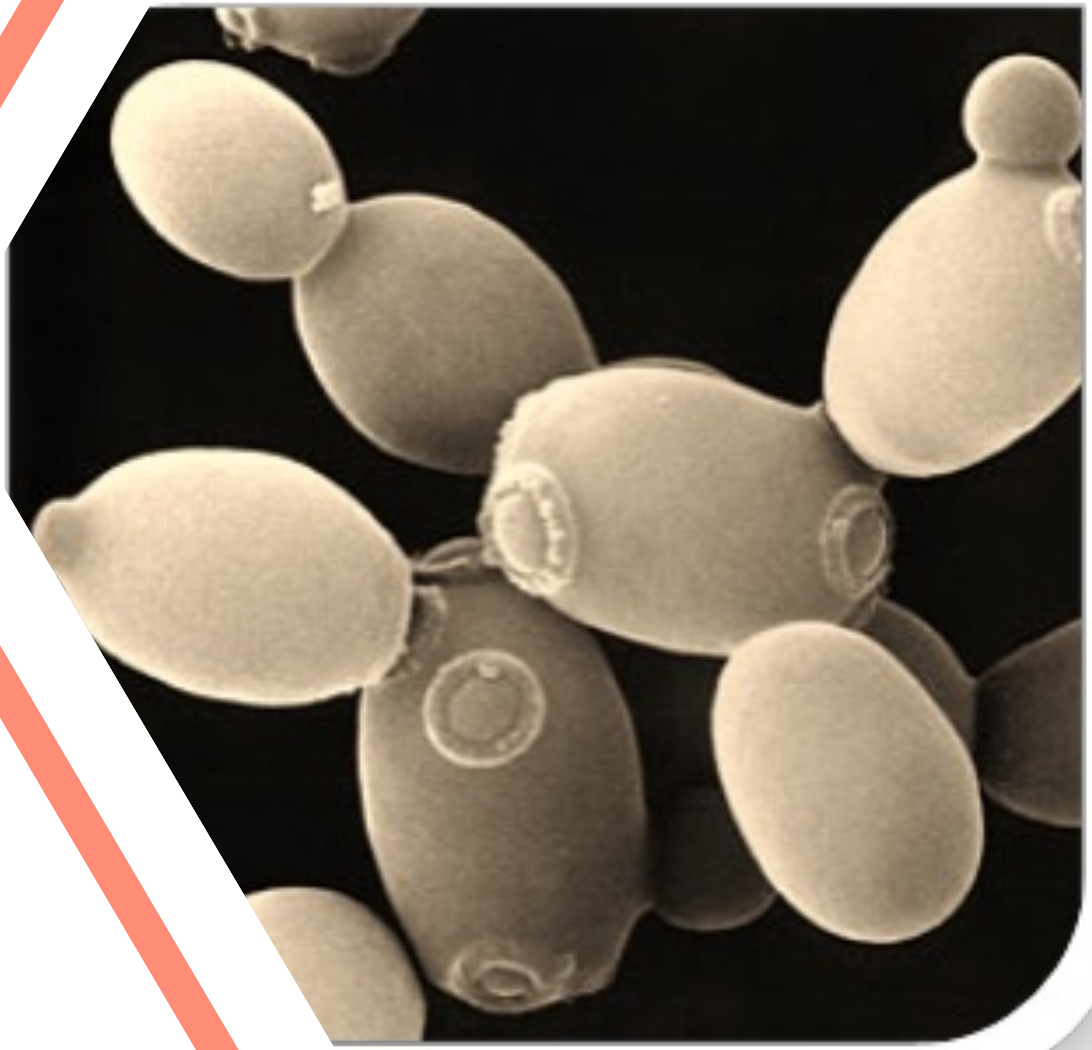
A Discussion of Genetic Engineering in the Brewing Industry

The Potential of Bioengineered Brewing Yeast



Chaz Rice
Senior Research Scientist
Mascoma LLC – a Lallemand subsidiary

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Outline

- How to engineer a brewing yeast and common vernacular
  - Homologous recombination versus CRISPR
- Traits of interest for brewing yeast
  - Examples of bioengineered brewing yeast
- Regulatory and Labeling
- Path Forward

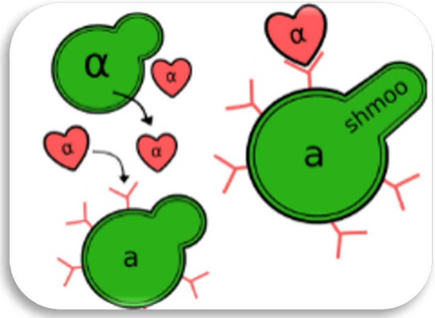
Methods for Generating "New" Brewing Yeast

Organism discovery – "Bioprospecting"

- Isolate new or adapted yeast from relevant ecosystems
- Explore new species for fermentation

Crafting yeast via genetic manipulation

- Classical genetics
  - Mating
  - Hybridization
  - Protoplast fusions
  - Mutagenesis



- Genetic engineering
  - Yeast-mediated ligation
  - CRISPR
  - Transgenic vs self-cloned

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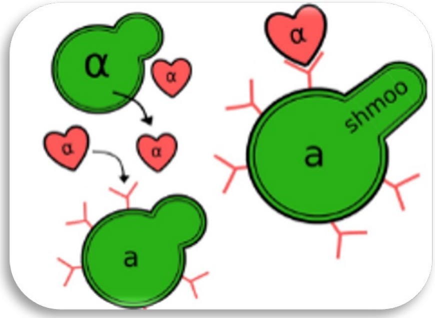
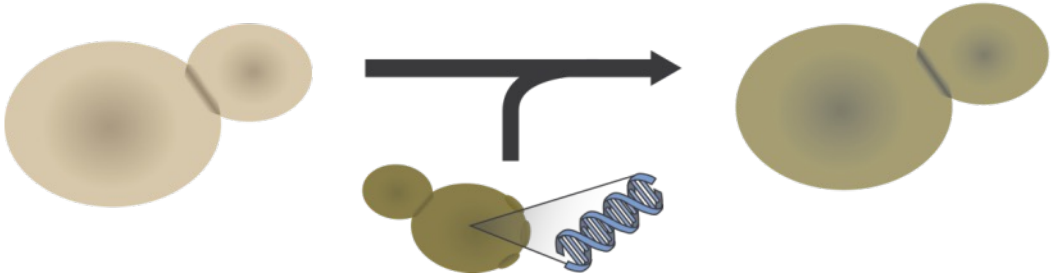


- Genetic engineering
  - Yeast-mediated ligation
  - CRISPR
  - Transgenic vs self-cloned

