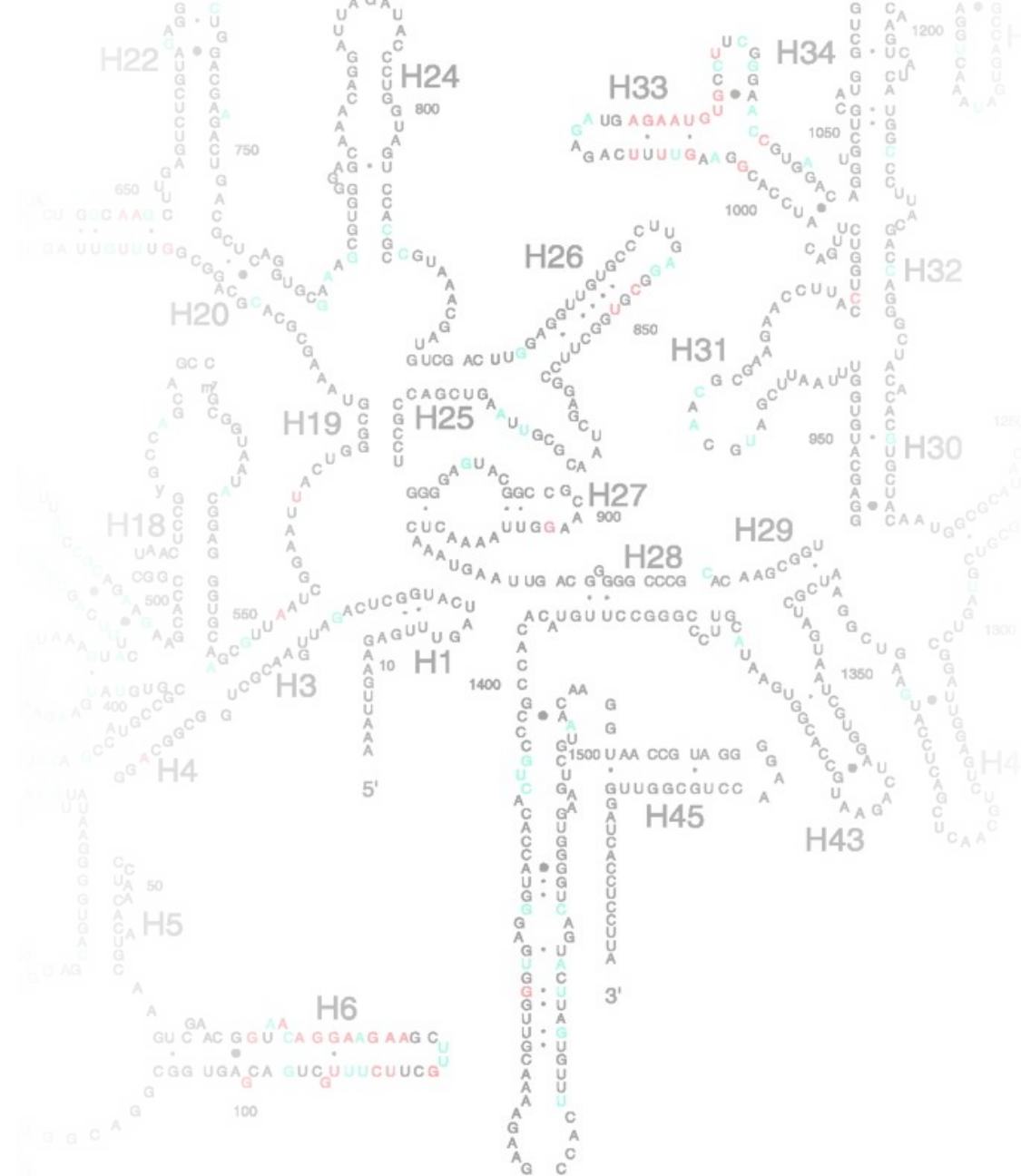
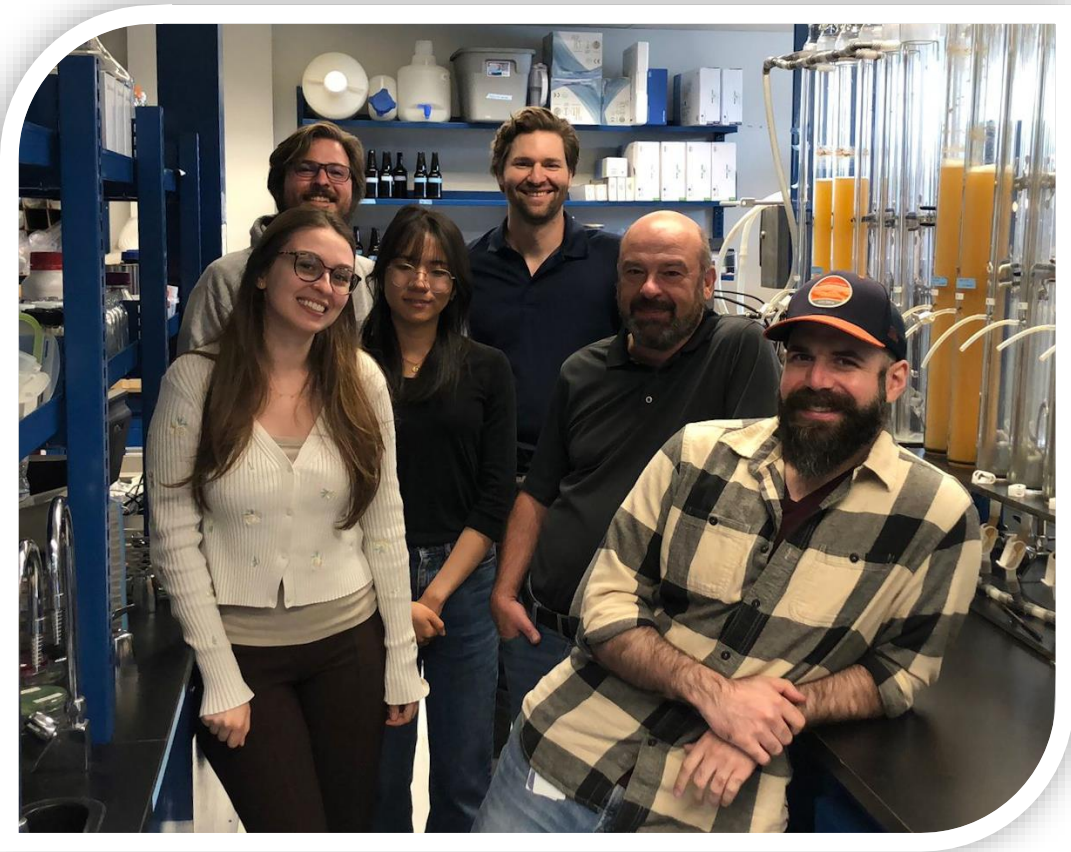


