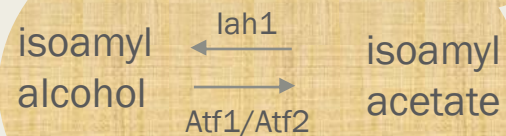


Investigating the Aroma-Producing Genetics of Brewing Yeast: A Productive Collaboration between Beer and Undergraduates



Joost Monen, PhD
Associate Professor of Biology
Ramapo College of New Jersey
Theoretical and Applied Science

Faculty Scholarship Symposium
October 30, 2024



The Impact of Involving Undergraduates in Research

- My experience mentoring students in research projects.
 - *There is no substitute for the learning gains and growth that takes place in the lab.*
- The “Vision and Change in Undergraduate Biology Education: A Call to Action” Report emphasizes the critical importance of research experiences.

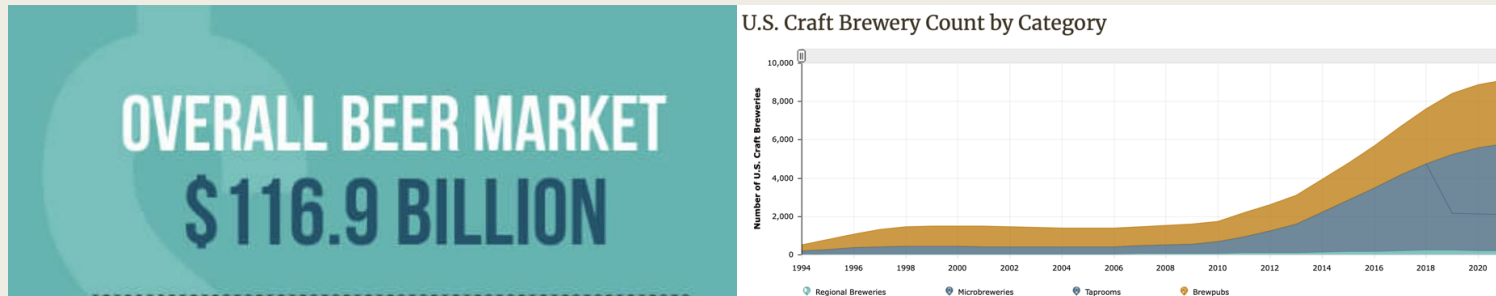


Why Study Yeast and Beer?

- Beer plays an important role in society.
 - *Bringing people together for millennia.*
 - *The brewing industry has a significant economical impact.*



Mesopotamian Beer Rations Tablet
(<https://www.worldhistory.org/article/222/the-hymn-to-ninkasi-goddess-of-beer/>)



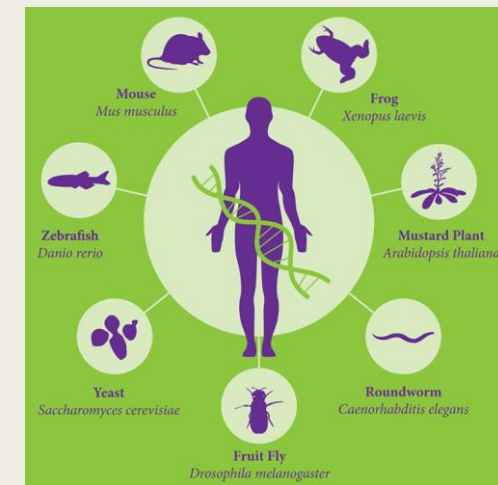
(<https://www.brewersassociation.org/statistics-and-data/national-beer-stats/>)

10 fold increase in craft breweries since 2010.

- Yeast are an important model organism.
 - *Yeast offer many advantages for studying gene function.*
 - Single celled eukaryote with a relatively simple genome.
 - Molecular tools for genetic manipulation have been developed.



(yeastgenome.org)