Self-cloned vs Transgenic Engineering

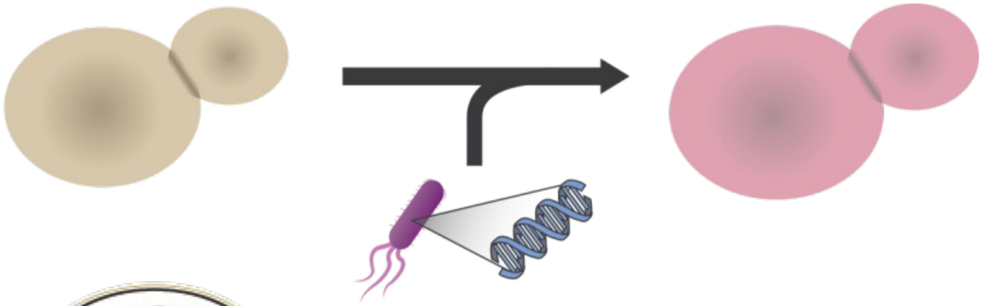
Cisgenic or "self-cloned"

DNA from the same species



Transgenic or "Heterologous"

DNA from a different genus



Self-cloned vs Transgenic Engineering

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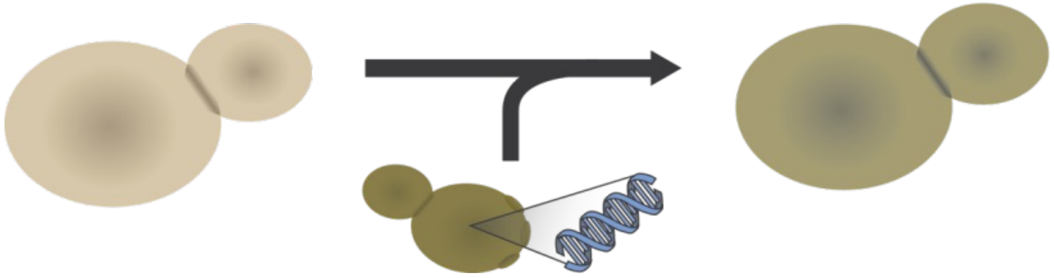
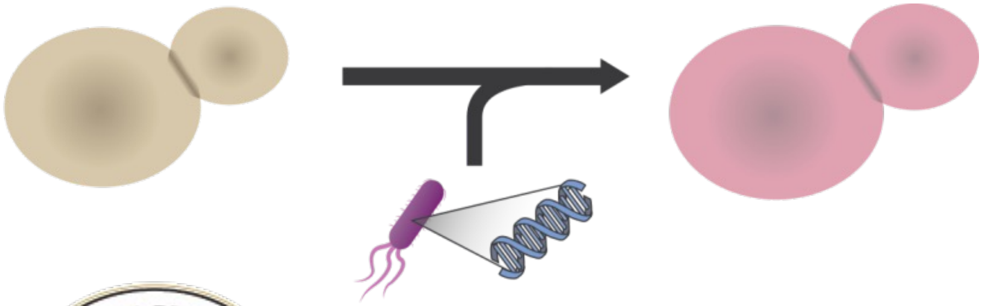


Overexpression or deletion of a native yeast gene or pathway

Transgenic or "Heterologous"

DNA from a different genus



Introduction of a new gene/pathway from a non-yeast organism to enhance a desired trait or introduce a novel trait

Genetic Engineering in *S. cerevisiae*

Promoter | **Gene (open reading frame)** | **Terminator**

Controls production of RNA → when & how much

Designates the order of amino acids and the resulting protein function

Stops transcription

Typical Yeast Expression Cassette

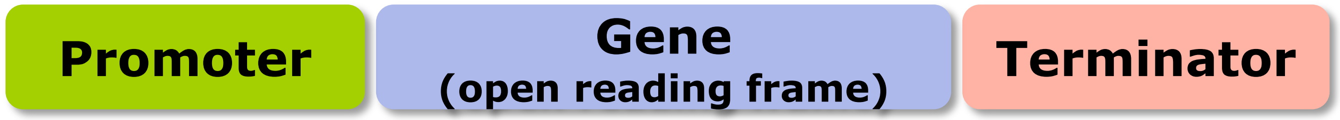
Genetic Engineering in *S. cerevisiae*

Promoter	Gene (open reading frame)	Terminator
Controls production of RNA → when & how much	Designates the order of amino acids and the resulting protein function	Stops transcription

Typical Yeast Expression Cassette

- Promoters and terminators are often taken from the *S. cerevisiae* genome for "native" control of gene expression
- Gene of interest can either be from the yeast genome (self-cloned) or from a new organism such as bacteria, mammals, plants, fungi (transgenic)

Genetic Engineering in *S. cerevisiae*



Identify a target sequence in the genome to introduce the new expression cassette

Genetic Engineering in *S. cerevisiae*

PCR clone each genetic element with overlapping homology, including the flanking regions of the yeast genomic target

