Genomic changes associated with improved maltotriose utilization in a kveik beer yeast

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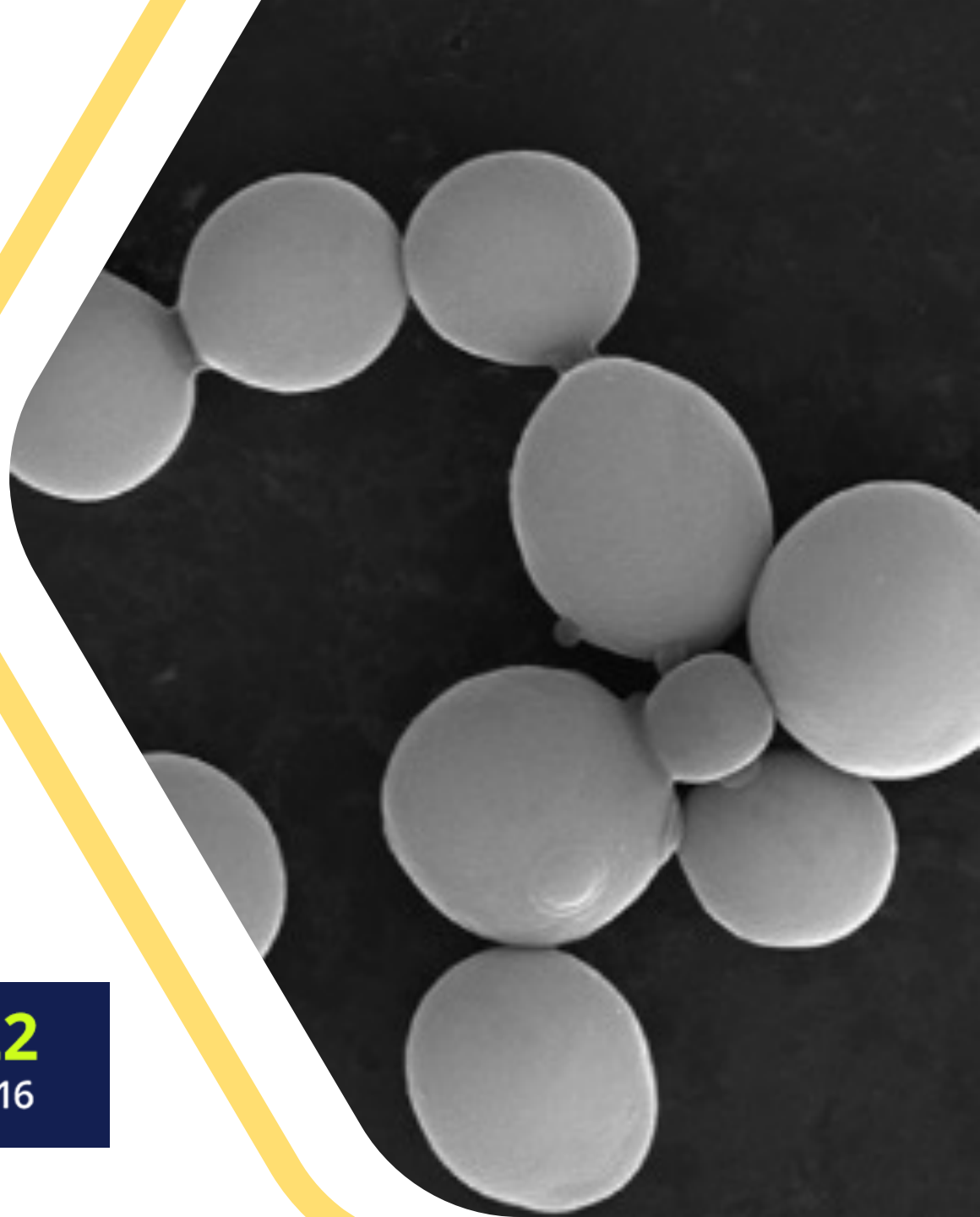

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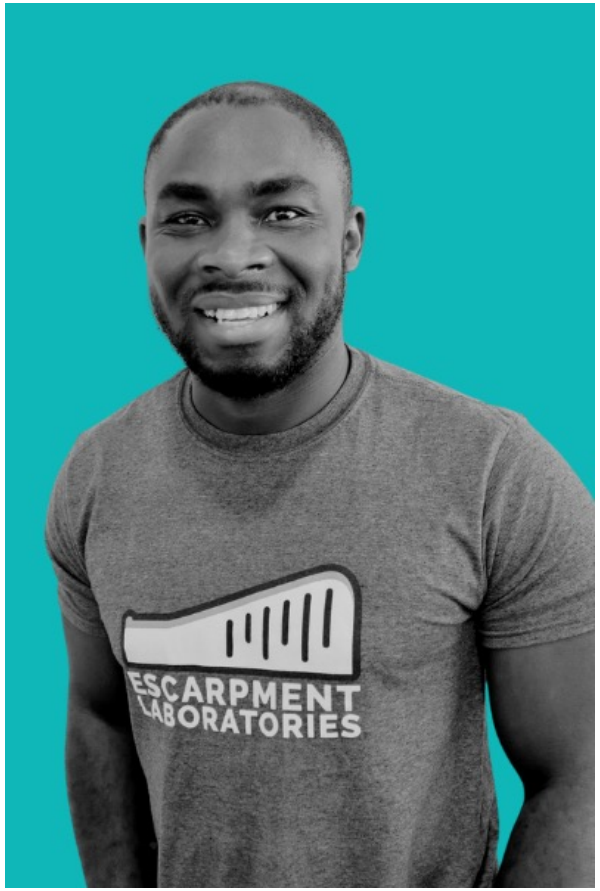
BREWING SUMMIT 2022
Providence, Rhode Island | August 14–16

Project Team

ESCARPMENT LABORATORIES

Yeast company based in Guelph, Ontario, Canada



Eugene Fletcher

PhD Cell & Molecular Biology

- Lab Evolution trials
- Screening
- Strain validation

Richard Preiss

MSc Molecular Biology & Genetics

- Genomics/ bioinformatics
- Partner Brewery testing

What is Kveik yeast & why is it special?