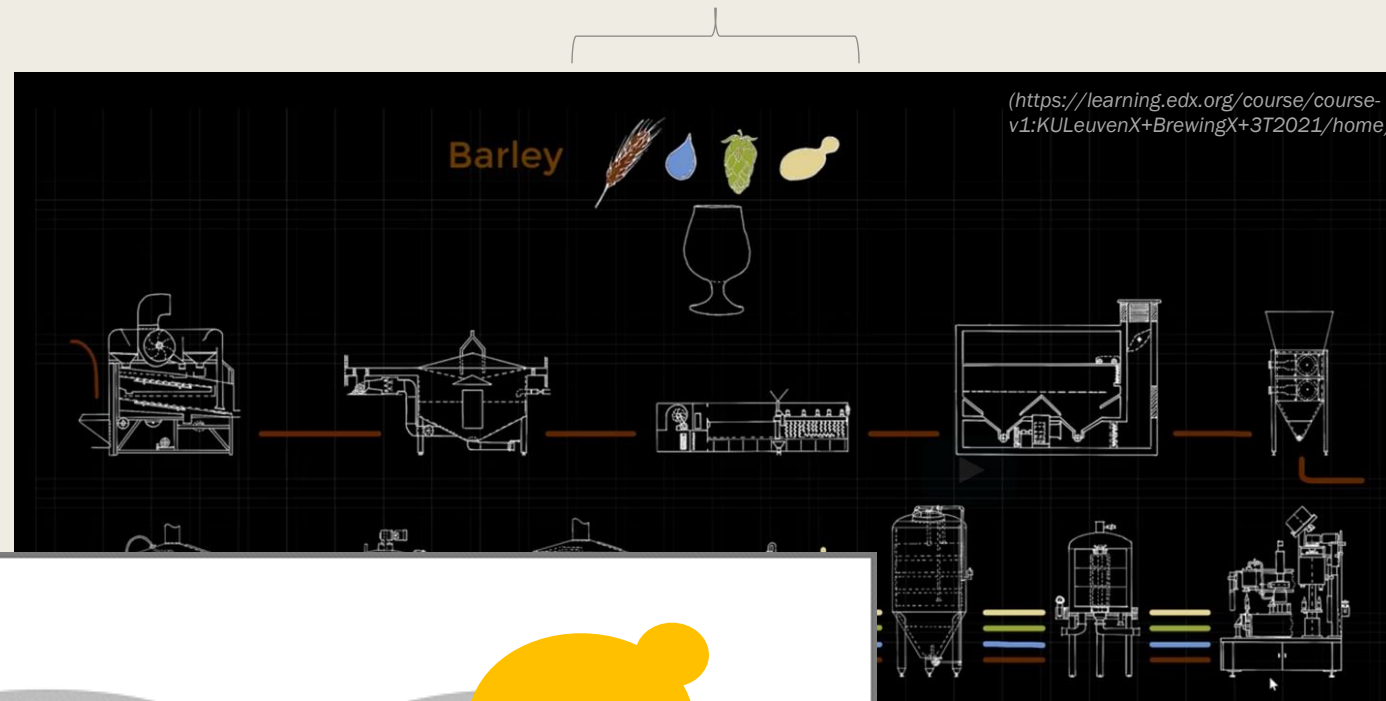


(bionomous.ch/articles/small-model-organisms/)

Yeast's Role in Brewing

4 Main Ingredients:
Malted Grains, Water, Hops, Yeast

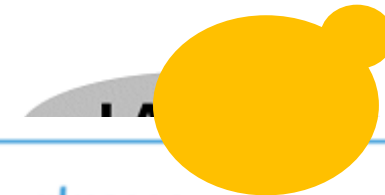
5 Main Steps in Brewing:
Milling, Mashing, Lautering,
Boiling, Fermentation



