OUTLOOK HAZY OR BRIGHT?

Yeast Strains to Dial in Haze

Vermont Craft Brewers Conference 2023

Lance Shaner
Owner
Omega Yeast Labs

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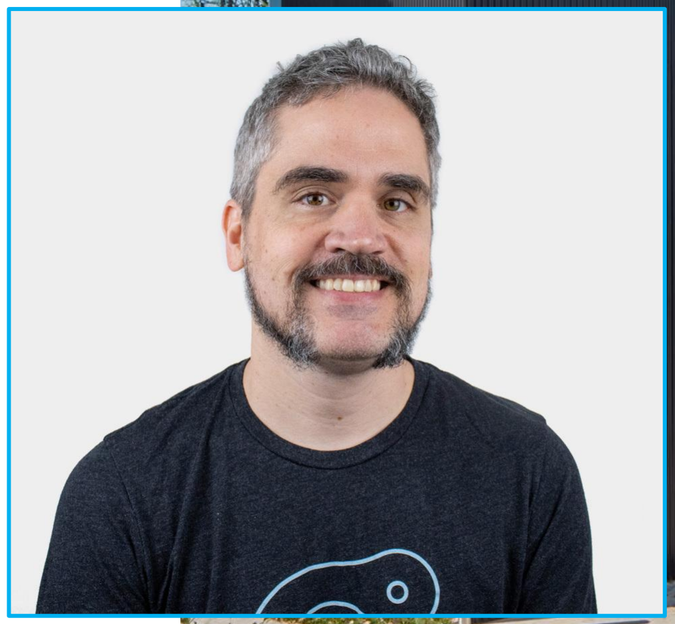
Who are we?

Omega Yeast labs
Chicago, IL / St. Louis, MO

High quality, pitch-ready liquid yeast. Handful of microbiologists, homebrewers, professional brewers and craft beer fans who made it our express purpose to make brewing easier and better for everyone.



Lance Shaner
Co-owner/Founder

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What is haze?

- Haze = Turbidity
- The cloudiness of a fluid cause by large numbers of individual particles that are generally invisible to the naked eye
- Analogous to fog or smoke in air

Maplewood Brewery & Distillery. Chicago, IL

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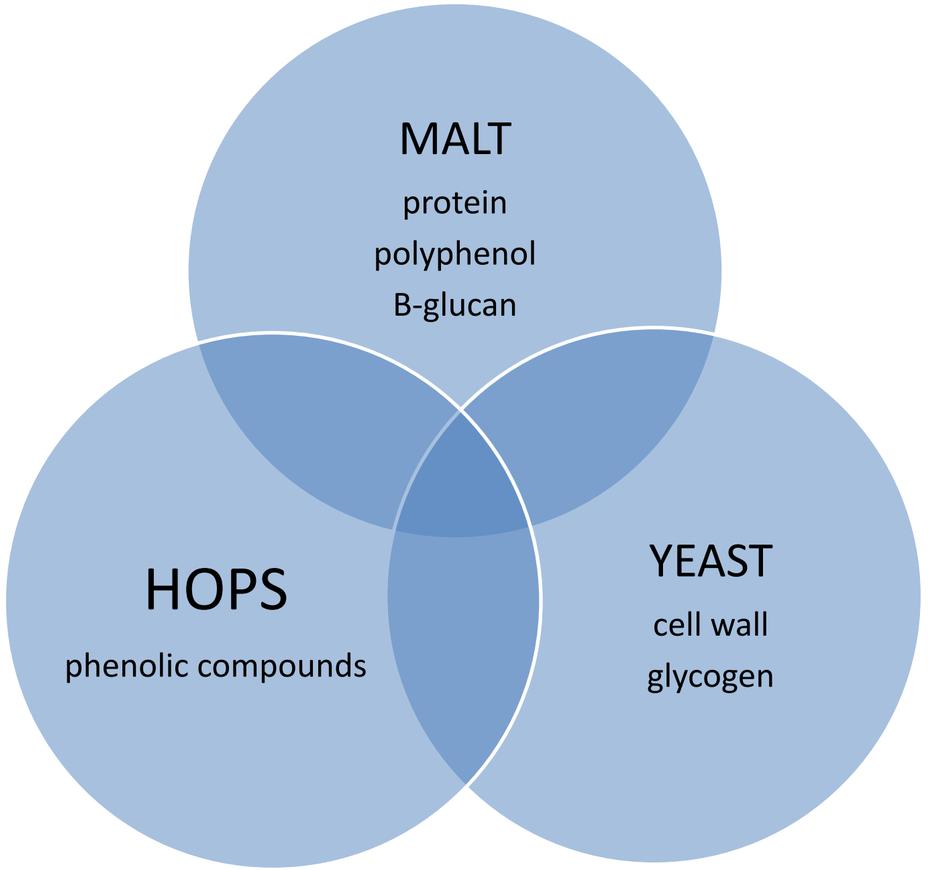
Where does haze come from?



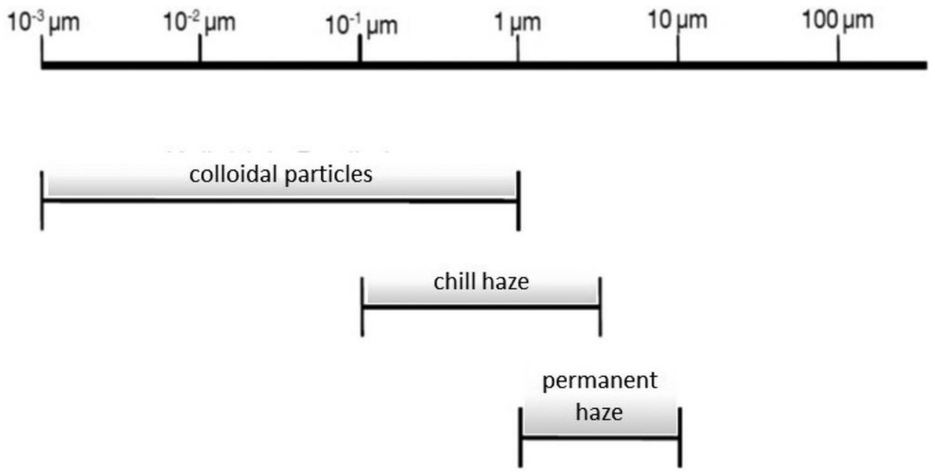
Types of haze

Chill Haze:

- Non-covalent interactions
  - hydrogen and hydrophobic bonding
- Reversible
  - Occurs at 0°C but goes away at 20°C

Permanent Haze:

- Covalent bonding
- Often occurring with increasing age, repeated cooling and warming, pasteurization, oxidation



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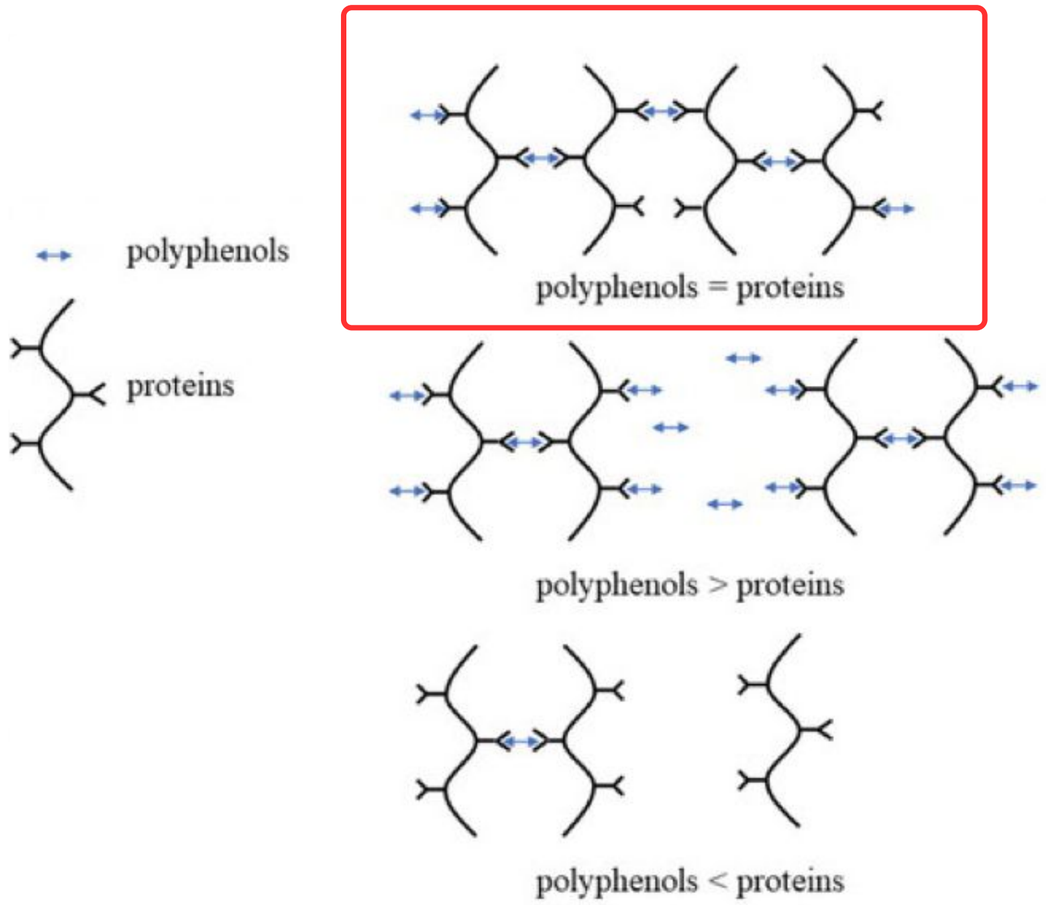
Malt and hops

Proteins

- Major malt-derived proteins
  - Proline-rich (different from foam stabilizing proteins)
  - Barley Hordein (20% proline)
  - Wheat Gliadins (15% proline)
  - Haze generating smaller <40kDa peptide/proteins

Polyphenols

- Proanthocyanidins
  - Monomers, dimers, trimers and higher polymers of catechin, epicatechin and gallocatechin



Kahle EM, Sarnkow M and Jacob F. JASBC 2020.