Traditional Norwegian Kveik Are a Genetically Distinct Group of Domesticated *Saccharomyces cerevisiae* Brewing Yeasts

Richard Preiss[1,2], Caroline Tyrawa[1], Kristoffer Krogerus[3,4], Lars Marius Garshol[5] and George van der Merwe[1*]

2018 paper

- Kveik are genetically distinct/unique from other beer yeasts
- Highly temperature tolerant and stress resistant

Kveik Brewing Yeasts Demonstrate Wide Flexibility in Beer Fermentation Temperature Tolerance and Exhibit Enhanced Trehalose Accumulation

Barret Foster[1], Caroline Tyrawa[1], Emine Ozsahin[1], Mark Lubberts[1], Kristoffer Krogerus[2], Richard Preiss[3] and George van der Merwe[1*]

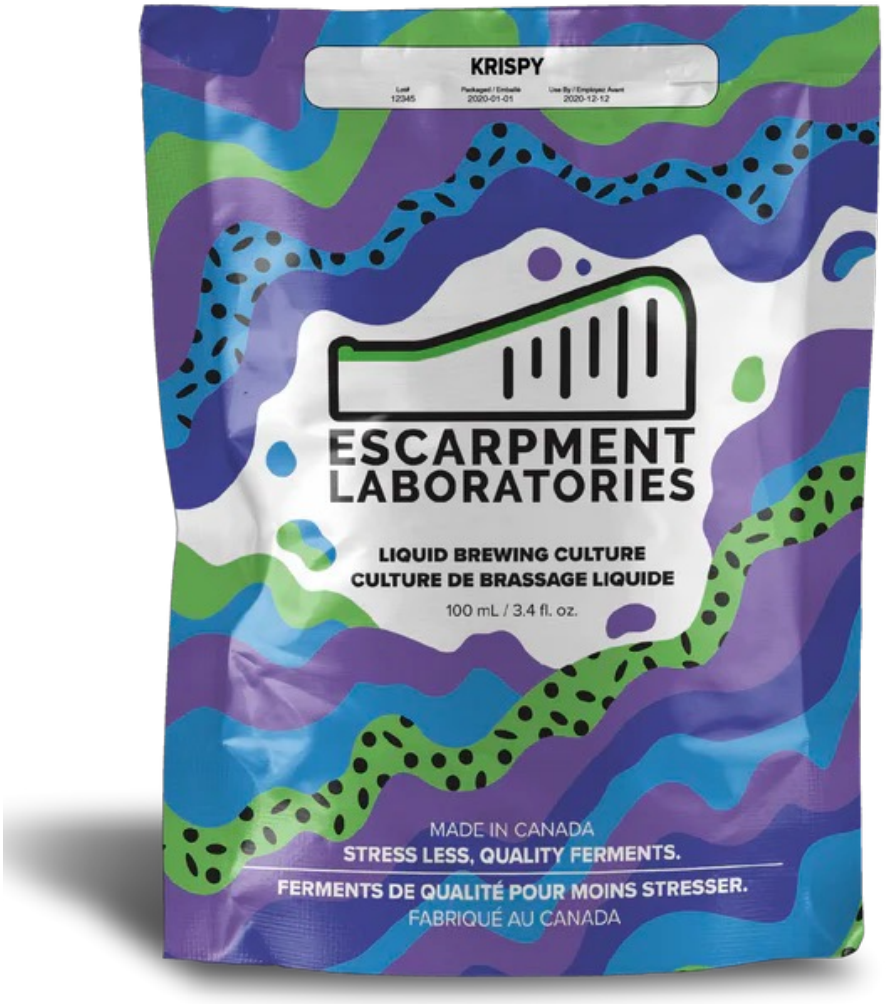
2022 paper

- Different temperatures drive different fermentation profiles in kveik
- Kveik stress tolerance may be linked to high levels of intracellular trehalose

The original KRISPY yeast

Features:

- A kveik yeast isolate (fast fermentation, short lag phase)
- Produces clean lager-like beer at 20-25°C
- Very low/no diacetyl formation
- Produces fruity esters that gives a prominent stonefruit aroma >25°C



Drawbacks:

- Attenuates slower below 25ºC.
- Lower attenuation than typical lager yeast.
- Blend of 2 strains (less consistent repitching and flocculation

Pseudolagers with kveik – Why?

Benefits:

- **Fast turnaround time (5-10 days total)**
- Less demand on glycol system
- Great choice for small brewers who need to increase throughput in the busy months

Drawbacks:

- Not the same flavor as lager yeast. Not ideal for traditional lager styles, best for “lite” lagers

Higher nutrient and oxygen requirements than lager yeast

How can we improve this yeast's attenuation?

OPTION 1: Rational approach using genetic engineering BUT…

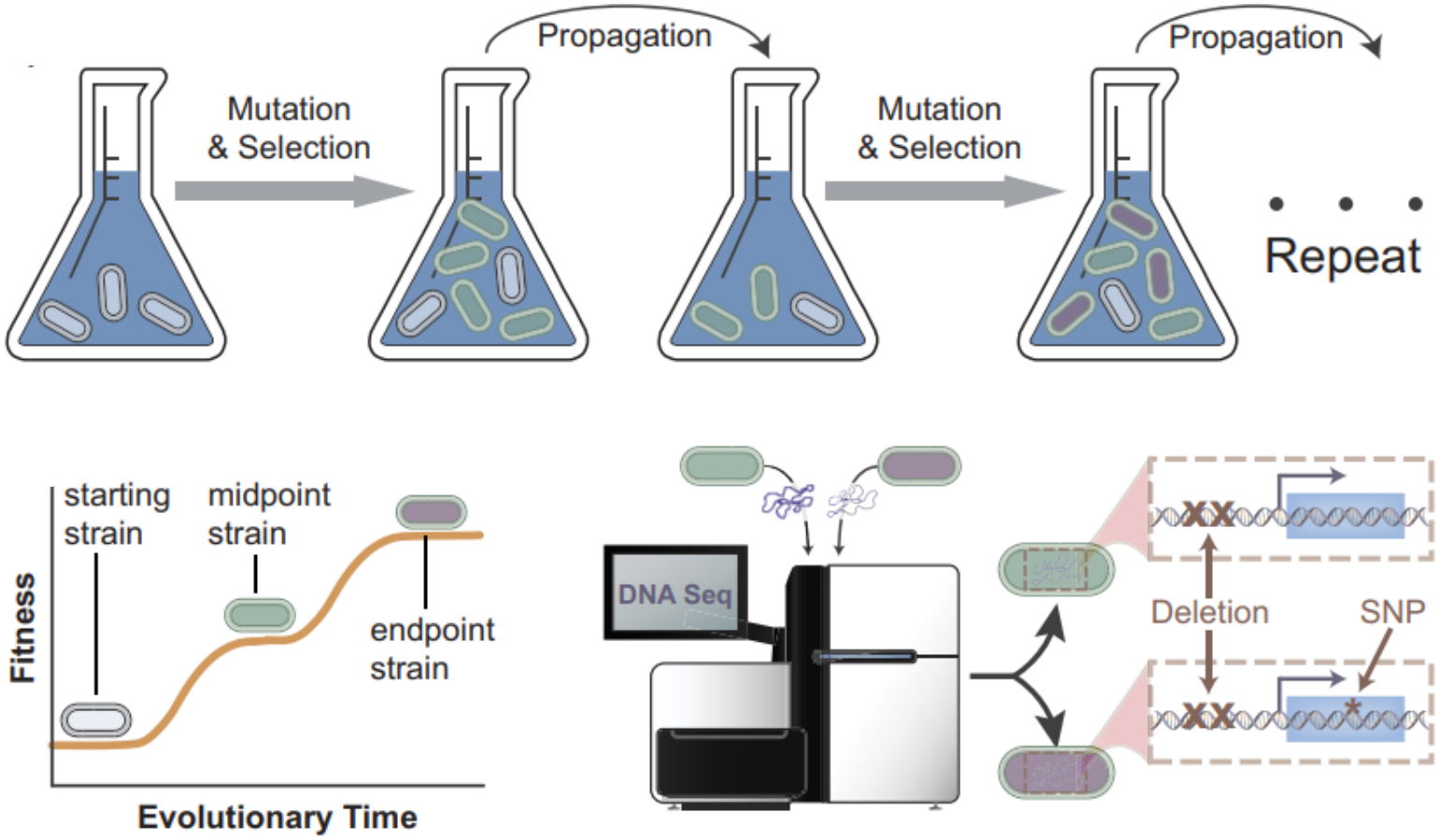
Jump all the regulatory hurdles

Figure out which genes to target

OPTION 2: Adaptive Laboratory Evolution approach.