Saccharomyces cerevisiae



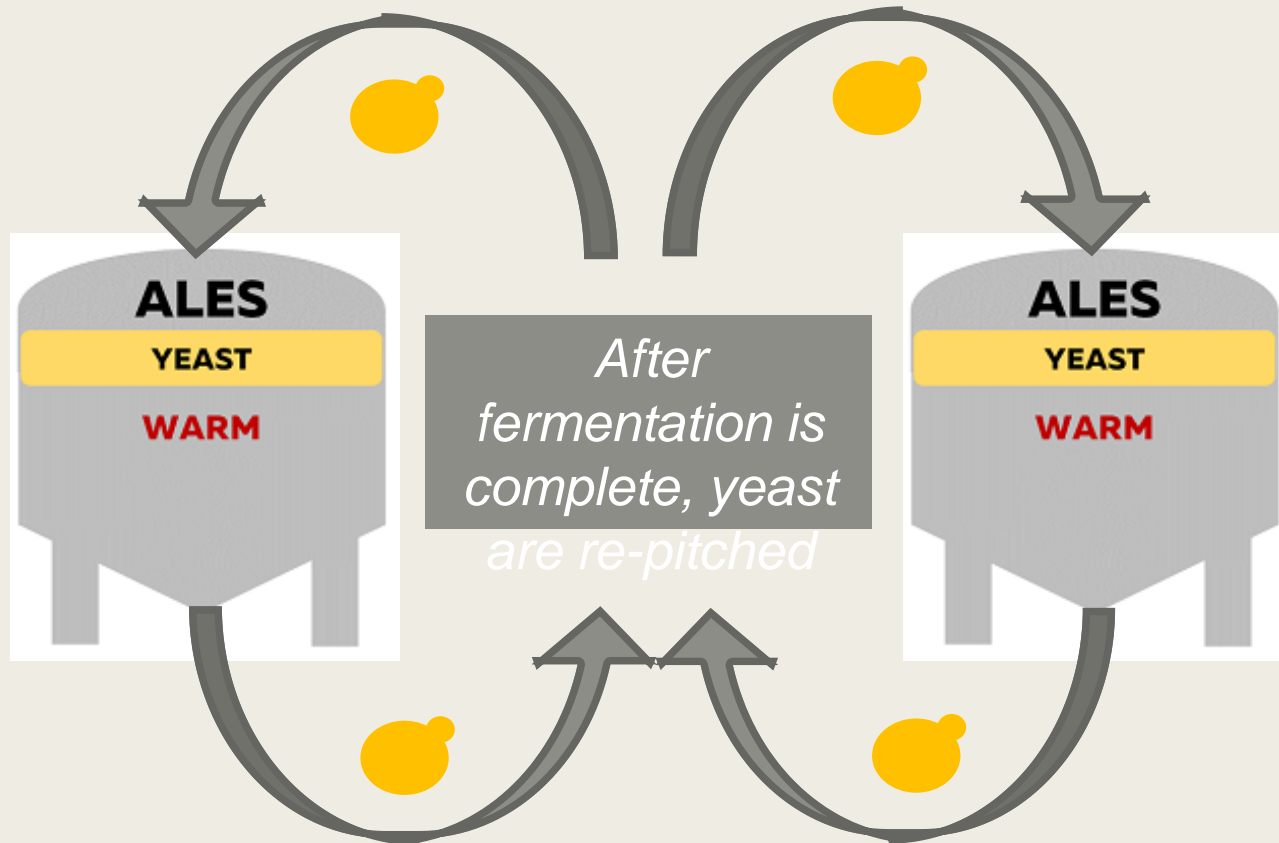
TOP FERMENTING



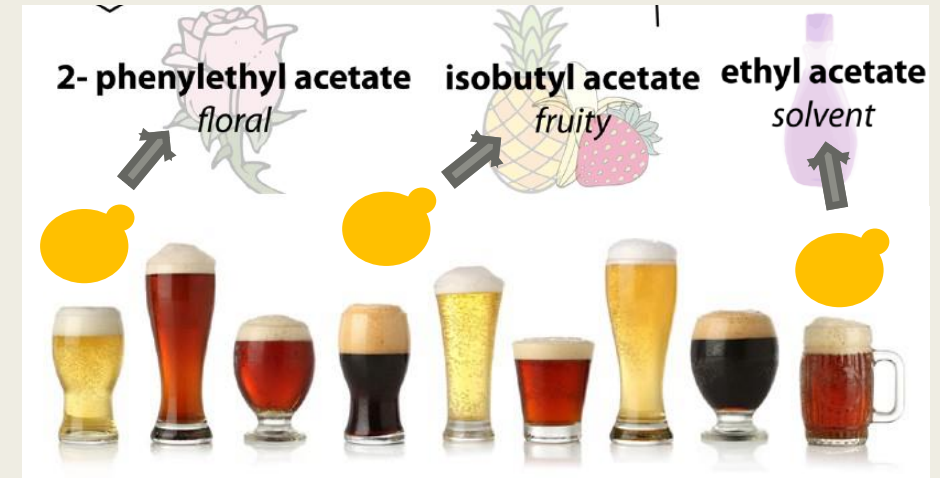
BOTTOM FERMENTING

torianus

The Brewery: Home of a 300-Year-Old Evolution Experiment



What are the genetic differences between strains that contribute to aroma production?

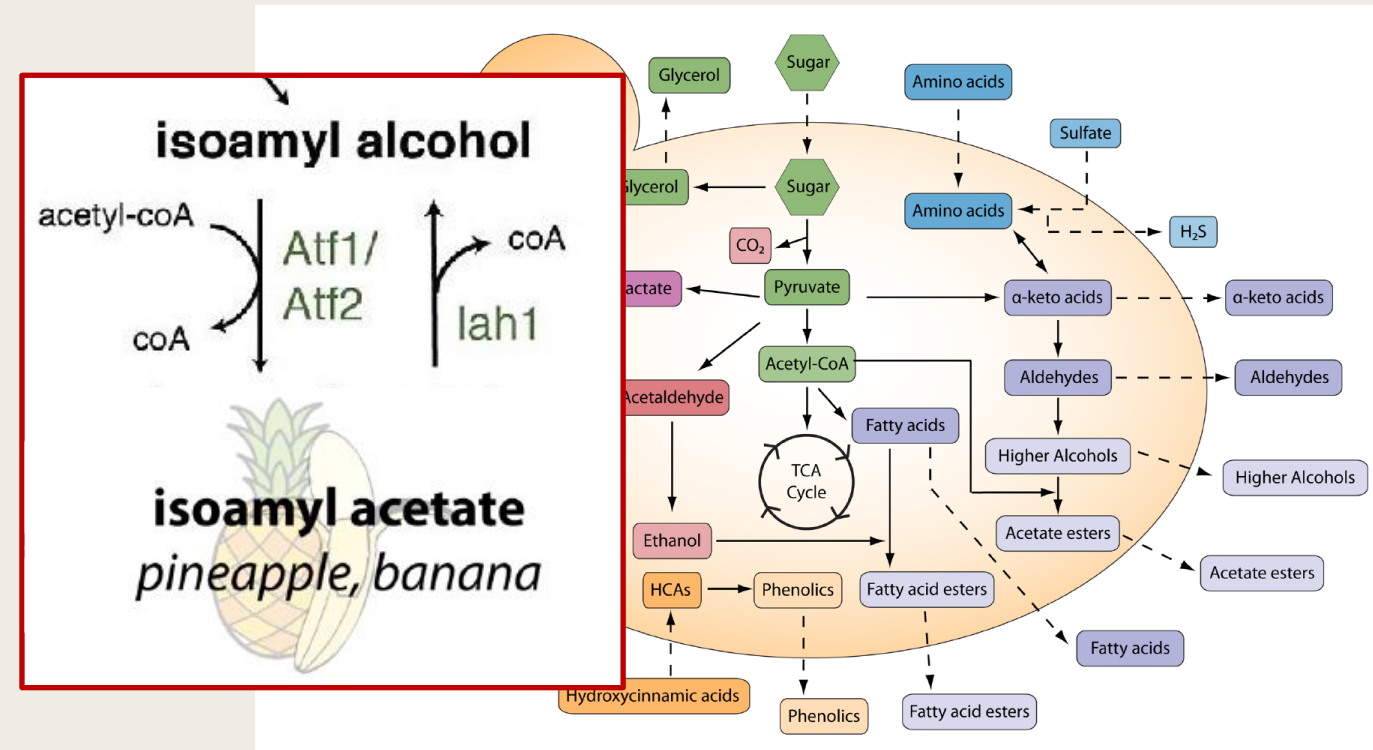


- Beer originating from different breweries gave rise to unique styles.
- Yeast contribute to many nuanced flavors and aromas.
- Serial re-pitching over 300 years has given rise to the divergence of hundreds of brewing yeast strains.