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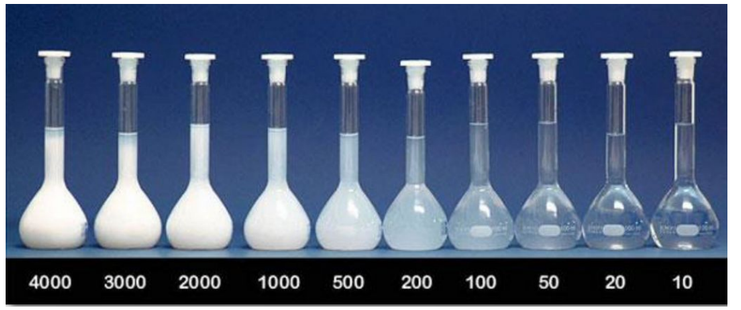
What kind of haze are we talking about?

NEIPA levels

NTUs are a measurement of haze



Sample	Turbidity Measurement
Drinking water	0.05–1.5 NTU
Lager	5–20 NTU
Porter / Pale Ale	20–200 NTU
Hazy IPA	200–1000 NTU
Orange juice	300–900 NTU
Milk	>4000 NTU

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Measuring Turbidity

Tubidimeters/nephelometers

In the lab:

Anton Paar HazeQC ME

- Measures 90º, 25º, and 0º

Hach Turbidimeter

- Measures 90º, and/or 0º

Optek Haze Control DT9011

- Measures 90º, 11º, and 0º

In the brewery:

Haffmans OptHaze-i

- Measures 90º, 25º, and 0º

Separators

- Measures 90º, and/or 0º

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Haze Assay

Wort: All barley malt (2-row) for target 15°P

Strains: Haze Positive OYL-011, Haze Neutral OYL-004

Pitch Rate: 10 million/ml

Temperature: 70°F

Fermentation End Point: 14 days

Dry Hop Amount: 2 lb/bbl (8g/L)

Dry Hop Addition:

- Control (no dry hop)
- Knockout (in fermentor pre-pitch)
- Day 1
- Day 2
- Day 3
- Day 4
- Day 7
- DDH (½ Day 1, ½ Day 7)

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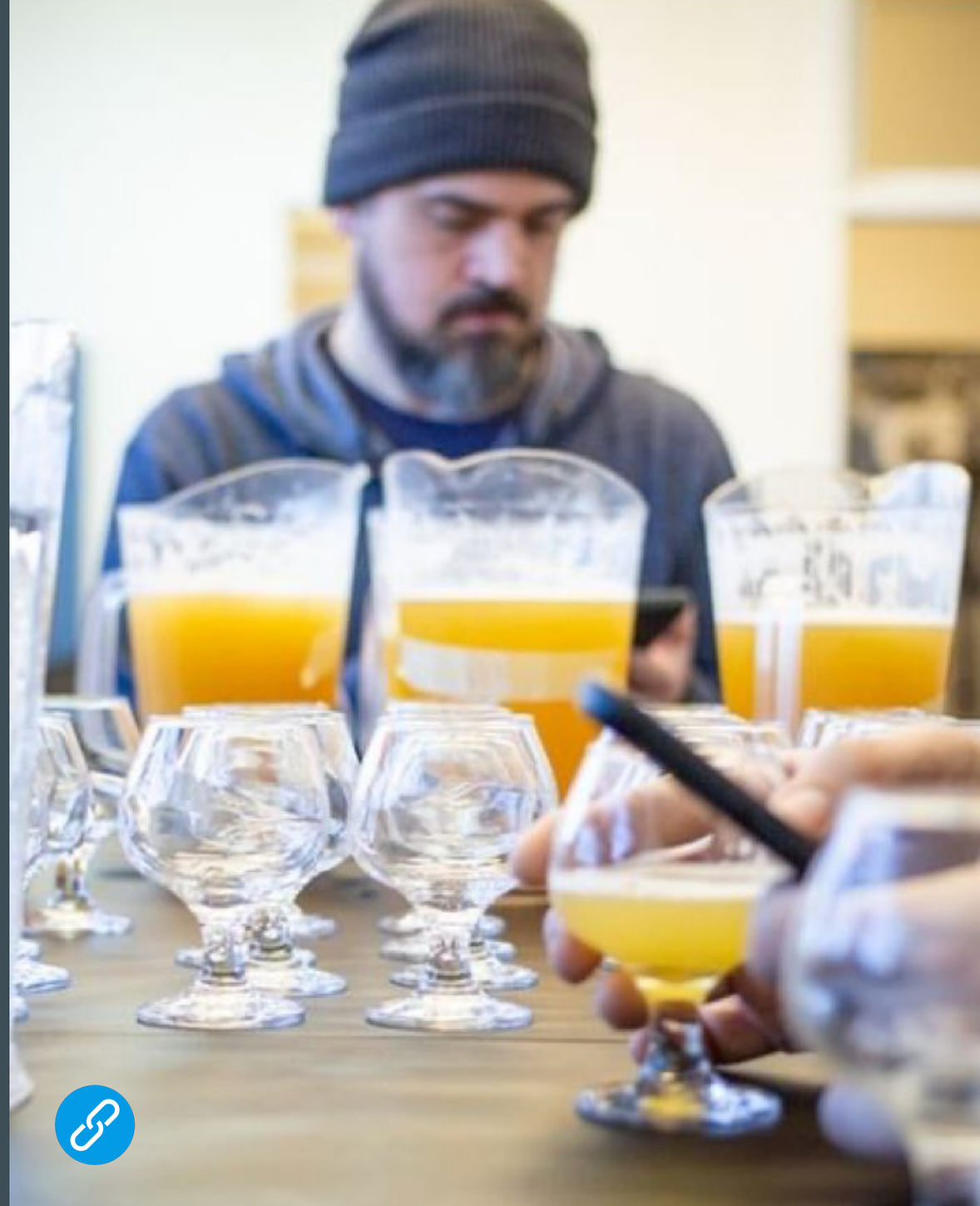
Talk Outline

1. Yeast Strain Specific:
   - “Haze-positive”
   - “Haze-neutral”
2. How does dry hopping play in?
   - Dry-hop timing
   - Hop variety
   - Hop products
   - Dose rates
3. *HZY1* - a gene for haze
4. Trial @ Georgetown Brewing



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Dry hop timing and yeast choice

This dramatically impacts degree of haze

Haze Positive

OYL-011 British V

Dry Hop Timing

Haze Neutral

OYL-004 West Coast Ale I

Dry Hop Timing

Pictures at 14 days. From left to right: Control (no dry hop), Knockout, Day 1, Day 2, Day 3, Day 4, Day 7, DDH (Day 1 and 7)