Genetic Engineering in *S. cerevisiae*

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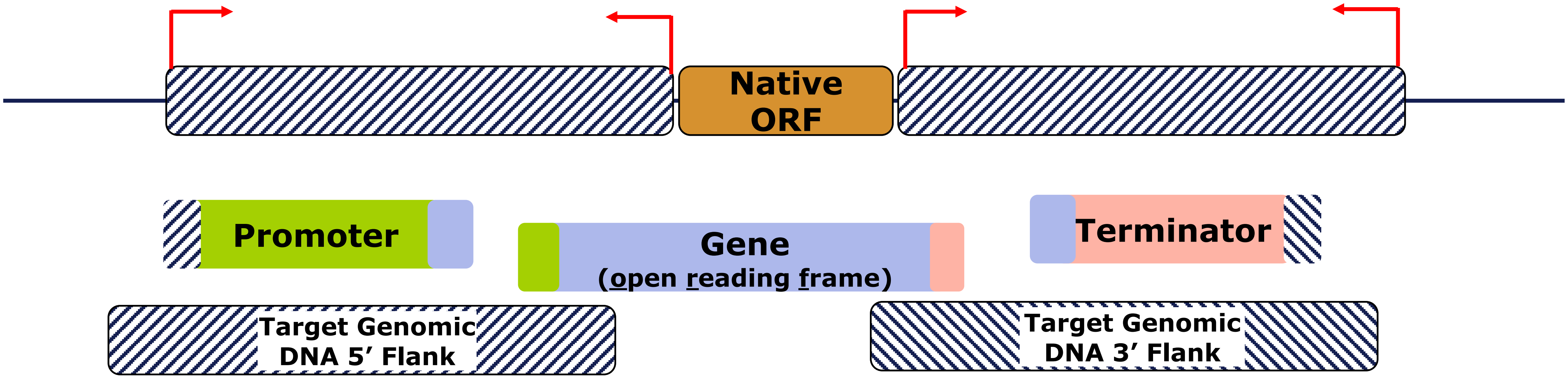
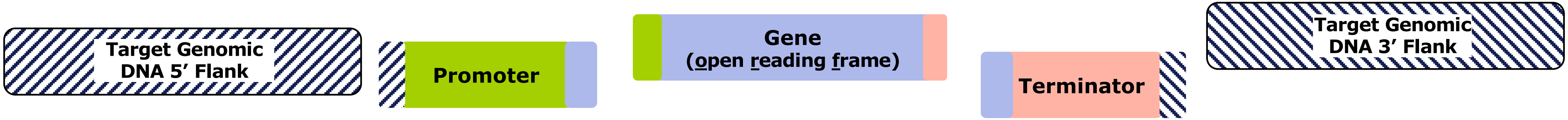
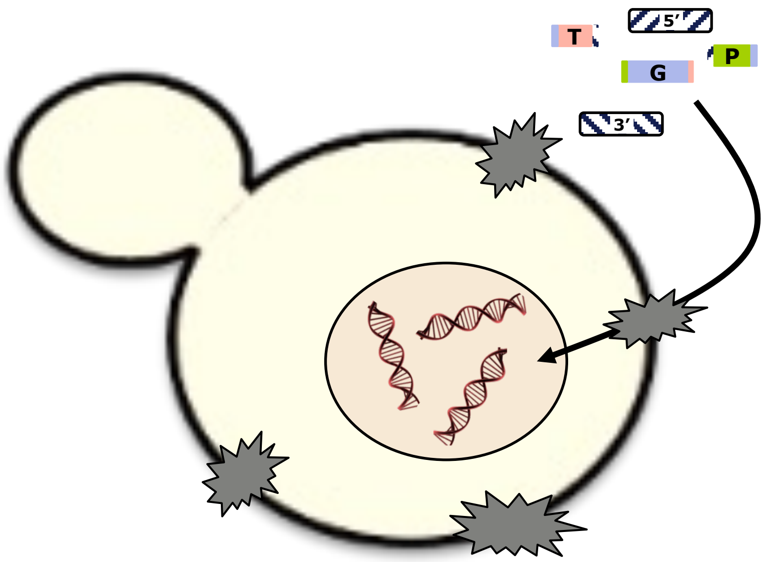
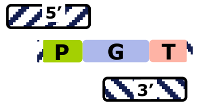


Genetic Engineering in *S. cerevisiae*



Transform the PCR cloned DNA fragments into yeast, typically via electrical shock to open membrane pores



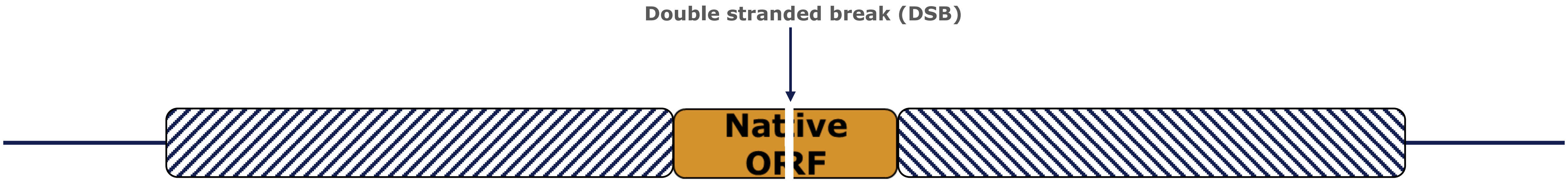


Expression cassette can also be pre-assembled via *in vitro* reactions

Genetic Engineering in *S. cerevisiae*

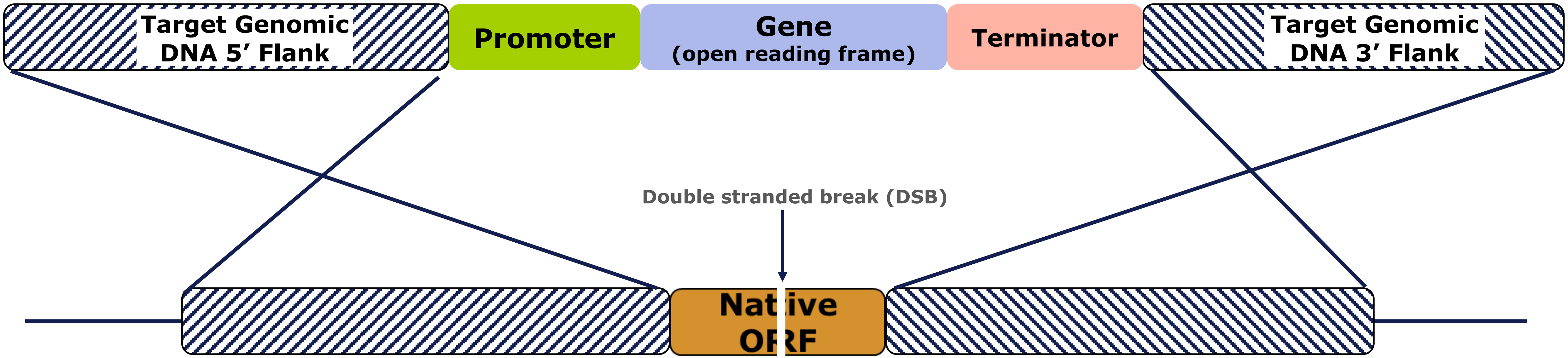
Yeast will "stitch" together or ligate the DNA fragments by homologous recombination which is a native yeast DNA repair mechanism

Genetic Engineering in *S. cerevisiae*



The expression cassette can be used to fix random double strand breaks at the targeted site through a double crossover event with the gDNA flanking regions

