OMEGA YEAST

4720 W Pensacola Ave
Chicago IL 60641

info@omegayeast.com
773.657.5438

Christopher Kampmeyer
Regulatory Review Scientist
Office of Food Additive Safety, HFS-200
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835

Dear Christopher Kampmeyer:

We received your letter on June 23th, 2022 regarding our submission dated November 17th, 2020, received on February 9th, 2022 for the intended use of Saccharomyces cerevisiae strain OYR-243 in food that we view to be generally recognized as safe (GRAS).

We are hereby submitting a revision of our Generally Recognized as Safe (GRAS) Notification in accordance with Title 21 C.F.R. §170.30, for Omega Yeast Labs Saccharomyces cerevisiae strain OYR-243. This revision has expanded Part 2 in response to your June 23rd email and letter to include batch analyses.

Please do not hesitate to contact me at any time by email at lance@omegayeast.com to discuss details or to request supplemental information as needed.

Thank you for your consideration.

Sincerely,

Lance Shaner & Mark Schwarz

Owners, Omega Yeast

GRAS Determination for *Saccharomyces cerevisiae* strain OYR-243

PREPARED FOR:
Office of Food Additive Safety (HFS-200)
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835

DATE: June 24th, 2022

List of Acronyms

NCYC – National Collection of Yeast Cultures
CRISPR – clustered regularly interspaced short palindromic repeats
sgRNA – small guide RNA
PAM – protospacer adjacent motif
ppt – parts per trillion
ppb – parts per billion
bp – base pair
YPD – yeast peptone dextrose
3SH – 3-sulfanylhexan-1-ol
4MSP – 4-methyl-4-sulfanylpentan-2-one
3S4MP – 3-sulfanyl-4-methylpentan-1-ol

§ 170.225 Part 1, GRAS Notice: Signed Statements and Certification

(1) GRAS Notice Submission

Omega Yeast Labs, LLC., in accordance with 21 CFR § 170.225(c)(1), has determined that its *Saccharomyces cerevisiae,* that has been genetically edited using the CRISPR-Cas9 system to insert an overexpressed native *IRC7* allele, is Generally Recognized as Safe ("GRAS") for the intended food application in accordance with Subpart E of 21 CFR § 170.

(2) Name and Address

Omega Yeast Labs, LLC
4720 W Pensacola Ave
Chicago, IL 60641
USA

(3) Name of Notified Substance

The name of the notified substance is a brewing yeast obtained from a *S. cerevisiae* strain that has been genetically edited using the CRISPR-Cas9 system to insert an overexpressed native allele of the yeast *IRC7* gene. This novel yeast strain is called OYR-243.

(4) Intended Use in Food

The OYR-243 strain was developed by Omega Yeast Labs to provide a novel flavor profile to beer by enhancing the activity and expression of yeast β-lyase gene, *IRC7. S. cerevisiae* produces many aroma-active metabolites that contribute to the complexity of a beer's flavor including phenolics, esters, carbonyl compounds, higher alcohols, aldehydes and sulfur compounds. Some of these metabolites are highly sought after for tropical fruit flavors. Polyfunctional thiols are a class of sulfur compounds that are extremely odorant and exist in both free and bound forms in brewing raw materials including barley and hops. These bound thiols are non-aromatic but are released by *S. cerevisiae* in part by the activity of the β-lyase Irc7 to produce grapefruit, passion fruit and guava flavors. The majority of industrial wine and brewing *Saccharomyces cerevisiae* strains contain mutated and inactive alleles of *IRC7* and thus are unable to release free thiols from bound forms during fermentation. There are a multitude of industrial strains that are marketed for high β-lyase activity with functional *IRC7* alleles and are used to enhance the thiol levels in wine. However, in beer fermentations this is a more complicated scenario. The abundance of nitrogen leads to repression of the yeast *IRC7* gene and to date, evidence for thiol release from native *Saccharomyces* strains in brewer's wort is extremely limited/non-existent. Thus, a functional *IRC7* allele with constitutive expression was introduced into the OYL-011 host strain to produce a strain with enhanced Irc7 β-lyase activity, OYL-243.

By enhancing Irc7 activity in the host strain, fermentation with the resulting OYR-243 strain produces a combination of aroma-active metabolites that is unique to this strain and highly desirable. The flavor characteristics of OYR-243 provide a complement to hops and malt that would be costly to obtain with other combinations of raw materials.

The OYR-243 yeast strain is intended for use as a commercial liquid yeast culture for the production of fermented beverages in accordance with Good Manufacturing Practices (GMPs). This liquid yeast strain performs alcoholic fermentation in the same manner as its host strain and other traditional brewing strains. The enhanced Irc7 activity of OYR-243 provides a well-suited brewing yeast strain for American craft beers, hoppy beers, and other "clean" beer styles.

(5) Statutory Basis for GRAS Determination

Omega Yeast Labs, LLC has determined that the OYR-243 yeast strain is "Generally Recognized As Safe" (GRAS) for the use as a starter culture in alcoholic beverage fermentation. This determination is based on scientific procedures and conforms to the regulations in accordance with 21 CFR § 170.30(a) and (b). Based on the design of OYR-243, it is substantially equivalent to the host strain, with the sole difference being the insertion of an activated form of the naturally occurring *S. cerevisiae* gene *IRC7*. The resulting levels of free polyfunctional thiols are in line with other fermented products such as wine where nitrogen is limited. The precise insertion of the *IRC7* allele at the inactive locus *fdc1* means there is a negligible chance of unintended effects in the OYR-243 strain.

(6) Premarket Approval Statement

Omega Yeast Labs further asserts that the use of the OYR-243 yeast strain ingredient, as described herein, is exempt from the pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act, based on a conclusion that the substance is GRAS under the conditions of its intended use.

(7) Availability of Information

The data and information that serve as the basis for this GRAS determination, as well any information that has become available since the GRAS determination, will be sent on request, or are available for the FDA's review and copying during customary business hours at the following address:

Omega Yeast Labs, LLC
Attention: Lance Shaner
4720 W Pensacola Ave
Chicago, IL 60641
USA

(8) Data and Information Confidentiality Statement

None of the data and information in the GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

(9) GRAS Certification

To the best of our knowledge, this GRAS notice for Saccharomyces cerevisiae OYR-243 and its use in fermentation of alcoholic beverages in accordance with Good Manufacturing practices (cGMP) is a complete, representative and balanced submission that includes both favorable and unfavorable information known to Omega Yeast Labs, LLC and pertinent to the evaluation of the safety and GRAS status of the use of the strain. Recent reviews of the scientific literature revealed no potential adverse health concerns.

(10) Name/Position of Notifier

6/24/2022

Lance Shaner, Ph.D. Date
Co-owner and Founder
Omega Yeast Labs, LLC

§ 170.230 Part 2, Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1. IDENTITY OF THE NOTIFIED SUBSTANCE

The subject of this notification is an industrial brewing *Saccharomyces cerevisiae* strain called OYR-243. This strain was modified with CRISPR-Cas9 introducing the insertion of an *IRC7* allele under the regulation of the *TDH3* promoter and *CYC1* terminator to promote expression in beer fermentations. This modification results in the insertion of only DNA derived from *S. cerevisiae*. Technical details regarding the modification are detailed in sections below. The use of OYR-243 in brewing enhances the hoppy aroma characteristics in beer during fermentation. Hops that exhibit elevated thiol levels are often desired for the tropical fruit flavors they impart. OYR-243 with enhanced Irc7 activity will release thiol compounds from precursor forms found in barley and hops. The resulting thiol levels will be similar to amounts found in heavily hopped beer styles or white wine varieties such as Sauvignon Blanc.

2.2 HOST MICROORGANISM

The host yeast strain, OYL-011, is an industrial brewing strain of *S. cerevisiae* that is widely used in commercial beer production of traditional English beer styles and modern American hoppy beer styles. The host strain is NCYC1318, from the National Collection of Yeast Cultures in Norwich, UK. It is commonly referred to by American craft brewers as London Ale III. The host strain was deposited by a British Brewery in 1971 listing it as an ale production strain.

2.2.1. History of use

S. cerevisiae is widely used in industrial fermentation of many food products. It is in the air that we breathe and it grows naturally on many foods that we eat regularly. It is likely that most humans are exposed daily to foods containing millions of live *S. cerevisiae* through consumption of wine, beer, fruits, salads, and cheese and other dairy foods, as well as other traditional and ethnic fermented foods. This yeast species has been used in food production for over five thousand years. Common industrial applications include; baked goods, beer, wine, cider, sake, spirits, coffee and chocolate (Boekhout and Robert, 2014).

S. cerevisiae serves as a model organism for scientific research. Over 30 years of yeast research has contributed to our fundamental understanding of cell and molecular biology. The *S. cerevisiae* genome size is small, is highly homologous with the human genome, genetics are very tractable, and DNA repair pathways are robust allowing for ease of accurate genetic manipulation (Botstein and Fink, 2011). A long history of research and industrial use has indicated that *S. cerevisiae* is safe for use in food and beverage manufacturing (Boekhout and Robert, 2014).

There are over 150 *S. cerevisiae* strains that are commercially sold as brewing yeast. These strains have been isolated from brewery and farmhouse origins, where the re-pitching or backslopping practices allowed for the propagation and selection of unique brewing characteristics (Gallone et al., 2016). The origin of the host strain, OYL-011, traces back to a British Brewery. It is a widely popular ale strain used by many US craft breweries for traditional English styles and modern American hoppy styles.

The FDA considers *Saccharomyces cerevisiae* and several derived products safe for consumption. Indeed, FDA has approved dried yeast as an ingredient for food (21 C.F.R. §172.896), and Baker's yeast extract has been affirmed by the FDA as a GRAS flavoring agent and adjuvant (21 C.F.R. §184.1983). The FDA has also approved various yeast-derived products for their use in food. These include Baker's yeast protein (21 C.F.R. §172.325), Yeast-malt sprout extract (21 C.F.R. §172.590) and Baker's yeast glycan (21C.F.R. §172.898). *Saccharomyces cerevisiae* is also considered Generally Recognized as Safe as noted in several GRAS Notices; GRNs 120, 175, 239, 260, 284, 350, 353.

2.2.2. Taxonomy

The OYL-011 host strain's taxonomy has been confirmed to be *S. cerevisiae* by the NCYC and through multiple whole genome sequencing studies. Furthermore, whole genome sequencing of OYL-011 confirms that it is closely related to other Belgian/ German/ English/ American "Beer 2" industrial brewing strains (Gallone et al., 2016).

2.2.3. Characteristics

The host strain exhibits standard industrial brewing characteristics. It is maltose-fermenting, and is easily propagated in wort produced from malted barley. Fermentations with this strain produce a desirable ale fermentation profile attributed to the production of esters. This strain sporulates, but exhibits low spore viability and thus is not well-suited for traditional breeding strategies.

2.3. DONOR ORGANISM

2.3.1. Taxonomy

Saccharomyces cerevisiae

The history of the use of *S. cerevisiae* is reviewed in Section 2.2.1 above. The *IRC7* DNA sequence is derived from the OYL-004 strain originating from an isolate of the commercial yeast from Sierra Nevada Brewery. This OYL-004 strain is commonly used in breweries around the world to produce American beer styles. The *TDH3* promoter and *CYC1* terminator regulatory elements are derived from the highly characterized S288C strain that serves as the reference sequence strain in the Saccharomyces Genome Database.

2.3.2. Genetic material synthesized based on sequences from donor organisms

Promoter Sequence

The *TDH3* promoter region -673 to -1 (i.e., region upstream of the *TDH3* gene) is a well characterized promoter that is used to drive constitutive expression of heterologous genes in *S. cerevisiae* (Partow et al., 2010). The *TDH3* promoter is a native yeast promoter that controls expression of Glyceraldehyde-3-phosphate dehydrogenase. The sequence was obtained from the S288C reference genome.

IRC7 allele

The *IRC7* donor DNA sequence is derived from the OYL-004 strain originating from an isolate of the commercial yeast from Sierra Nevada Brewery. Though many industrial wine and brewing strains have mutated versions of *IRC7* that result in loss of function phenotypes, OYL-004 contains a full length *IRC7* allele encoding a functional β-lyase (Cordente et al., 2019; Santiago and Gardner, 2015). The Irc7 cysteine-S-conjugate β-lyase enzyme functions in sulfur metabolism and L-cysteine catabolism. In wine fermentations, the function of this enzyme has been well characterized to release 3SH and 4MSP varietal thiol compounds from amino acid-bound precursors found in wine grapes (Roncoroni et al., 2011). The *IRC7* gene is under nitrogen catabolite repression and is induced under conditions of nitrogen limitation (Santiago and Gardner, 2015).

Terminator Sequence

The 248 bp sequence that directly follows the *CYC1* gene known as the *CYC1* terminator. This sequence is commonly used in *S. cerevisiae* to terminate transcription and allow for efficient heterologous gene expression. The sequence was obtained from the S288C reference genome (Curran et al., 2015).

2.4. THE MODIFIED MICROORGANISM

2.4.1. Final construct used in the CRISPR-Cas9 strategy

2.4.1.1. Construction strategy

To generate OYR-243, the *TDH3-IRC7-CYC1* cassette was targeted to the *FDC1*, which is commonly inactivated in brewing strains with a naturally occurring *fdc1-C460T* loss of function mutation. This cytosine to thymine mutation at position 460 of the *FDC1* coding sequence, replaces a glutamine residue (CAA) with a stop codon (UAA). CRISPR-Cas9 genome editing technology was used to insert the *TDH3-IRC7-CYC1* cassette specifically at this *fdc1-C460T* nucleotide in the OYL-011 host strain (Mans et al., 2015). Briefly, Cas9 (bacterial RNA-guided endonuclease) was expressed and targeted to the *fdc1-C460T* loci with a small guide RNA (sgRNA) that is homologous to the sequence surrounding the position 460 in *fdc1-C460T*. In addition, a protospacer adjacent motif (PAM) sequence (NGG) is in close proximity, which is crucial for the correct guidance of Cas9 to its target. Once recruited, Cas9 generates a double strand break adjacent to the cytosine at position 460 of *fdc1-C460T*. A repair template with homology to each end of the double strand break is supplied in excess and is utilized by the yeast cell's endogenous homologous recombination pathway for repair. This repair template contains the *TDH3-IRC7-CYC1* cassette as well as 100 bp *fdc1* homology

upstream and downstream of the cytosine at position 460. Once the repair occurs, the *fdc1-C460T* sgRNA targeting sequence is disrupted with the *TDH3-IRC7-CYC1* insertion and thus Cas9 is unable to efficiently target the *fdc1-C460T* locus. Successful CRISPR-Cas9 targeting results in the editing of each *fdc1-C460T* loci. The resulting *TDH3-IRC7-CYC1* insertion retains a nonfunctional *fdc1* allele with a premature stop codon at the insertion site. Transformation events were screened by colony PCR using oligos downstream of the fdc1 homology and internal to the *TDH3-IRC7-CYC1* cassette identifying clonal isolates that successfully incorporated the *TDH3-IRC7-CYC1* insertion at the *fdc1-C460T* loci. No genes encoding virulence factors, protein toxins or enzymes involved in the synthesis of mycotoxins, or any other toxic substances are expected based on our knowledge of these strains, the *IRC7* sequence, *TDH3* promoter and *CYC1* terminator. The insertion sites and flanking regions were examined for potential open reading frames. A bioinformatics search for 10 potential open reading frames spanning the site of insertion (*fdc1-C460T*) and the inserted DNA (*TDH3-IRC7-CYC1*) was performed using NCBI blastp (http://www.ncbi.nlm.nih.gov/BLAST/) on January 7th, 2022. The criteria used for blastp were default parameters: BLOSUM92 scoring matrix, Word size 6, Expect value 10, hitlist 100, Gapcosts 11,1, window size 40, threshold 21. Blast results for each of the 10 open reading frames were manually evaluated for any suggested toxicity and a search for the word toxin was performed on each list of subjects identified. No subjects of these blast searches were known toxins, leading us to conclude that the *TDH3-IRC7-CYC1* insertion does not raise any oral toxicity concerns.