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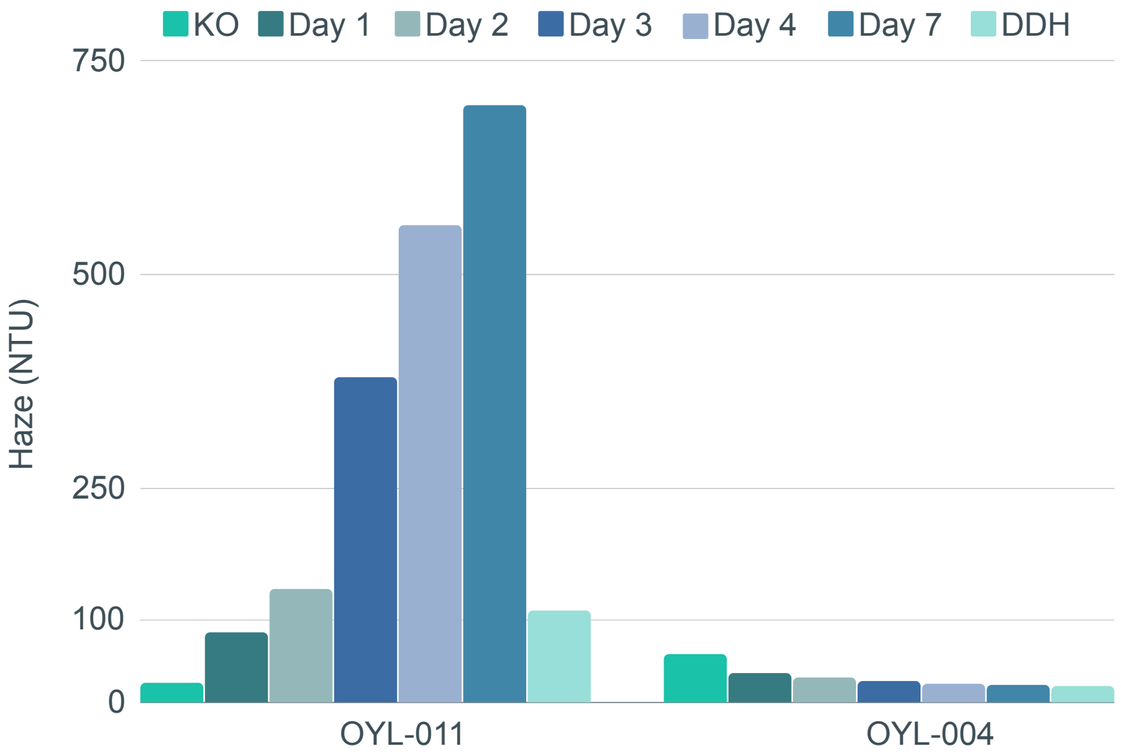
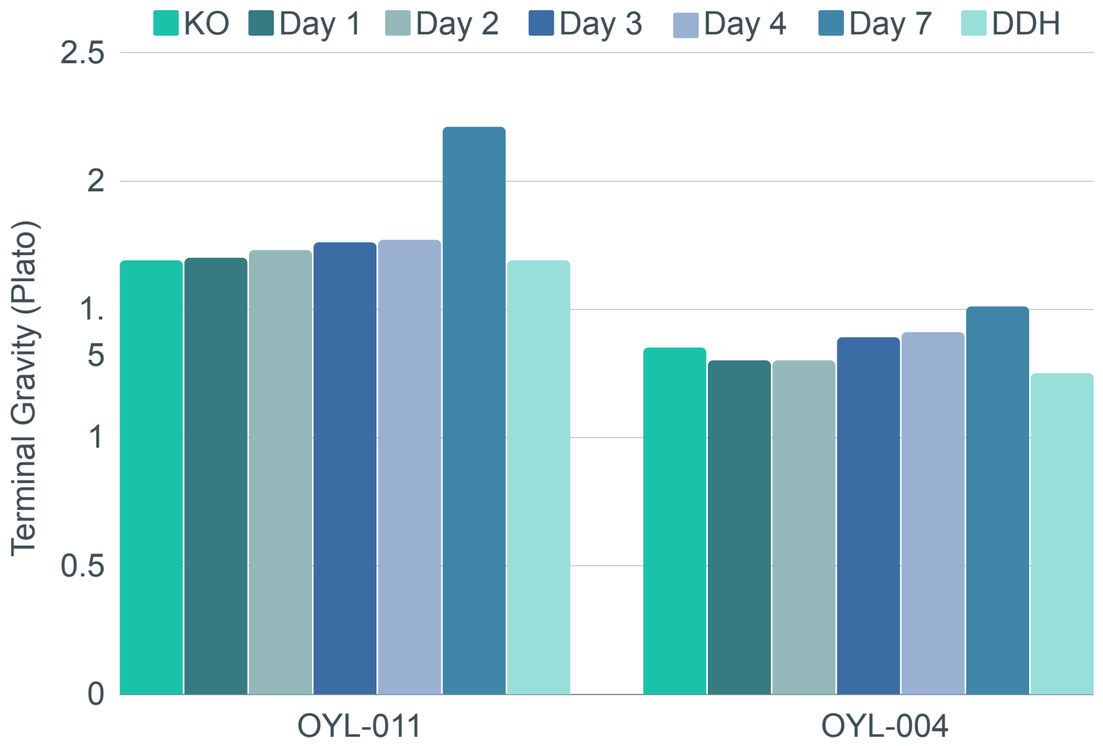
Dry hop timing and yeast choice

Haze independent of terminal plato





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Grouping strains:

Haze Positive

Hazy IPA strains/Hefe/Kolsch

- OYL-011 British V (Juice/London III/Foggy London)
- OYL-017 Kolsch I
- OYL-032 East Coast Ale
- OYL-061 Voss Kveik
- OYL-043 Point Loma
- OYL-015 Scottish Ale
- OYL-005 Irish Ale
- OYL-021 Hefeweizen
- OYL-044 Kolsch II
- OYL-006 British I

Haze Neutral

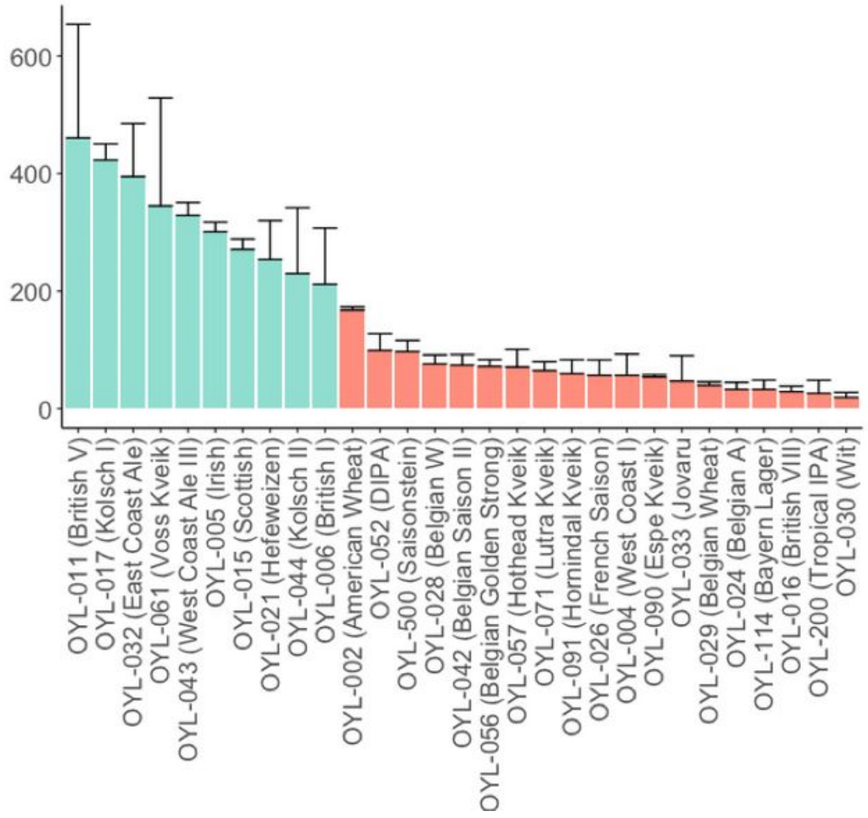
American IPA strains

- OYL-004 West Coast I (Chico)
- OYL-052 DIPA Ale (Conan)
- OYL-200 Tropical IPA

Other Strains

- OYL-030 Belgian Wit
- OYL-024 Belgian Ale
- OYL-071 Lutra Kveik
- OYL-016 British Ale VIII (Fullers)
- OYL-091 Hornindal Kveik
- OYL-057 Hothead Kveik
- OYL-114 Bayern Lager

Haze (NTU) with 2 lb/bbl dry-hop addition at day 7



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How does dry hopping play in?

- Hopping Rate
- Hop Variety
- Hop Products
- Early Dry Hoping vs. Late Dry Hopping?

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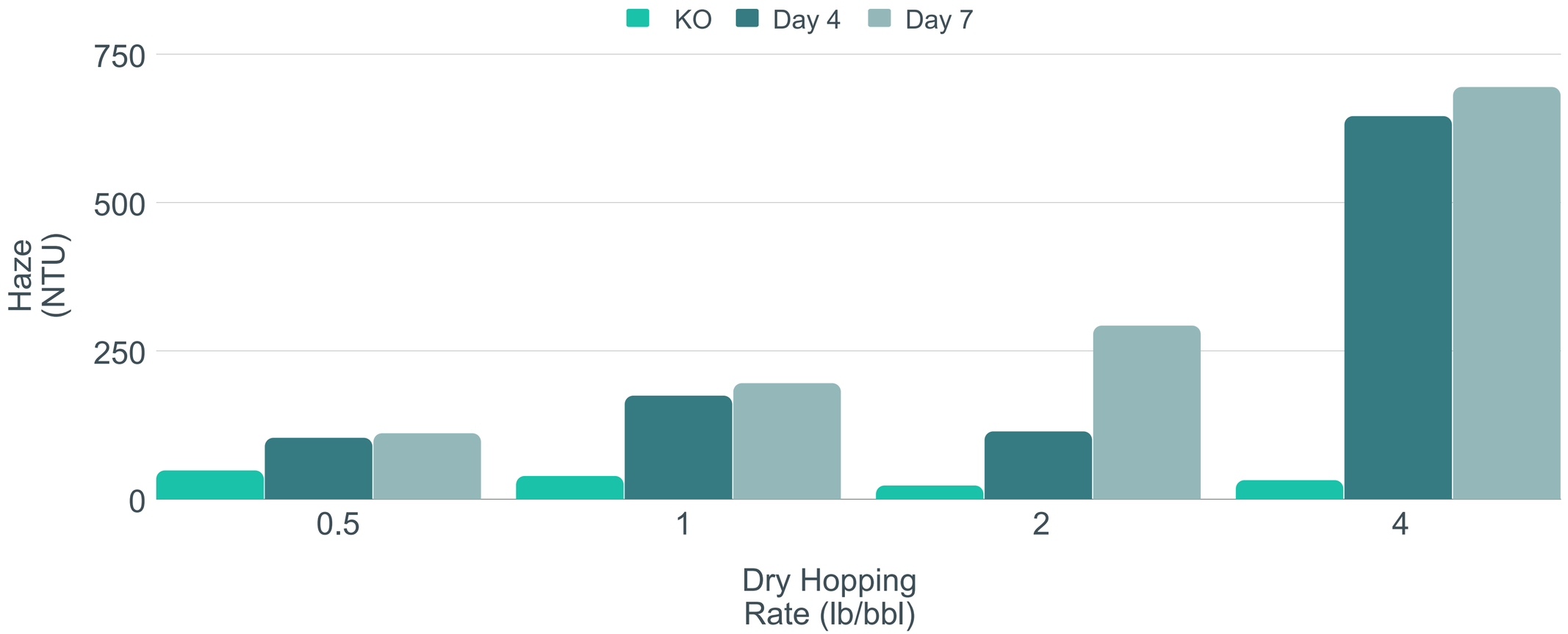
Haze increases with dry hopping rate