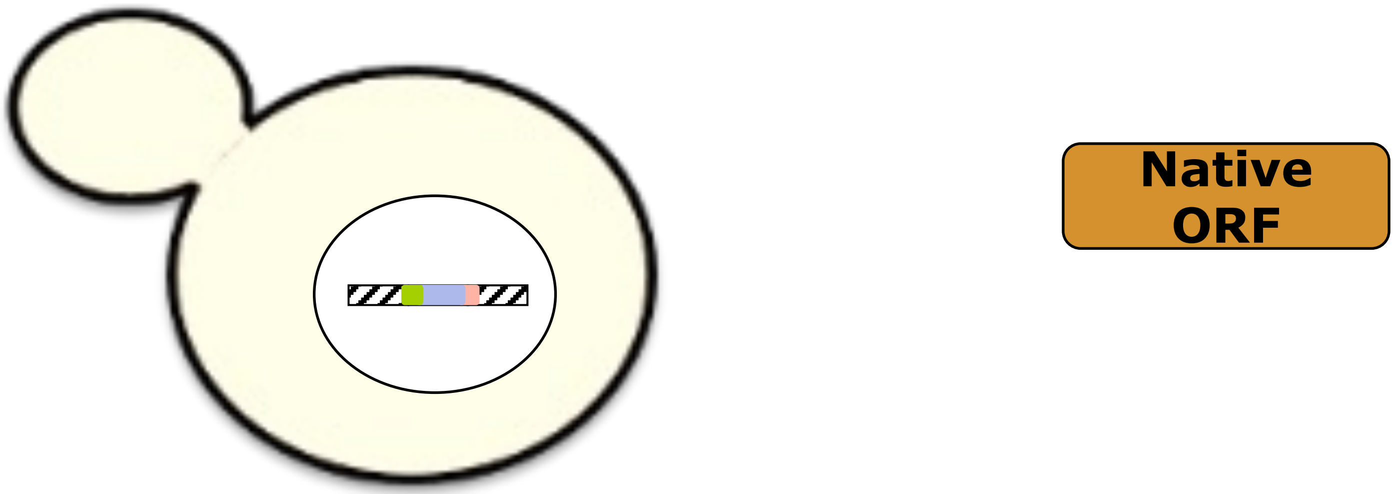
Genetic Engineering in *S. cerevisiae*



The expression cassette can be used to fix random double strand breaks at the targeted site through a double crossover event with the gDNA flanking regions

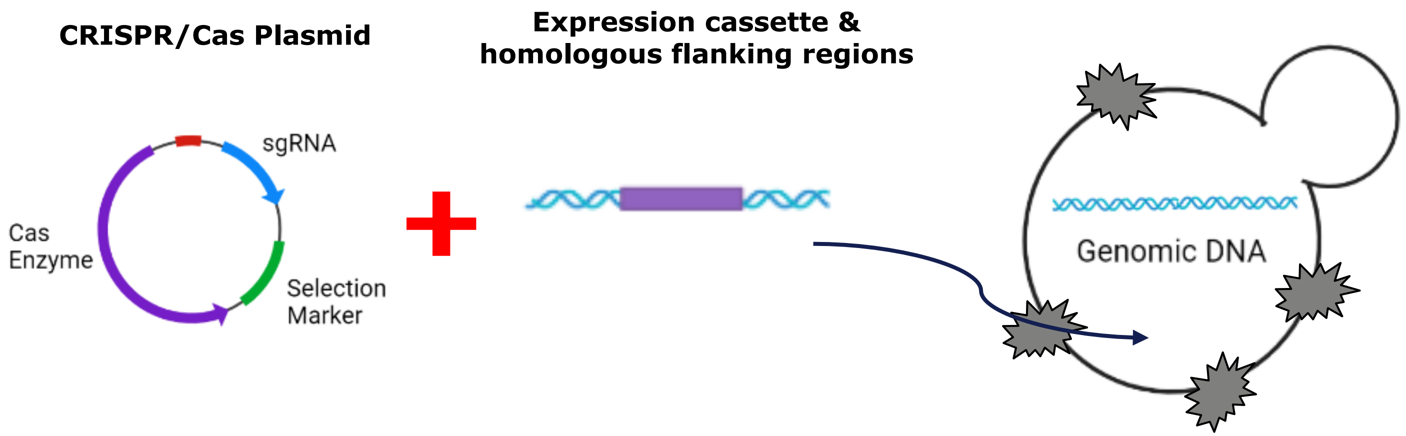
Genetic Engineering in *S. cerevisiae*

Chromosome is fixed while introducing the new expression cassette and typically deleting a gene that can be used as a selection



CRISPR/Cas System

<u>C</u>lustered <u>R</u>egularly <u>I</u>nterspaced <u>S</u>hort <u>P</u>alindromic <u>R</u>epeats



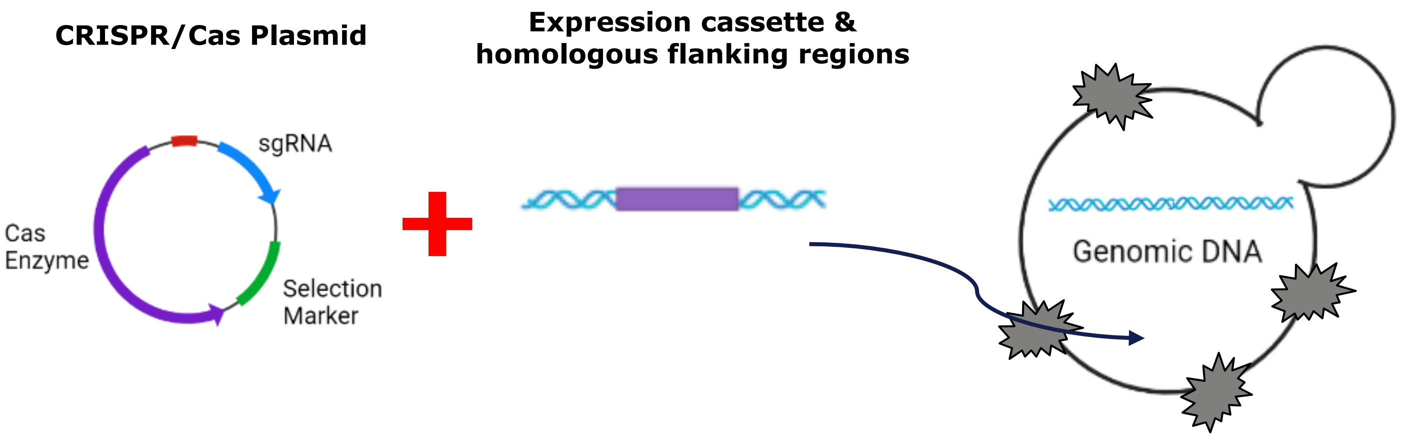
Nuclease-mediated system to introduce specific double stranded DNA breaks