Narrowing down the question

Overview of Aroma Compound Production in Yeast



Are there differences in *ATF1*, *ATF2*, or *IAH1* between strains that contribute to isoamyl acetate production?

- ** High levels of isoamyl acetate production).
- * Moderate levels of isoamyl acetate. (*whitelabs.com*)

The Experimental Approach

Yeast Strains

White Labs Code	Yeast Strain Name
WLP001	California Ale
WLP007	Dry English
WLP029	German/Kolsch Ale
WLP090	San Diego Super
WLP300	** Hefe Ale
WLP550	* Belgian Ale
WLP400	* Belgain Wit
WLP775	* English Cider
WLP500	* Monestary Ale
WLP565	* Belgian Saison I Ale
WLP036	Dusseldorf Alt Ale
WLP644	Saccharomyces brux-like Trois

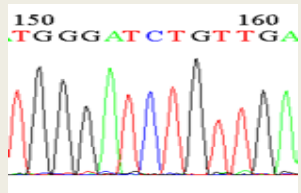
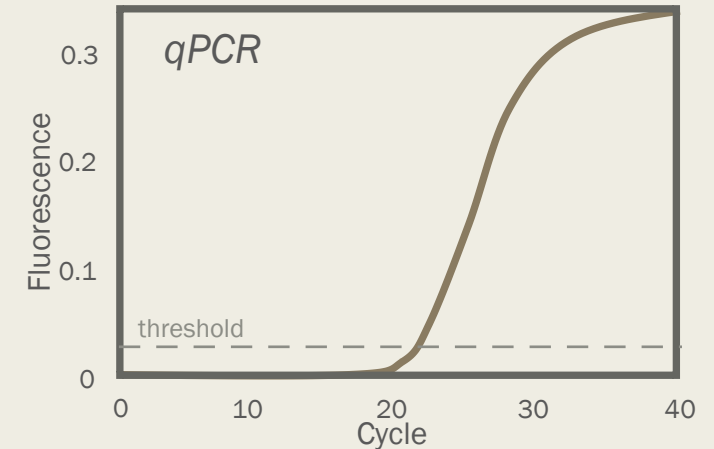
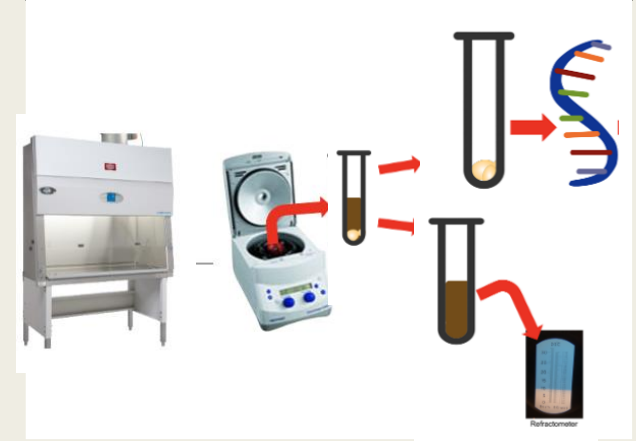
Determine yeast counts & Inoculate into small-batch fermentation vessels.

Culture Yeast



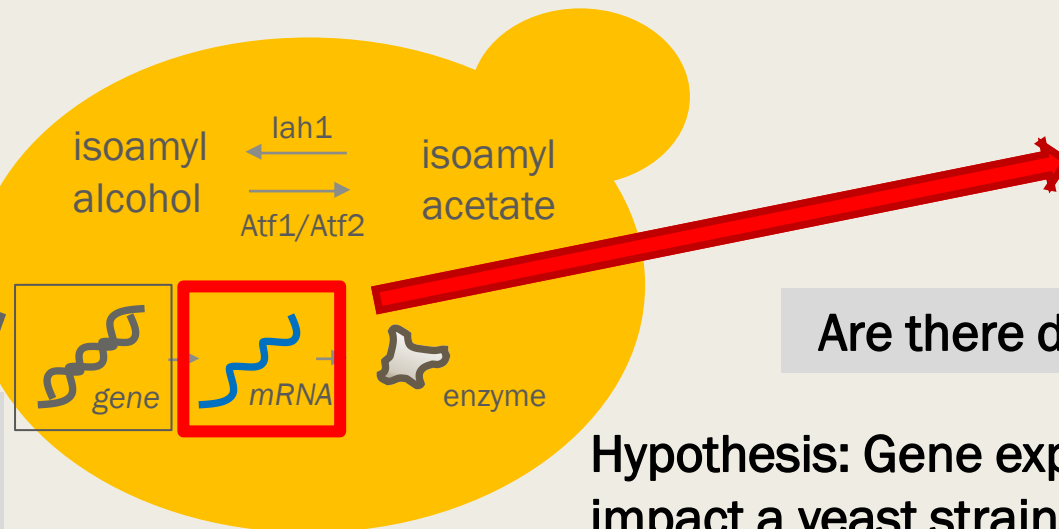
Ferment @ 20 °C

Samples collected during fermentation and yeast and beer are separated for analysis.