HARNESSING MODERN BIOTECHNOLOGY:

Conceptual development of high-value strains to
address industry challenges



INTRODUCTION



Lallemand Inc. Corporate BRW Team, Montreal, QC, Canada

- Who I am:
 - Avi Shayevitz
 - R&D Scientist for Lallemand Inc. (almost 5 years)
 - Primary focus on brewing yeast development, brewing process aids, and life cycle analyses
 - Interconnected team of different research units

INTRODUCTION: WHAT WE DO

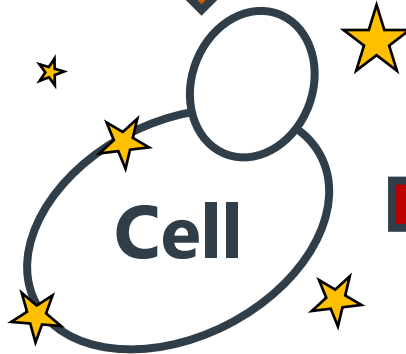
Nutrients

Carbon, nitrogen, minerals, vitamins



Metabolic Products

(ethanol, secondary metabolites)



Extracellular Macromolecules

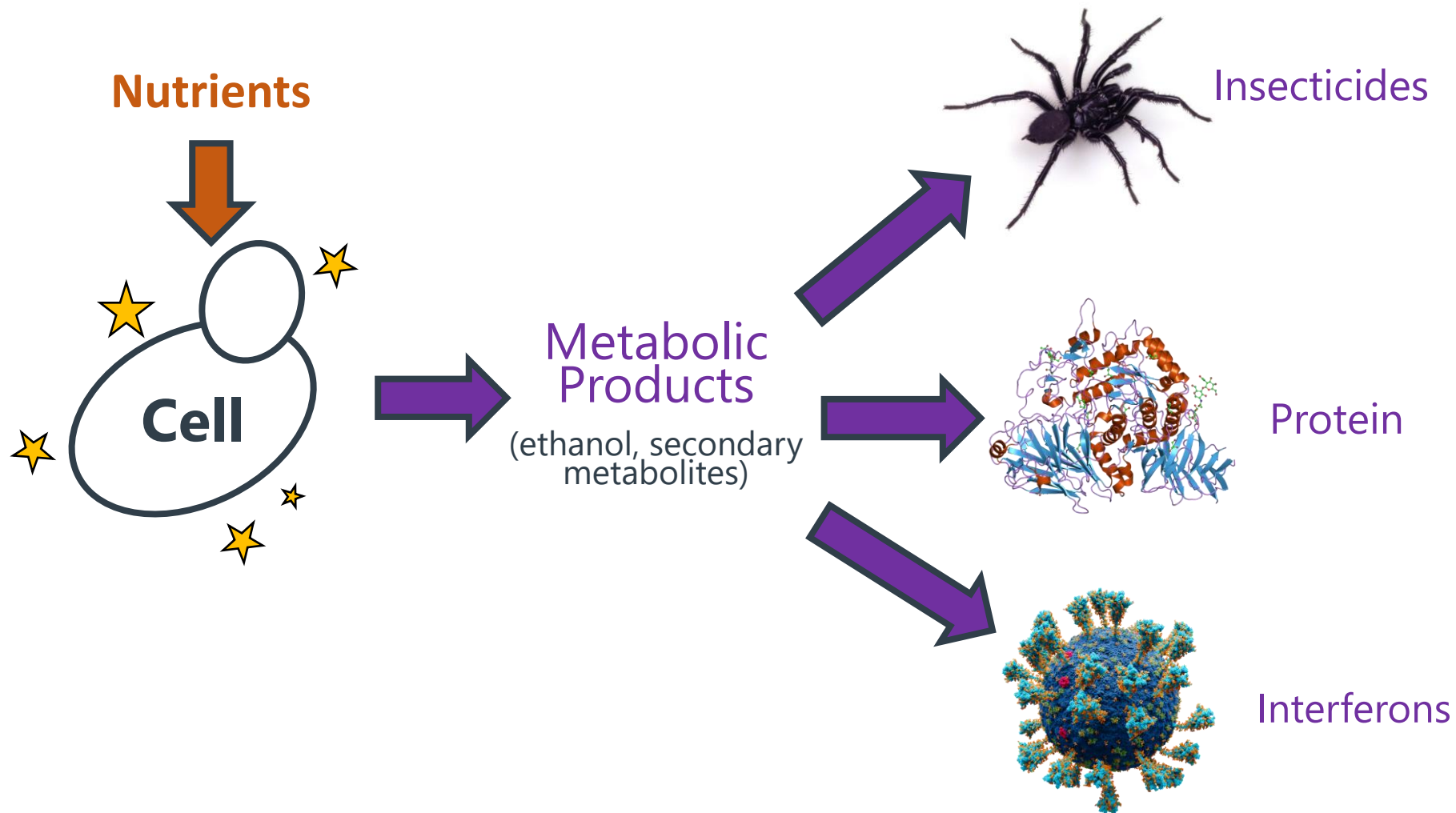
(membrane-bound proteins, chitin/cell wall components)

Biomass/Cell Components

(whole yeast cells, chitin, nucleotides)



INTRODUCTION: YEAST AS A BIOFACTORY



INTRODUCTION: EARLY WORK INTO MORE EFFICIENT BREWERY PROCESSES

The Genetic Modification of Brewing Yeast with Recombinant DNA¹

Edward Hinchliffe and Christine J. Daubney, *Research Department, Bass Public Limited Company, Burton-on-Trent, England*

intermittant process problems. One enzyme that has received considerable attention in recent years is β -glucanase, which can be used to obtain process benefits in wort production, fermentation, and conditioning (3). For example, an endo-1,3-1,4- β -glucanase from the Gram-positive bacterium *Bacillus subtilis* has been shown to produce a quantitative improvement in beer filtration performance (11,12). In view of the cost of adding commercial

INTRODUCTION: EARLY WORK INTO MORE EFFICIENT BREWERY PROCESSES

[European Food Research and Technology](#)

November 2012, Volume 235, [Issue 5](#), pp 951–961 | [Cite as](#)

Construction of recombinant industrial brewer's yeast with lower diacetyl production and proteinase A activity

Authors

[Authors and affiliations](#)

Jun Lu, Jian Dong, Deguang Wu, Yefu Chen, Xuewu Guo, Yu Shi, Xi Sun, Dongguang Xiao 

INTRODUCTION: EARLY WORK INTO MORE EFFICIENT BREWERY PROCESSES

Article | [OPEN ACCESS](#) | Published: 20 March 2018

Industrial brewing yeast engineered for the production of primary flavor determinants in hopped beer