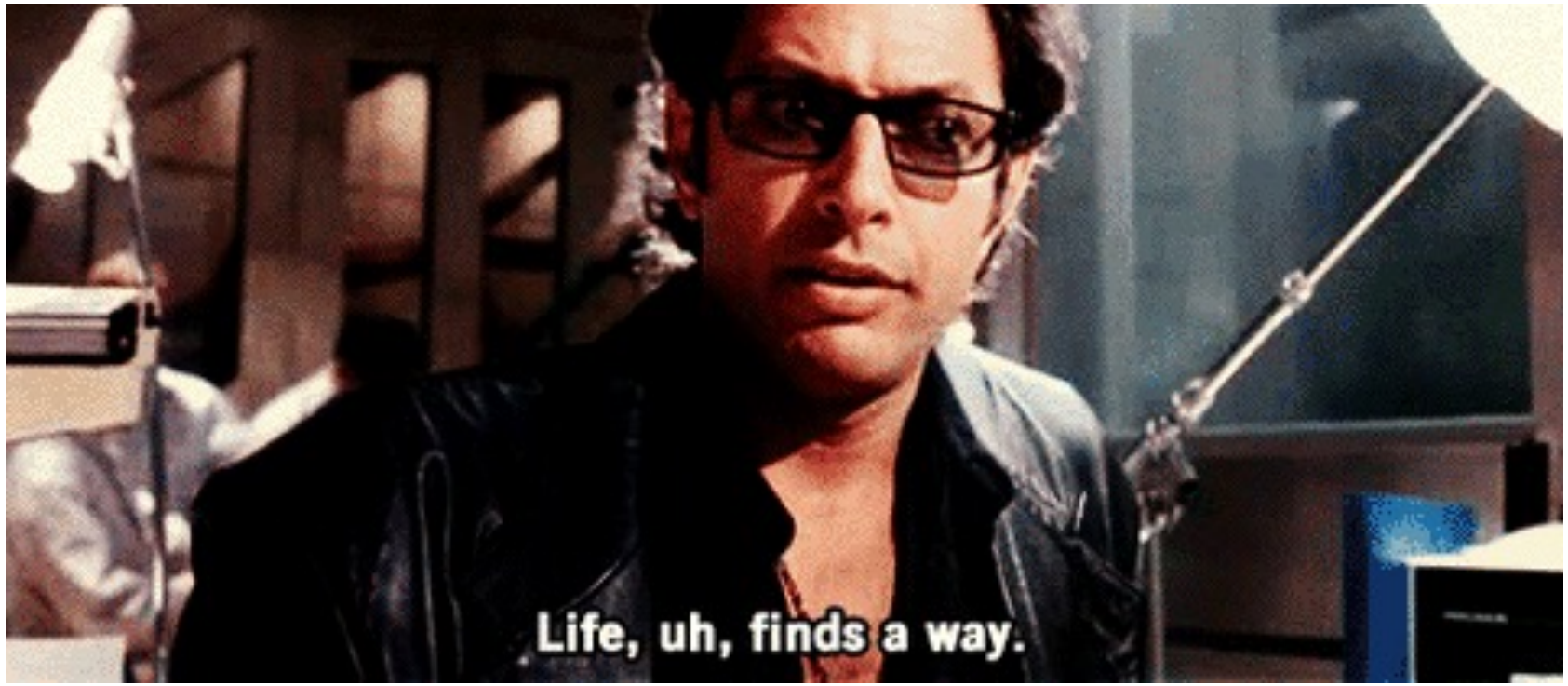
Adaptive Laboratory Evolution (ALE)

ALE allows us to replicate and fast-track evolution in the lab to select for useful traits in yeasts.

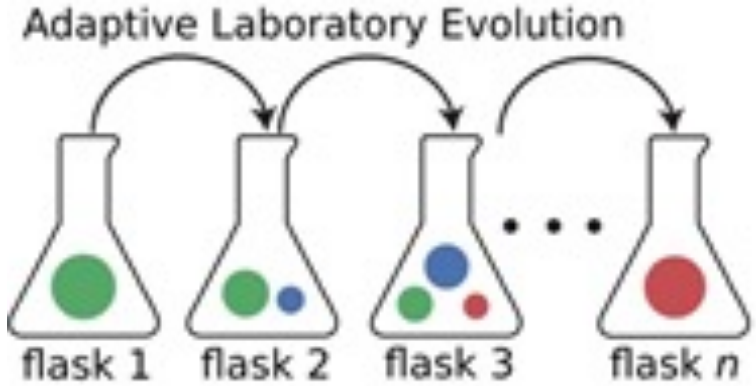


Sanberg et al. (2019). ***Metabolic Engineering*** 56:1-16

Adaptive laboratory evolution (ALE)