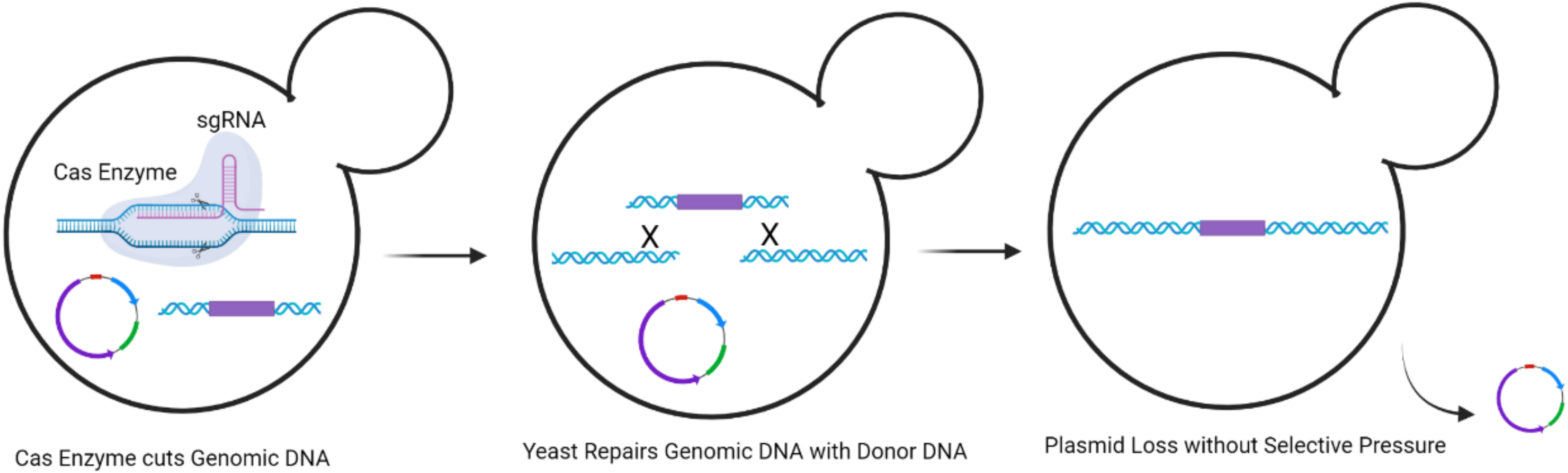
- **Single guide RNA (sgRNA) contains:**
  - **A unique 20 nucleotide targeting sequence to the site of interest**
  - **Cas nuclease-recruiting sequence (tracrRNA)**
  - **Proto-spacer adjacent motif (PAM site)**

- **Cas nuclease enzyme is recruited to the gRNA-DNA complex and makes a very specific cleavage in the chromosome**

- **The break can be repaired by homologous recombination with introduced homologous DNA (ie, an expression cassette)**

CRISPR/Cas System





*Graphic provided by Laura Burns – Omega Yeast

What traits would we want to target in brewing yeast?

Peformance, Stress tolerance, Enzymes

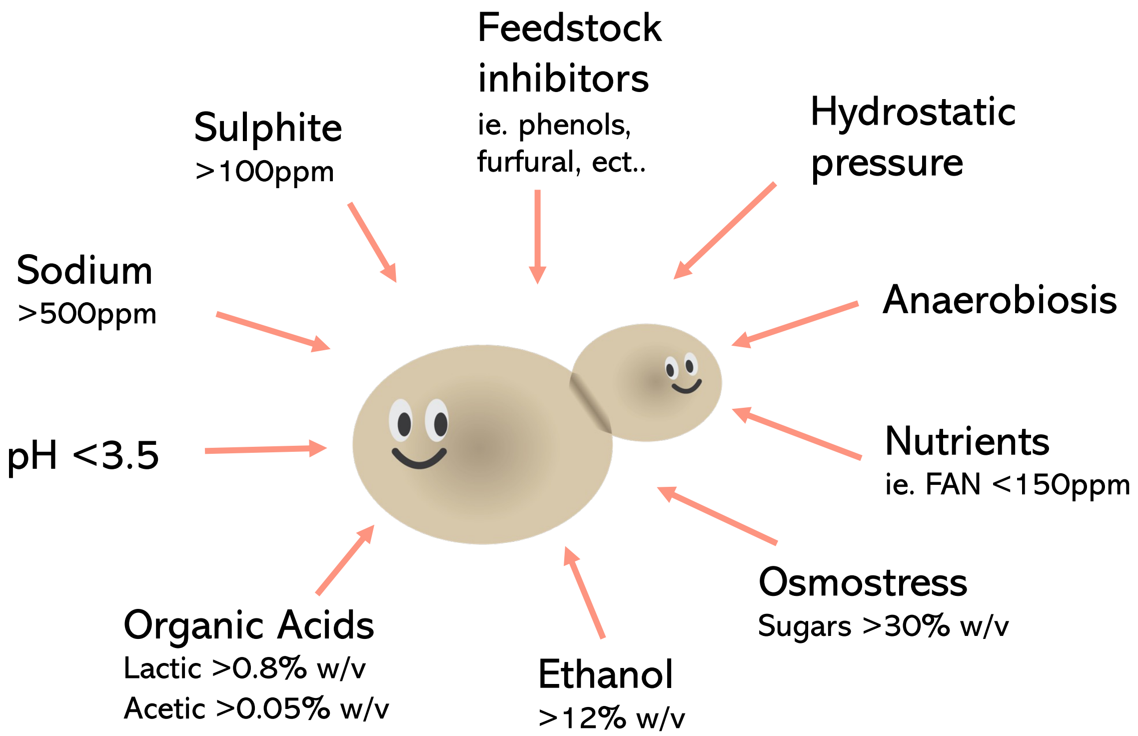
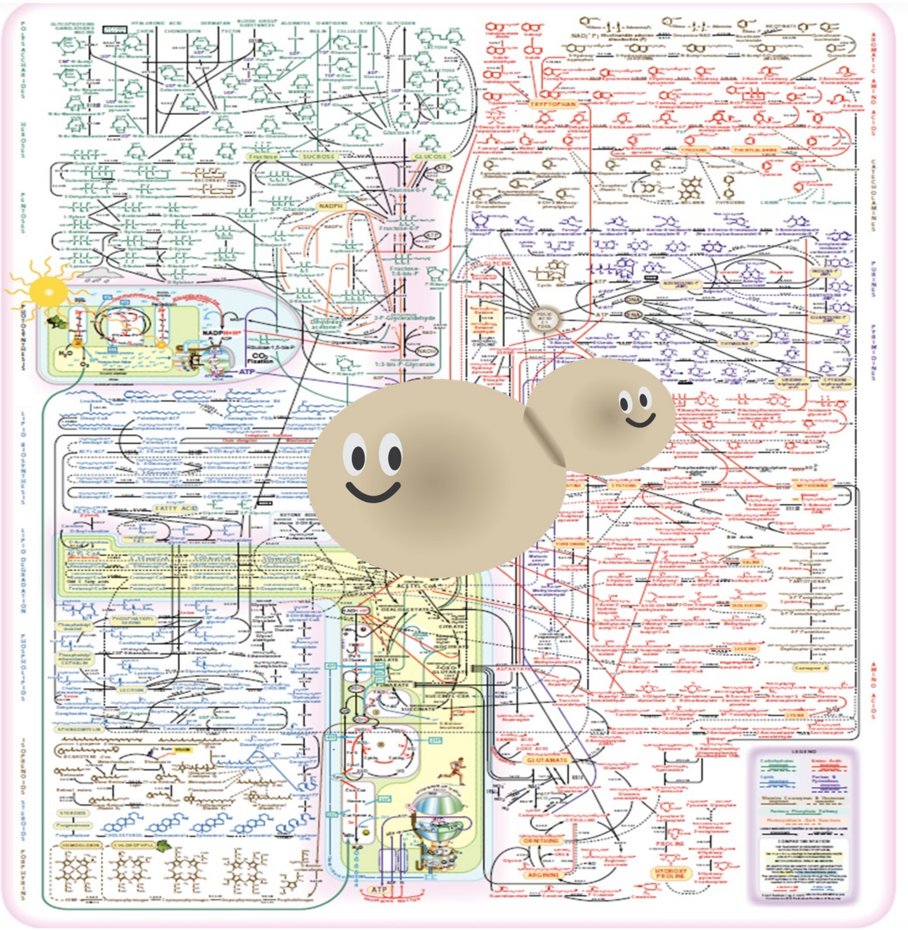