2.4.1.2. Generating the *fdc1-C460T* CRISPR plasmid and *TDH3-IRC7-CYC1* repair template

The final DNA constructs used for transformation of the host organism are the *fdc1-C460T* CRISPR plasmid and *TDH3-IRC7-CYC1* repair template. To generate the *fdc1-C460T* CRISPR plasmid, the *fdc1-C460T* targeting sgRNA was cloned into a parent yeast shuttling vector containing Cas9 regulated by *S. cerevisiae PGK1* promoter and terminator. The parent yeast shuttling vector was digested with BsmBI. An *fdc1-C460T* sgRNA expression cassette containing the tRNA phe promoter, tRNA phe, *fdc1-C460T* sgRNA, and the tSRN52 sequences with homology to the linearized ends of the parent yeast shuttling vector was synthesized. The gap repair yeast cloning method was used where two linear fragments with overlapping homology are transformed into *S. cerevisiae* and repaired through homology-directed repair pathways. The parent yeast shuttling vector also contains the *KanMX* gene, and thus successful gap repair results in G418-resistant colonies. The resulting *fdc1-C460T* CRISPR plasmid was isolated from the *S. cerevisiae* G418 transformants and confirmed through restriction digestion and sequencing. The *fdc1-C460T* CRISPR plasmid allows for co-expression of Cas9 and the *fdc1-C460T* sgRNA. The plasmid map and FASTA sequence is provided in Figure 1 and Appendix 1.

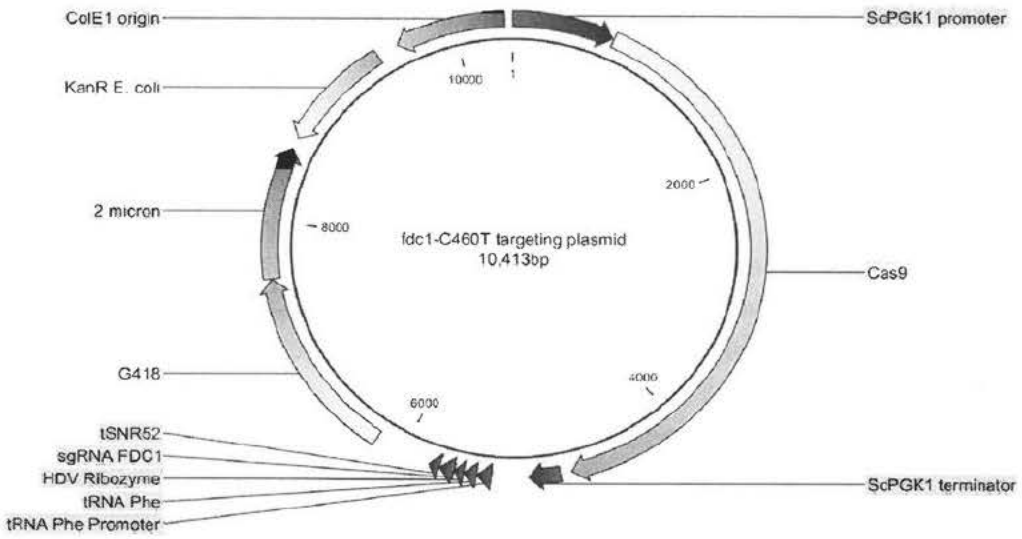


Figure 1: Schematic of *fdc1-C460T* targeting CRISPR-Cas9 plasmid

The *TDH3-IRC7-CYC1* cassette was constructed by Integrated DNA Technologies as a custom gene synthesis supplied in the pUCIDT vector. The gene cassette sequence was verified on both strands through Sanger sequencing. Phusion high fidelity polymerase was used to amplify the *TDH3-IRC7-CYC1* repair template. The resulting sequence is supplied in Figure 2 and Appendix 2.

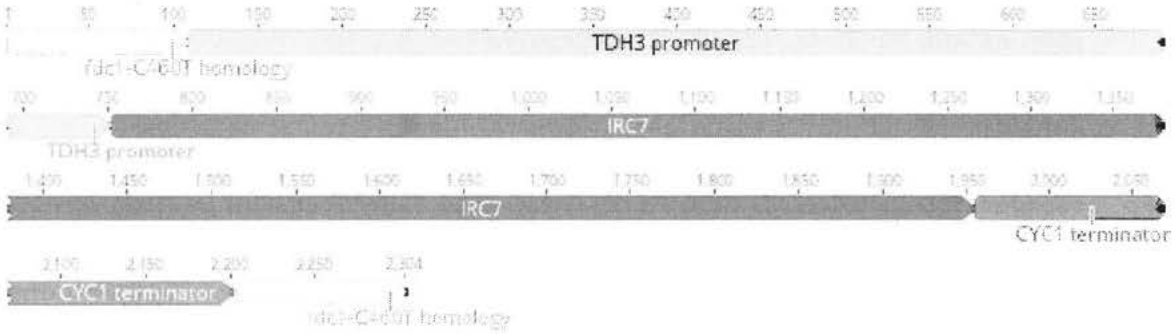


Figure 2: Schematic of *TDH3-IRC7-CYC1* repair template

2.4.1.3. Detailed description of the final *fdc1-C460T* CRISPR plasmid and *TDH3-IRC7-CYC1* repair template

The *fdc1-C460T* CRISPR plasmid is 10,413 bp long. The sequence contains the Cas9 ORF flanked by the ScPGK1 promoter and terminator (2-5,111 bp), the *fdc1-C460T* sgRNA expression cassette containing the tRNA phe promoter, tRNA phe, *FDC1* sgRNA, and the tSRN52 (5,362-5,799 bp), the *KanMX* gene for G418-selection in *S. cerevisiae* (8,631-9,446 bp), the yeast 2 micron multi-copy

selfish DNA element (7,591-8,553 bp), the *KanR* gene for Kanamycin-selection in E. coli (8,631-9,446 bp), and the ColE E. coli origin of replication (9,595-10,358 bp). The plasmid map and FASTA sequence is provided in Figure 1 above. The sequence of the repair template containing *TDH3-IRC7-CYC1* is provided in Figure 2 above.

2.4.2. The transformation event

2.4.2.1. Genetic material used for the transformation method

The *fdc1-C460T* CRISPR plasmid and *TDH3-IRC7-CYC1* repair template were the only DNA sequences used for the transformation of the host OYL-011 and generation of the modified OYR-243.

2.4.2.2. Screening method for transformants

Logarithmically growing cultures of OYL-011 were transformed with 500 ng of the *fdc1-C460T* CRISPR plasmid and 25 µg of the *TDH3-IRC7-CYC1* repair template using standard lithium acetate transformation protocol.[10] The transformation reaction was outgrown in YPD overnight and plated onto YPD-G418 agar plates to select for successful transformants. Colonies were restruck to YPD-G418 agar plates to confirm resistance and then individual transformants were screened by colony PCR. Briefly, yeast genomic DNA was prepared by lithium acetate-SDS method and primers specific to the insert and downstream region of *FDC1* were used to identify transformants with the successful CRISPR-Cas9 editing event.

2.4.3. Genetic characterization of the modified microorganism

2.4.3.1. The loss of the Cas9 plasmid containing the antibiotic resistance gene

Successful transformants were inoculated into liquid YPD, grown to saturation and subsequently diluted and plated onto YPD agar plates. Once individual colonies were observed, YPD plates were replica-plated to YPD-G418. Colonies that lost the *fdc1-C460T* CRISPR plasmid showed G418 sensitivity and were re-struck to YPD and YPD-G418 to confirm the loss of the antibiotic resistance gene (Figure 3).

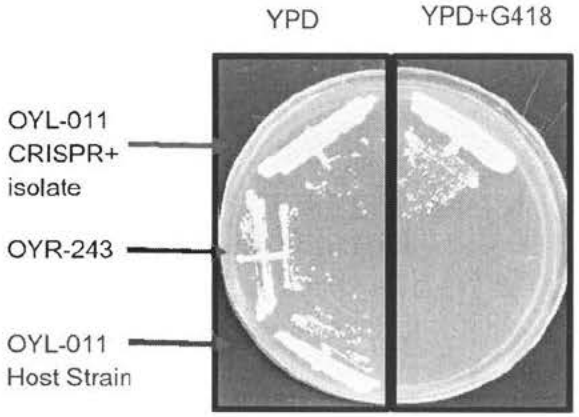


Figure 3: Loss of *fdc1-C460T* targeting CRISPR-Cas9 plasmid confirmed by loss of G418 antibiotic resistance.

2.4.3.2. PCR to confirm the *TDH3-IRC7-CYC1* insertion

Colony PCR of the resulting isolates was performed to amplify the insertion at *fdc1-C460T*. The PCR product was predicted to be 592 bp. Confirmation of the 592 bp insertion is reported in Figure 4.

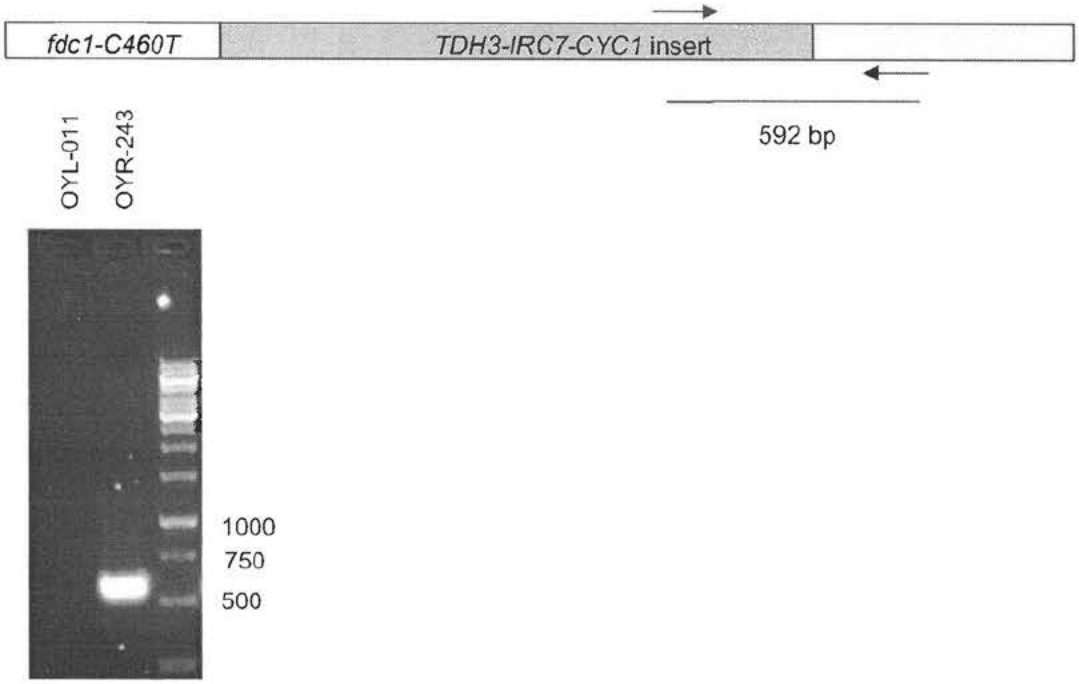


Figure 4: Colony PCR results confirming the targeted CRISPR-Cas9 editing to insert *TDH3-IRC7-CYC1* at *fdc1-C460T*

2.4.4. Absence of difference between genetic profiles of the transformed and the host strain

The genome of *S. cerevisiae* contains long terminal repeat sequences known as δ elements. These δ elements are the remnants of Ty1 transposon integration events. The number and location of these δ elements are specific to a strain and have been used to fingerprint and differentiate between strains of *S. cerevisiae*. Using PCR and primers δ12 (5'-TCAACAATGGAATCCCAAC-3') and δ21 (5'-CATCTTAACACCGTATATGA-3') to amplify these δ sequences, we observed identical PCR fingerprint and have verified the genetic relationship between the host strain OYL-011 and OYR-243 (Figure 5). This strategy is widely used to differentiate between industrial strains of *S. cerevisiae* (Legras and Karst, 2003). The interdelta PCR experiments were performed after serial passages YPD agar plates confirming the genome is stable. Furthermore, the expected genotype and phenotype of OYR-243 remains unchanged when propagated through the liquid yeast manufacturing process (Figure 6) representing greater than 30 cell divisions. The OYR-243 strain is not expected to be any more likely to undergo genomic rearrangement events within the normal environmental conditions of fermenting beer then the parent OYL-011 strain.

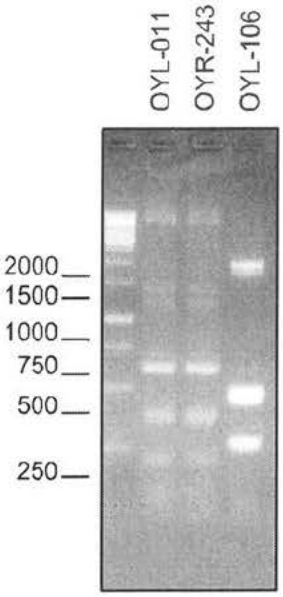


Figure 5: Interdelta PCR patterns of the OYL-011 and OYR-243 yeast strains

2.5. METHOD OF MANUFACTURE OF THE MODIFIED MICROORGANISM

Propagation of the OYR-243 does not require any additional selection or alterations to the propagation methods of the host strain OYL-011 or any other industrial brewing strains. The manufacturing of OYR-243 is identical to that of all liquid yeast used in brewing and is in accordance with cGMP. Packaged product is >98% viable on ship date, and free from detectable levels of contaminants (zero colony forming units (CFUs) of bacteria or yeast detected per 20 million yeast cells). We do not anticipate dangerous levels of heavy metals. A summary of the methods used in manufacturing of OYR-243 can be found in Figure 6.

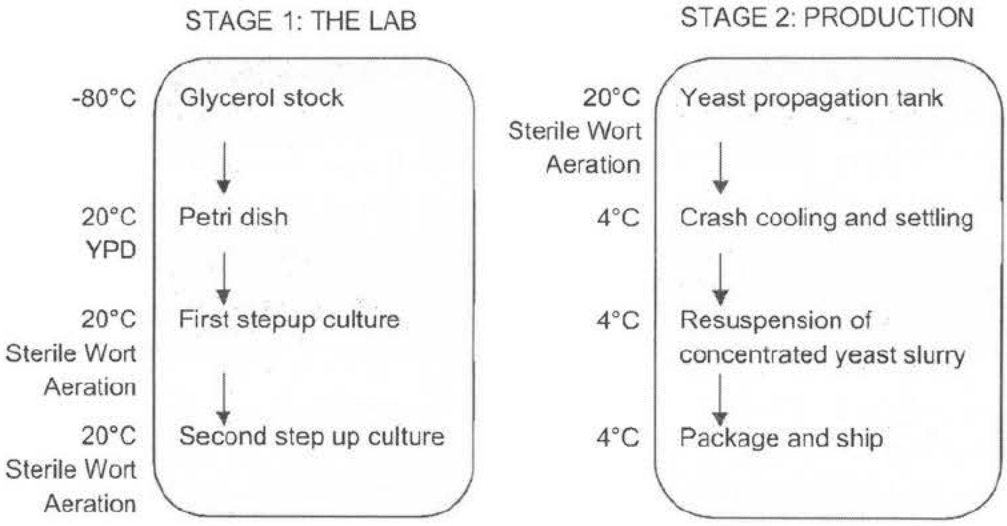


Figure 6: Flow diagram of liquid yeast manufacturing. Strains are started from glycerol stocks by streaking directly to YPD growth media plates. The YPD colonies are inoculated into the first step up culture. After reaching saturation, this culture is inoculated into the second step up culture. The second step up culture is

grown to saturation and brought to the production facility to inoculate the propagation tank. This large-scale propagation is the final stage. When the culture has depleted all fermentable sugar, the temperature is lowered to 4°C and the cells are separated through natural processes of flocculation and settling. The propagation media is decanted and the remaining yeast cell mass is resuspended as a concentrated liquid slurry. This concentrated yeast slurry is packaged and shipped to consumers.

2.5.2. Raw Materials

All raw materials are standard food grade ingredients used in the manufacturing of liquid brewing yeast. These ingredients are free of the 8 major food allergens specified under the Food Allergen Labeling and Consumer Protection Act.

2.5.3. Lab Stage

OYL-243 is inoculated onto nutrient agar plates from frozen stock cultures maintained at -80°C in glycerol. The yeast colonies are transferred with a sterile loop from the nutrient agar plate into sterilized flasks of propagation medium and grown with aeration at 20°C to saturation. These starter cultures are inoculated into larger sterilized flasks of propagation medium and grown to cell numbers that are sufficient for the inoculation of a yeast propagation tank.