DNA Sequencing

Are there genomic differences?

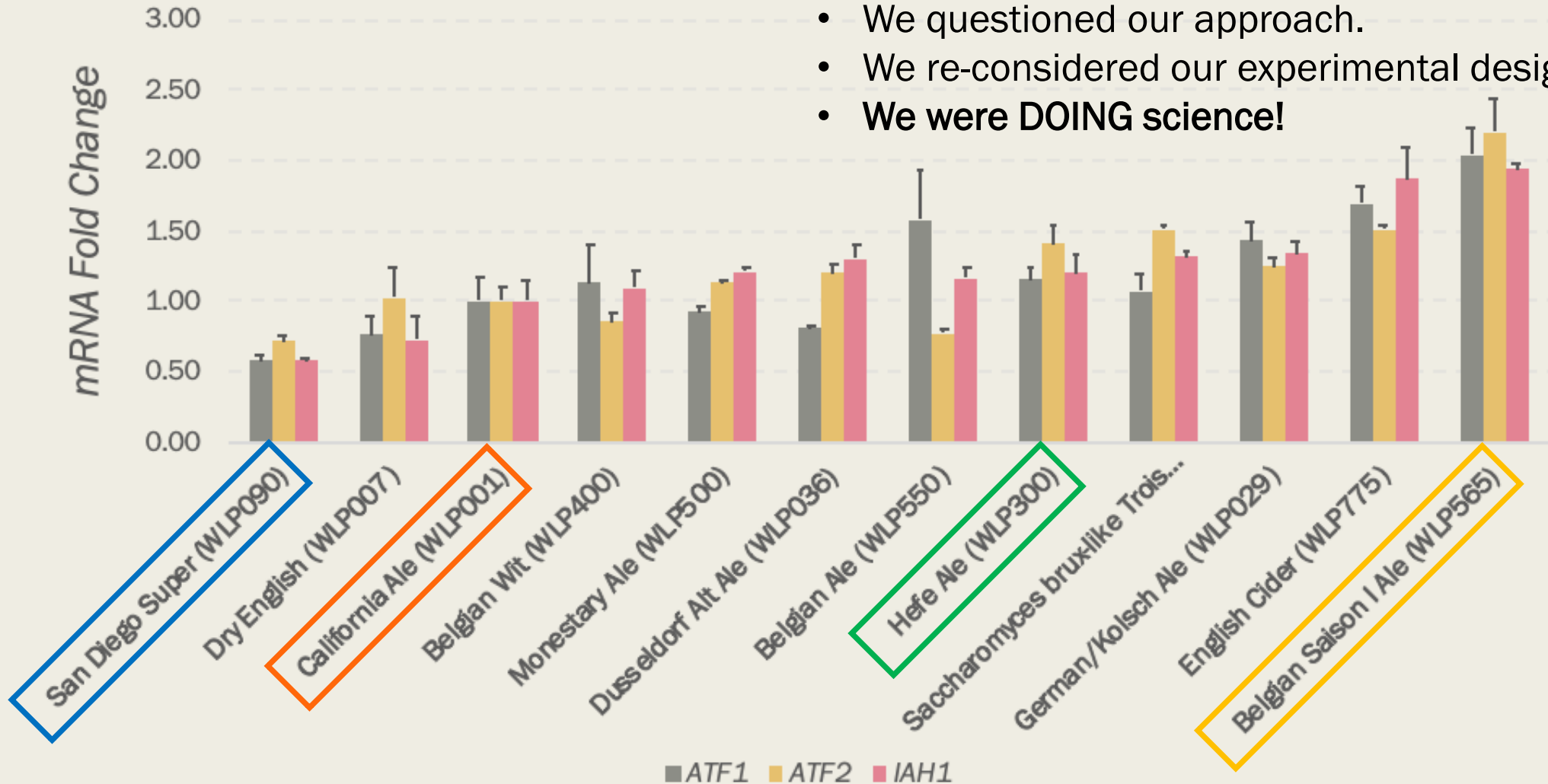


Are there differences in gene expression?

Hypothesis: Gene expression of *ATF1*, *ATF2*, and *IAH1* will impact a yeast strains' production of isoamyl acetate.

Our initial gene expression study led to more questions.