*fermented with OYL-011 haze-positive strain

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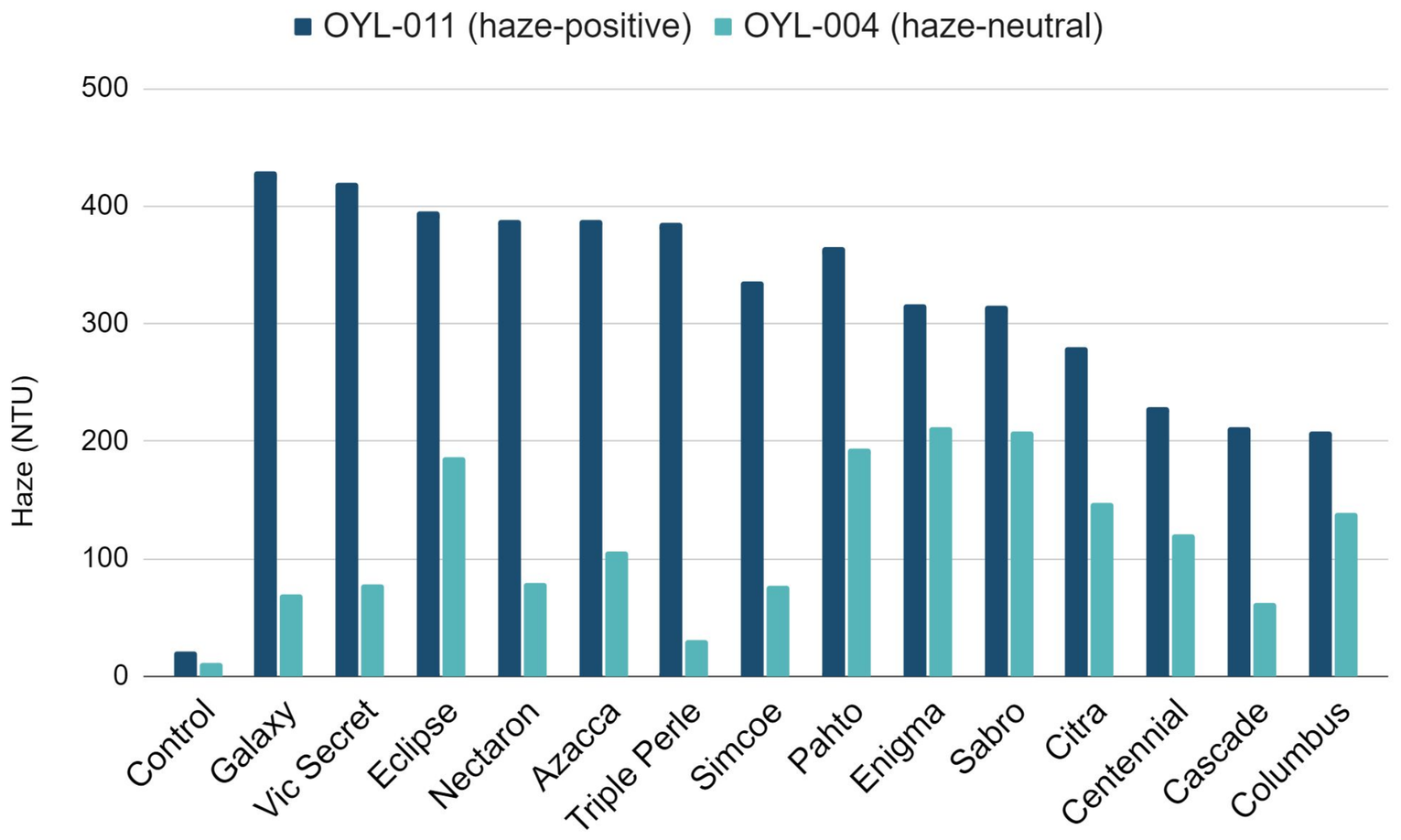
Haze is dependent on hop variety



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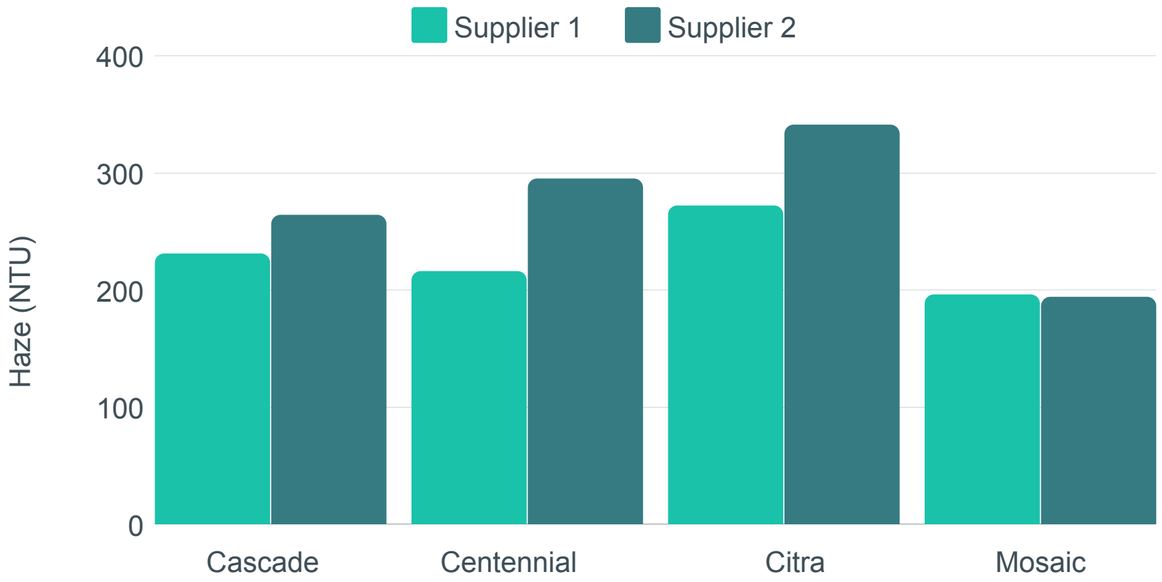
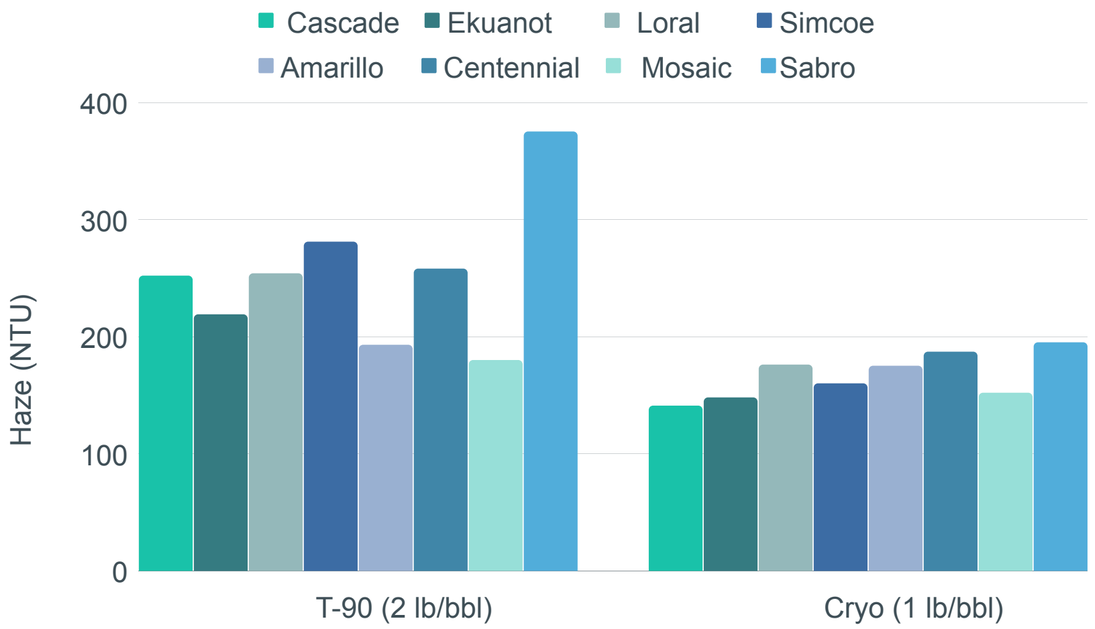
More haze variability between hop varieties and hop product types