2.5.4. Fermentation and Recovery

The yeast from the lab is inoculated into propagation tanks in production. The propagation is aerated and grown at 20°C. Once fermentation is completed, the temperature is lowered to 4°C for settling. The yeast slurry is resuspended to a slurry concentrate of 30% yeast solids (percent pellet weight to total volume). The slurry concentrate is transferred to sterile packaging and stored at 4°C until used to inoculate a beer fermentation.

To prevent contamination of foreign microorganisms, the propagation medium is sterilized and all equipment is carefully cleaned and sanitized. The propagation tanks are cleaned in place with acid and base and rinsed to neutral pH. The cleaning is validated with microbiological testing and ATP swabs. The tanks are then sanitized with food grade sanitizer prior to filling with sterile propagation medium. The propagations are sampled throughout for microbiological controls and the yeast percent solids is determined at the end of propagation to insure proper and consistent growth.

2.6. APPLICATION AND USE LEVELS

It is recommended that brewers use approximately 1 million cells of OYR-243 per milliliter of wort per degree Plato for brewing wort fermentation, as is standard industrial practice. At this inoculation level, alcoholic fermentation will be efficiently conducted and thiol compounds will be present in the range of nd-1500 ng/L, levels similar to heavily hopped beers produced with OYL-011 and other traditional brewing strains.

2.7. PRODUCT SPECIFICATIONS

The specifications for liquid yeast are measured on every batch and subsequently approved for release by Quality Control. The liquid yeast slurry is plated on microbiological growth mediums to detect contaminating bacteria and wild yeast. Any observation of growth results in the product being discarded. At the end of fermentation, cell growth is monitored by percent yeast solids and anything under 3% is discarded. The growth medium and liquid yeast product does not contain any of the 8 major food allergens specified under the Food Allergen Labeling and Consumer Protection Act. Product specifications are listed in the Table 1.

Table 1. Liquid Yeast Specifications

Parameter	Specification Range
Percent Yeast Solids	>3%
Total Viable Cells	>98%
Total Bacteria	<1 per 2 x 10^6 yeast cells
Total Wild Yeast	<1 per 2 x 10^6 yeast cells

If a liquid yeast propagation does not meet the Quality Control specifications, then the batch is rejected. Table 2 contains data for three batches of the OYR-243 liquid yeast.

Table 2. Data for three batches of OYR-243 liquid yeast

Parameter	Specification Range	4/12/2022	5/5/2022	5/9/2022
Percent Yeast Solids	>3%	5.40	5.17	5.09
Total Viable Cells	>98%	100%	100%	100%
Total Bacteria	<1 per 2 x 10^6 yeast cells	n.d.	n.d.	n.d.
Total Wild Yeast	<1 per 2 x 10^6 yeast cells	n.d.	n.d.	n.d.

n.d. not detected

§ 170.235 Part 3, Dietary Exposure

3.1. INTENDED USE OF OYR-243

Omega Yeast Labs, LLC proposes the use of OYR-243 in brewing to enhance hoppy aroma characteristics in beer during fermentation. Hops that exhibit elevated thiol levels are often desired for the tropical fruit flavors they impart. OYR-243 with enhanced Irc7 activity will release thiol compounds from precursor forms found in barley and hops. The resulting thiol levels will be similar to amounts found in heavily hopped beer styles or white wine varieties such as Sauvignon Blanc. OYR-243 is intended to be added at approximately 1 million cells per milliliter of wort per degree Plato for brewing wort fermentation, as is standard industrial practice.

3.2. ESTIMATED DIETARY EXPOSURE

3.2.1. History of consumption

Saccharomyces cerevisiae has been used in food production for thousands of years (Boekhout, 2014). Bread and alcoholic beverages fermented by *S. cerevisiae* have been at the heart of many ancient civilizations. The use of yeast in fermentation can be dated as far back as ancient Egypt. Today the industrial food and beverage applications of *S. cerevisiae* range from bread, alcoholic beverages, coffee, and chocolate to name a few. The manufacturing of *S. cerevisiae* is a global industry with millions of tons of industrial *S. cerevisiae* manufactured each year.

Thiol compounds including 3SH, 4MSP and 3S4MP are widely found in foods including hops, wine grapes and fruits. These compounds are highly aromatic and contribute to passion fruit, grapefruit and guava flavors in beer and wine. These thiol compounds are in the parts per billion (ug/kg) concentrations in tropical fruits (Cannon and Ho, 2018), the parts per billion (ug/L) in wines (Roland et al., 2011), and parts per billion in hops (ug/kg) (Roland et al., 2017). Hop addition rates up to 32 g/L are common in heavily dry hopped beer styles, resulting in parts per trillion (ng/L) to parts per billion (ug/L) amounts of these compounds in beer.

3.2.2. Estimated consumption

Beer produced with OYR-243 will contain comparable levels of alcohol and flavor metabolites as the host strain, with elevated levels of 3SH from the fermentation of wort in the absence of hops. In the presence of hops, the levels of thiols in beer brewed with OYL-243 are similar to those found in heavily hopped beer styles.

Exposure to the engineered OYR-243 will not differ from the exposure to industrial yeast used in other commercial beer applications. This yeast is flocculant and rapidly declines in viability at the end of fermentation. The settled, non-viable yeast is discarded at the completion of fermentation. The resulting beer contains trace levels of yeast (<3 million cells/ml) and any yeast remaining will have a limited viability and metabolic activity in the packaged beer (Boulton and Quain, 2001).

§ 170.240 Part 4, Self-Limiting Levels of Use

The use of the proposed yeast in brewing is considered to be self-limiting for technological reasons, such as product flavor profile, which could affect consumer acceptance.

§ 170.245 Part 5, Experience Based on Common Use in Food

While there exists an extensive history of the safe consumption of *S. cerevisiae* yeast and yeast products by both humans and animals, and *S. cerevisiae* products have FDA GRAS status, the statutory basis for our conclusion of its GRAS status in the notice is based on scientific procedures and not common use in food.

§ 170.250 Part 6, GRAS Narrative

6.1. SAFETY ASSESSMENT OF THE HOST STRAIN

The host strain, OYL-011 is an industrial brewing strain of *Saccharomyces cerevisiae*. The origin of OYL-011 is a British Brewery. OYL-011 is widely used in commercial beer production of traditional English beer styles and modern American hoppy beer styles.

S. cerevisiae is considered GRAS for use in brewing, baking and winemaking industries (21 CFR §172.896). The genome of *S. cerevisiae* has been extensively studied and it has been determined that the yeast is free of known pathogenicity traits. In addition, several genetically modified *S. cerevisiae* strains for use in fermented beverages have received letters of no objection from the US FDA (GRN 120, GRN 175, GRN 350, GRN 798 and GRN 841).

In the 27th report of The Scientific Committee for Human Food of the European Community the authors state that *Saccharomyces cerevisiae* has a safe history of use in food and belongs to a species that is known not to produce toxins. In addition, the Environment Protection Agency (EPA) has included *S. cerevisiae* as a recipient microorganism that is eligible for exemptions from EPA review under the Toxic Substances Control Act and/or expedited EPA review (40 CFR 725.420). The inclusion of *S. cerevisiae* in the list of recipient microorganisms that are eligible for exemption is based upon the demonstrated lack of adverse effects for this species. They also determined that the introduction of genetic material would not increase the potential for adverse effects, provided that the genetic material is limited in size, well-characterized, free of certain sequences and is poorly mobilizable.

According to the European Food Safety Agency (EFSA), yeasts used in food production, particularly baker's/brewer's yeast, are considered among the safest of microorganisms (EFSA, 2007; EFSA, 2013) *S. cerevisiae* has been designated Qualified Presumption as Safe (QPS) status in Europe, which indicates that no additional safety assessment is needed according to the established guidelines (EFSA, 2007; EFSA, 2008).

The OYL-011 host strain belongs to the *Saccharomyces cerevisiae* species which has been used for more than 7,000 years by humans in fermented beverages. It can therefore be concluded that species will remain GRAS, even after genetic transformation, as long as the transformation does not introduce new or changed capabilities for harmful effects.

6.2. SAFETY ASSESSMENT OF THE GENETIC MATERIAL USED TO CONSTRUCT THE MODIFIED ORGANISM

6.2.1. Foreign genetic material source and product

No foreign (non-*Saccharomyces*) genetic material exists in the OYR-243 yeast strain. The same basis of assessment applied in Section 6.1 would apply to this section.

6.2.2. Native genetic material source and product: The *TDH3-IRC7-CYC1* repair template

Source: *Saccharomyces cerevisiae*

The sequence of the *IRC7* allele that was introduced is naturally occurring in industrial brewing strains of *S. cerevisiae*, including the widely used Chico ale strain. The *TDH3* promoter and *CYC1* terminator sequences are native to *S. cerevisiae* and are well-characterized, commonly used non-coding regulatory elements.

Product: Irc7 beta-lyase

The amino acid sequence of Irc7 is identical to the sequence found in industrial brewing strains, and only differs in that it is expressed highly in beer fermentations. The native Irc7 enzyme is not expressed when nutrients such as nitrogen are in excess through a regulatory feedback system in yeast termed nitrogen catabolite repression (Thibon et al., 2008; Michel et al., 2019). The Irc7 beta lyase however is active in wine fermentations where nitrogen is limited (Harsch and Gardner, 2013; Howell et al., 2004; Swiegers and Pretorius, 2007; Tominaga et al., 1998; Roncoroni et al., 2011). The relative amounts of thiols measured in beer fermentations with enhanced Irc7 levels (500 ppt) are 10-fold lower than those found in wine fermentations (up to 5-10 ppb) and, thus, the resulting thiol levels are not unprecedentedly elevated (Cannon and Ho, 2018; Pinu et al., 2012). Furthermore, the *IRC7* allele present in the OYR-243 strain resulting from the genetic modification does not code for either toxic proteins, nor proteins implicated in the formation of undesirable compounds. A literature search performed on Jan 7th 2022 with the words "Irc7 AND toxin" and "Irc7 AND toxic" produced 1 result on pubmed.gov. The 1 result, described a genetic analysis of IRC7 overexpression resulting in yeast that were hypersensitive to a toxic analogue, but no evidence to toxicity of the protein itself (Santiago et al., 2015).

Given the reported activity of Irc7 in wine fermentations, enhancing the expression of Irc7 in beer fermentation can be reasonably considered as safe.

6.2.3. Construction of the modified organism

6.2.3.1. Vector and repair template

The *fdc1-C460T* CRISPR plasmid (Figure 1) was constructed using the gap repair subcloning procedure in *S. cerevisiae*. A parent yeast shuttling vector containing Cas9 regulated by *S. cerevisiae PGK1* promoter and terminator was digested with BsmBI. An *fdc1-C460T* sgRNA expression cassette containing the tRNA phe promoter, tRNA phe, *FDC1* sgRNA, and the tSRN52 sequences with homology to the linearized ends of the parent yeast shuttling vector was synthesized. The two linear fragments with overlapping homology were transformed into *S. cerevisiae* and repaired through homology-directed repair pathways.

A repair template for homology-directed repair of the induced double strand break was prepared by PCR from a vector containing the synthesized gBlock. The sequence of the repair template contains the *TDH3-IRC7-CYC1* sequence and homology to the 100 bp regions upstream and downstream of the *fdc1-C460T* CRISPR-Cas9 cut site (provided in Figure 2).

6.2.3.2. Transformation of the host strain

The *fdc1-C460T* CRISPR plasmid and *TDH3-IRC7-CYC1* repair template were the only DNA sequences used in the transformation method.

OYL-011 was transformed with *fdc1-C460T* CRISPR plasmid and *TDH3-IRC7-CYC1* repair template and plated onto YPD-G418 media to select for transformed cells. Single colonies were chosen and re-streaked to YPD-G418 media. These transformants were screened with PCR primers that anneal to the CYC1 promoter and a region of *fdc1-C460T* downstream of the homology used to direct homology repair. Thus, only successful repair events directed to the *fdc1-C460T* resulted in a 592 bp PCR product. The resulting OYR-243 was passaged in liquid YPD media, plated to single colonies and replica plated on YPD-G418 plates. Colonies that no longer displayed G418-resistance were re-streaked to YPD and YPD-G418 medias. The lack of G418-resistance confirmed the loss of the *fdc1-C460T* CRISPR plasmid (Figure 3).

The results show that the *fdc1-C460T* CRISPR plasmid containing the G418 selection marker is not present in OYR-243 and that the CRISPR-Cas9 editing event was specifically targeted to the *fdc1-C460T* locus.

6.3. SAFETY ASSESSMENT OF THE MODIFIED ORGANISM

The safety assessment evaluates the exposure to OYR-243, though the presence of OYR-243 in the final beer product is minimal and is further reviewed in section 6.4.2.3.

6.3.1. Characterization of the transformation event

The genome of *Saccharomyces cerevisiae* contains long terminal repeat sequences known as δ elements. These δ elements are the remnants of Ty1 transposon integration events. The number and location of these δ elements are specific to a strain and have been used to fingerprint and differentiate between strains of *S. cerevisiae*. Using PCR and primers δ12 (5'-TCAACAATGGAATCCCAAC-3') and δ21 (5'-CATCTTAACACCGTATATGA-3') to amplify these δ sequences, we have verified the genetic relationship between the host strain OYL-011 and OYR-243 (Figure 5).

Loss of the G418 antibiotic resistance marker was confirmed by streaking OYR-243 onto YPD-G418 media and confirming sensitivity. Figure 3 clearly shows that OYR-243 is sensitive to this antibiotic like the parental strain OYL-011.

Colony PCR of the resulting isolates was performed to confirm the insertion of *TDH3-IRC7-CYC1* at position 460 in *fdc1-C460T*. The presence of the 592 bp PCR fragment in OYR-243 and absence in the OYR-011 is shown in Figure 4.

6.3.2. Effect of the genetic modification on the physiology of the OYR-243

6.3.2.1. Overview of the function of the *IRC7* gene

IRC7 is a non-essential gene in *Saccharomyces cerevisiae*, encoding a beta-lyase enzyme responsible for the cleavage of carbon sulfur bonds as a mechanism for acquiring sulfur for amino acid biosynthesis. Irc7 has also been confirmed to release volatile thiol compounds from amino acid precursors (Cys-thiol, CysGly-thiol, GluCys-thiol, Glut-thiol) found in wine grapes, hops, malt and a variety of natural sources. In beer, *IRC7* is transcriptionally repressed through nitrogen catabolite repression (Harsch and Gardner, 2013), but in wine the release of varietal thiols in fermentation is linked to functional *IRC7* alleles. Irc7 is 400 amino acids long and 44 kDa in weight. The thiols released by *S. cerevisiae* by the activity of the β-lyase Irc7 produce grapefruit, passion fruit and guava flavors.

6.3.2.2. Growth and fermentation rates of OYL-011 and OYR-243 during laboratory-scale fermentations

Brewing trials with OYL-011 and OYR-243 showed similar fermentation rates, degree of fermentation, and terminal pH. Propagation trials with OYL-011 and OYR-243 resulted in similar peak cell densities. No growth advantages were observed in OYR-243 relative to the OYL-011 host strain. All data indicate that the only difference between OYL-011 and OYR-243 is the enhanced expression of *IRC7*.

Therefore, it can be concluded that the OYR-243 strain is substantially equivalent to the host strain OYL-011 except for the enhanced expression of *IRC7*.

6.3.2.3. Sensory and chemical analysis of beer brewed with OYR-243 and OYL-011

Fermentations were performed for sensory analysis of the OYR-243 and OYL-011 host strain. Wort was prepared from barley malt (15°P extract). Fermentations were carried out at 23°C for 14 days. Descriptive sensory analysis was performed by a panel of 6 trained tasters. All 6 tasters were able to discern between the OYR-243 and OYL-011 parent strain, indicating a significant difference. Sensory descriptors for the OYR-243 strain included passionfruit, guava and grapefruit. The samples were analyzed by Nyseos in Montpellier, France, an expert contract lab with validated LC-MS/MS methods for quantification of 3SH, 4MSP and 3S4MP. The level of 3SH reached 417 ng/L in the fermentation with OYL-243 relative to 16 ng/L in OYL-011 parent strain (Figure 7), though significantly less than the reported level for 3SH in wine (up to 5-10 ug/L).