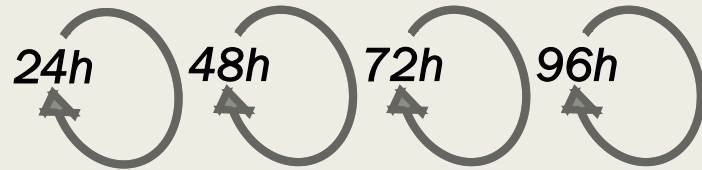
- We analyzed the data.
- We got some unexpected results.
- We questioned our approach.
- We re-considered our experimental design.
- **We were DOING science!**



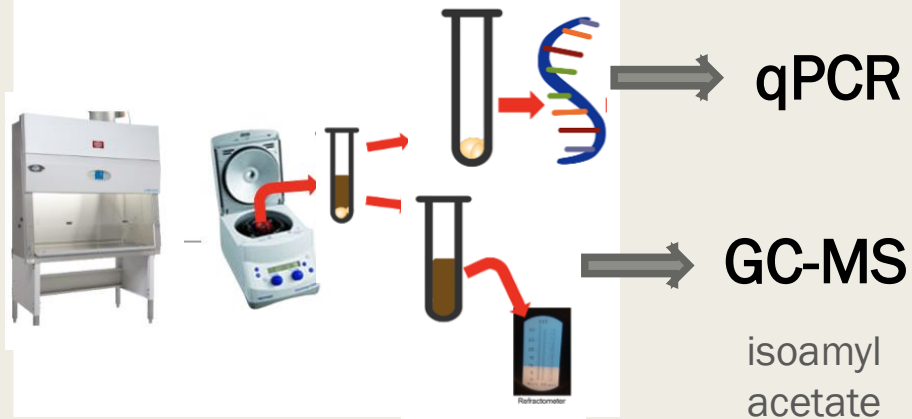
Re-designing the Approach

- Hefe ●
- Belgian Saison ●
- California ●
- San Diego Super ●

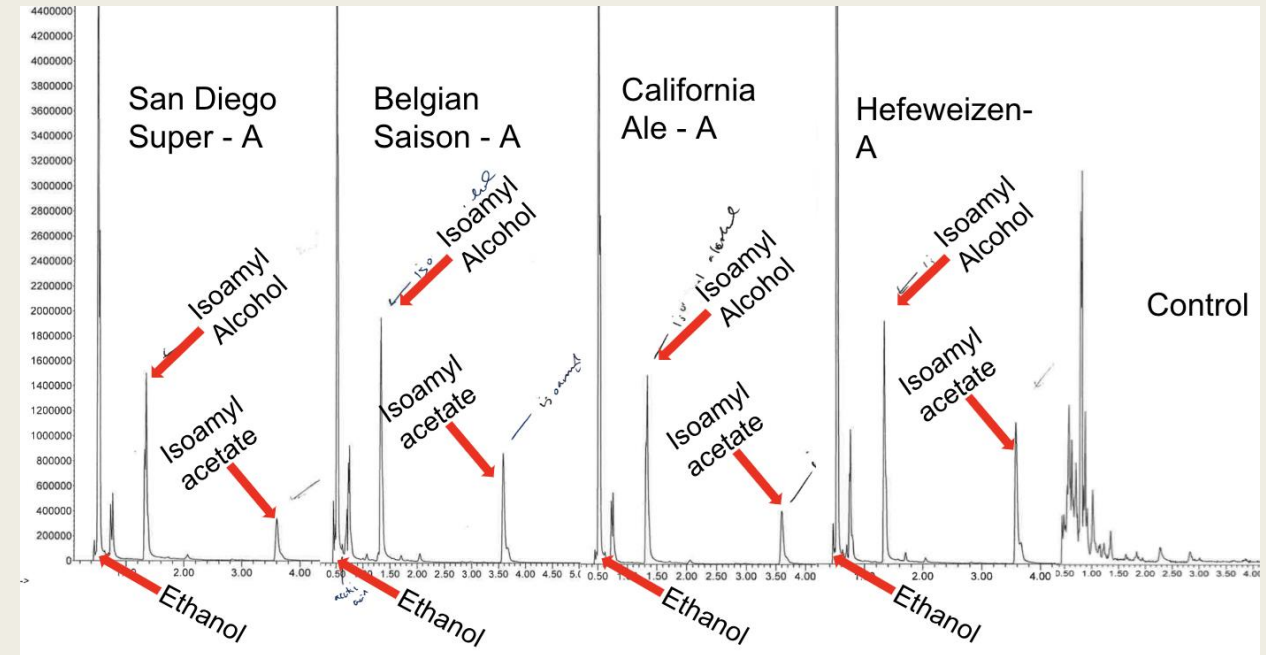
Repeat, Repeat, Repeat...