Figure adapted from Ingledew (1999)

Flavors and metabolites



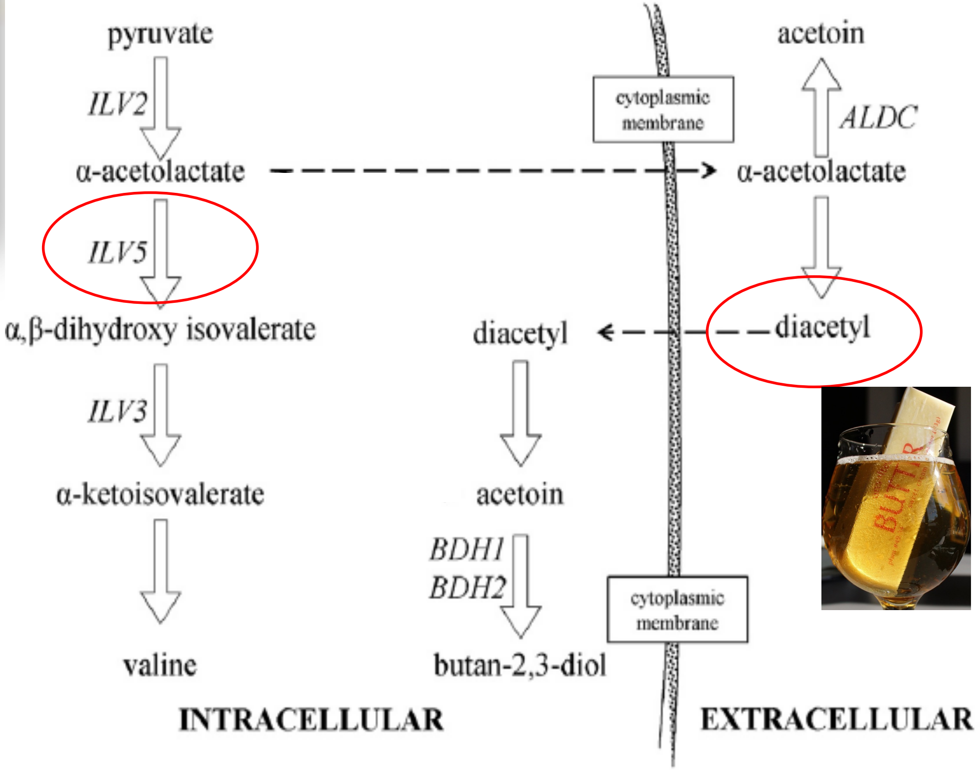
Low Diacetyl production and improved foam stability

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

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

- Overexpression of the ILV5 gene to increase acetolactate utilization (thereby preventing it from spontaneously forming into diacetyl)

- Partial removal of the proteinase A gene to decrease extracellular protease activity to improve foam stability



Graphic: Krabin et al 2017

Lu et al 2012

Vanillin pathway engineered into *S. cerevisiae*

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2009, p. 2765–2774 Vol. 75, No. 9
0099-2240/09/$08.00+0 doi:10.1128/AEM.02681-08

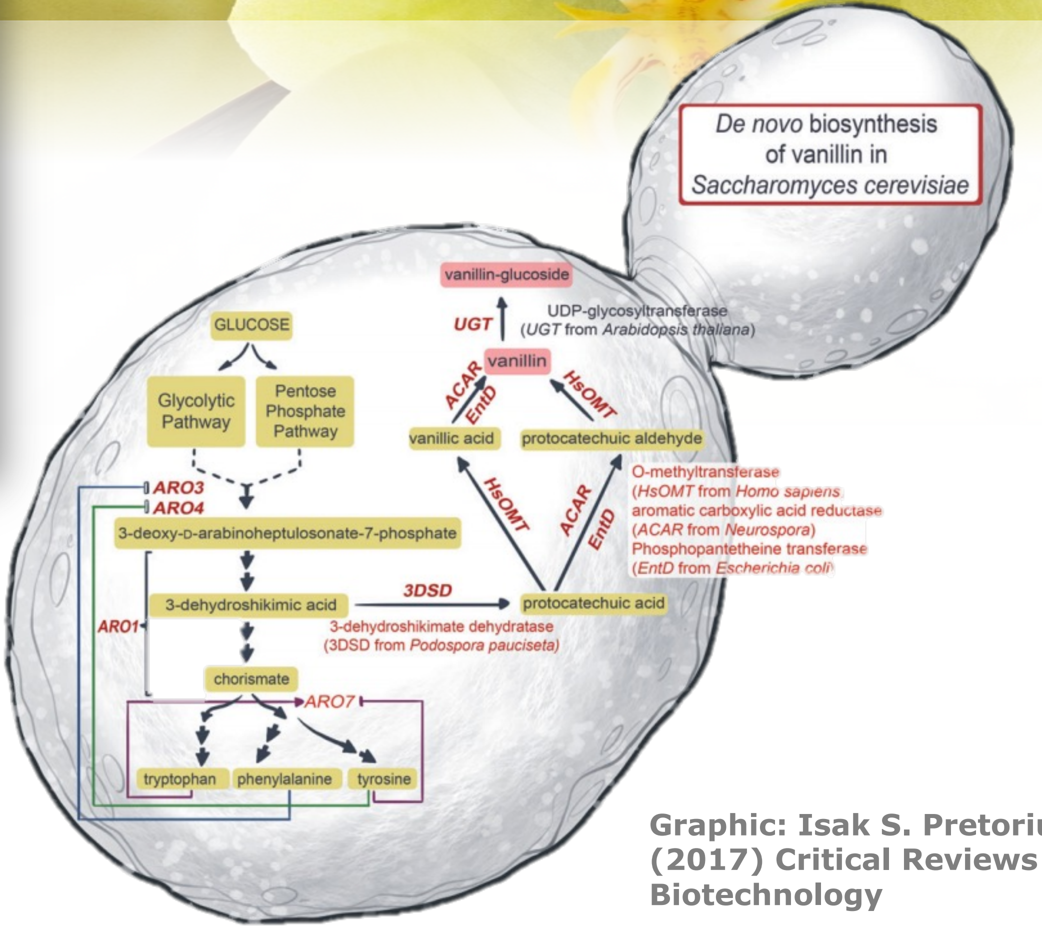
De Novo Biosynthesis of Vanillin in Fission Yeast (*Schizosaccharomyces pombe*) and Baker's Yeast (*Saccharomyces cerevisiae*)▽