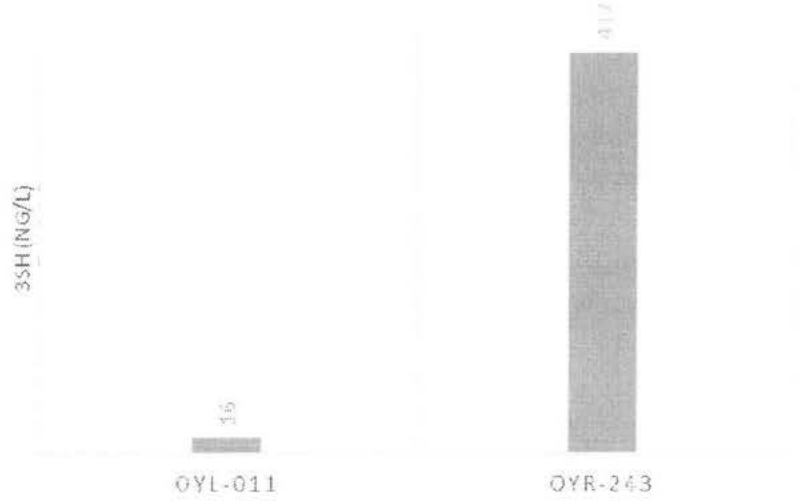


Figure 7: 3SH levels measured in the OYL-011 and OYL-243 fermentations.

A subset of tasters identified additional sulfur aromas of rotten-egg at a low odor threshold. Irc7 functions as a cysteine desulfhydrase (Santiago and Gardner, 2015), and thus the release of hydrogen disulfide was also detected at very low levels in beer brewed with OYR-243 relative to OYL-011. Hydrogen disulfide is a common off flavor described as rotten-egg in beer and wine due to slow/sluggish fermentation rates or inadequate nutrients. An identical beer recipe was brewed at commercial scale and split for fermentations with OYL-011 and OYL-243. The resulting beer was analyzed by ETS laboratories for sulfides by headspace gas chromotography mass spectrometry (HS-GC-MS). The results are displayed in Figure 8.

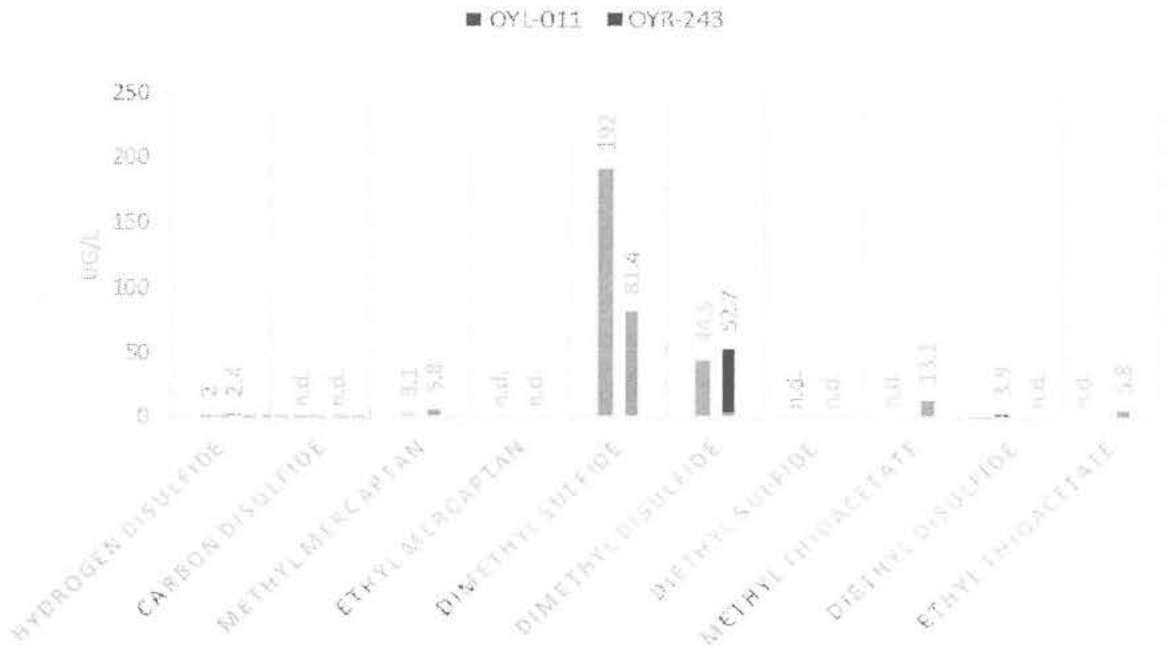


Figure 8: Sulfide Analysis by HS-GC/MS in OYL-011 and OYR-243 fermentations

Several sulfides varied between the two fermentations, but the amounts detected were within ranges reported in beer. The slightly elevated level of hydrogen sulfide (from 2.0 to 2.4 ug/L), methyl thioacetate (< 5 to 13.1 ug/L) and ethyl thioacetate (<5 to 5.8 ug/L) could be a result of enhanced cysteine desulfhydrase activity of Irc7. Though the odor threshold of these compounds are 4 ug/L, 85 ug/L and 10 ug/L, these compounds would be predicted to produce the slight difference in reductive odors observed between OYL-011 and OYR-243. Notably, the major difference between the OYR-243 and OYL-011 host strain was the targeted enhancement of passionfruit and guava aromas due to elevated levels of 3SH.

6.3.3. Allergenic/Toxigenic potential

The *IRC7* allele present in the OYR-243 strain resulting from the genetic modification does not code for either toxic or allergenic proteins, nor proteins implicated in the formation of undesirable compounds. A literature search performed with the words "Irc7 AND allergen", "Irc7 AND toxin" and "Irc7 AND toxic" produced 1 result on pubmed.gov. The 1 result, described a genetic analysis of IRC7 overexpression resulting in yeast that were hypersensitive to a toxic analogue, but no evidence to toxicity of the protein itself (Santiago et al., 2015). A bioinformatics comparison of Irc7 to known allergens was performed on January 7th, 2022 using a well-documented allergen database (AOL version 21, http://www.allergenonline.org/) with an 80mer sliding window. Of the 261- 80mers in Irc7, no matches were found with greater than 35% identity. A bioinformatics search for Irc7 protein was performed using NCBI blastp on January 7th, 2022. The criteria used for blastp were default parameters: BLOSUM92 scoring matrix, Word size 6, Expect value 10, hitlist 100, Gapcosts 11,1, window size 40, threshold 21. No sequence alignments with reported toxins were identified supporting the conclusion that the Irc7 protein has no known associated allergen or toxin risks.

These considerations lead us to conclude that allergenic or toxic risks related to the presence of this *IRC7* allele within the OYR-243 strain are negligible.

6.3.4. Presence of unintended gene products as a result of the transformation event

The *fdc1-C460T* allele encodes for a truncated fdc1 protein. The C to T mutation at position 460 of the FDC1 coding sequence replaces a glutamine residue (CAA) by a stop codon (UAA). This truncated fdc1 protein disrupts the dimerization domain and the catalytic pocket of the protein is not formed anymore. Industrial brewing strains with this naturally occurring *fdc1-C460T* allele would also express this truncated fdc1 protein (Mukai et al., 2014), however there are no known adverse effects. The insertion of the *TDH3-IRC7-CYC1* at this residue preserves the truncated fdc1 protein in the parent strain OYL-011. Therefore, we conclude that to the best of our knowledge, the presence of the truncated fdc1 protein poses no known-associated risk. The insertion sites and flanking regions were examined for potential open reading frames. A bioinformatics search for 10 potential open reading frames spanning the site of insertion (*fdc1-C460T*) and the inserted DNA (*TDH3-IRC7-CYC1*) was performed using NCBI blastp (http://www.ncbi.nlm.nih.gov/BLAST/) on January 7th, 2022. The criteria used for blastp were default parameters: BLOSUM92 scoring matrix, Word size 6, Expect value 10, hitlist 100, Gapcosts 11,1, window size 40, threshold 21. Blast results for each of the 10 open reading frames were manually evaluated for any

suggested toxicity and a search for the word toxin was performed on each list of subjects identified. No subjects of these blast searches were known toxins, leading us to conclude that the *TDH3-IRC7-CYC1* insertion does not raise any oral toxicity concerns.

6.4. SAFETY ASSESSMENT OF THE PRODUCT DERIVED FROM THE MODIFIED ORGANISM

6.4.1. Changes in brewing procedures as a result of OYR-243 fermentation

The use of the OYR-243 yeast strain leads to no significant changes in brewing procedures, as outlined in Section 6.3.2.3

6.4.2. Changes in beer composition as a consequence of OYR-243 fermentation

6.4.2.1. Global Characteristics of beer

The OYR-243 fermentation enhances the release of volatile thiols to levels that have been previously reported in heavily hopped beers. Sulfides were also detected, but at levels commonly observed in beer. Residual extract, pH, and additional sensory descriptors remain consistent to the OYL-011 host strain. Thus, OYR-243 fermentation will have limited impact on the global beer composition.

6.4.2.2. Flavor modification

Laboratory and pilot scale brewing trials showed that the use of OYR-243 beer yeast strain led to enhanced tropical aromas of passion fruit, guava, and grapefruit. Descriptive characters of the OYR-243 beer were similar to Sauvignon Blanc wines and beers brewed with hop varieties known to have elevated thiol levels (e.g., Nelson Sauvin, Citra and Simcoe).

6.4.2.3. Yeast cells and release of yeast products during brewing

After alcoholic fermentation, the viable yeast population starts to decrease in beer and yeast autolysis occurs. While many of the solid particles of the beer as well as the yeast have settled to the bottom of the fermented by this point, the majority of beers are also clarified by one or more of three processes: cooling (which leads to accelerated flocculation), centrifugation, and filtration.

Beer clarification is often performed by letting the solid particles of the beer sediment by gravity, followed by elimination of the sediments. This process is initiated by a "cold crash"—a drop in the temperature of the beer to 33-40 degrees Fahrenheit. Clarification depends on the flocculation ability of the yeast strain: a strong flocculator will result in clear beer, a weak flocculator will result in turbid beer. Both the parent strain OYL-011 and OYR-243 efficiently flocculate, thus the majority of the yeast is purified from the resulting finished beer.

Native and heterologous proteins may be liberated if cells are allowed to lyse. However,

clarification processes will remove the bulk of the proteins, as well as larger polypeptide fragments. Hence, only hydrolysis products such as smaller polypeptides and amino acids of these proteins will remain in the beer. The cellular content of an autolyzing yeast is rich in nucleases and phosphatases. As a result of yeast autolysis, nucleic material will normally be found in beer as single nucleic bases or small nucleic base chains.

6.4.2.4. Formation of unwanted substances in beer

S. cerevisiae is considered GRAS for use in brewing, baking and winemaking industries (21 CFR §172.896) and the introduced material has been reviewed for known toxin and allergen sequences. Moreover, to the best of our knowledge, the use of the OYR-243 strain in brewing will not lead to the release or the enhancement of undesirable compounds in beer.

6.5. BASIS FOR THE GRAS DETERMINATION Basis for the GRAS Determination

6.5.1. Introduction

The regulatory framework for determining whether a substance can be considered GRAS in accordance with section 201(s) (21 U.S.C. § 321(s)) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 301 et. Seq.) ("the Act"), is set forth at 21 CFR 170.30, which states:

> General recognition of safety may be based only on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.
>
> General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies, which may be corroborated by unpublished studies and other data and information.

These criteria are applied in the analysis below to determine whether the use of the modified *S. cerevisiae* yeast ingredient in brewing that is the subject of this GRAS determination is GRAS based on scientific procedures. All data relied upon in this GRAS determination are publicly available and generally known, and therefore meet the "general recognition" standard under the Federal Food, Drug, and Cosmetics Act.

6.5.2. Safety Determination

The proposed Omega Yeast Labs brewing ingredient (from *S. cerevisiae*) that is the subject of the current GRAS determination is proposed to be added at approximately 1 million cells per milliliter of wort per degree Plato for brewing wort fermentation, as is standard industrial practice. The proposed yeast ingredient is similar to many other yeast products using *S. cerevisiae* and all have a long history of safe consumption by humans as well as animals. Humans have consumed *S. cerevisiae* through the consumption of its fermentative products, which include bread, beer, and wine.

S. cerevisiae is widely used in industrial fermentation of many food products. It is in the air that we breathe, and it grows naturally on many foods that we eat regularly. It is likely that most humans are exposed daily to foods containing millions of live *S. cerevisiae* through consumption of wine, beer, fruits, salads, and cheese and other dairy foods, as well as other traditional and ethnic fermented foods. This yeast species has been used in food production for over five thousand years. Common industrial applications include baked goods, beer, wine, cider, sake, spirits, coffee and chocolate (Boekhout and Robert, 2014.

Brewer's yeast serves as a model organism for scientific research. Over 30 years of yeast research has contributed to our fundamental understanding of cell and molecular biology. The *S. cerevisiae* genome size is small, is highly homologous with the human genome, genetics are very tractable, and DNA repair pathways are robust allowing for ease of accurate genetic manipulation (Botstein and Fink, 2011). A long history of research and industrial use has indicated that *S. cerevisiae* is safe for use in food and beverage manufacturing (Boekhout and Robert, 2014).

There are over 150 *S. cerevisiae* strains that are commercially sold as brewing yeast. These strains have been isolated from the brewery and farmhouse origins, where the re-pitching or backslopping practices allowed for the propagation and selection of unique brewing characteristics (Gallone et al., 2016). The origin of the host strain, OYL-011, traces to a British Brewery. OYL-011 is a popular strain used by many US craft breweries for traditional English styles and modern American hoppy styles.

The FDA considers *S. cerevisiae* and several derived products safe for consumption. Indeed, FDA has approved dried yeast as an ingredient for food (21 C.F.R. §172.896), and Baker's yeast extract has been affirmed by the FDA as a GRAS flavoring agent and adjuvant (21 C.F.R. §184.1983). The FDA has also approved various yeast-derived products for their use in food. These include Baker's yeast protein (21 C.F.R. §172.325), Yeast-malt sprout extract (21 C.F.R. §172.590) and Baker's yeast glycan (21C.F.R. §172.898). *S. cerevisiae* is also considered Generally Recognized as Safe as noted in several GRAS Notices; GRNs 120, 175, 239, 260, 284, 350, 353.

S. cerevisiae has a long history of being considered non-pathogenic. Regulatory authorities have evaluated the safety of *S. cerevisiae* and consider the organism safe for use in food manufacturing. Several GRAS notifications for modified strains *of S. cerevisiae* used in winemaking have been sent to FDA, and the agency has responded that they had no further questions on the GRAS determinations. In summary,

regulatory authorities have reviewed the extensive safety database on *S. cerevisiae* and found no issues of concern with respect to its use in human food or human food production.

6.5.3. General recognition of the safety of a modified *Saccharomyces cerevisiae* for use in brewing

The intended use of the modified *S. cerevisiae* has been determined to be safe through scientific procedures, as set forth in 21 CFR § 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination, and this determination is based on the following:

- The Omega Yeast Labs modified yeast ingredient (OYR-243 strain) that is the subject of the current GRAS determination is derived from *S. cerevisiae*, also (also known as "bakers" or "brewers" yeast).

- The OYR-243 strain was developed by Omega Yeast Labs to provide a novel flavor profile to beer by enhancing the activity and expression of yeast β-lyase gene, *IRC7*. *S. cerevisiae* produces many aroma-active metabolites that contribute to the complexity of a beer's flavor including phenolics, esters, carbonyl compounds, higher alcohols, aldehydes and sulfur compounds. Some of these metabolites are highly sought after for tropical fruit flavors. This strain was modified with CRISPR-Cas9 introducing the insertion of an *IRC7* allele under the regulation of the *TDH3* promoter and *CYC1* terminator to promote expression in beer fermentations. This modification results in the insertion of only DNA derived from *S. cerevisiae*.

- The OYR-243 yeast strain is intended for use as a commercial liquid yeast culture for the production of fermented beverages in accordance with Good Manufacturing Practices (GMPs). This liquid yeast strain performs alcoholic fermentation in the same manner as its host strain and other traditional brewing strains. The enhanced Irc7 activity of OYR-243 provides a well-suited brewing yeast strain for American craft beers, hoppy beers and other "clean" beer styles.

- The proposed Omega Yeast Labs OYR-243 (from *S. cerevisiae*) is proposed to be added at approximately 1 million cells per milliliter of wort per degree Plato for brewing wort fermentation, as is standard industrial practice.