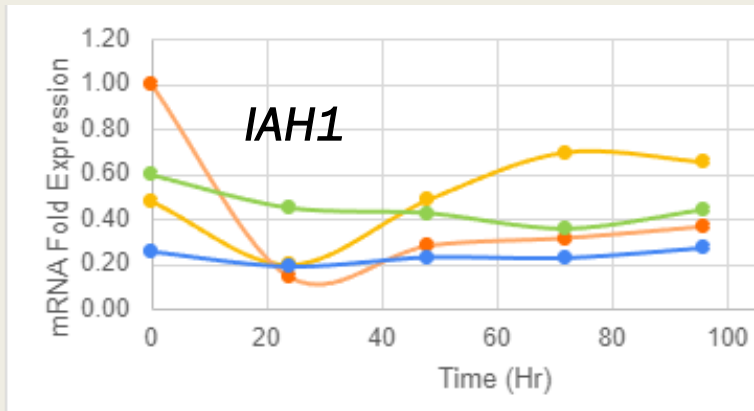
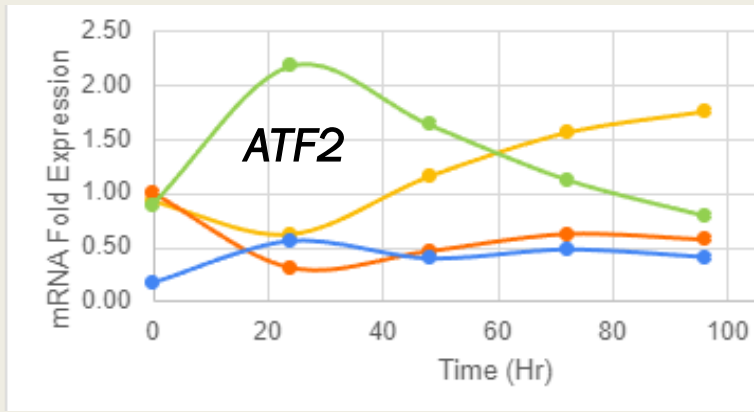
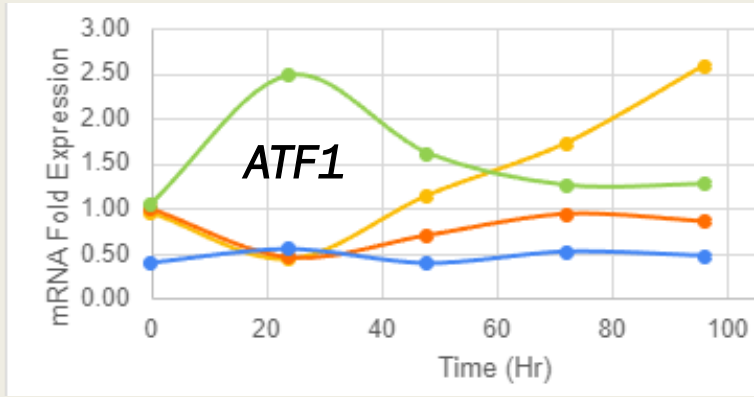
Collect samples every 24 hours



Gas Chromatography-Mass Spectrometry (GC-MS)

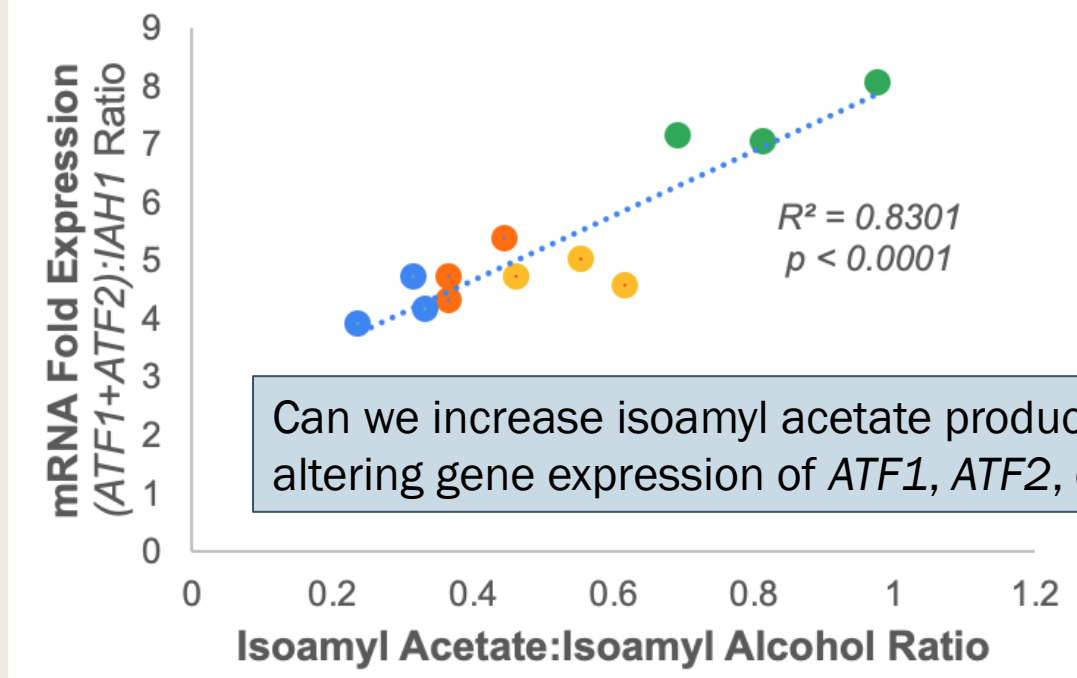
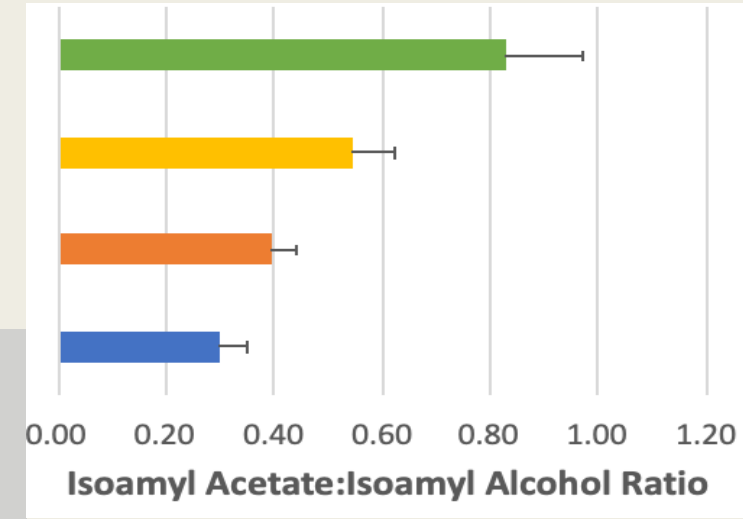