Charles M. Denby , Rachel A. Li, Van T. Vu, Zak Costello, Weiyin Lin, Leanne Jade G. Chan, Joseph Williams, Bryan Donaldson, Charles W. Bamforth, Christopher J. Petzold, Henrik V. Scheller, Hector Garcia Martin & Jay D. Keasling 

Nature Communications **9**, Article number: 965 (2018) | [Download Citation](#) 

INTRODUCTION: MODIFICATION OF COMPLEX METABOLIC PATHWAYS

Complete biosynthesis of cannabinoids and their unnatural analogues in yeast

Xiaozhou Luo, Michael A. Reiter, Leo d’Espaux, Jeff Wong, Charles M. Denby, Anna Lechner, Yunfeng Zhang, Adrian T. Grzybowski, Simon Harth, Weiyin Lin, Hyunsu Lee, Changhua Yu, John Shin, Kai Deng, Veronica T. Benites, George Wang, Edward E. K. Baidoo, Yan Chen, Ishaan Dev, Christopher J. Petzold & Jay D. Keasling 

Nature **567**, 123–126 (2019) | [Download Citation](#) 

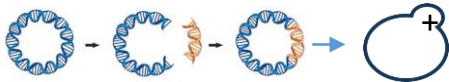
OPTIMIZATION ORIENTED GOALS

Workflow dynamics:

- Assessing industry need
- Clear phenotype in mind
- Cross-discipline/intra-industry coordination
- Viability
- Execution

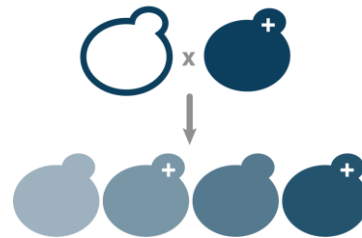
OPTIMIZATION ORIENTED GOALS

Genetic Engineering



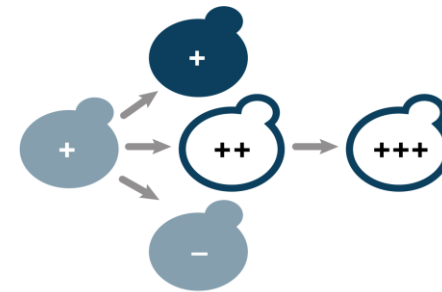
Selective Breeding/Hybridization

non
GMO



Adaptive Evolution

non
GMO



HOW CAN THIS BE ACCOMPLISHED?

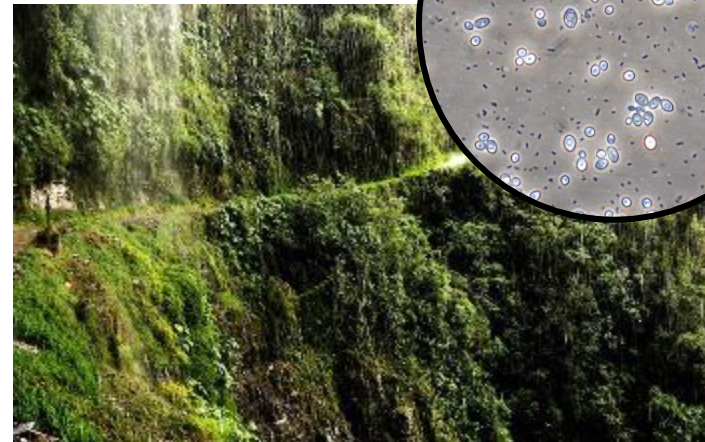
Generating new yeast for modern applications:

- Organism discovery
 - New genes!
- Breeding new strains using classical genetics
 - Mixing genes!
- Direct manipulation of genome with biotechnology
 - Custom genes!

SEARCHING FOR THAT NOVELTY

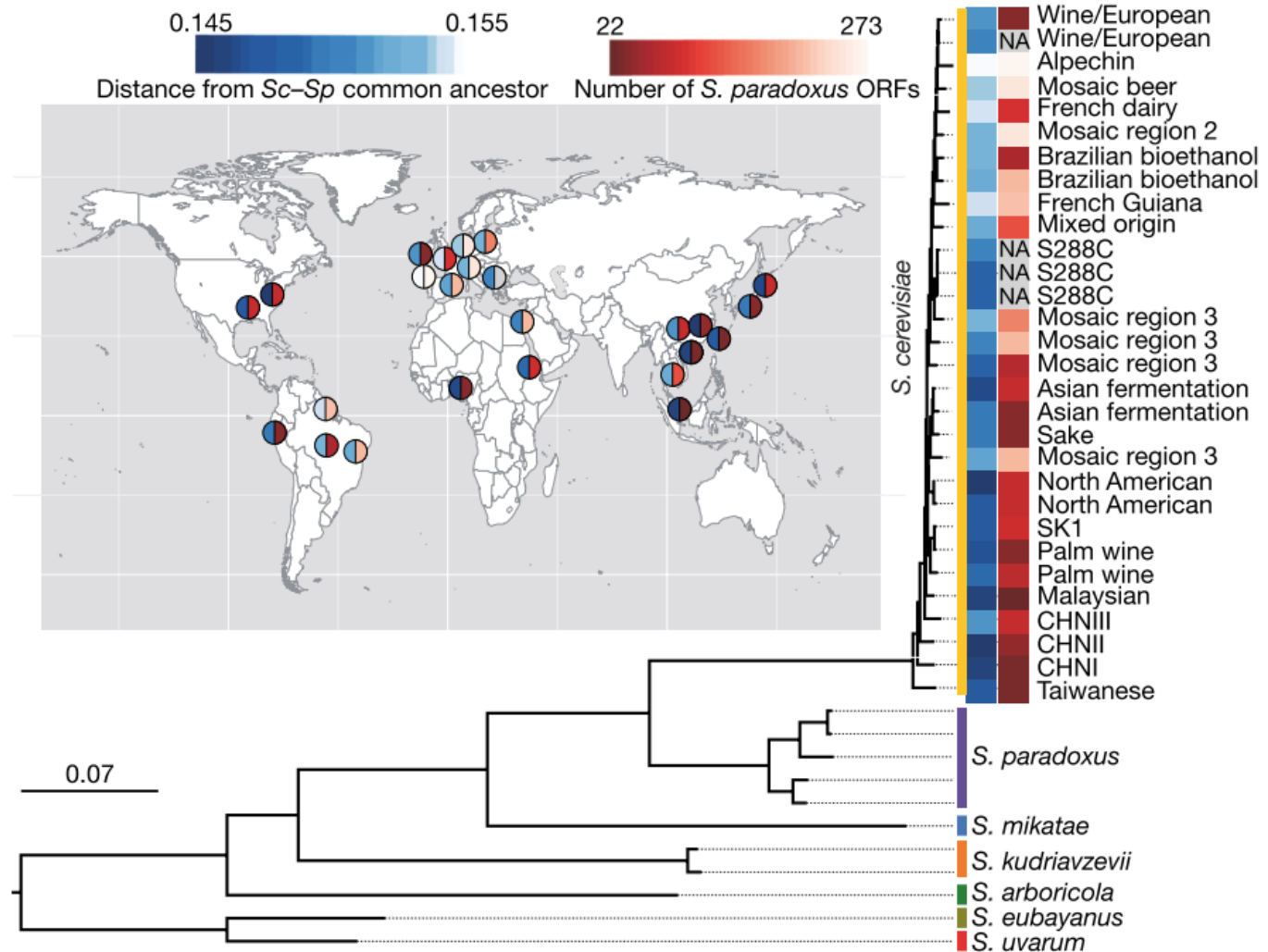
Organism discovery:
Prospecting for novel genetic material