Laboratory evolution of KRISPY1 Kveik Yeast



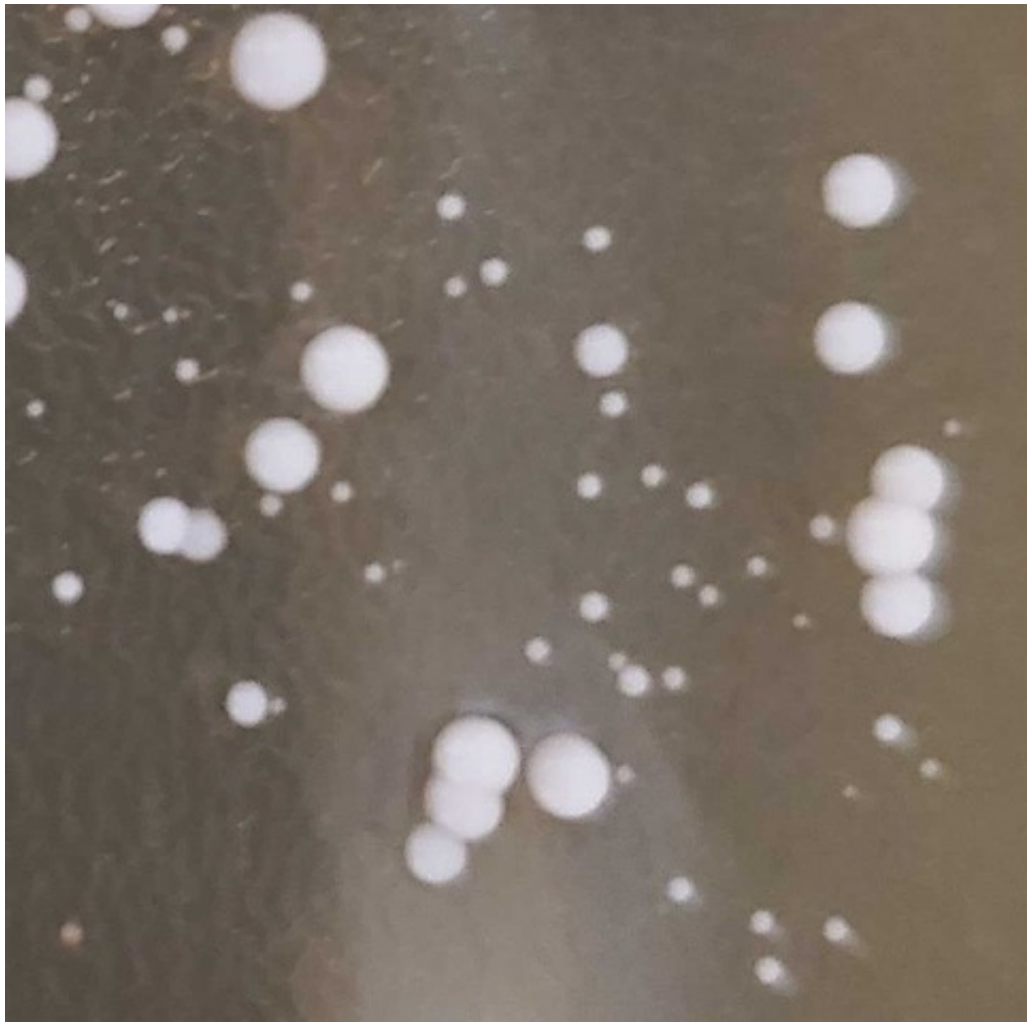
Isolation of clonal isolates on YP-Maltose agar

Selection pressure: High gravity wort (17°P >> 21°P >> 24°P)

# Days	# Yeast Generations	# Serial dilutions
97	425	55

Generations = log2 (final OD/initial OD)

Selecting isolates with higher maltose affinity

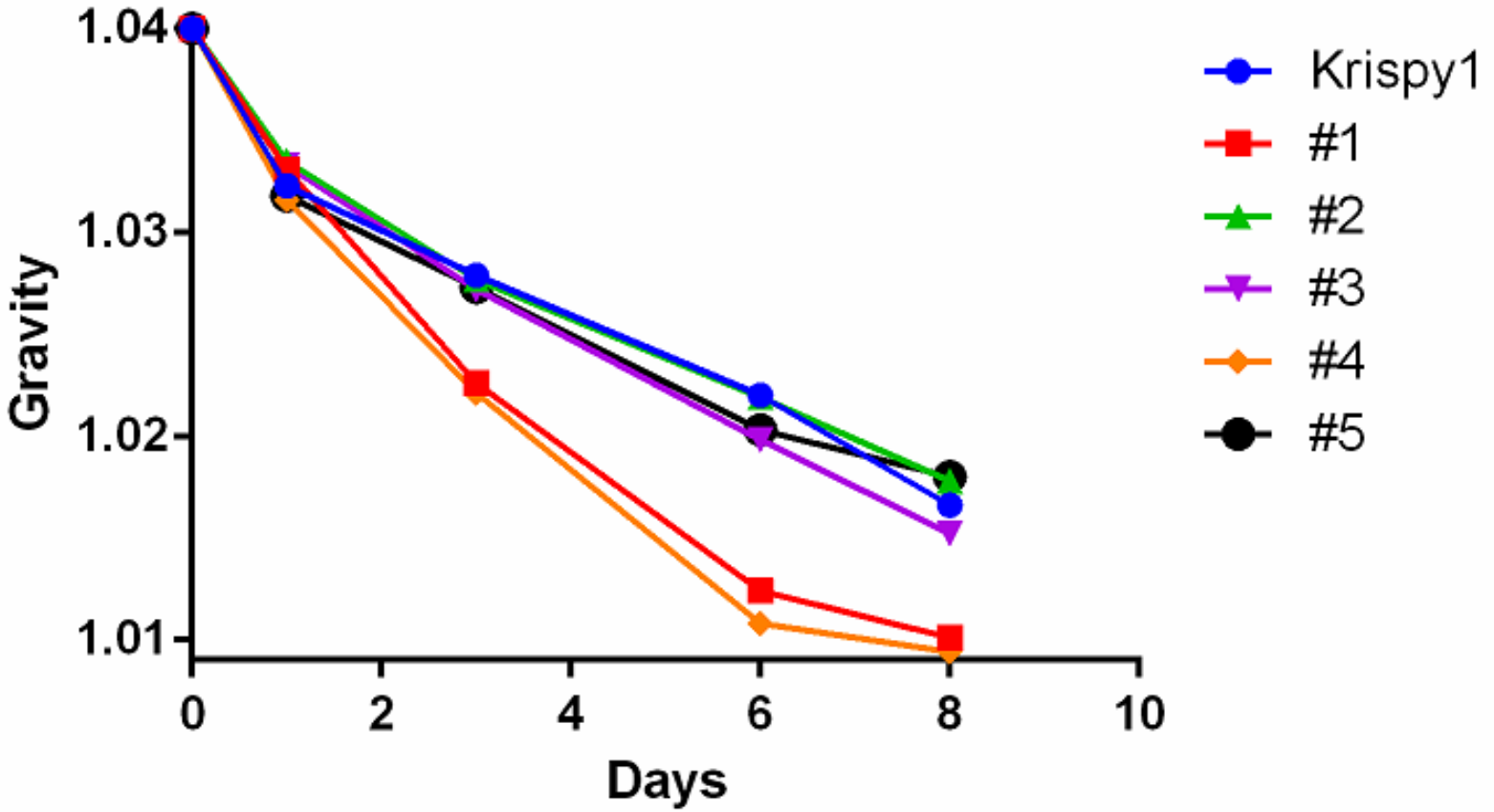


Five large clonal isolates were picked from YP-Maltose agar plates.