Esben H. Hansen,[1¶] Birger Lindberg Møller,[2] Gertrud R. Kock,[1¶] Camilla M. Bünner,[1#] Charlotte Kristensen,[1¶] Ole R. Jensen,[1‡] Finn T. Okkels,[1§] Carl E. Olsen,[3] Mohammed S. Motawia,[2] and Jørgen Hansen[1*]

Poalis A/S, Bülowsvej 25, DK-1870 Frederiksberg C, Denmark[1]; Plant Biochemistry Laboratory, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Copenhagen[2]; and Department of Natural Sciences, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Copenhagen[3]

Received 24 November 2008/Accepted 6 March 2009

- **4 enzymes engineered into yeast for *de novo* production of natural vanillin**



Graphic: Isak S. Pretorius (2017) Critical Reviews in Biotechnology

Hansen et al 2009

Hop flavor (monoterpene) production in *S. cerevisiae*

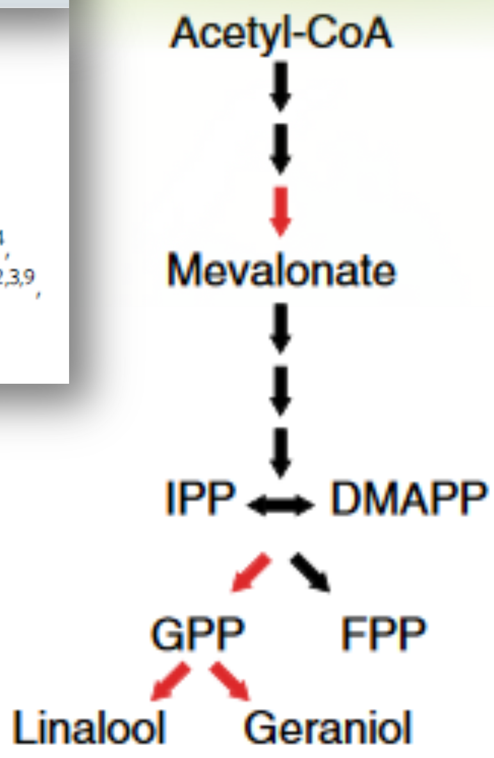
nature COMMUNICATIONS

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

Charles M. Denby[1,2], Rachel A. Li[2,3,4], Van T. Vu[5], Zak Costello[2,4,6], Weiyin Lin[1,2], Leanne Jade G. Chan[2,4], Joseph Williams[7], Bryan Donaldson[8], Charles W. Bamforth[7], Christopher J. Petzold[2,4], Henrik V. Scheller[2,3,9], Hector Garcia Martin[2,4,6] & Jay D. Keasling[1,2,4,5,10,11]

BERKELEY YEAST

- Overexpressed a **linalool synthase** from mint and a **geraniol synthase** from basil to produce essential hop oils in brewing yeast



https://berkeleyyeast.com

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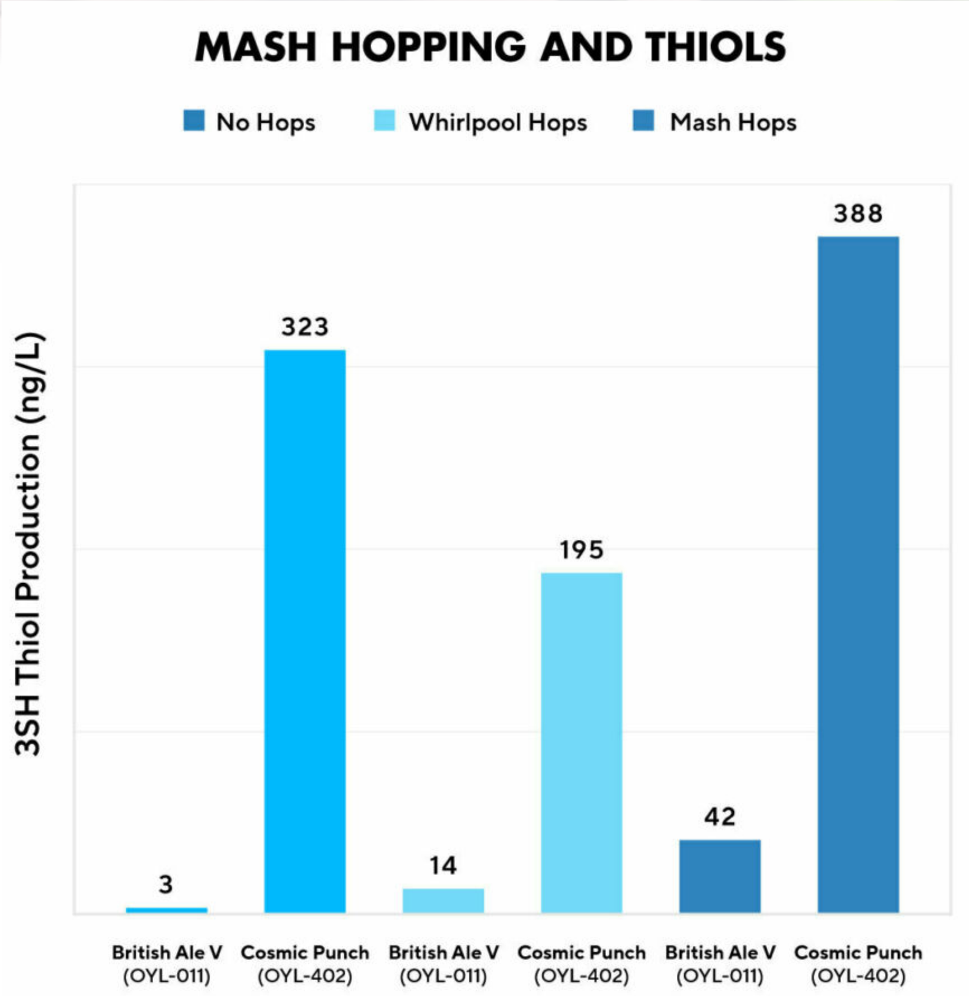
Denby et al 2018

Increased β-Lyase expression for enhanced thiol release

- β-lyase activity results in the release of volatile sulfur compounds called thiols associated with tropical aroma and are active at very low flavor thresholds

- Omega repaired the functionality of the native *S. cerevisiae* β–lyase gene IRC7, to enhance the biotransformation of thiol precursors to there volatile and aromatic free forms