- Exploring new species for fermentation



FOLLOWING THE CLUES

Fig. 2 | Chinese origin of *S. cerevisiae*. Maximum-likelihood rooted tree of the *Saccharomyces* complex, based on the alignment of 2,018 concatenated conserved genes. Heat maps display the distance from the last common ancestor of *S. cerevisiae* (*Sc*)–*S. paradoxus* (*Sp*) (white–blue), and the number of introgressed *S. paradoxus* ORFs (white–red). The map shows the geographical origins of the strains.



Peter J. et al. 2018. Genome evolution across 1,011 *Saccharomyces cerevisiae* isolates Species-wide genetic and phenotypic diversity. *Nature*. 556:339–347.

HOW CAN THIS BE ACCOMPLISHED?

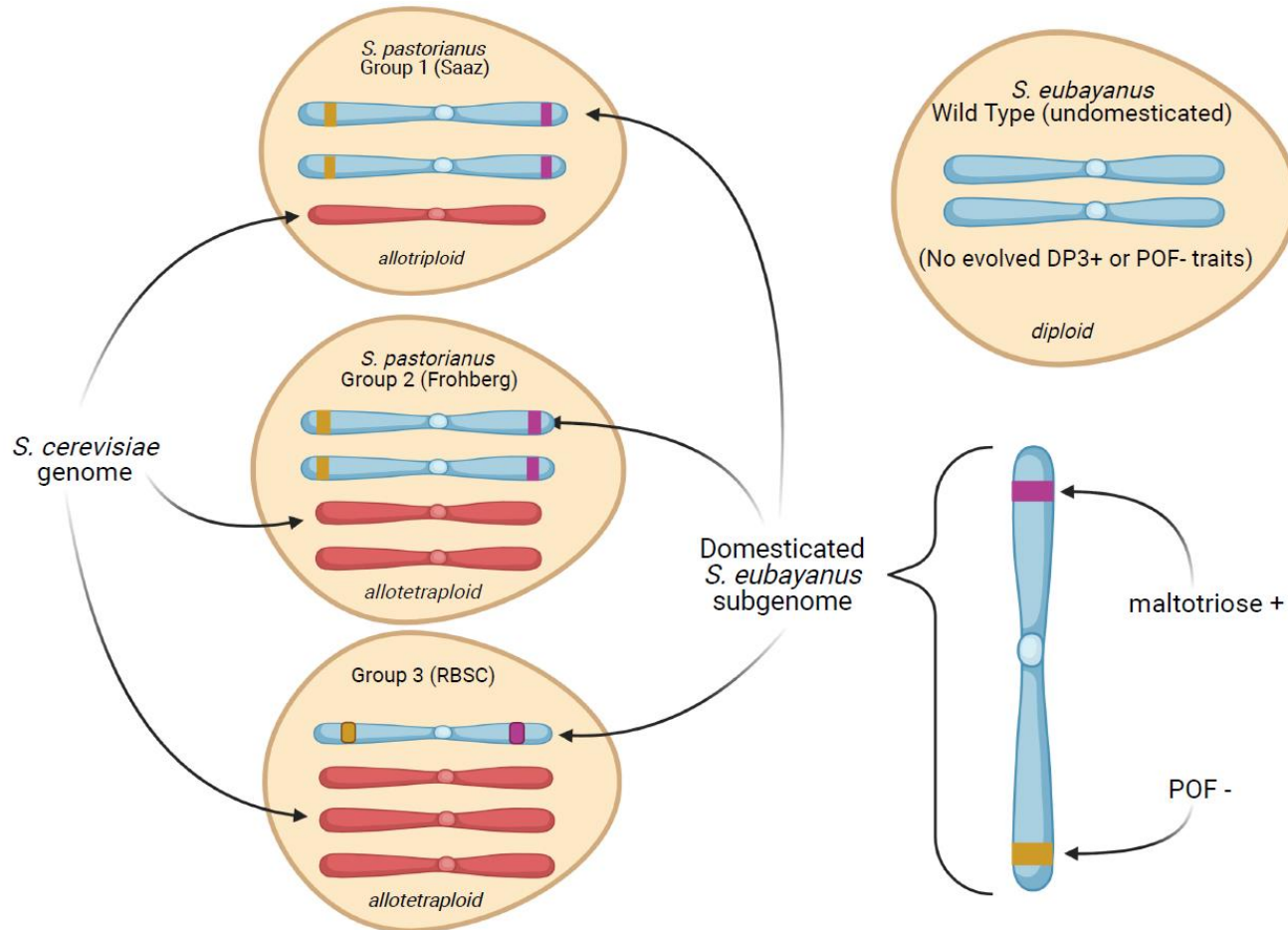
Hybridization:

- Well accepted method as an alternative to direct genetic engineering
- Excellent method of artificially enriching genetic diversity
- Can happen when in nature when conditions are right

However...

- Relies on mating-competent meiotic segregants
- Extensive characterization of parent organisms for optimum results

HYBRIDIZATION SCHEMATIC:



- “Group 3” – proposed by Turgeon *et al.* (2021) taxonomic subgrouping of brewing yeast into new family of artificially derived lager yeast
- Genetically distinct from Group 1 and 2 lager yeast with higher proportion of *S. cerevisiae* genome while retaining classical traits defining domesticated *S. eubayanus*.