These clonal isolates or variants were faster consumers of malto(trio)se

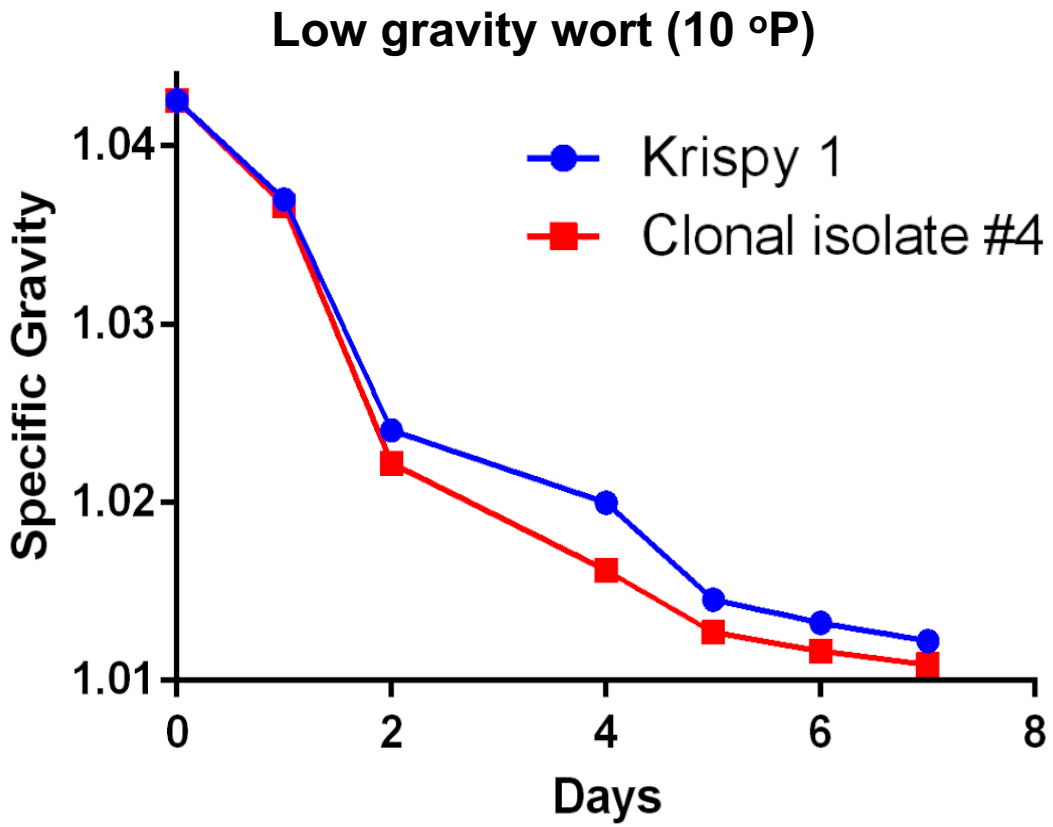
The making of "KRISPY 2.0"

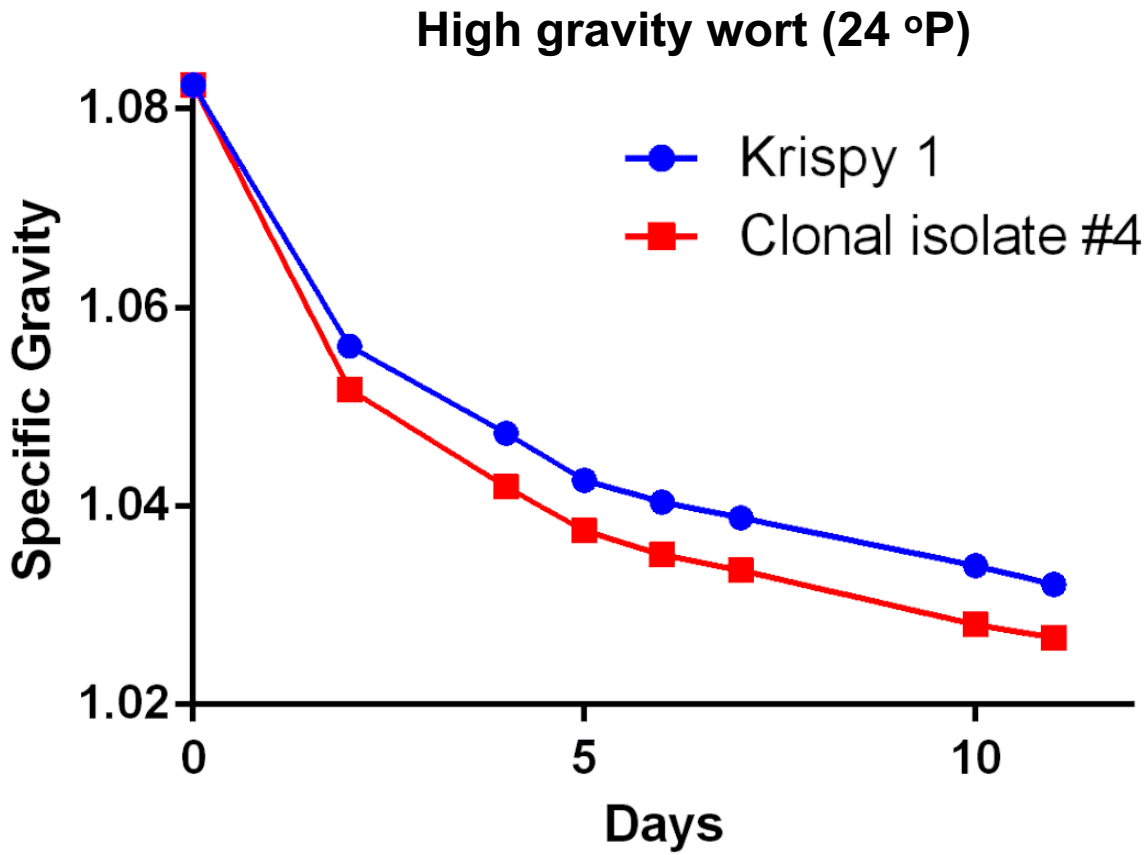
Top performing isolates with improved attenuation were identified in lab scale fermentations (400mL) at 25ºC using hopped wort (10 ºP)