- Significant enhancement of grapefruit, passion fruit and guava notes



OMEGA YEAST

https://omegayeast.com

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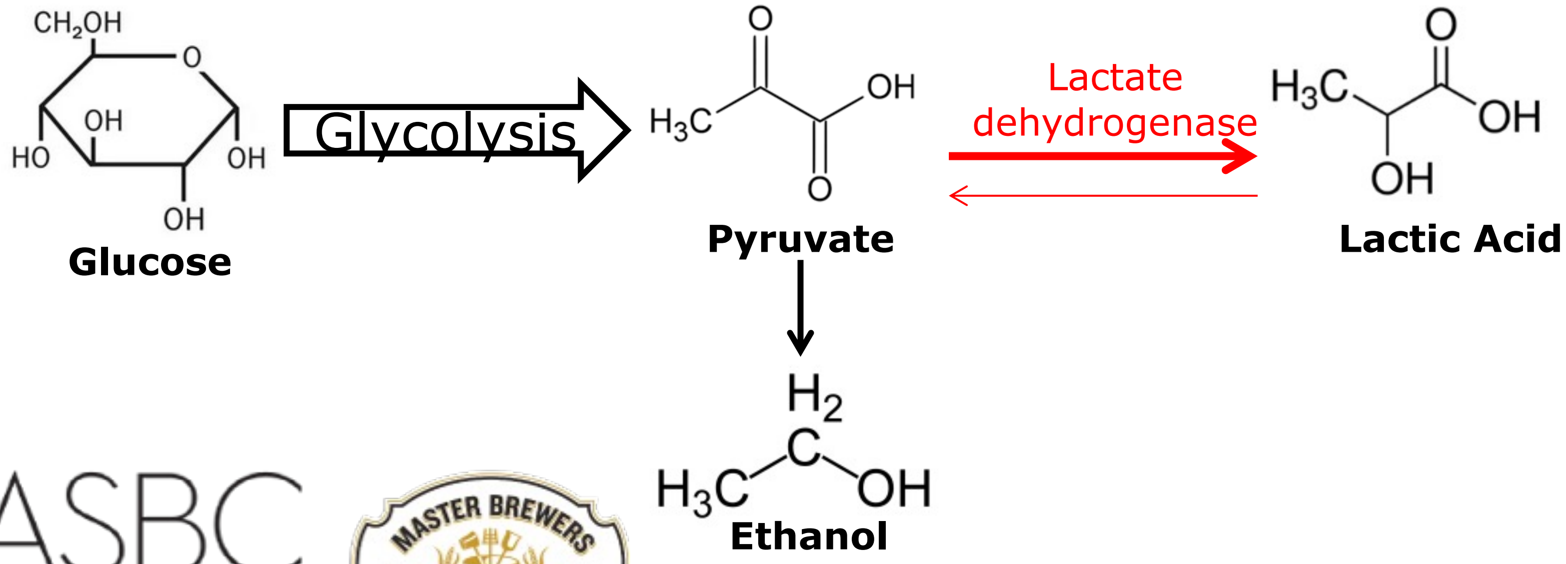
Lactic acid production in *S. cerevisiae* for sour beer production

- *S. cerevisiae* engineered with a heterologous lactate dehydrogenase sourced from a food microbe
- Produces lactic acid during primary fermentation to provide brewers with an easy, reproducible, and mono-culture product for sour-style beer production



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https://www.lallemandbrewing.com

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Regulatory - Generally Recognized As Safe (GRAS)

- A typical regulatory process requires thorough evaluation of the bioengineered organism with a documented dossier detailing:

Extensive descriptions of:

- ✓ How the product was developed
- ✓ How it will be used
- ✓ How it will be manufactured
- ✓ Byproducts or coproducts from both manufacturing and industrial process

Extensive safety evaluation on:

- ✓ Toxicity/Allergenicity
- ✓ Environmental impacts
- ✓ Antibiotic resistance
- ✓ Inactivation
- ✓ Genetic stability

- GRAS is a U.S. Food and Drug Administration (FDA) designation that a substance added to food is considered safe under its intended use by a general recognition among qualified experts, and so, is exempted from pre-market review and approval under the Federal Food, Drug, and Cosmetic Act.

- GRAS determination based on scientific procedures, requires the same quantity and quality of scientific evidence as would be required to obtain approval of a food additive.

Labeling of bioengineered ingredients in the US

- Currently, beer with bioengineered ingredients **does not have** to be labeled according to the National Bioengineered Food Disclosure Standard (NBFDS)
  – NBFDS is a national mandate for food manufacturers, importers and other entities that label foods for retail, to disclose information about bioengineered food/ingredients with a mandatory compliance by Jan 1, 2022

  - Excerpt from the NBFDS:
    - "Distilled spirits, wines, or malt beverages as defined by the Federal Alcohol Administration Act (FAA Act) are foods under the Federal Food, Drug, and Cosmetic Act (FDCA), but **are not subject to the NBFDS** because they are subject to the labeling provisions of the FAA Act rather than the labeling requirements of the FDCA."
    - https://www.federalregister.gov/documents/2018/12/21/2018-27283/national-bioengineered-food-disclosure-standard
  - However, important to point out that beer without barley and hops may be subject to the labeling rules
    - "Alcoholic beverages not subject to the labeling provisions of the FAA Act, such as wines with less than seven percent alcohol by volume and beers brewed without malted barley and hops, would be subject to the NBFDS."

- Beer using bioengineered ingredients can not be certified or labeled as organic
  - "*Excluded methods.* A variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes and are not considered compatible with organic production."
  - https://www.usda.gov/media/blog/2013/05/17/organic-101-can-gmos-be-used-organic-products

Path Forward for Bioengineered Brewing Yeast

- Engineered brewing yeast offer many advantages to the industry
  - Consistent products
    - Less reliant on process changes to generate optimal flavors/performance
    - Engineering can directly target desired phenotypes
    - Potential for monoculture fermentations
  - Reduced process times and cost savings
    - Reduced enzyme costs, expensive flavor additives, lost product
  - Accelerating the expansion of biodiversity
    - Continue to push beverage industries with new novel products!
    - Could GE attract consumers? Current consumer trends targeting the experience and trying something new!

- Important to continue the education
  - Consumers are much more informed about what they put in their body
    - Omnipotence of GE foods in daily life, years of research on GE products, and the information revolution have all improved consumer education which has reduced the stigma and alleviated health concerns
  - But it is important to continue to hold companies to a high standard to ensure health and environmental safety are top priorities when introducing new products

Thank you!

References:

- Committee on Genetically Engineered Crops: Past Experience and Future Prospects, Board on Agriculture and Natural Resources, Division on Earth and Life Studies & National Academies of Sciences, Engineering, and Medicine. *Genetically Engineered Crops: Experiences and Prospects*. (National Academies Press, 2016).
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