Overall trends show variety specific impacts on haze



Cryo and T-90 pellets show different haze potential



*fermented with OYL-011 haze-positive strain

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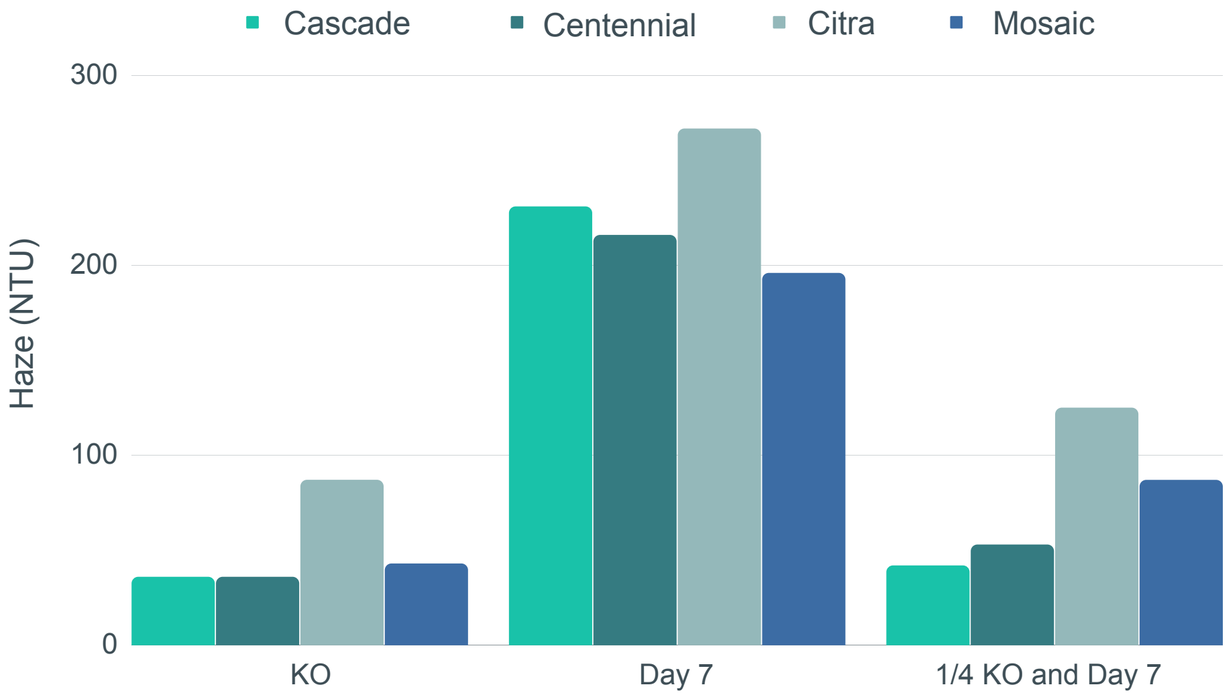
Double dry hop experiments

Early hop additions limit haze potential



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Pilot Brew

Can we make a bright NEIPA with an addition of a 0.5 lb/bbl of knockout hops?

Recipe Details

Wort: 100% 2-row, 16.7ºP

Strain : Haze Positive OYL-011

Pitch Rate: 10 million/ml

Temperature: 70°F

Fermentation End Point: 14 days

Whirlpool Hops: 1 lb/bbl Citra, Wai-iti, and Mandarina Bavaria

Dry Hop:

- Hazy Control: Day 4 alone
- Experimental: Knockout + Day 4

Cold crashed at Day 14 for one week and transferred to keg

	Hazy Control Pictured Left	Experimental Pictured Right
Knockout	-	0.5 lb/bbl
Day 4	2 lb/bbl	2 lb/bbl
Haze	487 NTUs	34 NTUs
ABV	7.60 %	7.61%
AE	2.93	2.86
pH	4.66	4.7

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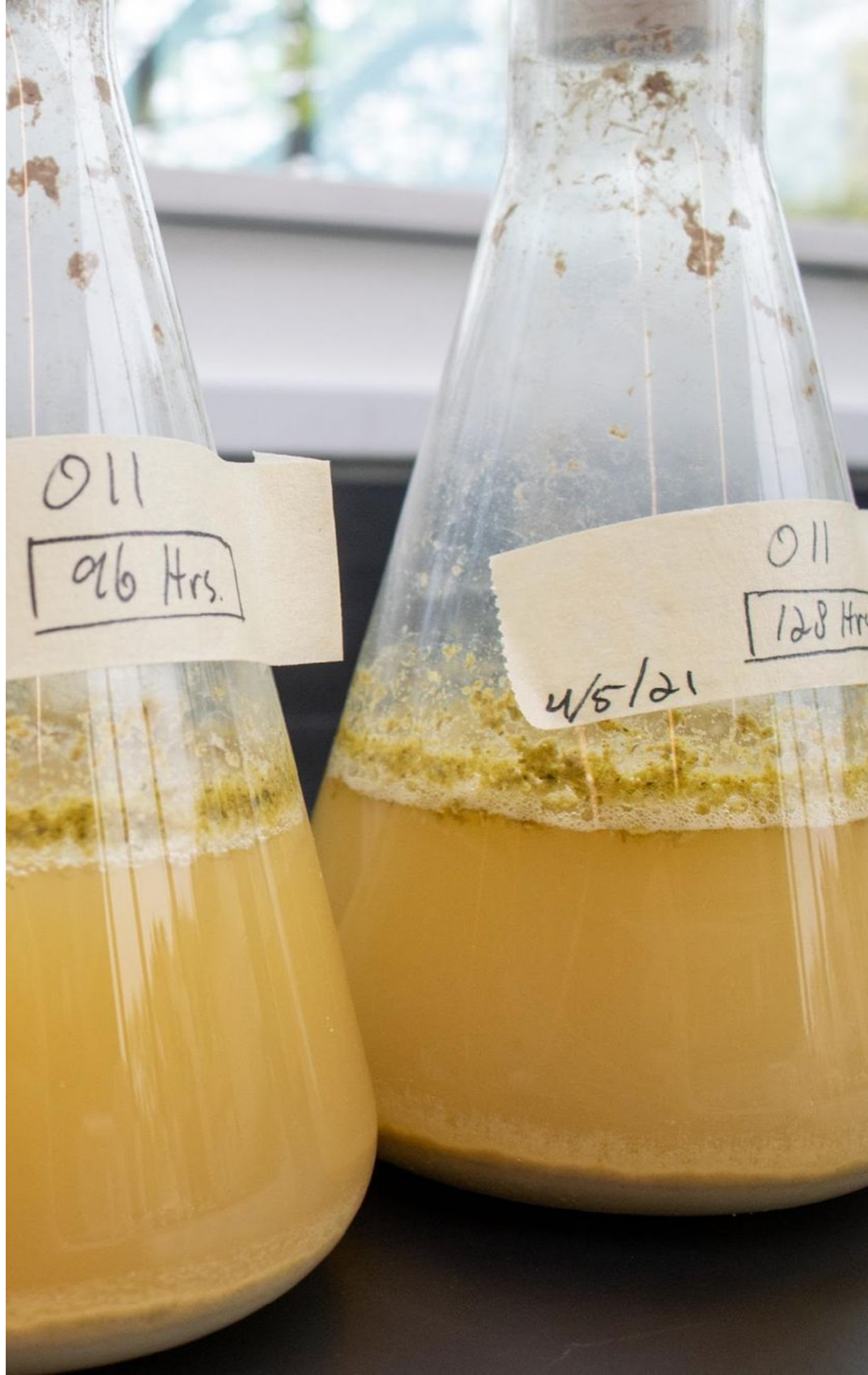
Summary of initial work

	Haze	No Haze
Yeast	OYL-011 or haze-positive strains	OYL-004 or haze-neutral strains
Dry-hop timing	Mid-late	Early
Dry-hop rate	High	Low
Hop Product/Variety	*Variable, more to learn!*	

…what makes a yeast haze-positive?



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HZY1 makes yeast haze-positive!

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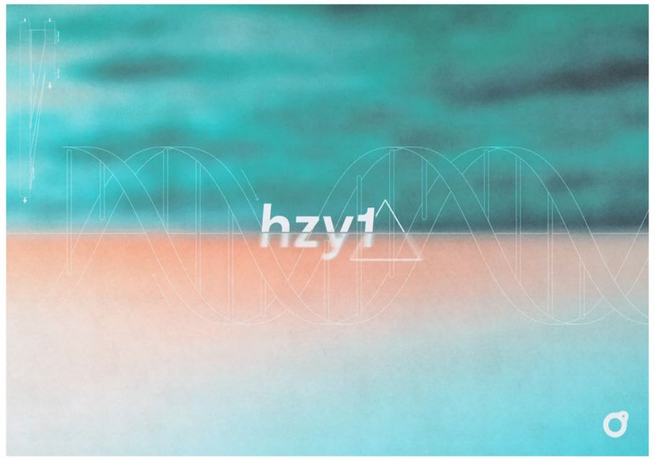
Uncovering a Gene for Haze

The *HZY1* gene from brewing yeast plays an important role in making the haze in hazy IPAs

By Shana Solarte
Jul 12, 2023

70 Cheers Share



Cold Spring Harbor Laboratory

bioRxiv
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New Results

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Identification of the yeast mannoprotein gene *HZY1* as a key genetic determinant for yeast-derived haze in beer

Keith Lacy, Rita Mormando, Jeremy R. Smith, Patrick A. Gibney, Lance M. Shaner, Laura T. Burns

doi: https://doi.org/10.1101/2023.07.10.548400

This article is a preprint and has not been certified by peer review [what does this mean?].

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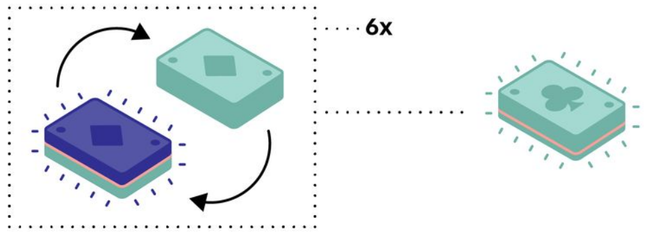
How we found HZY1

Genetic backcrossing experiment analogy

1. This card represents the haze gene and has the ability to glow (haze). When located in a full deck of cards, it allows the entire deck to glow.

2. When you take this purple deck and shuffle it with a green deck, you get one large deck that you can then split in half. These two decks have an equal amount of cards, but only one deck holds the magic card.

3. You can then take the glowing deck that is made up of half purple and half green cards, and then shuffle it with another full deck of green cards. If you repeat this seven times, you'll get a deck that is almost 100% green but still includes the magic glowing card.