- Beer produced with OYR-243 will contain comparable levels of alcohol and flavor metabolites as the host strain, with elevated levels of 3SH from the fermentation of wort in the absence of hops. In the presence of hops, the levels of thiols in beer brewed with OYL-243 will be similar to those found in heavily hopped beer styles. Exposure to the engineered OYR-243 will not differ from the exposure to industrial yeast used in other commercial beer applications. This yeast is flocculant and rapidly declines in viability at the end of fermentation. The settled, non-viable yeast is discarded at the completion of fermentation. The resulting beer contains trace levels of yeast (<3 million cells/ml) and any yeast remaining will have a limited viability and metabolic activity in the packaged beer.

- The current US daily intake of yeast in the diet for the US population 2 years and older is as follows: the per-capita average daily intake for yeast from all foods was 1.3 g/day (2.9 g/day, 90th

percentile), and the per-user average daily intake was 1.5 g/day (3 g/day, 90th percentile) (FDA, 2021).

- *S. cerevisiae* has a long history of being considered non-pathogenic. It is used in many food manufacturing processes, including bread making, beer brewing, and grape fermentation for wine. The FDA, EPA, and EFSA have evaluated the safety of *S. cerevisiae* and consider the organism safe for use in food manufacturing. Several GRAS notifications for modified strains of *S. cerevisiae* used in winemaking have been sent to FDA, and the agency has responded that they had no further questions on the GRAS determinations.

- The body of publicly available scientific literature on the consumption and safety of *S. cerevisiae* is sufficient to support the safety and GRAS determination of the proposed yeast ingredient. Because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination.

Determination of the safety and GRAS status of OYR-243 that is the subject of this evaluation has been made by Omega Yeast Labs. Omega Yeast Labs has critically reviewed and evaluated the publicly available information summarized in this document and has concluded that the yeast ingredient, produced in a manner consistent with cGMP and meeting the specifications described herein, is safe under its intended conditions of use.

Omega Yeast Labs further concludes that use of OYR-243 in brewing described herein is GRAS based on scientific procedures, and that other experts qualified to assess the safety of food and food ingredients for human consumption would concur with these conclusions.

It is also Omega Yeast Labs' opinion that other qualified scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Omega Yeast Labs has concluded that OYR-243 is GRAS under the intended conditions of use on the basis of scientific procedures; and therefore, it is excluded from the definition of a food additive and may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

Omega Yeast Labs is not aware of any information that would be inconsistent with a finding that the proposed use of the yeast ingredient in brewing, meeting appropriate specifications, and used according to cGMP, is GRAS. Recent reviews of the scientific literature revealed no potential adverse health concerns.

§ 170.250 Part 7, Supporting Data and Information

The following references are all generally available, unless otherwise noted. Appendices 1 and 2 (*fdc1-C460T* CRISPR-Cas9 plasmid sequence and *TDH3-IRC7-CYC1* repair template) are not generally available but are attached for reference.

References

Boekhout T, Robert V. *Yeasts In Food*. London: Elsevier; 2014.

Botstein D, Fink GR. Yeast: an experimental organism for 21st Century biology. *Genetics*. 2011;189(3):695-704. doi:10.1534/genetics.111.130765

Boulton C, Quain D. *Brewing Yeast And Fermentation*. Oxford, UK: Blackwell Science Ltd.; 2001:244.

Cannon R, Ho C. Volatile sulfur compounds in tropical fruits. *J Food Drug Anal*. 2018;26(2):445-468. doi:10.1016/j.jfda.2018.01.014

Cordente A, Borneman A, Bartel C et al. Inactivating Mutations in Irc7p Are Common in Wine Yeasts, Attenuating Carbon-Sulfur β-Lyase Activity and Volatile Sulfur Compound Production. *Appl Environ Microbiol*. 2019;85(6). doi:10.1128/aem.02684-18

Curran K, Morse N, Markham K, Wagman A, Gupta A, Alper H. Short Synthetic Terminators for Improved Heterologous Gene Expression in Yeast. *ACS Synth Biol*. 2015;4(7):824-832. doi:10.1021/sb5003357

European Food Safety Agency (EFSA). (2007) Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA - Opinion of the Scientific Committee: Introduction of a Qualified Presumption of Safety (QPS approach for the assessment of selected microorganisms referred to EFSA. ESFA Journal, 5(12), 587. https://doi.org/10.2903/j.efsa.2007.587.

European Food Safety Agency (EFSA). (2008). The maintenance of the list of QPS microorganism intentionally added to food or feed - Scientific Opinion of the panel on Biological Hazards: The maintenance of the list of QPS microorganism intentionally added to food of feed - Scientific Opinion of the Panel on Biological Hazards. EGSA journal, 6(12), 923. https://doi.org/10.2903/j.efsa.2008.923.

European Food Safety Agency (EFSA) Panel on Biological Hazards (BIOHAZ). (2013). Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). ESFA Journal, 11(11). https://doi.org/10.2903/j.efsa.2014.3449

FDA. 2021. GRAS Notification: For the use of dried *Saccharomyces cerevisiae* yeast fermentate. Prepared by Cargill, Inc., May 14.

Gallone B, Steensels J, Prahl T et al. Domestication and Divergence of Saccharomyces cerevisiae Beer Yeasts. *Cell*. 2016;166(6):1397-1410.e16. doi:10.1016/j.cell.2016.08.020

Harsch M, Gardner R. Yeast genes involved in sulfur and nitrogen metabolism affect the production of volatile thiols from Sauvignon Blanc musts. *Appl Microbiol Biotechnol*. 2013;97(1):223-235. doi:10.1007/s00253-012-4198-6

Howell K, Swiegers J, Elsey G et al. Variation in 4-mercapto-4-methyl-pentan-2-one release by Saccharomyces cerevisiae commercial wine strains. *FEMS Microbiol Lett*. 2004;240(2):125-129. doi:10.1016/j.femsle.2004.09.022

Kawai S, Hashimoto W, Murata K. Transformation of *Saccharomyces cerevisiae* and other fungi. *Bioeng Bugs*. 2010;1(6):395-403. doi:10.4161/bbug.1.6.13257

Legras, J. L.; Karst, F., Optimisation of interdelta analysis for Saccharomyces cerevisiae strain characterisation. *FEMS Microbiol Lett* 2003, 221 (2), 249-55.

Mans R, van Rossum H, Wijsman M et al. CRISPR/Cas9: a molecular Swiss army knife for simultaneous introduction of multiple genetic modifications in *Saccharomyces cerevisiae*. *FEMS Yeast Res*. 2015;15(2). doi:10.1093/femsyr/fov004

Michel, Maximilian & Haslbeck, Korbinian & Ampenberger, Friedrich & Meier-Dörnberg, Tim & Stretz, Dominique & Hutzler, Mathias & Coelhan, Mehmet & Jacob, Fritz & Liu, Yang. (2019). Screening of brewing yeast β-lyase activity and release of hop volatile thiols from precursors during fermentation. BrewingScience. 72:179-186. 10.23763/BrSc19-26michel.

Mukai, N.; Masaki, K.; Fujii, T.; Iefuji, H., Single nucleotide polymorphisms of PAD1 and FDC1 show a positive relationship with ferulic acid decarboxylation ability among industrial yeasts used in alcoholic beverage production. *J Biosci Bioeng* 2014, 118 (1), 50-5.

Partow S, Siewers V, Bjorn S, Nielsen J, Maury J. Characterization of different promoters for designing a new expression vector in *Saccharomyces cerevisiae*. *Yeast*. 2010;27:955–64

Pinu F, Jouanneau S, Nicolau L, Gardner R, Villas-Boas S. Concentrations of the Volatile Thiol 3-Mercaptohexanol in Sauvignon blanc Wines: No Correlation with Juice Precursors. *Am J Enol Vitic*. 2012;63(3):407-412. doi:10.5344/ajev.2012.11126

Roland A, Schneider R, Razungles A, Cavelier F. Varietal Thiols in Wine: Discovery, Analysis and Applications. *Chem Rev*. 2011;111(11):7355-7376. doi:10.1021/cr100205b

Roland A, Delpech S, Dagan L. A Powerful Analytical Indicator to Drive Varietal Thiols Release in Beers: The Thiol Potency. *BrewingScience* 2017; 11(12), 170-175.

Roncoroni M, Santiago M, Hooks D et al. The yeast IRC7 gene encodes a β-lyase responsible for production of the varietal thiol 4-mercapto-4-methylpentan-2-one in wine. *Food Microbiol.* 2011;28(5):926-935. doi:10.1016/j.fm.2011.01.002

Santiago M, Gardner RC. The *IRC7* gene encodes cysteine desulphydrase activity and confers on yeast the ability to grow on cysteine as a nitrogen source. *Yeast.* 2015 Jul;32(7):519-32. doi: 10.1002/yea.3076.

Stewart, Graham. Brewing and Distilling Yeasts. Springer; 2017. 10.1007/978-3-319-69126-8.

Swiegers J, Pretorius I. Modulation of volatile sulfur compounds by wine yeast. *Appl Microbiol Biotechnol.* 2007;74(5):954-960. doi:10.1007/s00253-006-0828-1

Thibon C, Marullo P, Claisse O, Cullin C, Dubourdieu D, Tominaga T. Nitrogen catabolic repression controls the release of volatile thiols by Saccharomyces cerevisiae during wine fermentation. *FEMS Yeast Res.* 2008;8(7):1076-1086. doi:10.1111/j.1567-1364.2008.00381.

Tominaga T, Furrer A, Henry R, Dubourdieu D. Identification of new volatile thiols in the aroma of Vitis vinifera L. var. Sauvignon blanc wines. *Flavour Fragr J.* 1998;13(3):159-162. doi:10.1002/(sici)1099-1026(199805/06)13:3<159::aid-ffj709>3.0.co;2-7

APPENDIX 1

fdc1-C460T CRISPR-Cas9 plasmid

>

GGTGAGTAAGGAAAGAGTGAGGAACTATCGCATACCTGCATTTAAAGATGCCGATTTGGGCGCGAATCCTTTATTTTGGCTTCACC
CTCATACTATTATCAGGGCCAGAAAAAGGAAGTGTTTCCCTCCTTCTTGAATTGATGTTACCCTCATAAAGCACGTGGCCTCTTATC
GAGAAAGAAATTACCGTCGCTCGTGATTTGTTTGCAAAAAGAACAAAACTGAAAAAACCCAGACACGCTCGACTTCCTGTCATCCT
ATTGATTGCAGCTTCCAATTTCGTCACACAACAAGGTCCTAGCGACGGCTCACAGGTTTTGTAACAAGCAATCGAAGGTTCTGGAA
TGGCGGGAAAGGGTTTAGTACCACATGCTATGATGCCCACTGTGATCTCCAGAGCAAAGTTCGTTCGATCGTACTGTTACTCTCTCT
CTTTCAAACAGAATTGTCCGAATCGTGTGACAACAACAGCCTGTTCTCACACACTCTTTTCTTCTAACCAAGGGGGTGGTTTAGTTT
AGTAGAACCTCGTGAAACTTACATTTACATATATATAAACTTGCATAAATTGGTCAATGCAAGAAATACATATTTGGTCTTTTCTAAT
TCGTAGTTTTTCAAGTTCTTAGATGCTTTCTTTTTCTCTTTTTTACAGATCATCAAGGAAGTAATTATCTACTTTTTACAACAAATATA
AAACAAGATCTATGGACAAGAAGTATTCTATCGGACTGGATATCGGGACTAATAGCGTCGGGTGGGCCGTGATCACTGACGAGTA
CAAGGTGCCCTCTAAGAAGTTCAAGGTGCTCGGGAACACCGACCGGCATTCCATCAAGAAAAATCTGATCGGAGCTCTCCTCTTTG
ATTCAGGGGAAACCGCTGAAGCAACCCGCCTCAAGCGGACTGCTAGACGGCGGTACACCAGGAGGAAGAACCGGATTTGTTACC
TTCAAGAGATATTCTCCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCATAGGCTGGAAGAATCATTCCTCGTGGAAGAGGA
TAAGAAGCATGAACGGCATCCCATCTTCGGTAATATCGTCGACGAGGTGGCCTATCACGAGAAATACCCAACCATCTACCATCTTC
GCAAAAAGCTGGTGGACTCAACCGACAAGGCAGACCTCCGGCTTATCTACCTGGCCCTGGCCCACATGATCAAGTTCAGAGGCCA
CTTCCTGATCGAGGGCGACCTCAATCCTGACAATAGCGATGTGGATAAACTGTTCATCCAGCTGGTGCAGACTTACAACCAGCTCT
TTGAAGAGAACCCCATCAATGCAAGCGGAGTCGATGCCAAGGCCATTCTGTCAGCCCGGCTGTCAAAGAGCCGCAGACTTGAGAA
TCTTATCGCTCAGCTGCCGGGTGAAAAGAAAAATGGACTGTTCGGGAACCTGATTGCTCTTTCACTTGGGCTGACTCCCAATTTCAA
GTCTAATTTCGACCTGGCAGAGGATGCCAAGCTGCAACTGTCCAAGGACACCTATGATGACGATCTCGACAACCTCCTGGCCCAGA
TCGGTGACCAATACGCCGACCTTTTCCTTGCTGCTAAGAATCTTTCTGACGCCATCCTGCTGTCTGACATTCTCCGCGTGAACACTG
AAATCACCAAGGCCCCTCTTTCAGCTTCAATGATTAAGCGGTATGATGAGCACCACCAGGACCTGACCCTGCTTAAGGCACTCGTC
CGGCAGCAGCTTCCGGAGAAGTACAAGGAAATCTTCTTTGACCAGTCAAAGAATGGATACGCCGGCTACATCGACGGAGGTGCCT
CCCAAGAGGAATTTTATAAGTTTATCAAACCTATCCTTGAGAAGATGGACGGCACCGAAGAGCTCCTCGTGAAACTGAATCGGGA
GGATCTGCTGCGGAAGCAGCGCACTTTCGACAATGGGAGCATTCCCCACCAGATCCATCTTGGGGAGCTTCACGCCATCCTTCGGC
GCCAAGAGGACTTCTACCCCTTTCTTAAGGACAACAGGGAGAAGATTGAGAAAATTCTCACTTTCCGCATCCCCTACTACGTGGGA
CCCCTCGCCAGAGGAAATAGCCGGTTTGCTTGGATGACCAGAAAGTCAGAAGAAACTATCACTCCCTGGAACTTCGAAGAGGTGG
TGGACAAGGGAGCCAGCGCTCAGTCATTCATCGAACGGATGACTAACTTCGATAAGAACCTCCCCAATGAGAAGGTCCTGCCGAA
ACATTCCCTGCTCTACGAGTACTTTACCGTGTACAACGAGCTGACCAAGGTGAAATATGTCACCGAAGGGATGAGGAAGCCCGCA
TTCCTGTCAGGCGAACAAAAGAAGGCAATTGTGGACCTTCTGTTCAAGACCAATAGAAAGGTGACCGTGAAGCAGCTGAAGGAG
GACTATTTCAAGAAAATTGAATGCTTCGACTCTGTGGAGATTAGCGGGGTCGAAGATCGGTTCAACGCAAGCCTGGGTACTTACC
ATGATCTGCTTAAGATCATCAAGGACAAGGATTTTCTGGACAATGAGGAGAACGAGGACATCCTTGAGGACATTGTCCTGACTCTC
ACTCTGTTCGAGGACCGGGAAATGATCGAGGAGAGGCTTAAGACCTACGCCCATCTGTTCGACGATAAAGTGATGAAGCAACTTA
AACGGAGAAGATATACCGGATGGGGACGCCTTAGCCGCAAACTCATCAACGGAATCCGGGACAAACAGAGCGGAAAGACCATTC
TTGATTTCCTTAAGAGCGACGGATTCGCTAATCGCAACTTCATGCAACTTATCCATGATGATTCCCTGACCTTTAAGGAGGACATCC
AGAAGGCCCAAGTGTCTGGACAAGGTGACTCACTGCACGAGCATATCGCAAATCTGGCTGGTTCACCCGCTATTAAGAAGGGTAT
TCTCCAGACCGTGAAAGTCGTGGACGAGCTGGTCAAGGTGATGGGTCGCCATAAACCAGAGAACATTGTCATCGAGATGGCCAG
GGAAAACCAGACTACCCAGAAGGGACAGAAGAACAGCAGGGAGCGGATGAAAAGAATTGAGGAAGGGATTAAGGAGCTCGGG
TCACAGATCCTTAAAGAGCACCCGGTGGAAAACACCCAGCTTCAGAATGAGAAGCTCTATCTGTACTACCTTCAAAATGGACGCGA
TATGTATGTGGACCAAGAGCTTGATATCAACAGGCTCTCAGACTACGACGTGGACCATATCGTCCCTCAGAGCTTCCTCAAAGACG
ACTCAATTGACAATAAGGTGCTGACTCGCTCAGACAAGAACCGGGGAAAGTCAGATAACGTGCCCTCAGAGGAAGTCGTGAAAA
AGATGAAGAACTATTGGCGCCAGCTTCTGAACGCAAAGCTGATCACTCAGCGGAAGTTCGACAATCTCACTAAGGCTGAGAGGGG
CGGACTGAGCGAACTGGACAAAGCAGGATTCATTAAACGGCAACTTGTGGAGACTCGGCAGATTACTAAACATGTCGCCCAAATC
CTTGACTCACGCATGAATACCAAGTACGACGAAAACGACAAACTTATCCGCGAGGTGAAGGTGATTACCCTGAAGTCCAAGCTGG
TCAGCGATTTCAGAAAGGACTTTCAATTCTACAAAGTGCGGGAGATCAATAACTATCATCATGCTCATGACGCATATCTGAATGCC