Adapted from Turgeon et al. 2021

HYBRIDIZATION SCHEMATIC:

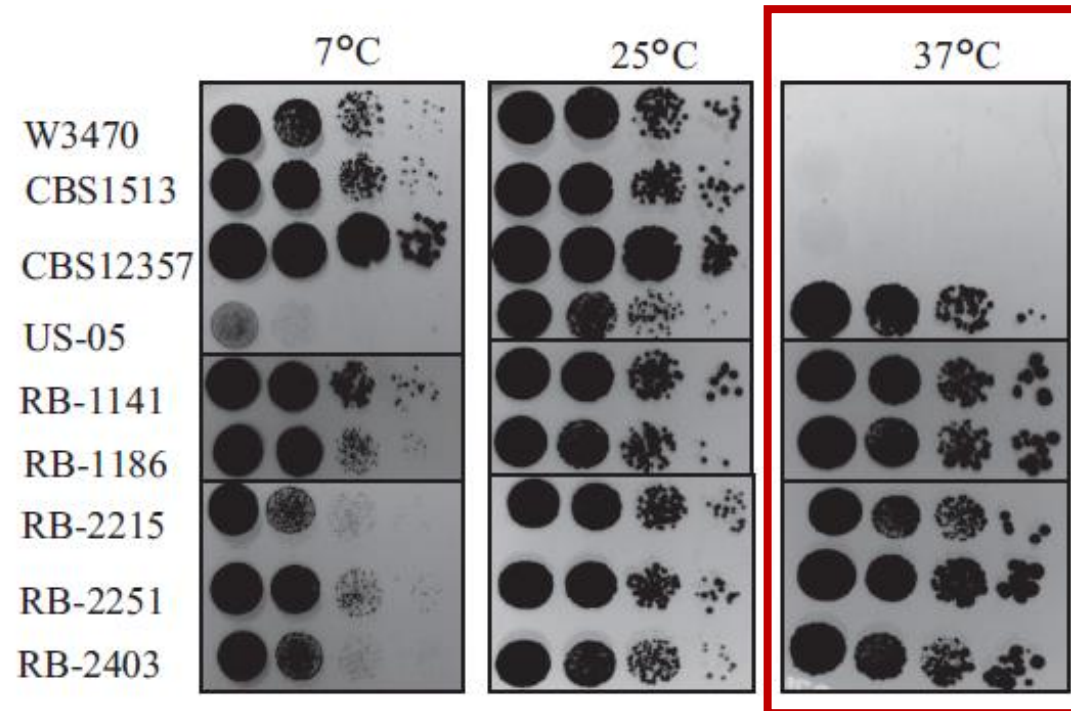
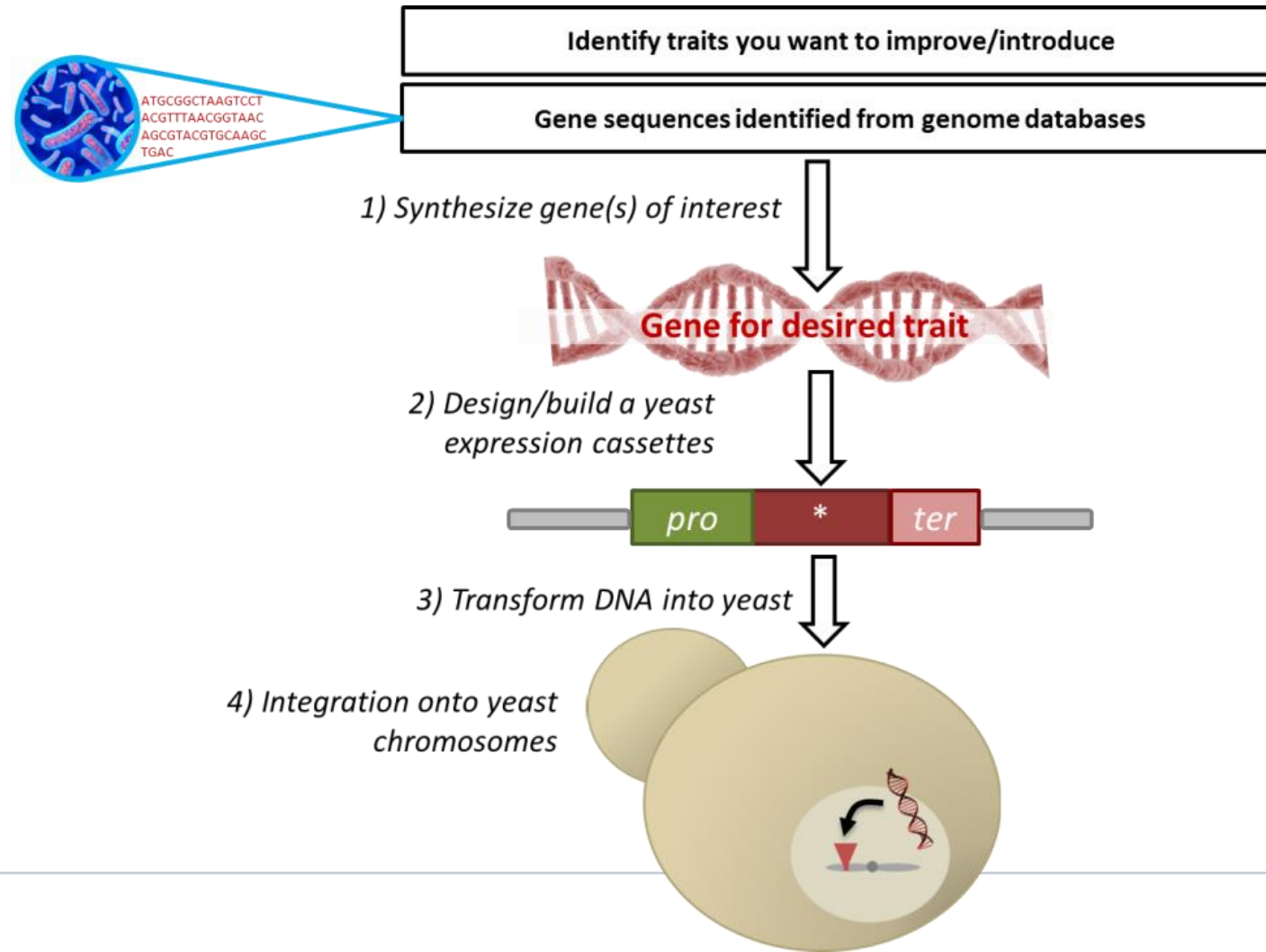


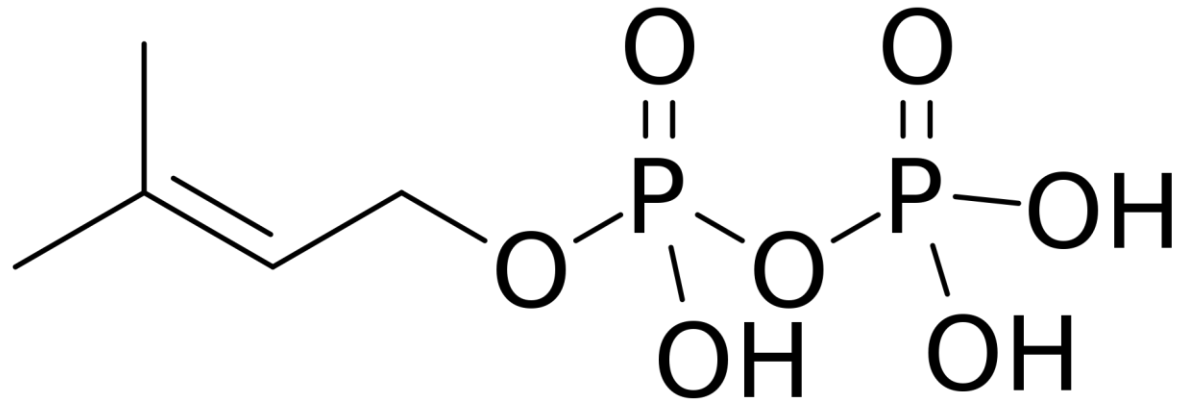
FIG 1 Temperature tolerance of novel hybrids compared to that of group I (CBS1513), group II (W3470), *S. eubayanus* (CBS12357), and *S. cerevisiae* (US-05) controls at 7°C, 25°C, and 37°C. Each spot represents a 10-fold serial dilution (10⁴ to 10¹ cells/spot) on YEG medium.