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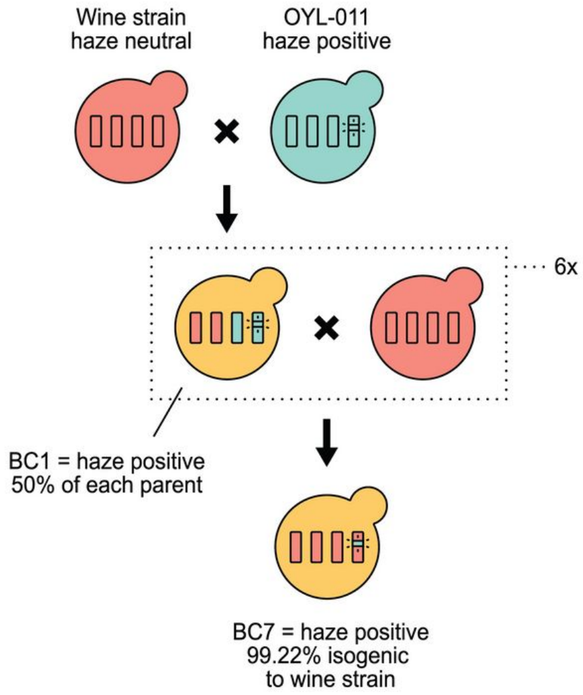
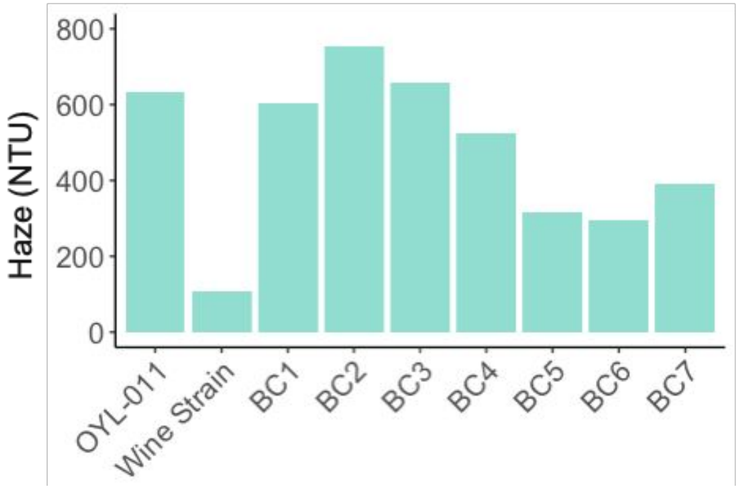
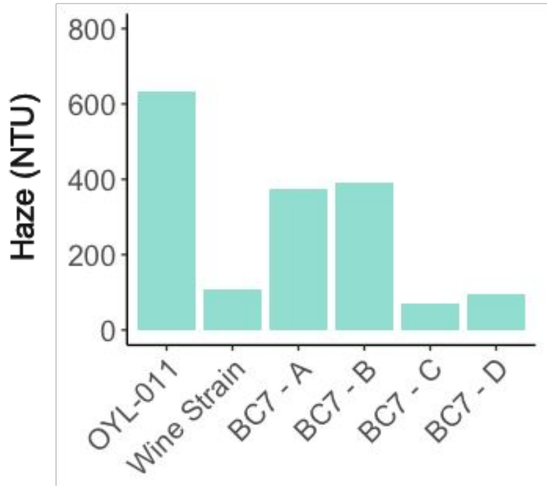
How we found HZY1

Following the genetics







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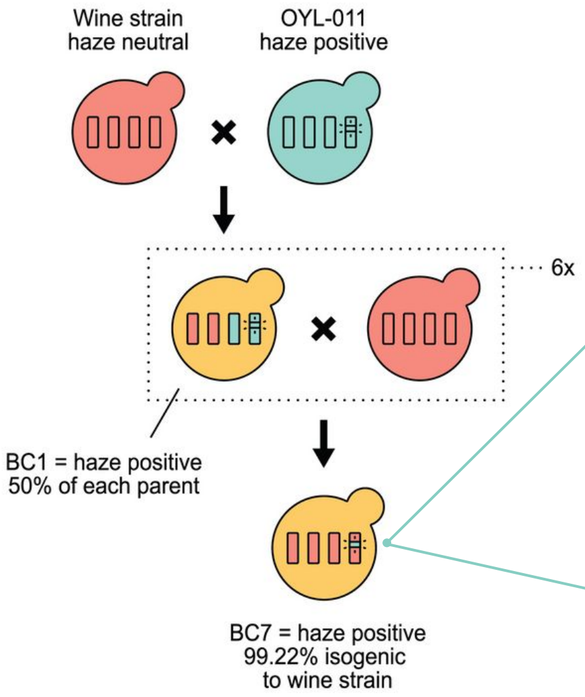
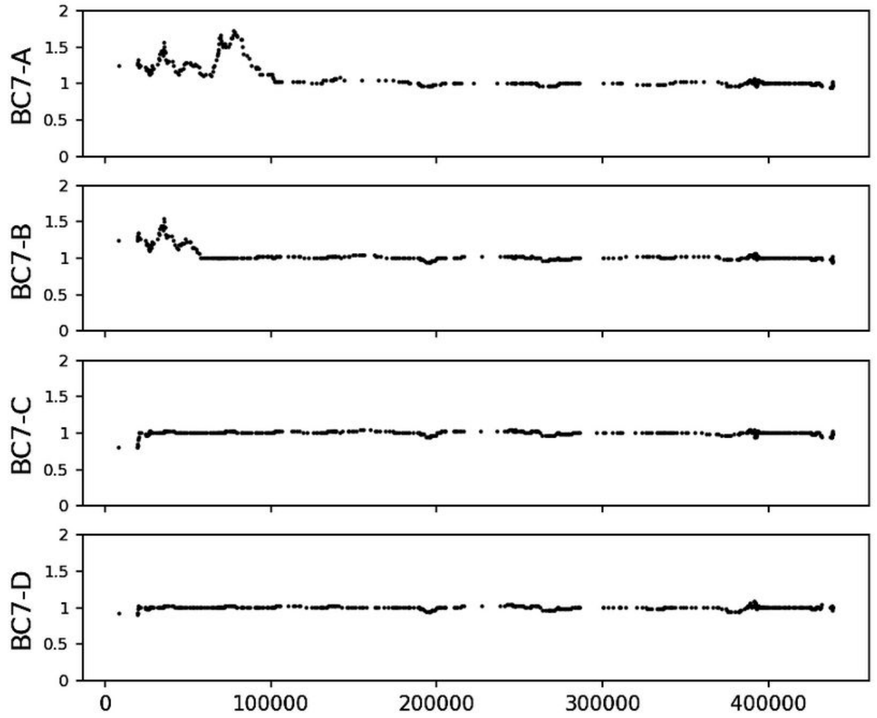
How we found HZY1

Following the genetics





Haze-positive isolate

Haze-positive isolate

Haze-neutral isolate

Haze-neutral isolate

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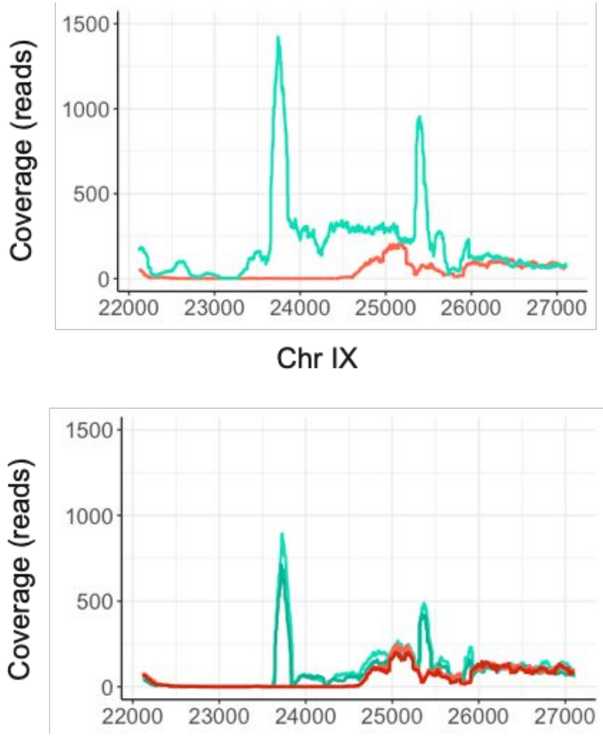
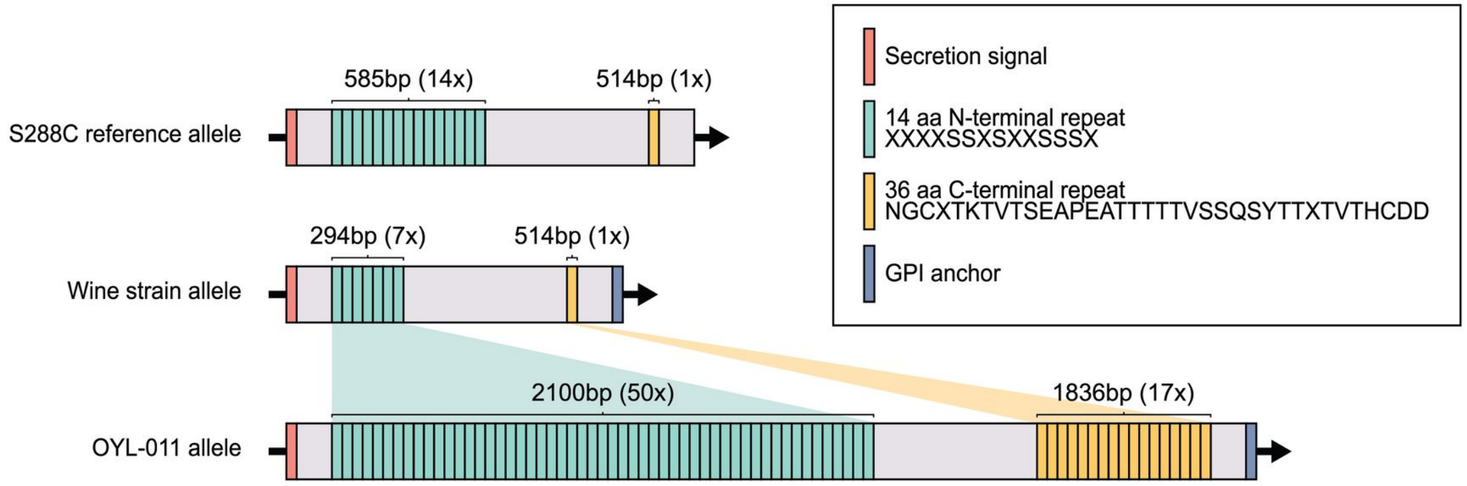
Large repeat expansions in *HZY1* gene in haze-positive strains