The making of KRISPY 2.0

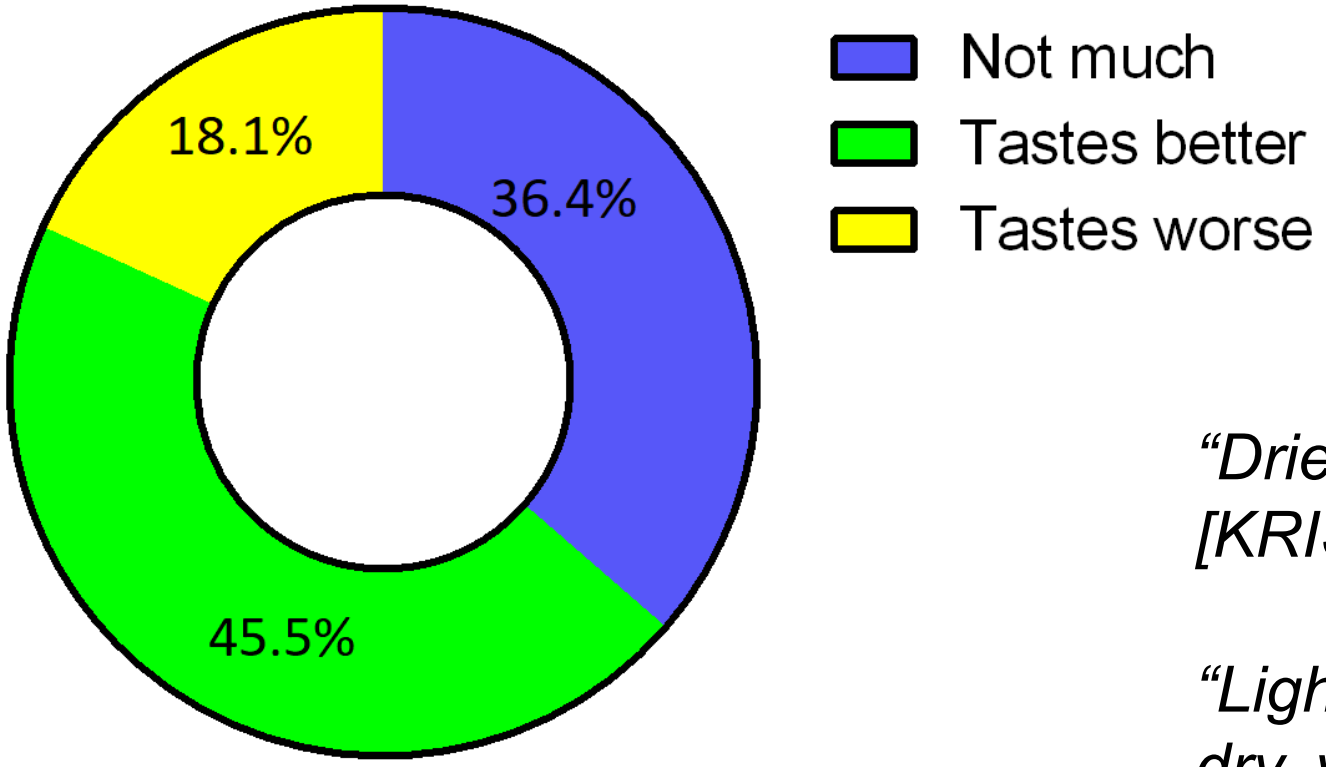
Fermentation performance of the top variant (Clonal isolate #4) was assessed in 400 mL fermentations





****The data shown are an average of four biological replicates.*

In-house sensory evaluation (KRISPY1 vs KRISPY 2.0)



"Drier and slightly less tart than sample A [KRISPY 1]."

"Light orchard and apricot notes, crisp and dry, very pleasant"

"Slightly fruity taste, very crisp"

Hedonic evaluation by trained tasters

Feedback from commercial breweries

"Very drinkable lager style beer in 7.5 days. This one is a keeper!"

"The new KRISPY exceeded our expectations and resulted in a clean, crisp and clear beer in less than two weeks."

"We have now found the ideal Kveik yeast to replace W34/70 in all our light beers."

What may have changed? Genetic features of kveik yeast

Phenotype

Compared to typical beer yeasts:

- Slower consumption of maltotriose
  - Not ideal for high wort attenuation
- Higher accumulation of trehalose
  - Storage carbohydrate, stress protectant
  - Shortens lag phase

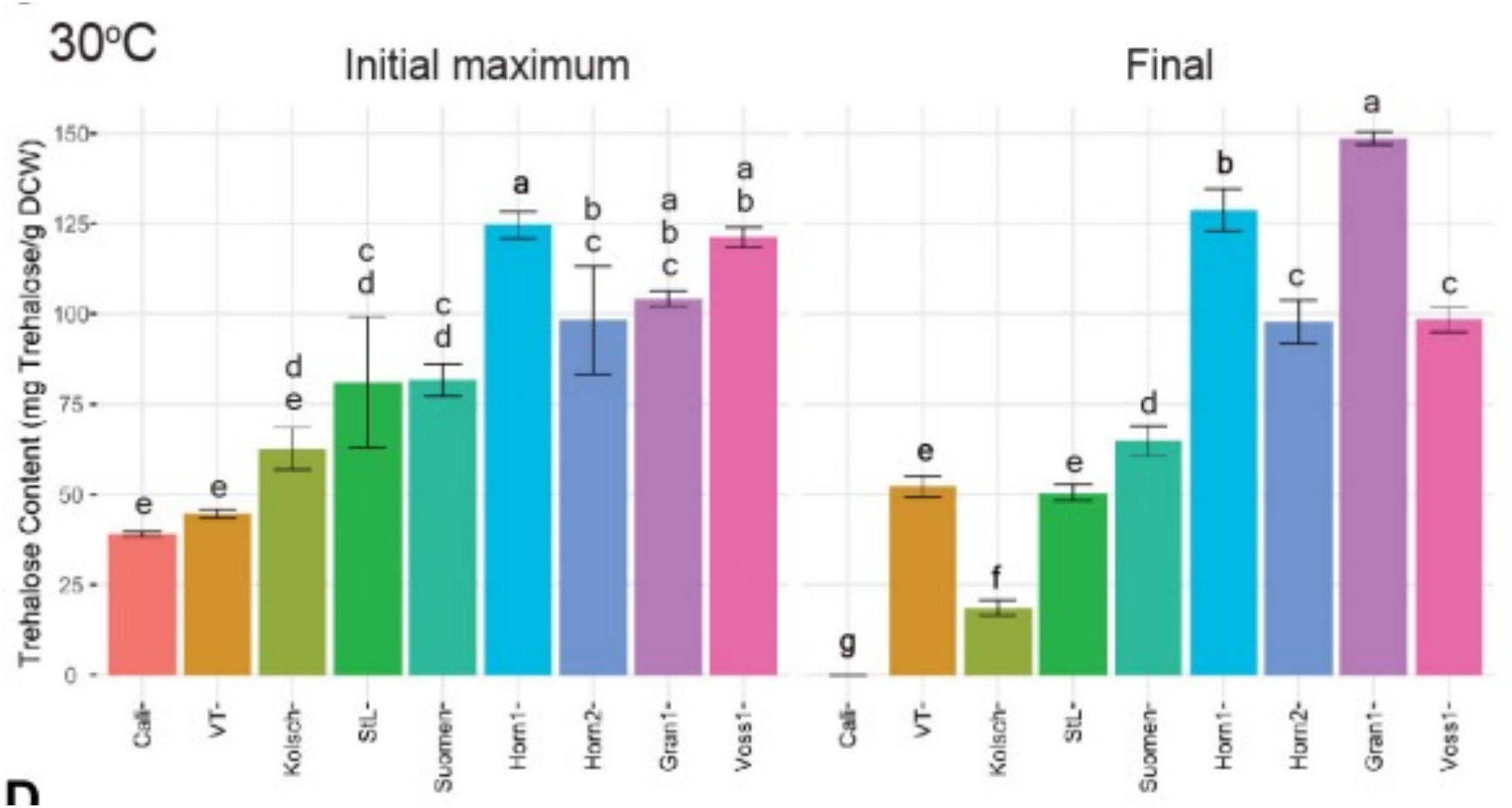
Genotype

Compared to typical beer yeasts:

- Mutations in *AGT1* maltotriose transporter (also trehalose symporter)
- Mutations in *NTH* (neutral trehalase) genes

Fast fermentation and short lag phase in kveik yeasts has a tradeoff: weaker maltotriose consumption.