Adapted from Turgeon et al. 2021

BIOENGINEERING: SIMPLIFIED WORKFLOW

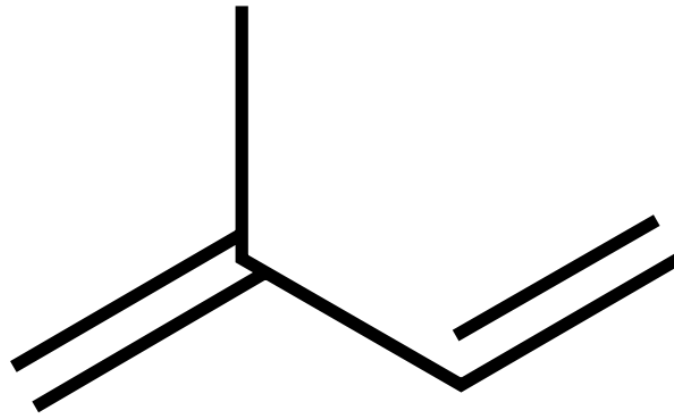


BIOENGINEERING: SIMPLIFIED WORKFLOW



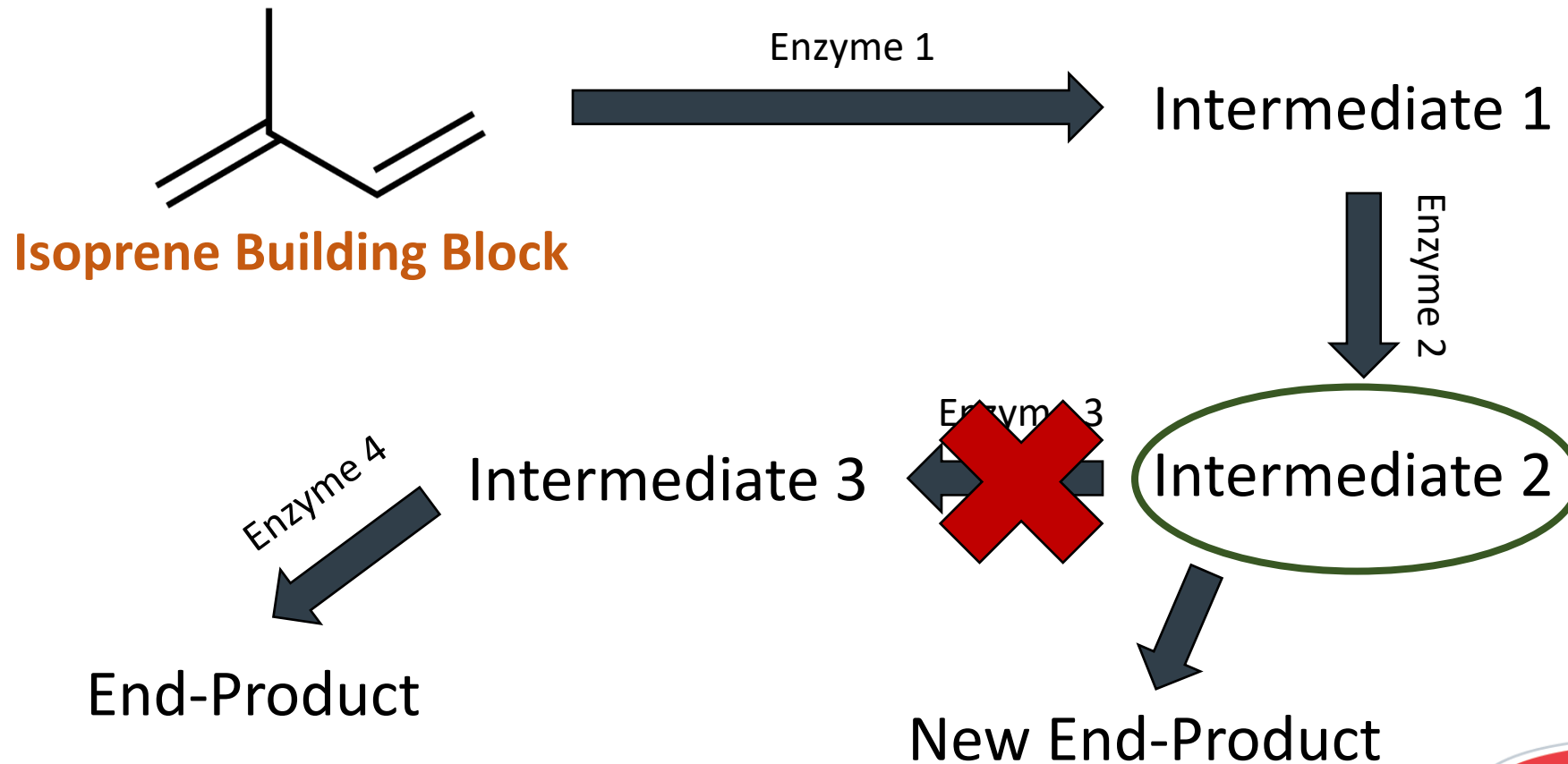
Dimethylallyl pyrophosphate: Terpene-Building Compound