The *HZY1* gene has large repeat expansions in OYL-011





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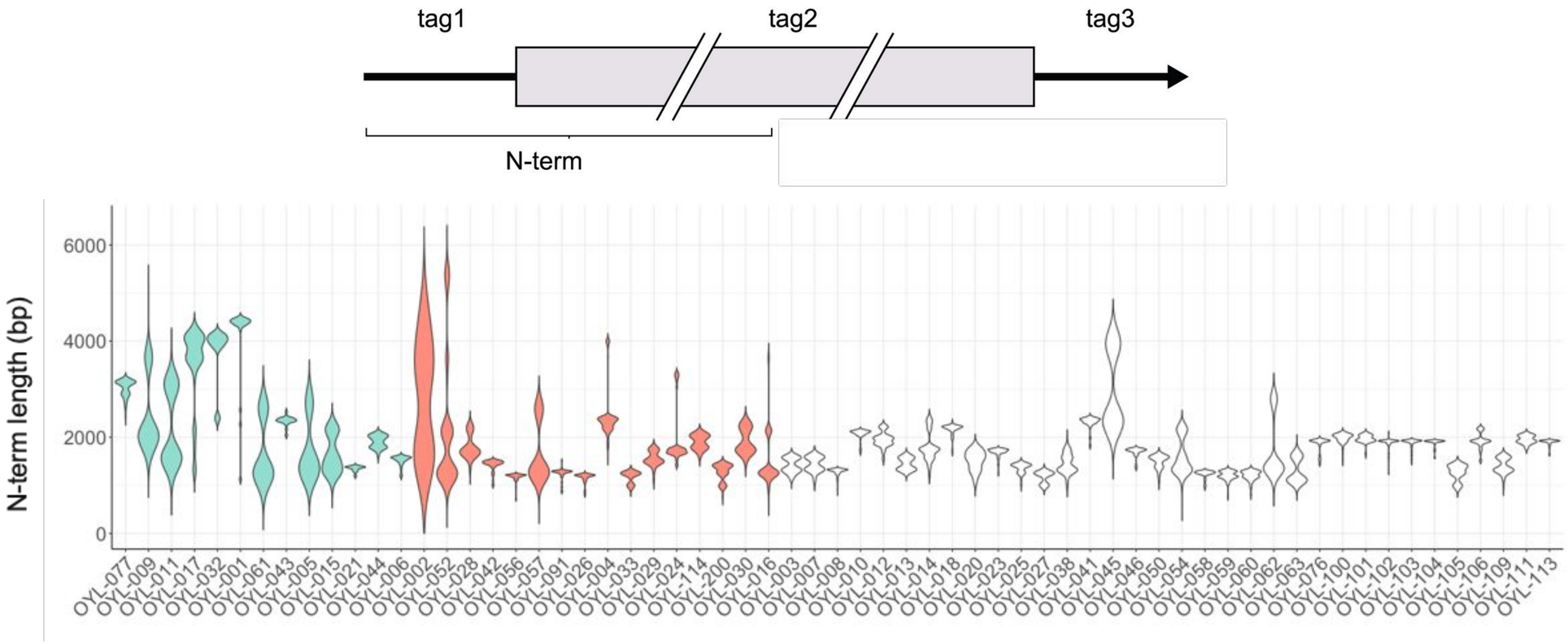
Large repeat expansions in *HZY1* gene in haze-positive strains



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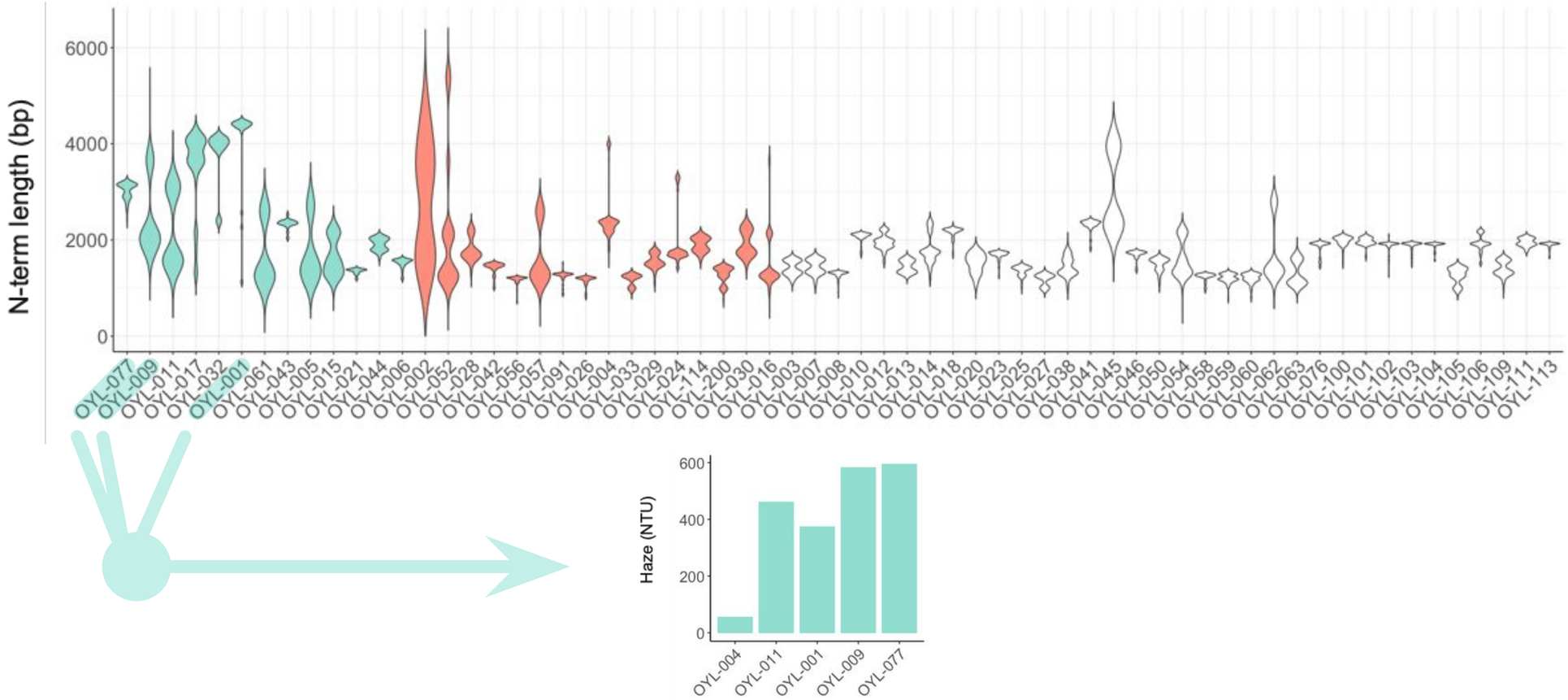
Large repeat expansions in HZY1 gene in haze-positive strains



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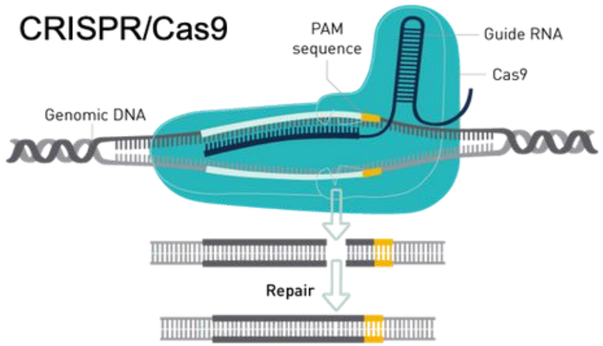
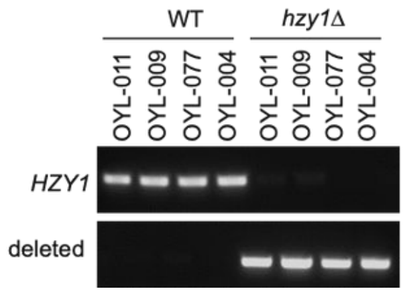
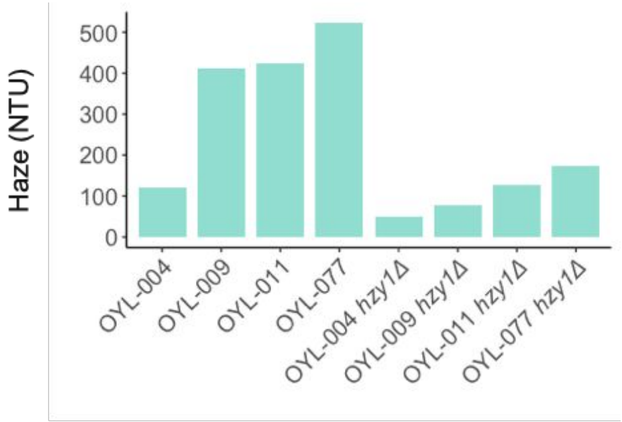
Deleting HZY1 in Brewing Strains Reduces Haze

Delete HZY1





Reduce Haze



WT hzy1Δ

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What about sensory when the haze difference is obvious?