What may have changed?
Genetic features of kveik yeast



Foster et al. (2022). ***Frontiers in Microbiology*** 13:747546

Whole genome sequence analysis

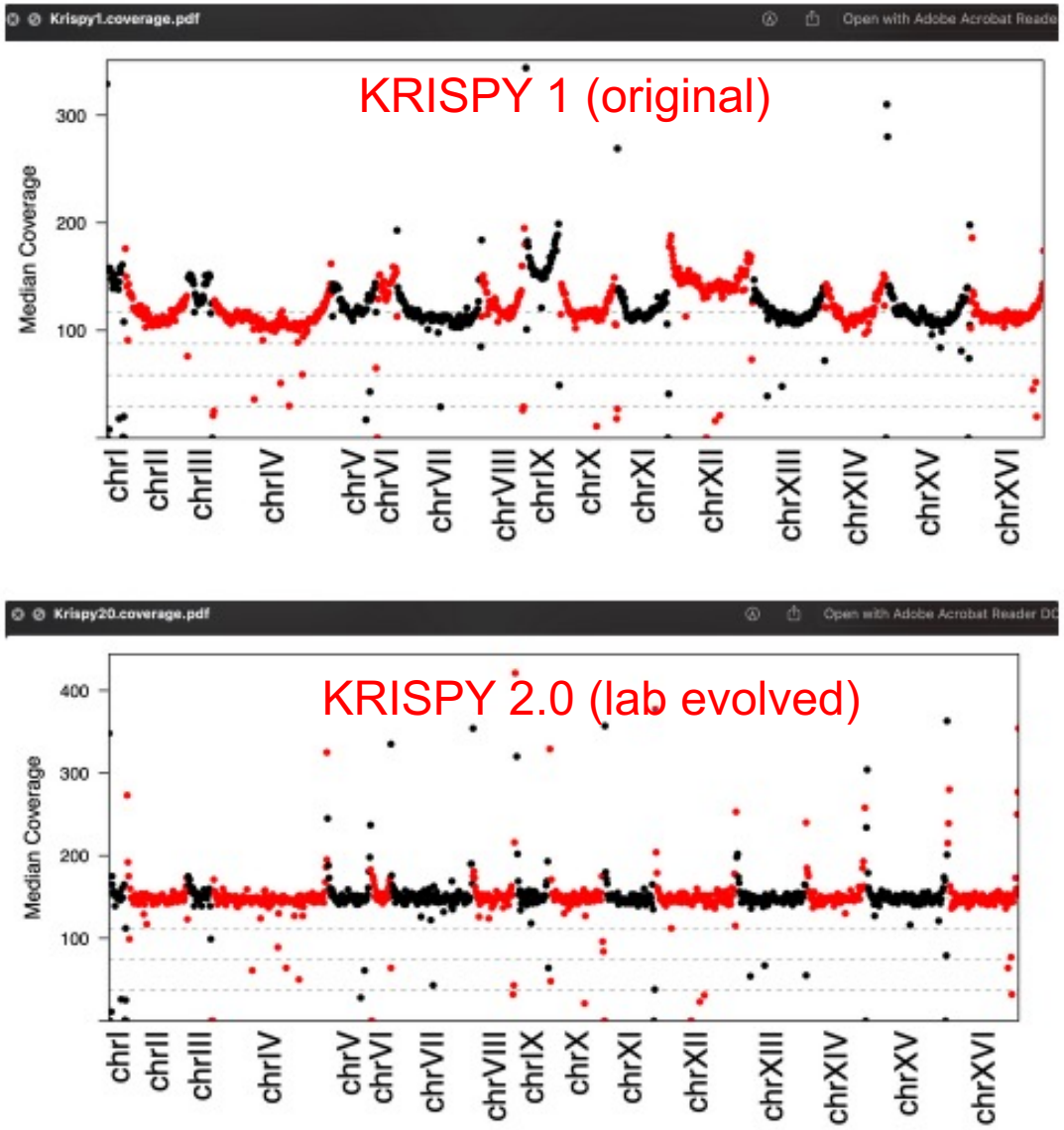
Method: Illumina NextSeq 2000 (short read DNA sequencing) of original strain vs. evolved strain

Sequencing by MiGS Pittsburgh

Genomic data processing in-house as per Foster *et al.* (2022)

Whole genome sequence analysis: allele frequency

- Both strains predicted to be tetraploid (4 copies of each chromosome)
- Changes in predicted copy number of genes near telomeres (ends of chromosomes)
- Lab evolution changed Aneuploid (odd numbered chromosome copies) to Euploid