GTGGTGGGAACCGCCCTGATCAAGAAGTACCCAAAGCTGGAAAGCGAGTTCGTGTACGGAGACTACAAGGTCTACGACGTGCGC
AAGATGATTGCCAAATCTGAGCAGGAGATCGGAAAGGCCACCGCAAAGTACTTCTTCTACAGCAACATCATGAATTTCTTCAAGAC
CGAAATCACCCTTGCAAACGGTGAGATCCGGAAGAGGCCGCTCATCGAGACTAATGGGGAGACTGGCGAAATCGTGTGGGACAA
GGGCAGAGATTTCGCTACCGTGCGCAAAGTGCTTTCTATGCCTCAAGTGAACATCGTGAAGAAAACCGAGGTGCAAACCGGAGGC
TTTTCTAAGGAATCAATCCTCCCCAAGCGCAACTCCGACAAGCTCATTGCAAGGAAGAAGGATTGGGACCCTAAGAAGTACGGCG
GATTCGATTCACCAACTGTGGCTTATTCTGTCCTGGTCGTGGCTAAGGTGGAAAAAGGAAAGTCTAAGAAGCTCAAGAGCGTGAA
GGAACTGCTGGGTATCACCATTATGGAGCGCAGCTCCTTCGAGAAGAACCCAATTGACTTTCTCGAAGCCAAAGGTTACAAGGAA
GTCAAGAAGGACCTTATCATCAAGCTCCCAAAGTATAGCCTGTTCGAACTGGAGAATGGGCGGAAGCGGATGCTCGCCTCCGCTG
GCGAACTTCAGAAGGGTAATGAGCTGGCTCTCCCCTCCAAGTACGTGAATTTCCTCTACCTTGCAAGCCATTACGAGAAGCTGAAG
GGGAGCCCCGAGGACAACGAGCAAAAGCAACTGTTTGTGGAGCAGCATAAGCATTATCTGGACGAGATCATTGAGCAGATTTCC
GAGTTTTCTAAACGCGTCATTCTCGCTGATGCCAACCTCGATAAAGTCCTTAGCGCATACAATAAGCACAGAGACAAACCAATTCG
GGAGCAGGCTGAGAATATCATCCACCTGTTCACCCTCACCAATCTTGGTGCCCCTGCCGCATTCAAGTACTTCGACACCACCATCGA
CCGGAAACGCTATACCTCCACCAAAGAAGTGCTGGACGCCACCCTCATCCACCAGAGCATCACCGGACTTTACGAAACTCGGATTG
ACCTCTCACAGCTCGGAGGGGATGAGGGAGCTGATCCAAAAAAGAAGAGAAAGGTAGATCCAAAAAAGAAGAGAAAGGTATAA
GGATCCTAACTCGAGATTGAATTGAATTGAAATCGATAGATCAATTTTTTTCTTTTCTCTTTCCCCATCCTTTACGCTAAAATAATAGT
TTATTTTATTTTTTGAATATTTTTTATTTATATACGTATATATAGACTATTATTTATCTTTTAATGATTATTAAGATTTTTATTAAAAAAA
AATTCGCTCCTCTTTTAATGCCTTTATGCAGTTTTTTTTTCCCATTCGATATTTCTATGTGCTGCCAAGATATCAACAACAGAGGACAT
ATGCCCTACCTCCATGATCTACTGACGTCCCTGAGGCTGCAATTCATGTAATGGGGCAGTATCCGCGGCAAGTCCTAGTGCAATGG
CGGTTTTTTACCCTCGTTCTGAAGAAGAGGCGACGCGGGTGCGGTCATCACTAATGTGGAAATTGGTAAGACTCTCGGGCCTCCG
CCTTTAGGCGGTGCTTACTCTTTCATAAAGGGGCTGTTAGTTATGCAACGATGTGCTTCAGTATTACATTTTTTGCCTTCAACGCCTT
GATTGTTCTATTTTTGCTAATAATAAATCTATTTCATCGGACTAAAAGTCCATTAGTTGTAAGCGGATTTAGCTCAGTTGGGAGAGC
GCCAGACTGAAGAAAAACTTCGGTCAAGTCATCTGGAGGTCCTGTGTTCGATCCACAGAATTCGCAGATGGCCGGCATGGTCCCA
GCCTCCTCGCTGGCGCCGGCTGGGCAACACCTTCGGGTGGCGAATGGGACTTTGGCAAGTACTTATAAACGTAGTTTTAGAGCTA
GAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTATTTTTTGTCACTATT
GTTATGTAAAATGCCACCTCTGACAGTATGGAACGCAAACTTCTGTCTAGTGGATAGCTGGACATTCCAGGCAGTGCCTCTGCCGC
CGGACCCCTCTCGTGATTGGGTAGCTGGACATGCCCTTGTAAGATATAACAAGAGCCTGCCTGTCTAATGATCTCACGGCGAAAGT
CGGGGAGACAGCAGCGGCTGCAGACATTATACCGCAACAACACTAAGGTGAGATAACTCCGTACATTGACTACGCGTTCCTCTAG
ACCTTACTTGACCGGATACAGTGTCTTTGACACGTTTATGGGTTACAGCAATCACATCCAAGACTGGCTATGCACGAAGCAACTCTT
GAGTGTTAAAATGTTGACCCCTGTATTTGGGATGCGGGTAGTAGATGAGTGCAGGATATCAGCAGTTACAGAGATGTTACGAACC
ACTAGTGCACTGCAGTACAGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGGCCCAGAATACCCTCCTTG
ACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAGCCCATACATCCCCATGTATAATCATTTGCAT
CCATACATTTTGATGGCCGCACGGCGCGAAGCAAAAATTACGGCTCCTCGCTCCAGACCTGCGAGCAGGGAAACGCTCCCCTCAC
AGACGCGTTGAATTGTCCCCACGCCGCGCCCCTGTAGAGAAATATAAAAGGTTAGGATTTGCCACTGAGGTTCTTCTTTCATATACT
TCCTTTTAAAATCTTGCTAGGATACAGTTCTCACATCACATCCGAACATAAACAAAAATGGGTAAGGAAAAGACTCACGTTTCGAG
GCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCT
ATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGAT
GGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCAC
CACTGCGATCCCCGGCAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGT
TCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTGGCTCAGGCGCAATCACGAA
TGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAA
GCTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGT
TGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTC
ATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTC
TAAAGTAACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTAATCAA
ATGTTAGCGTGATTTATATTTTTTTTCGCCTCGACATCATCTGCCCAGATGCGAAGTTAAGTGCGCAGAAAGTAATATCATGCGTCA
ATCGTATGTGAATGCTGGTCGCTATACTGGAGTAACGAAGCATCTGTGCTTCATTTTGTAGAACAAAAATGCAACGCGAGAGCGCT
AATTTTTCAAACAAAGAATCTGAGCTGCATTTTTACAGAACAGAAATGCAACGCGAAAGCGCTATTTTACCAACGAAGAATCTGTG

CTTCATTTTTGTAAAACAAAAATGCAACGCGAGAGCGCTAATTTTTCAAACAAAGAATCTGAGCTGCATTTTTACAGAACAGAAAT
GCAACGCGAGAGCGCTATTTTACCAACAAAGAATCTATACTTCTTTTTTGTTCTACAAAAATGCATCCCGAGAGCGCTATTTTTCTAA
CAAAGCATCTTAGATTACTTTTTTTCTCCTTTGTGCGCTCTATAATGCAGTCTCTTGATAACTTTTTGCACTGTAGGTCCGTTAAGGTT
AGAAGAAGGCTACTTTGGTGTCTATTTTCTCTTCCATAAAAAAAGCCTGACTCCACTTCCCGCGTTTACTGATTACTAGCGAAGCTG
CGGGTGCATTTTTTCAAGATAAAGGCATCCCCGATTATATTCTATACCGATGTGGATTGCGCATACTTTGTGAACAGAAAGTGATA
GCGTTGATGATTCTTCATTGGTCAGAAAATTATGAACGGTTTCTTCTATTTTGTCTCTATATACTACGTATAGGAAATGTTTACATTT
TCGTATTGTTTTCGATTCACTCTATGAATAGTTCTTACTACAATTTTTTTGTCTAAAGAGTAATACTAGAGATAAACATAAAAAATGT
AGAGGTCGAGTTTAGATGCAAGTTCAAGGAGCGAAAGGTGGATGGGTAGGTTATATAGGGATATAGCACAGAGATATATAGCAA
AGAGATACTTTTGAGCAATGTTTGTGGAAGCGGTATTCGCAATATTTTAGTAGCTCGTTACAGTCCGGTGCGTTTTTGGTTTTTTGA
AAGTGCGTCATCAGAGCGCTTTTGGTTTTCAAAAGCGCTCTGAAGTTCCTATACTTTCTAGCTAGAGAATAGGAACTTCCCGAGCG
GCCGCGTGTTACAACCAATTAACCAATTCTGATTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTA
TCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTA
TCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACC
ATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGCTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGT
CATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCGAGGCGAAATACGCGATCGCTGTTAAAAGGA
CAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTC
TAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTC
GGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGA
AACAACTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCA
TATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTGGAGCAAGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTA
CTGTTTATGTAAGCAGACAGTTTTATTGTTCATGATGATATATTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACACAACGT
GGCTTTGTTGAATAAATCGAACTTTTGCTGAGTTGAAGGATCAGTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGC
GTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACC
GCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAA
ATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTT
ACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCG
GGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGA
GAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGG
AGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGT
CAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTC
TTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTGCGGCCGCCCCTGAATTCGCATCTAGACTGAACTGGCCGATAATTGCAGAC
GAAC

APPENDIX 2

TDH3-IRC7-CYC1 repair template

>

CCTGTGTCATCTGCACCTTGTAAAACACATATACTTTCTGAAGAAAAAATACATCTACAAAGCCTGCCAACACCATATCTACATGTTT
CAGACGGTGGCAAGTACTTATCATTATCAATACTCGCCATTTCAAAGAATACGTAAATAATTAATAGTAGTGATTTTCCTAACTTTAT
TTAGTCAAAAAATTAGCCTTTTAATTCTGCTGTAACCCGTACATGCCCAAAATAGGGGGCGGGTTACACAGAATATATAACATCGT
AGGTGTCTGGGTGAACAGTTTATTCCTGGCATCCACTAAATATAATGGAGCCCGCTTTTTAAGCTGGCATCCAGAAAAAAAAAGAA
TCCCAGCACCAAAATATTGTTTTCTTCACCAACCATCAGTTCATAGGTCCATTCTCTTAGCGCAACTACAGAGAACAGGGGCACAAA
CAGGCAAAAAACGGGCACAACCTCAATGGAGTGATGCAACCTGCCTGGAGTAAATGATGACACAAGGCAATTGACCCACGCATGT
ATCTATCTCATTTTCTTACACCTTCTATTACCTTCTGCTCTCTCTGATTTGGAAAAAGCTGAAAAAAAAGGTTGAAACCAGTTCCCTG
AAATTATTCCCCTACTTGACTAATAAGTATATAAAGACGGTAGGTATTGATTGTAATTCTGTAAATCTATTTCTTAAACTTCTTAAAT
TCTACTTTTATAGTTAGTCTTTTTTTTAGTTTTAAAACACCAGAACTTAGTTTCGATGATTGATCGTACCGAGTTATCGAAGTTTGGT
ATTACTACGCAACTGTCTGTTATTGGACGTAATCCAGATGAACAAAGTGGCTTTGTTAATCCACCTTTGTATAAGGGGTCAACCATC
ATTCTTAAAAAACTTAGTGATTTAGAACAAAGGAAAGGAAGATTTTACGGGACAGCAGGTTCTCCAACTATTGACAATTTAGAAAA
TGCCTGGACGCATTTAACCGGCGGCGCTGGGACAGTGCTATCAGCTTCTGGGCTTGGTTCTATCTCTTTGGCGCTATTGGCCCTTTC
GAAAGCTGGTGATCATATCTTGATGACTGATAGTGTCTACGTGCCAACACGTATGCTATGTGATGGTTTATTGGCCAAGTTCGGTG
TTGAAACGGATTATTATGACCCATCAATAGGGAAGGATATAGAAAAACTAGTTAAGCCAAATACAACCGTCATTTTCCTCGAAAGC
CCGGGTTCTGGGACCATGGAAGTACAGGATATTCCAGCTTTGGTCTCTGTTGCCAAAAAGCATGGGATAAAGACAATTCTAGACA
ACACATGGGCAACGCCACTCTTTTTTGATGCTCATGCGCATGGTATCGATATTTCGGTAGAAGCTGGGACAAAATATTTGGGTGGT
CATTCAGATCTTCTTATTGGTCTGGCCTCCGCAAATGAAGAATGTTGGCCGCTATTACGGTCAACTTATGATGCAATGGCAATGTTA
CCAGGTGCCGAGGACTGTCAATTAGCATTGCGAGGAATGCGTACATTGCACTTAAGATTGAAAGAGGTAGAAAGAAAAGCCCTG
GATTTGGCTGCTTGGCTCGGAAATCGAGATGAGGTTGAAAAAGTGCTTCACCCCGCCTTTGAAGATTGTCCCGGACATGAATACTG
GGTTCGTGACTACAAAGGTTCTTCAGGCTTATTTTCCATTGTCCTTAAAAATGGGTTCACAAGAGCTGGTCTGGAGAAAATGGTAG
AAGGGATGAAAGTTTTGCAATTGGGATTTTCATGGGGTGGCTACGACTCCTTGATTACCCCTTTAAATCCTTGTAATAATAGAAAA
GCTTCAACATGGCCTTACAAAGGTTTTGCACTAAGAATACAAGTGGGTCTCGAAGAATTTGAAGATTTAAAAAGGGATTTTGAGTT
AGGCTTTGAACGTCTCGCAGAAGAAATTTCTTTGAATCCTTTACAAATCTGATCATGTAATTAGTTATGTCACGCTTACATTCACGCC
CTCCCCCCACATCCGCTCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTTATGTTAG
TATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTT
GAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCAAACGTACGGAATGTGGATTCTTCAAACTCCAGATAAAAAATGGACTAA
TTGGTCAATTGCTAGAGGTATGGTTGTAGATGACAAGCATATCACTGGTCTGG

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 07/31/2022
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*, OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3. Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: _____

4. For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier	Name of Contact Person Lance Shaner	Position or Title Owner		
	Organization *(if applicable)* Omega Yeast Labs, LLC			
	Mailing Address *(number and street)* 4720 W Pensacola Ave			
City Chicago	State or Province Illinois	Zip Code/Postal Code 60641	Country United States of America	
Telephone Number (773) 657-3438	Fax Number	E-Mail Address lance@omegayeast.com		

1b. Agent or Attorney *(if applicable)*	Name of Contact Person	Position or Title		
	Organization *(if applicable)*			
	Mailing Address *(number and street)*			
City	State or Province	Zip Code/Postal Code	Country	
Telephone Number	Fax Number	E-Mail Address		

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Saccharomyces cerevisiae strain OYR-243

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☐ Electronic files on physical media

☒ Paper

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes 1

Total number of pages 41

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8) and 170.250(d) and (e))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

Omega Yeast Labs, LLC proposes the use of OYR-243 in brewing to enhance hoppy aroma characteristics in beer during fermentation. Hops that exhibit elevated thiol levels are often desired for the tropical fruit flavors they impart. OYR-243 with enhanced Irc7 activity will release thiol compounds from precursor forms found in barley and hops. The resulting thiol levels will be similar to amounts found in heavily hopped beer styles or white wine varieties such as Sauvignon Blanc. OYR-243 is intended to be added at approximately 1 million cells per milliliter of wort per degree Plato for brewing wort fermentation, as is standard industrial practice.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

Did you include this other information in the form of electronics?