qPCR Timepoint Data Reveals Significant Genetic Expression Pattern Differences



- Hefe ●
- Belgian Saison ●
- California ●
- San Diego Super ●

GC-MS Data Confirm that the Hefe Ale Yeast Strain Produces Higher Levels of Isoamyl Acetate



Can we increase isoamyl acetate production by altering gene expression of ATF1, ATF2, or IAH1?

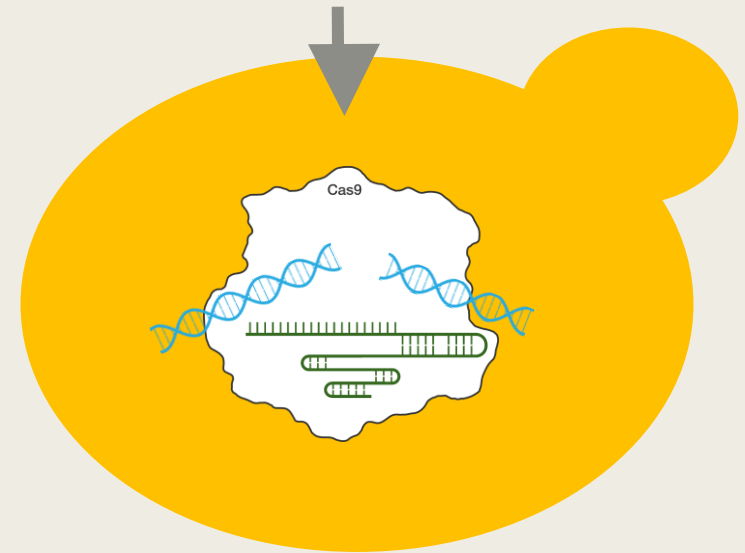


A strong correlation exists between the ratio of gene expression and isoamyl acetate production

Ongoing and Future Directions

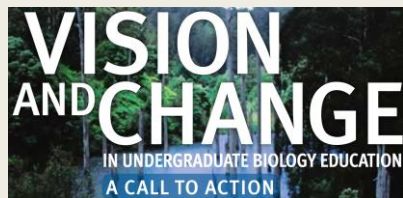
- Genetically engineer yeast to decrease *IAH1* expression and test to see if isoamyl acetate production is increased.
- Expand on these studies to examine more genes and aromatics.
 - *Incorporate Next Generation Sequencing (NGS) to look at the entire genome.*
- Piloting a Course-Based Undergraduate Research Experience.

Knockout *IAH1* using CRISPR/
Cas-9 genome engineering



The Impact of Involving Undergraduates in Research → Lessons Learned

- There is no substitute for the learning gains and growth that take place than when doing science.
 - *Students involved in research develop and strengthen core competencies.*
 - Problem-solving, critical thinking, collaboration, communication, and independent learning/thinking/working.
 - Gain confidence, a passion for science, and appreciation for the scientific method.



“Appreciating the scientific process can be even more important than knowing scientific facts. People often encounter claims that something is scientifically known. If they understand how science generates and assesses evidence bearing on these claims, they possess analytical methods and critical thinking skills that are relevant to a wide variety of facts and concepts and can be used in a wide variety of contexts.”

—National Science Foundation, Science and Technology Indicators, 2008

- The research space is my most effective teaching grounds.

Acknowledgments

Dr. Jennifer Kennell, PhD
Vassar College
for helping with GC-MS

Dean Saiff and TAS staff
and faculty for supporting
student research

Dr. Thomas Owen

*TLTR funding for molecular
toolkit to support CRISPR-
Cas-9 genome editing*

Current Members

- Magdalena Nikolova
- Evelyn Voisekhovich
- Numa Quraishi
- Jestina Brown
- Cookie Wahba
- Victoria Martin
- Abdul Alhaddad
- Laura Barsamyan

Student Collaborators!

Past Members

- Natalie Chung
- Gabriela Tactuk
- Grace Byrne
- Serina Sajjad
- Alyssa Capasso
- Misha Mathai
- Avantiva Thakur
- Anagha Brahmajosyula
- Amravi Tarachandani
- Jenna Gleason
- Benjamin Wahba
- Isabela Torres
- Mahmoud Alhaddad
- **Jenna Vesey**



Past Members

- Maddy Maas
- JJ Fritsch
- Gabe Makar
- Matt Von Bargen
- Sam Davis
- Jenna-Marie Tracy
- Keimya Sadegh
- Lauren Schmidt
- Brianna Romer
- Meghann Herman
- Austin Marcelo
- Marco Salazar
- Dana McKenna
- Juliana Herman
- Daniel Pleskowics
- Melissa Ranzer
- Celine Decaro
- Ava Gomaa
- Jonathan Lopez
- James Costanzo