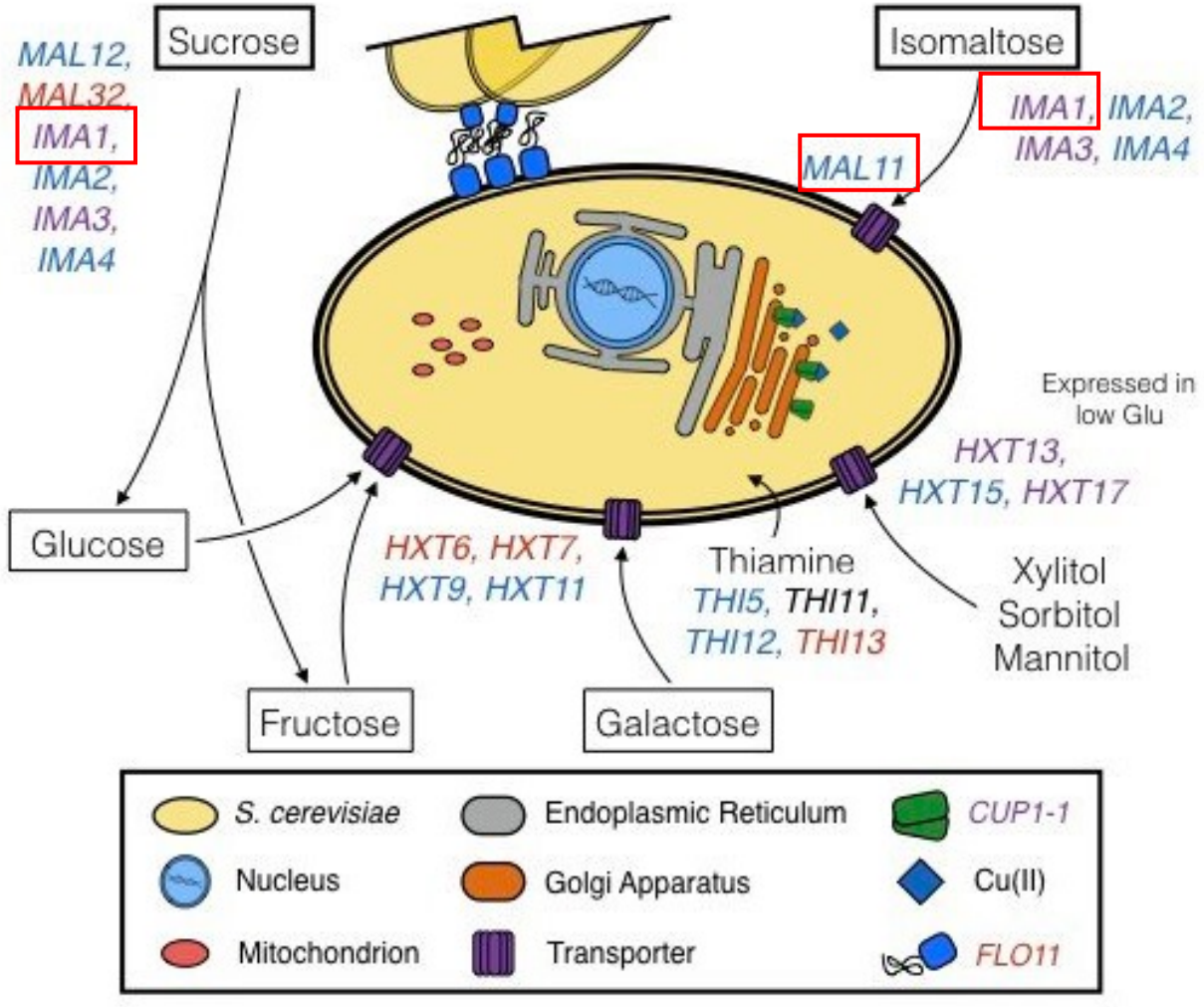
Whole genome sequence analysis: Single Nucleotide Polymorphisms (SNPs)

673 differences in high impact single nucleotide changes

Whole yeast genome is ~12 million nucleotides

Gene mutations of interest:

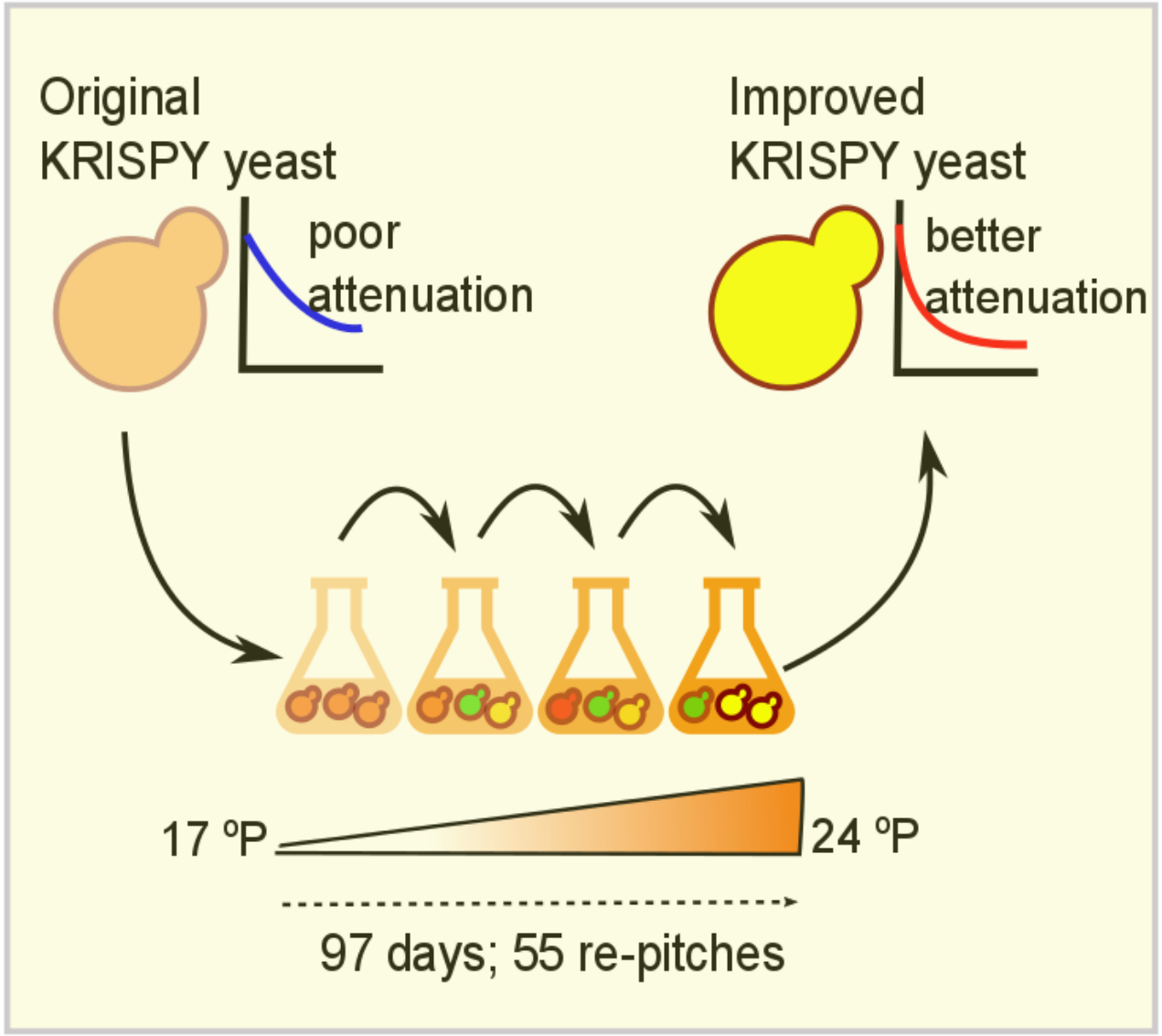
- *FLO1*, flocculation
- *MAL11, MAL13, IMA1* - sugar metabolism
- *ATF1* - ester production
- *SPG3* - high temperature tolerance



Steenwyk and Rokas (2017). ***G3*** *7(5):g3.117.040105*

Outcomes

- Adaptive Lab Evolution (ALE) over 97 days produced noticeable changes in the kveik yeast performance
- “KRISPY 2.0” showed higher affinity for maltotriose and higher wort attenuation
- This is potentially related to gene mutations in maltose metabolic genes (MAL11, MAL13, IMA1)
- Knowledge could be used to engineer precise attenuation in yeast strains
- **Adaptive Lab Evolution demonstrates potential to improve beer yeasts**



Q&A