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Omega Yeast Labs, LLC
(name of notifier)

has concluded that the intended use(s) of Saccharomyces cerevisiae strain OYR-243
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Omega Yeast Labs, LLC agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

4720 W Pensacola Ave., Chicago, IL 60641
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance. The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

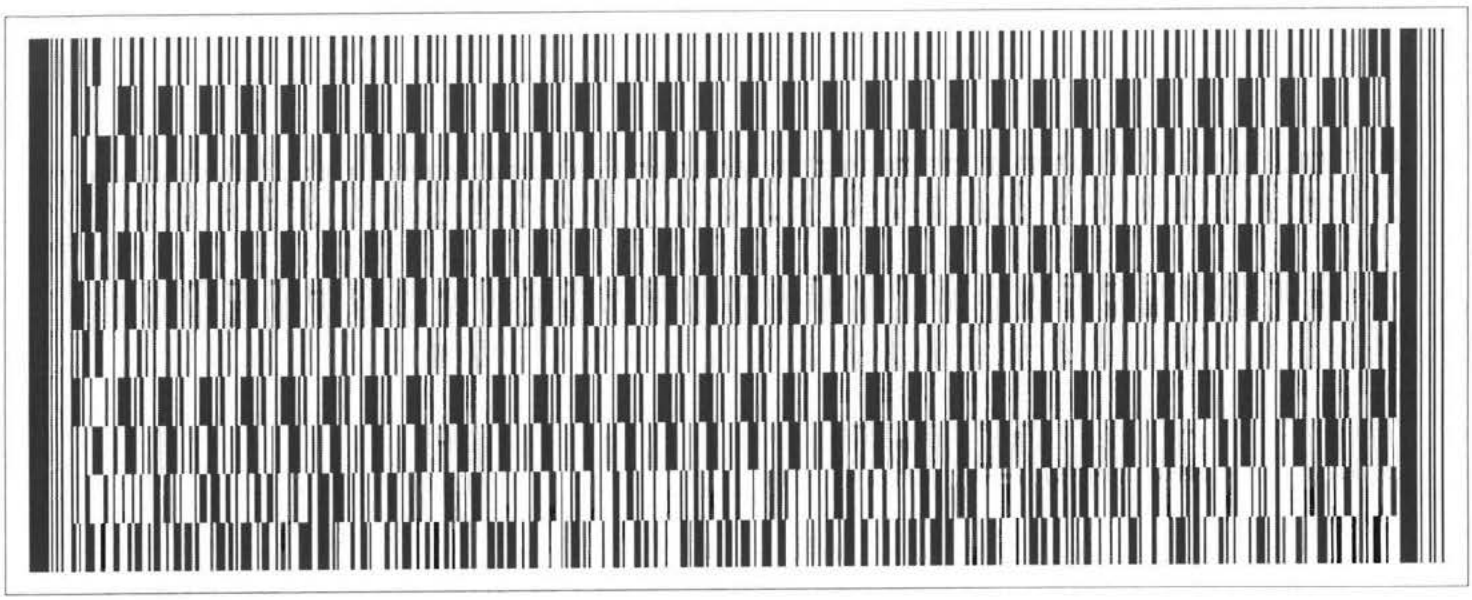
3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Lance Shaner	Lance Shaner	6/27/2022

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	OYR-243 GRAS Cover Letter	1
	GRAS Determination for Saccharomyces cerevisiae strain OYR-243	2-41

OMB Statement: Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, PRAStaff@fda.hhs.gov. (Please do NOT return the form to this address). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.



Questions/Comments for GRN 1096:

1. In Part 2.6 on page 15, you recommend that brewers use approximately 1 million cells of Saccharomyces cerevisiae strain "OYR-243" (*S. cerevisiae* "OYR-243") per milliliter (mL) of wort per degree Plato for brewing wort fermentation. Please confirm that *S. cerevisiae* "OYR-243" is not intended to be used at levels higher than 1 million cells/mL of wort per degree Plato for brewing wort fermentation.

The recommended use level is set as an intended use for a standard beer fermentation under good manufacturing practices.

The FDA has previously had no questions regarding this recommendation (GRN No 798). Dry yeast substances used in beer and wine fermentations (GRN No 841, GRN No 120) also have recommended use levels that are consistent with the proposed use level. There is a self-limiting use for our product as noted in "Part 4", as brewers would incur significant fermentation performance and quality issues *c*reating a self-limiting use for GMP compliance.

2. Please provide a statement that all processing aids used in the manufacture of *S. cerevisiae* "OYR-243" are used in accordance with applicable U.S. regulations, were concluded to be GRAS for their respective uses or are subjects of effective food contact notifications.

All processing aids used in the manufacture of *S. cerevisiae* "OYR-243" are food grade, concluded to be GRAS for their respective uses, and used in a manner consistent with current GMPs.

3. In Table 1 on page 17, you list the specifications for *S. cerevisiae* "OYR-243." However, you did not provide the analytical methods used. Please provide the methods used to ensure your ingredient meets the listed specifications. In addition, please confirm that all analytical methods are validated for their intended use.

The percent solids, viability, total bacteria and wild yeast protocols are provided by the American Society of Brewing Chemists (ASBC) Methods of Analysis.

Percent Solids by (Yeast 5 ASBC), Viable Count and Yeast Viability by (Yeast 3 ASBC) and (Yeast 4 ASBC) and Total Bacteria and Wild Yeast by (Microbiological Control 2. Detection of Microorganisms ASBC) and (Microbiological Control 5. Differential Culture Media ASBC).

All the analytical methods listed are validated for their intended uses.

4. In part 2.5 you state that you "do not anticipate dangerous levels of heavy metals."

- **Please specify the heavy metals that the statement above refers to.**
- **Please clarify if heavy metals are monitored and if so, specify the acceptance criteria for each heavy metal.**

- **Please include a specification for lead and provide the results from the analyses of at least three non-consecutive batches to demonstrate that the ingredient can meet this specification. Please note that the specification for lead should reflect the results of your batch analyses and be as low as possible. In addition, please provide the analytical method used to test for lead and confirm that the method is validated for the intended use.**

Heavy metals are potential risks identified in agriculture ingredients, as well as being present in certain water systems. Omega Yeast Labs controls these risks through cGMPs and other pre-requisite programs, requiring suppliers for agricultural ingredients to be verified by Omega Yeast and each lot of agricultural ingredient is accompanied by a Certificate of Analysis to be within food grade standards for heavy metals, herein defined as < 1.0 ppm lead, < 0.5 ppm arsenic, < 0.03 ppm cadmium, and < 10 ppm (as Pb) total heavy metals. Process water used in the facility is supplied by the municipality of Chicago, and monitored by our facility through collecting annual water quality reports from the city as well as submitting a process water sample to an external laboratory for yearly analysis for total metals (lead with a limit of < 1.1 ppb and mercury with a limit of <0.22 ppb) Thereby controlling the raw material and water inputs through cGMPs, heavy metals are not introduced during the manufacturing of OYR-243.

The specification limit for lead in OYR-243 is no greater than 5 ppb. The analytical results from a minimum of three non-consecutive batches of OYR-243 demonstrate that the ingredient can be manufactured to meet this specification limit and is included in the table below.

Data for three non-consecutive batches of OYR-243 liquid yeast

Parameter	Specification Limit	LC20A6 OYR-243 11/19/2022	LC20AA OYR-243 11/23/2022	LC20B0 OYR-243 11/29/2022
Heavy Metals (Lead, Pb)	5 ppb	<5.00 ppb	<5.00 ppb	<5.00 ppb

The analytical method used to test for lead in OYR-243 has method reference AOAC 2015.01 (modified) and this method is validated for the intended use. This method has a limit of detection (LOD) of 5 ppb and all samples had values below the method LOD and limit of quantification (LOQ).

5. In part 3.2.2 on page 18, you state that the dietary exposure to *S. cerevisiae* "OYR-243" will not differ from the exposure to industrial yeast used in other commercial beer applications. Please confirm that the use of *S. cerevisiae* "OYR-243" will be substitutional for the use of other *S. cerevisiae* strains used in commercial beer production and that its use is not expected to increase the dietary exposure to *S. cerevisiae.*

Omega Yeast confirms that the use of “OYR-243” will be substitutional for the use of other *S. cerevisiae* strains currently used in commercial beer brewing and the use of “OYR-243” is not expected to increase the dietary exposure to *S. cerevisiae*.

The dietary exposure estimates for 1 million cells/ml represents the trace levels of commercial *S. cerevisiae* in finished beer. The use levels stated are 7-15 times this amount, and the natural process of settling/flocculation results in levels <1 million cells/ml of *S. cerevisiae* in the finished beer. This estimate is consistent with the dietary exposure estimate provided in GRN No. 841

The dietary estimates of exposure for heavy drinking individuals is outlined below. There is no concern of safety with heavy drinking exposure based on the nature of OYR-243 being cisgenic to other brewing yeast.

Heavy Drinking Population	Daily Beer Consumption (ml)	Weight (kg)	Exposure to Yeast (cells/kg bw/day)
Men	1420	88.3	16.1×10^6
Women	1065	74.7	14.3×10^6

Reference:

Guenther PM, Ding EL, Rimm EB. Alcoholic beverage consumption by adults compared to dietary guidelines: results of the National Health and Nutrition Examination Survey, 2009-2010. J Acad Nutr Diet. 2013;113(4):546-550. doi:10.1016/j.jand.2012.12.015

6. Please address the levels of the thiol compounds in the final beer product that result from the intended use of *S. cerevisiae* “OYR-243” and indicate how the levels compare to that found in other beer products.

In American Craft IPAs, hops are added at rates varying from 2-40 g/L. Hops contain volatile thiols 4-methyl4-sulfanylpentan-2-one (4MSP), 3-sulfanyl-4-methylpentan-1-ol (3S4MPol), and 3-sulfanylhexan-1-ol (3SHol) at varying concentrations depending on the variety, growing region and harvesting practices. Hop varieties that have elevated levels of thiols are Amarillo, Citra, Nelson Sauvin and Simcoe. Amounts of 3SHol measured in raw hop pellets of US Amarillo and Citra varieties were 413 ug/kg and 616 ug/kg, respectively (Kishimoto et al. 2008). This translates to a range of 800-12,000 ng/L in finished beers at the hopping rate of 2-40 g/L. A Nelson Sauvin beer hopped at 0.4g/L the 3S4MPol measured 92 ng/L, which would result in 3S4MPol levels ranging from 46-9,200 ng/L with a typical IPA hopping rate. Overall, the levels of thiols contributed by hops are much more elevated then those released from thiol precursors by the OYR-243 strain in fermentation. The most significant source of thiol precursor in beer is the barley malt, which only provides the 3SHol precursor. The highest 3SHol level that we have measured from barley wort fermented with OYR-243 is 900 ng/L, which is <10% of what would

occur at the higher hopping rates used in American Craft IPAs. Thus the contribution of thiols from OYR-243 is not substantially elevated relative to the wide range of 3SHol levels that occurs naturally in hop varieties and with the varying hopping rates used in American Craft IPAs.

Reference:

Kishimoto et al. J. Agric. Food Chem. 2008, 56, 1051–1057.

7. There are numerous places in the notice that refer to "OYL-243." For example, on page 5 under the intended use, you state "a functional IRC7 allele was introduced into the OYL-11 host strain to produce a strain with enhanced Irc7 β-lyase activity, OYL-243," and on page 16 you state "OYL-243 is inoculated onto nutrient agar plates…" For the administrative record please clarify if "OYL-243" is different or the same as the production strain "OYR-243."

All instances of "OYL-243" were meant to refer to OYR-243. We apologize for any confusion that resulted from this labeling error.

8. Please provide a short narrative for the safety of the three flavor molecules (3-sulfanylhexan-1-ol; 4-methyl-4-sulfanylpentan-2-one; and 3-sulfanyl-4- methylpentan-1-ol) that will be present in the beer.

- **You may use publicly available information (e.g., JECFA, FEMA) for the safety assessment of these flavors using the published ADI values and comparing those numbers with the expected highest exposure to these flavor molecules (e.g., in 90th percentile consumers) through beer. Please also state whether the dietary exposure to these flavors will be substitutional or additive for these flavor molecules currently in beer.**
- **Alternatively, you may provide information/data showing that the concentrations of these three flavors in beer using your yeast strain will be similar to or less than already consumed beer varieties produced using highly aromatic hop preparations. Because the beer will have higher levels of these flavors, please indicate whether the exposure to these flavor molecules might exceed the safety threshold if they are consumed through multiple types of alcoholic drinks (additive exposure).**

The main thiol released in beer fermentations by OYR-243 is 3SHol or 3-mercaptohexanol (JECFA 545, FEMA 3850, GRAS Publication No 18). From the JECFA evaluation, 1999 (Session 53), the ADI value for 3SHol is 1 ug/day or 0.01 ug/kg bw per day. The maximum levels of thiols released in beer fermentations with OYR-243 is 900 ng/L, and would require a daily intake of 1.1 L of beer to reach 1 ug/day. The FDA GRAS publication No 18, indicates average and maximum food flavoring levels of 0.01-0.1 ppm in alcoholic beverages. With the maximum estimate of 3SHol in a heavily hopped beer fermented with OYR-243, the additive consumption would require 7.75 L of beer as compared to 1 L of alcoholic beverage at the

maximum food flavoring level of 0.1 ppm. This is well over the daily average intake and for the 90th percentile this would require 5 times the intake of heavy drinking men and 7 times the intake for heavy drinking women.

Similar to the response to question 6, hops have the potential to contribute substantially higher levels of thiols to beer then those released by OYR-243 in fermentation. Hops contain volatile thiols 4-methyl4-sulfanylpentan-2-one (4MSP), 3-sulfanyl-4-methylpentan-1-ol (3S4MPol), and 3-sulfanylhexan-1-ol (3SHol) at varying concentrations. Hops that have elevated thiol amounts are aroma varieties such as Amarillo, Citra, Nelson Sauvin and Simcoe. Amounts of 3SHol measured in raw hop pellets of US Amarillo and Citra varieties were 413 ug/kg and 616 ug/kg, respectively (Kishimoto et al. 2008). This translates to a range of 800-12,000 ng/L in finished beers at the hopping rate of 2-40 g/L. In a Nelson Sauvin beer hopped at 0.4g/L the 3S4MPol measured 92 ng/L, which would result in 3S4MPol levels ranging from 46-9,200 ng/L with a typical IPA hopping rate. Overall, the levels of thiols contributed by hops are much more elevated then those released from thiol precursors by the OYR-243 strain in fermentation. The most significant source of thiol precursor in beer is the barley malt, which only provides the 3SHol precursor. The highest 3SHol level that we have measured from barley wort fermented with OYR-243 is 900 ng/L. Similarly to hops that naturally contain high thiol levels, the amount of 3SHol found in fruit sources can reach levels similar to those found in beer. The 3SHol level in Green Guava fruit has been measured at 15,000 ug/kg (Cannon et al. 2018). One guava fruit weighing 50 grams would be an estimated exposure of 300 ng of 3SH. The level of 3SHol in Sauvignon blanc can measure up to 18,000 ng/L (Coetzee et al. 2012). Based on the evidence provided herein, 3SHol, 4MSP and 3S4MPol can be found at higher levels in natural sources than those that are released in beers fermented with OYR-243. We conclude that the levels of thiols released in beers fermented with OYR-243 are much lower than those found in fruit, wine and hops, and thus even additive exposure (i.e., multiple drinks) does not present an elevated exposure to thiols beyond those found in natural sources.

Kishimoto et al. J. Agric. Food Chem. 2008, 56, 1051–1057.
Cannon et al. J Food Drug Anal. 2018, 26 (2), 445-468.
Coetzee et al. Food Research International. 2012, 45, 287–298.