BIOENGINEERING: SIMPLIFIED WORKFLOW

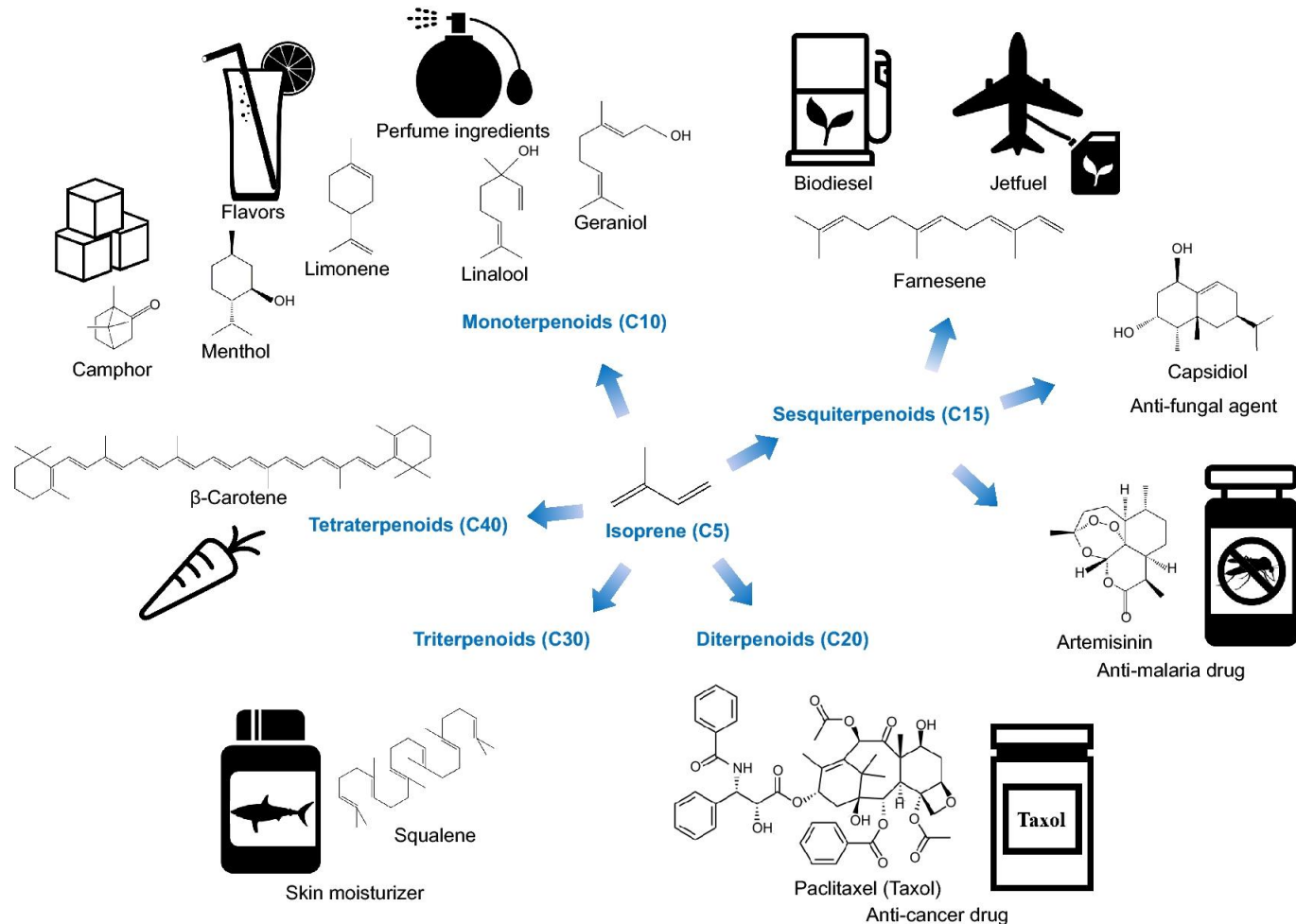


Isoprene – A Universal Biological Building Compound

BIOENGINEERING: SIMPLIFIED WORKFLOW



BIOENGINEERING: SIMPLIFIED WORKFLOW

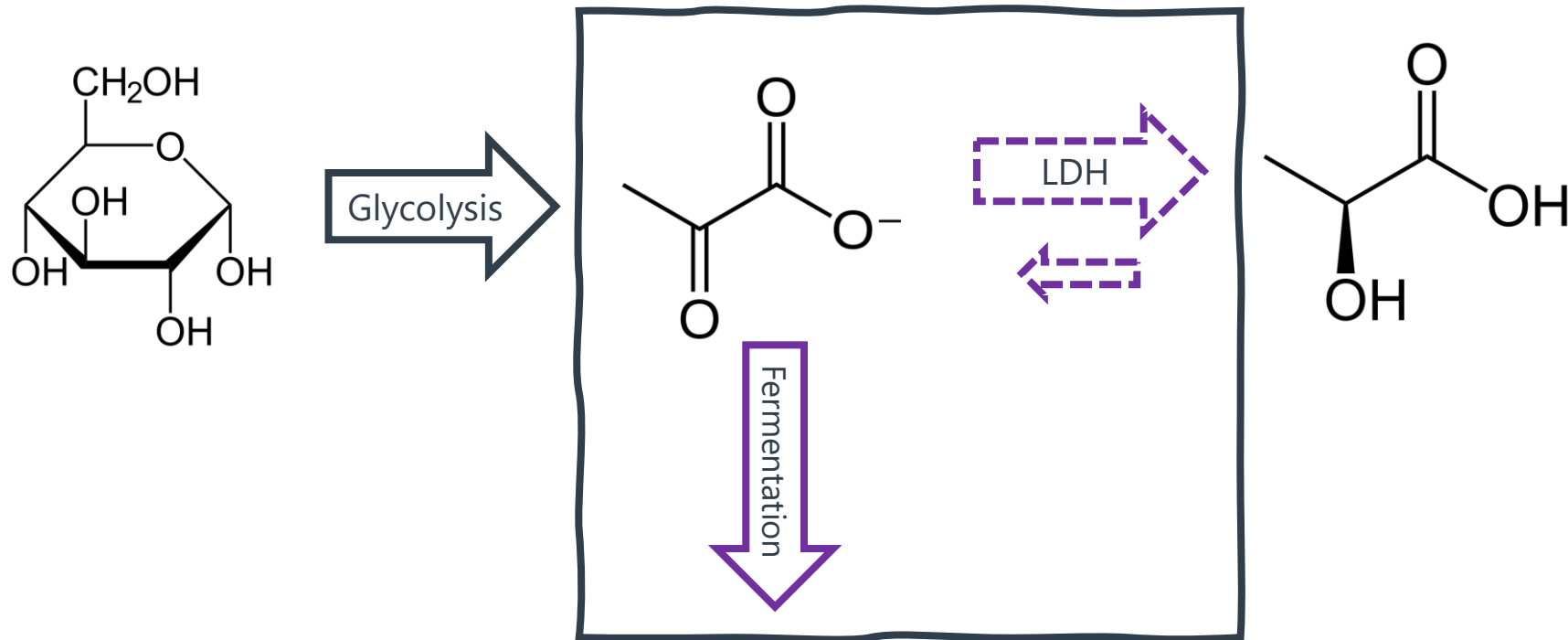


Terpene Pathways:

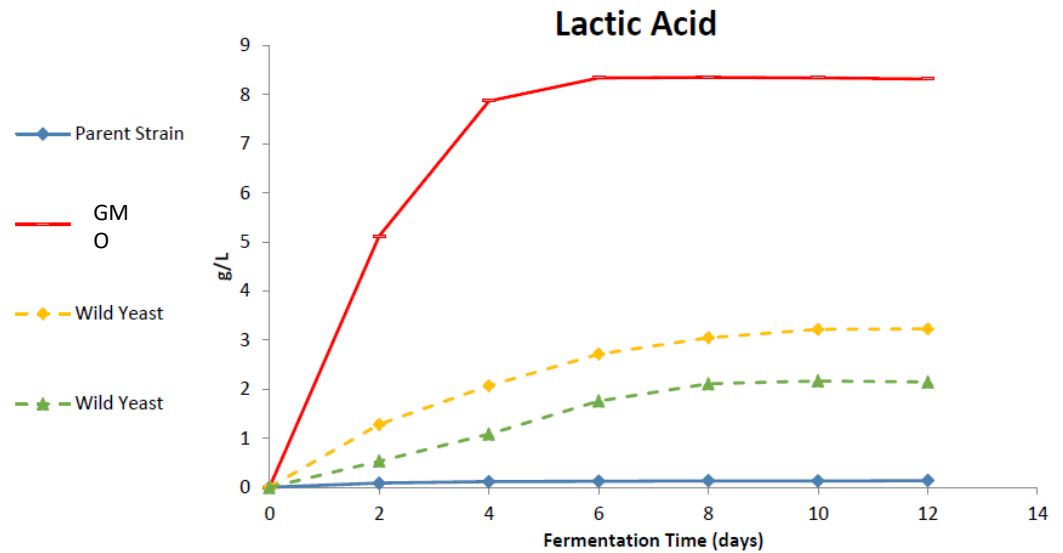
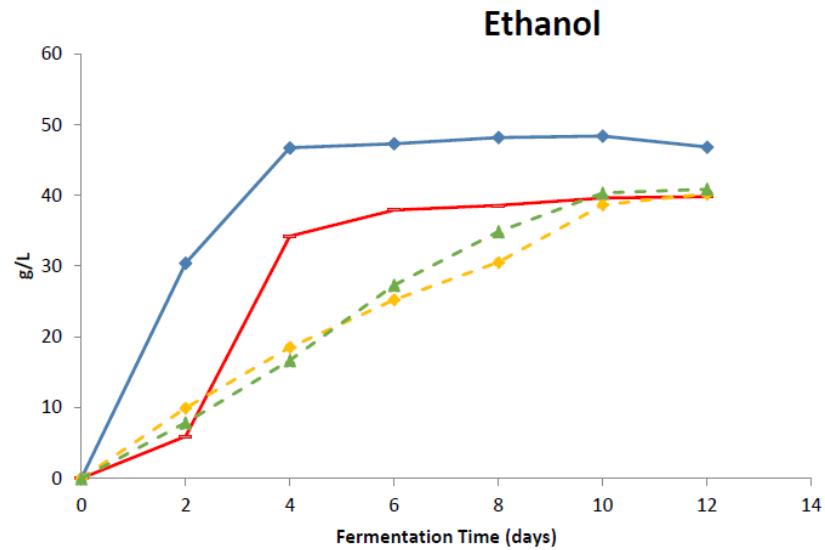
- Of particular interest
- Highly versatile
- Wide range of metabolic byproducts
- Conserved/universal

BIOENGINEERING: SIMPLIFIED WORKFLOW

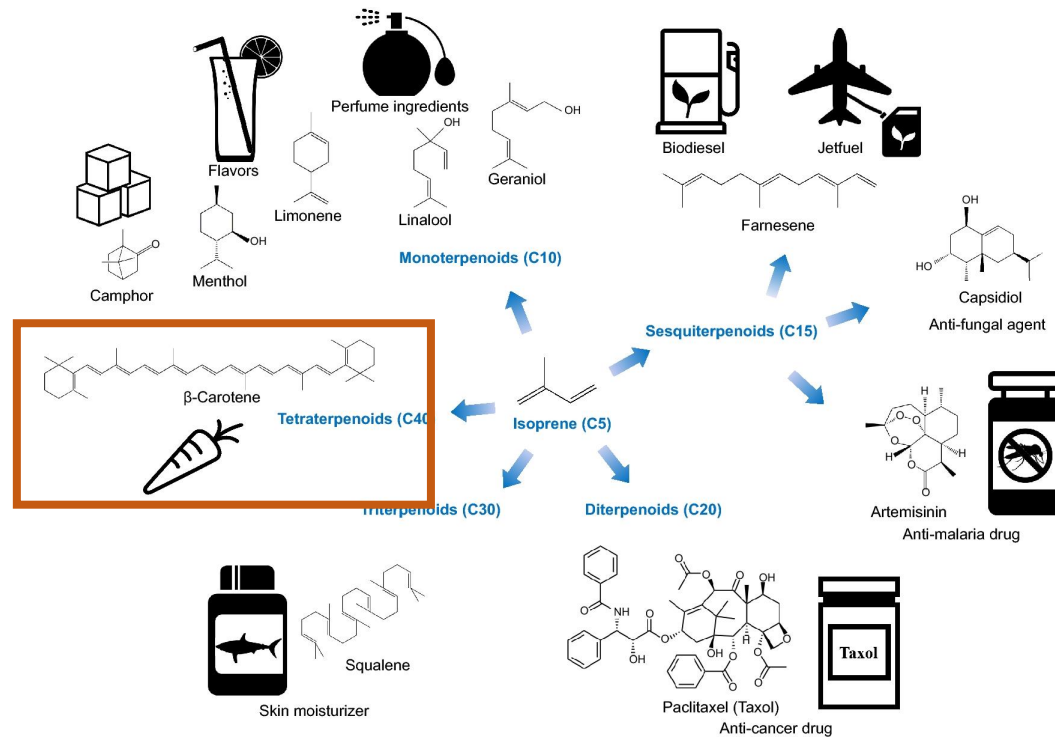
Proof-of-concept modification turned brewing product:



BIOENGINEERING: SIMPLIFIED WORKFLOW



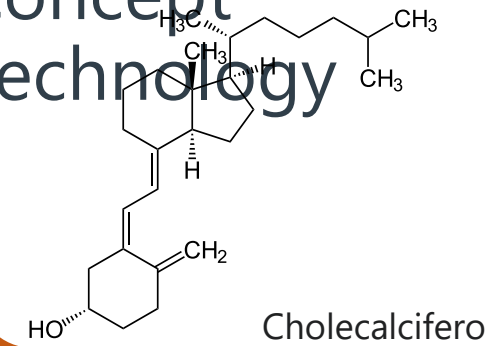
BIOENGINEERING: SIMPLIFIED WORKFLOW



Exploration of other pathways:

Further proof of

concept technology



FEMS Yeast Research, Volume 17, Issue 8, December 2017, fox080, <https://doi.org/10.1093/femsyr/fox080>

THANK YOU FOR YOU TIME!