Does Hazy = Juicy???

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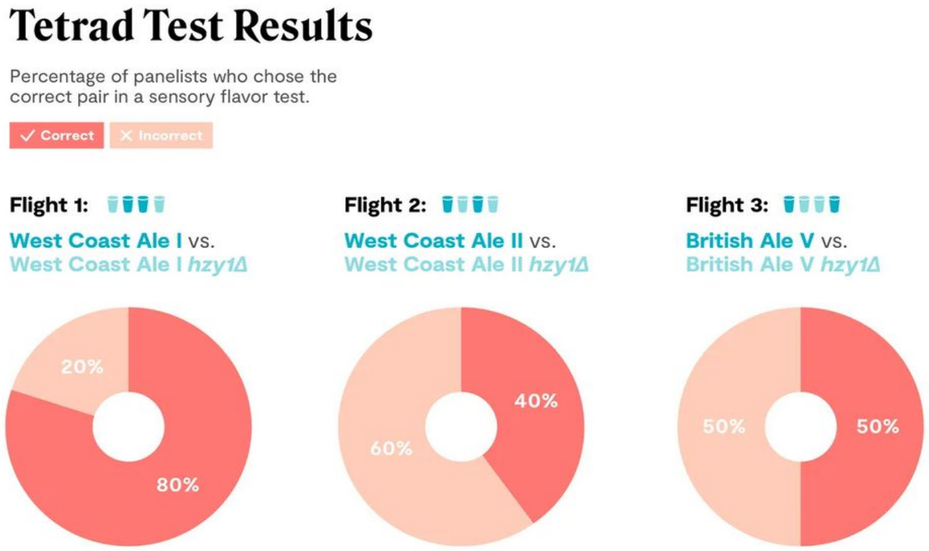
The differences were not obvious… more tasting required!

“Honestly a guess,” while another correct taster commented, “I’m having difficulty picking out similar beers.” Across the three panels, the theme of the comments was primarily that all four samples were incredibly similar.



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What does this all mean?

- Making hazy and non-hazy beers can be calculated!
- Know what yeast and hop combinations work best for the targeted style

Style	Yeast	Dry Hop
Pilsner	Haze-neutral (lager strains)	Consider an early dry hop and keep addition rates low
WC IPA	Haze-neutral (West Coast I, Lutra, even a lager strain)	Consider an early dry hop and hop varieties with minimal haze
Hazy IPA	Haze-positive (British V, Voss, Point Loma)	Only late dry hop and hop varieties with maximum haze
Hefe	Haze-positive (OYL-021)	Small late dry hop to help with haze stability

* Email me if you have questions or are interested in trialing strains!

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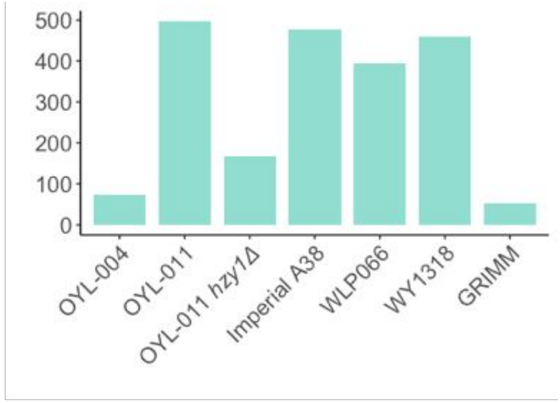
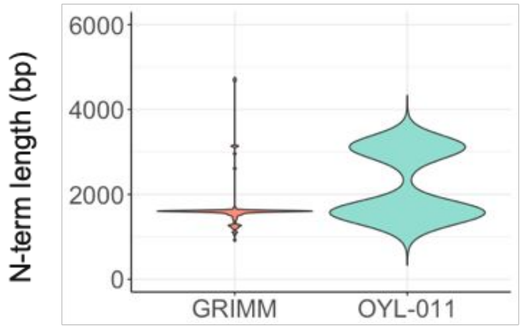
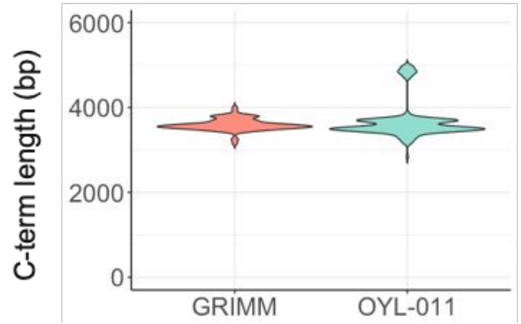
Evidence for *HZY1* changing in the brewery

- Grimm Artisanal Ales noticed their house strain was becoming less and less hazy. They had been serial repitching for 300 batches.
- Their strain proved to have lost its haze positive phenotype in our flask assay.
- After sequencing the Grimm house strain, we observed it had lost the N-terminal repeat expansion.
- What drives the selection for this change in HZY1?







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How did brewing yeast evolve to be hazy?

- Hzy1 is a yeast mannoprotein that is heavily glycosylated. The expansions could change the surface of the cell to promote it to be more “buoyant”
    - Maybe… Top Cropping Selected for Buoyant (HZY1)
    - Cone Harvesting Selected for Flocculant (FLO genes)
- Still more to learn about Hzy1, and the mechanisms for yeast-dependent haze in dry hopped beers!

Jamie Bogner skimming the open top fermentor at the RRBC pilot brewery

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THANKS!

Stay in Touch

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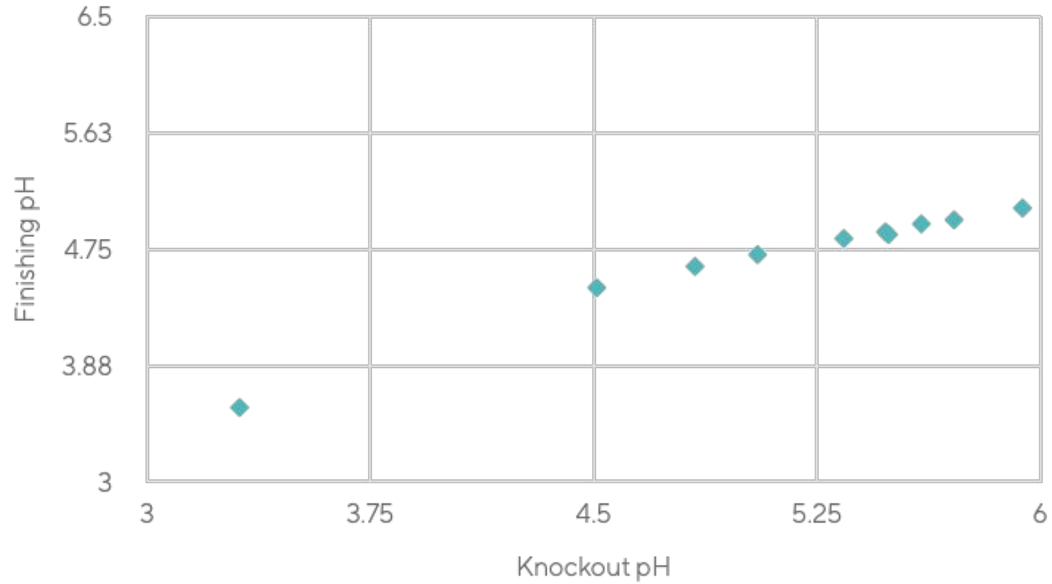
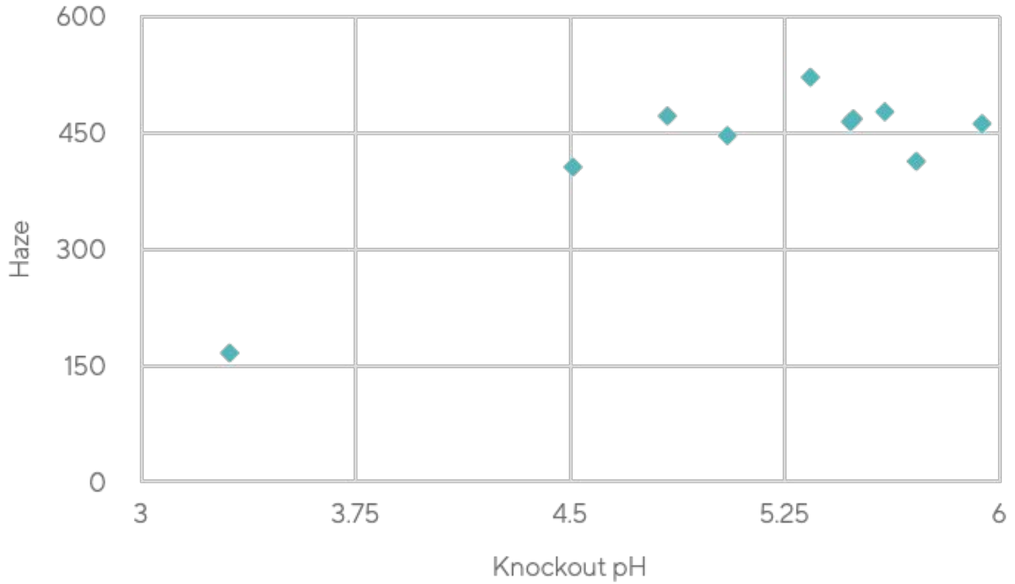
Haze occurs across a range of knockout pHs





Kncokout pH was adjusted with lactic acid and NaOH between pH 4.5 and 6 and fermentations were dry hopped on day 7

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Yeast blends to for stable haze