9. For the administrative record, please state whether *S. cerevisiae* "OYR-243" has been deposited in a recognized culture collection and provide the deposit designation. If it has not been deposited, please describe how the strain was taxonomically identified and verified.

It is not customary for brewing strains to be deposited in a recognized culture collection. Culture stocks are maintained in breweries through repitching from batch to batch and at companies that manufacture brewers yeast cultures. The initial source strain (OYL-011) has been confirmed to be *S. cerevisiae*, and thus the modified OYR-243 is *S. cerevisiae*. OYL-011 originates from the same source and is supplied by another yeast manufacturer under WY1318. The whole genome sequence can be found here and confirms the S. cerevisiae taxonomy.
https://www.ncbi.nlm.nih.gov/biosample/?term=WY1318

The OYR-243 ITS sequencing results confirmed *S. cerevisiae* taxonomy. Briefly, OYR-243 strain was struck to single colonies on YPD-agar plates and was submitted for ITS sequencing with Azenta Life Sciences (https://www.azenta.com/). The ITS sequencing results were queried in NCBI Blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and showed highest percent identity, query coverage and max score to *S. cerevisiae* ITS sequences confirming the OYR-243 *S. cerevisiae* taxonomy. This method is validated for its intended use.

Reference:

Langdon QK, Peris D, Baker EP, et al. Fermentation innovation through complex hybridization of wild and domesticated yeasts. Nat Ecol Evol. 2019;3(11):1576-1586. doi:10.1038/s41559-019-0998-8

10. On page 11, the notifier states, "No genes encoding virulence factors, protein toxins or enzymes involved in the synthesis of mycotoxins, or any other toxic substances are expected based on our knowledge of these strains, the IRC7 sequence, TDH3 promoter and CYC1 terminator." For the administrative record, please confirm that *S. cerevisiae* "OYR-243" is non-pathogenic and non-toxigenic.

The OYR-243 strain is non-pathogenic and non-toxigenic. The OYR-243 strain belongs to the *S. cerevisiae* species which have been used for more than 7,000 years by humans in fermented beverages. It can therefore be concluded that species will remain GRAS, even after genetic transformation, as long as no harmful DNA material is added to it. The historic use of S. cerevisiae in food and beverage fulfills criteria for GRAS. In addition, *S. cerevisiae* (bakers yeast protein/glycan and dried bakers yeast) are approved additives listed in Title 21 of the Code of Federal Regulations Part 172 and 173. The European Food Safety Agency considers bakers/brewer's yeast among the safest of microorganisms. *S. cerevisiae* also has Qualified Presumption as Safe (QFS) status in Europe.

There is no evidence for genotypic or phenotypic characteristics of OYR-243 that would pose a safety concern.

References:

Gallone B, Steensels J, Prahl T, et al. Domestication and Divergence of Saccharomyces cerevisiae Beer Yeasts. Cell. 2016;166(6):1397-1410.e16. doi:10.1016/j.cell.2016.08.020

Baker' s yeast extract (21 C.F.R. § 184.1983)

Baker' s yeast protein (21 C.F.R. § 172.325)

Dried yeast as an ingredient in food (21 C.F.R. § 172.896);

Baker' s yeast glycan (21 C.F.R. § 172.898)

European Food Safety Authority (EFSA). (2007). Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA - Opinion of the Scientific Committee: Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA - Opinion of the Scientific. EFSA Journal, 5(12), 587. https://doi.org/10.2903/j .efsa.2007 .587

European Food Safety Authority (EFSA). (2008). The maintenance of the list of QPS microorganisms intentionally added to food or feed - Scientific Opinion of the Panel on Biological Hazards: The maintenance of the list of QPS microorganisms intentionally added to food or feed - Scientific Opinion of the Panel on Biological Hazards. EFSA Journal, 6(12), 923. https://doi.org/10.2903/j .efsa.2008.923.

European Food Safety Agency (EFSA) Panel on Biological Hazards (BIOHAZ). (2013). Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). EFSA Journal, 11(1 l). https://doi.org/10.2903/j.efsa.2013.3449

11. Please describe how the notifier confirms the insertion point of the FDC1 plasmid and state how many copies are inserted into the host strain S. cerevisiae "OYL-011." Additionally, please describe how the notifier verifies that no other place in the genome has been affected by the insertion of the plasmid.

The FDC1 plasmid is not inserted into the genome of S. cerevisiae strain "OYR-243". The FDC1 plasmid is *S. cerevisiae* 2 micron plasmid (yeast small circular piece of DNA) that is maintained at a copy number of 20 per cell through G418 selection and is lost through mitotic segregation in the absence of G418 selection. The FDC1 plasmid is no longer in the "OYR-243" strain and was transiently expressed during the strain development and CRISPR/Cas9 editing event.

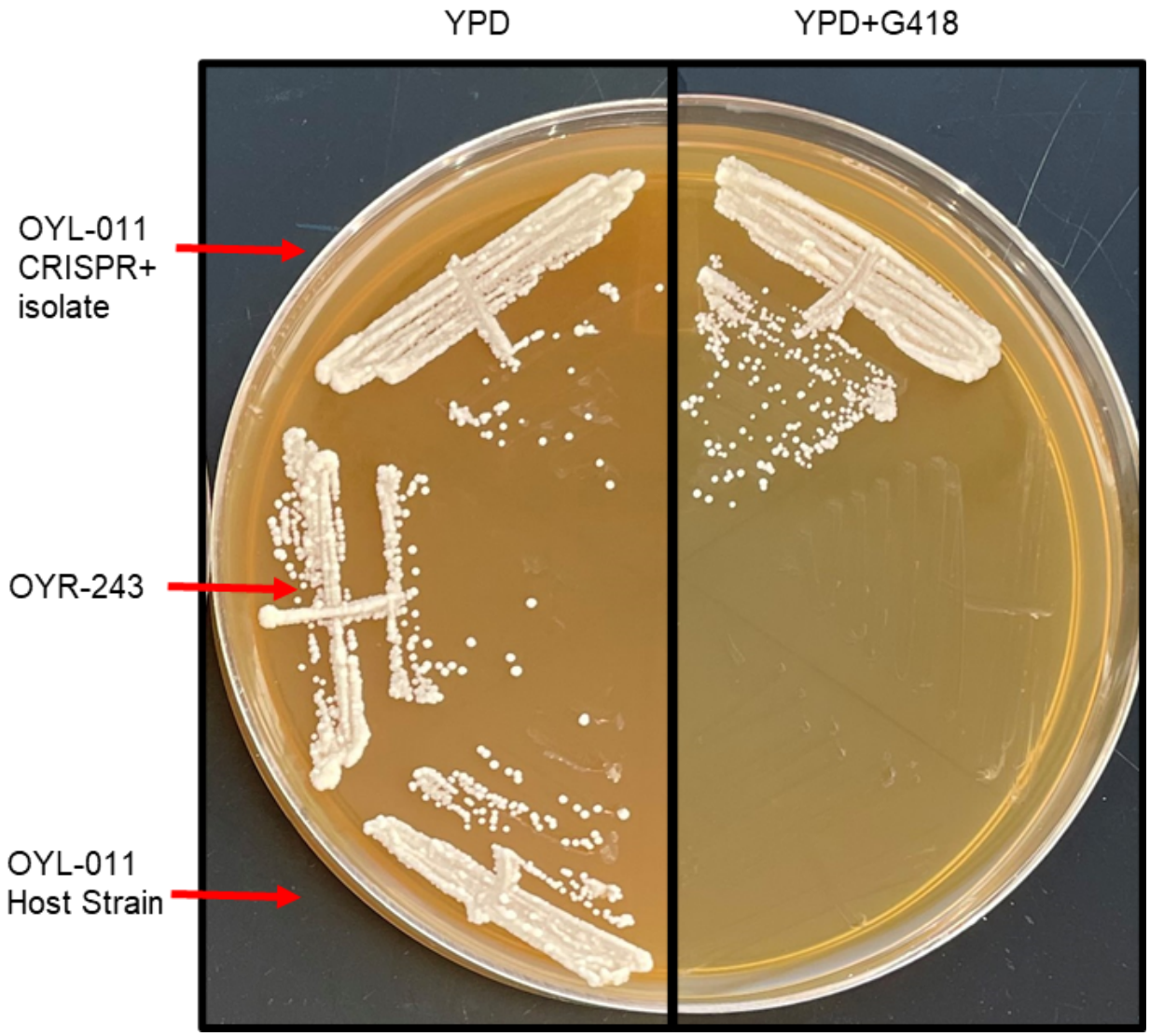
A bioinformatics tool (https://crispy-pop.glbrc.org/) was used to evaluate the specificity of the sgRNA and exclude any potential off site targets based on 1011 fully sequenced *S. cerevisiae* genomes. The interdelta PCR method confirmed the absence of any off-target effects that would result in structural alterations to the genome. We chose this method as it has been used in past applications (GRN No. 798) to ensure the specificity of the CRISPR/Cas9 gene editing event in the *S. cerevisiae* strain yBBS002.

Reference:

Christianson TW, Sikorski RS, Dante M, Shero JH, Hieter P. Multifunctional yeast high-copy-number shuttle vectors. Gene. 1992;110(1):119-122. doi:10.1016/0378-1119(92)90454-w

Stoneman HR, Wrobel RL, Place M, et al. CRISpy-Pop: A Web Tool for Designing CRISPR/Cas9-Driven Genetic Modifications in Diverse Populations. G3 (Bethesda). 2020;10(11):4287-4294. Published 2020 Nov 5. doi:10.1534/g3.120.401498

12. For the administrative record, please provide a higher resolution photo of Figure 3 on page 13.



13. For the administrative record, please state whether *S. cerevisiae* "OYR-243" is capable of DNA transfer to other organisms.

The "OYR-243" strain is capable of sexual reproduction and thus the transfer of DNA to other S. cerevisiae strains. The "OYR-243" strain however does not contain any DNA sequence that is foreign to the *S. cerevisiae* gene pool. The *TDH3* promoter, *IRC7* gene and *CYC1* terminator sequences are naturally occurring and common among industrial brewing yeast.

14. For the administrative record, please briefly specify how the purity of *S. cerevisiae* "OYR-243" inoculum is ensured.

OYR-243 was obtained from a single clonal isolate of the CRISPR/Cas9 gene editing event. The purity of the manufactured OYR-243 strain is ensured through GMPs and sterile technique and validated through microbiological testing procedures (details provided in response 16).

15. On page 16, the notifier discusses the raw materials used in the manufacturing process and states that the "ingredients are free of the 8 major food allergens specified under the Food Allergen Labeling and Consumer Protection Act. As of January 1, 2023, sesame is considered a major food allergen under the Food Allergy Safety, Treatment, Education, and Research (FASTER) Act (https://www.fda.gov/food/cfsan-constituent-updates/faster-act-video-foodindustry-and-other-stakeholders). For the administrative record, please state whether sesame or substances derived from sesame are used in your manufacturing process and whether this poses a safety concern.

All products sold from Omega Yeast have not been produced with sesame or substances derived from sesame.

16. On page 15, the notifier states, "Packaged product is >98% viable on ship date, and free from detectable levels of contaminants (zero colony forming units (CFUs) of bacteria or yeast detected per 20 million yeast cells)." Please describe how you distinguish between the final ingredient, *S. cerevisiae* "OYR-243," and yeast contaminants in the packaged product.

Omega Yeast tests all product prior to packaging on LCSM and MRS+ to test for Brettanomyces, non-Saccharomyces yeast, and other "wild yeasts" (yeasts not normally used in brewing), and quadrant streaks on WLN to ensure strain purity and that no cross contamination occurred.

References:

Laura T. Burns, Christine D. Sislak, Nathan L. Gibbon, Nicole R. Saylor, Marete R. Seymour, Lance M. Shaner & Patrick A. Gibney (2021) Improved Functional Assays and Risk Assessment for STA1+ Strains of Saccharomyces cerevisiae, Journal of the American Society of Brewing Chemists, 79:2, 167-180, DOI: 10.1080/03610470.2020.1796175

Deng, Y., Liu, J., Li, H., Li, L., Tu, J., Fang, H., Chen, J. and Qian, F. (2014), An improved plate culture procedure for the rapid detection of beer-spoilage lactic acid bacteria. J. Inst. Brew., 120: 127-132. https://doi.org/10.1002/jib.121

17. The notifier provides specifications for “Total Bacteria” and “Total Wild Yeast” on page 17.

- **Please provide the analytical methods used to test for these specifications and state whether these methods have been validated for their intended use.**
- **Please clarify what the specification for “Total Bacteria,” refers to.**
- **Common microbial tests for ingredients added to conventional food, such as coliforms, Enterobacteriaceae, or foodborne pathogens, such as Salmonella serovars, are not included in the provided list of specifications. Please discuss why testing for “Total Bacteria” and “Total Wild Yeast” is sufficient for your manufacturing process and intended use.**
- **Please clarify your use of the term “Wild Yeast.”**

The total bacteria and wild yeast protocols are based on American Society of Brewing Chemists (ASBC) methods of analysis Microbiological Control 2. Detection of Microorganisms and Microbiological Control 5. Differential Culture Media. These methods are validated for their intended uses and represent a culture that is free from bacteria or wild yeast contaminants

“Total Bacteria" and “Wild Yeast” refer to common beer spoilage organisms that can cause quality issues and often are more resilient to fermentation hurdles than certain foodborne pathogens. “Total Bacteria” refers to lactic acid bacteria, acetic acid bacteria, and other wort- and beer-spoiling bacteria, while “Wild Yeast” refers to yeasts not normally used in brewing or spoiling organisms such as Brettanomyces and non-Saccharomyces yeast.

Lactic acid bacteria (LAB) have a significantly higher heat-resistance in wort and beer when compared to *E.Coli* O157:H7 and *S. typhimurium*, and gram-negative LAB/acetic acid bacteria (AAB) and wild yeasts are highly adapted for growth in restrictive fermentation environments (pH < 4.2, alcohol content, hop compounds, yeast fermentation metabolites). These quality indicator organisms, “Total Bacteria” and “Total Wild Yeast,” represent undesired microbial growth during fermentation-based ingredient manufacturing that is inhibitive to common foodborne pathogens, and are appropriate indicator organisms for microbiological testing for our manufacturing process and intended use.

Additionally, we would like to state a specification limit for Enterobacteriaceae in OYR-243 as no greater than 10 cfu/g. The microbiological results from a minimum of three non-consecutive batches of OYR-243 demonstrate that the ingredient can be manufactured to meet this specification limit and is included in the table below.

Data for three non-consecutive batches of OYR-243 liquid yeast

Parameter	Specification Limit	LC20A6 OYR-243 11/19/2022	LC20AA OYR-243 11/23/2022	LC20B0 OYR-243 11/29/2022
Enterobacteriaceae	10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g

The analytical method used to test for Enterobacteriaceae in OYR-243 has method reference CMMEF Chapter 9.62 and this method is validated for the intended use.

References:

Roberts, T.A. et al. Fermented beverages. Micro-Organisms in Foods 6. Springer, Boston, MA. doi:10.1007/0-387-28801-5_17

Jeon SH, Kim NH, Shim MB, et al. Microbiological diversity and prevalence of spoilage and pathogenic bacteria in commercial fermented alcoholic beverages (beer, fruit wine, refined rice wine, and yakju). J Food Prot. 2015;78(4):812-818. doi:10.4315/0362-028X.JFP-14-431

Menz G, Vriesekoop F, Zarei M, Zhu B, Aldred P. The growth and survival of food-borne pathogens in sweet and fermenting brewers' wort. Int J Food Microbiol. 2010;140(1):19-25. doi:10.1016/j.ijfoodmicro.2010.02.018

Nancy C. L'Anthoën & W. M. Ingledew (1996) Heat Resistance of Bacteria in Alcohol-Free Beer, Journal of the American Society of Brewing Chemists, 54:1, 32-36, DOI: 10.1094/ASBCJ-54-0032

Suzuki K, Asano S, Iijima K, Kuriyama H, Kitagawa Y. Development of detection medium for hard-to-culture beer-spoilage lactic acid bacteria. J Appl Microbiol. 2008;104(5):1458-1470. doi:10.1111/j.1365-2672.2007.03669.x

Lin, Y. (1981), Formulation and testing of cupric sulphate medium for wild yeast detection. Journal of the Institute of Brewing, 87: 151-154. https://doi.org/10.1002/j.2050-0416.1981.tb04005.x