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Genetically Engineered Yeast—A Review of Terminology, Science, and Regulation

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ABSTRACT

The current offerings of genetically engineered yeast are expanding, and an effort is being made to provide accessible information to brewers surrounding this new wave of innovation. While in use for decades, imprecise descriptions of genetic engineering methods could potentially lead to misconceptions of how genetically modified organisms are developed, used, and regulated. This review discusses the preferred terminology currently used to describe genetically modified organisms and the use of genetic modification in foods and beverages and provides an overview of fundamental methods of engineering brewing yeast and of the regulatory aspects that relate to the safety and application of these organisms in brewing. The goal is to offer a starting point for product development specialists to make informed decisions about rapidly changing technology and nomenclature, as well as provide a perspective into regulatory affairs involved in bringing such technology to market. As routine use of genetically engineered brewing strains gains traction, they will undoubtedly have a resonating impact on how novel beers are produced and perceived.

Keywords: bioengineered, gene editing, genetic engineering, genetic modification, genetically engineered, *Saccharomyces cerevisiae*

Introduction

Enhancing and modifying desirable traits in economically essential organisms has been the driving force behind agricultural advancements for millennia (2,14). Since the advent of modern microbiology, the modification of microorganisms is of particular interest due to their ease of manipulation, ubiquity, and potential usefulness to humans. The production of foodstuffs like yogurt, soy sauce, wine, beer, spirits, and bread, among others, all rely on the use and optimization of microbial metabolism. Of these examples, the advent of modern beer fermentation is arguably the most reliant on high-purity monoculture fermentations, resulting in gradual divergence of diverse *Saccharomyces cerevisiae* strain types across the industry (9,11,22,23). These phenotypes play important stylistic roles in the production of conventional beers and in the continued development of new and innovative styles. Variations among strain metabolism dictate interactions with substrates, which alter how strain types behave under a variety of environmental conditions (i.e., stress response), providing a wide range of potential flavor and aroma outcomes (5,13,20).

Taking advantage of diverse phenotypes is arguably the primary impetus behind the development and breeding of novel yeast strains. As knowledge of biotechnology becomes more mainstream, the industry is slowly gravitating toward modern approaches to genetic engineering of strains for those willing to fund development. Indeed, the cost of such endeavors has dramatically decreased since the start of the 21st century, thanks in part to the rapid development of computational biology and more efficient selection techniques. A myriad of biotechnologies exists to develop custom brewing yeast, ranging from high-throughput mutagenesis screening to precise genome editing. In this review, a selection of common methods will be highlighted, with a strong emphasis on modern genetically modified organism (GMO) creation.

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Defining a GMO

Before moving into means of development, it is prudent to outline and discuss what the classification of GMO means to the average person, and the potential economic and ethical impacts this entails. Much of the historical conversation on GMO technology revolves around the topic of economically vital genetically modified crop plants. The majority of agriculture is highly visible and out in the open (e.g., grain growing and processing), and therefore, the ethical implications of introducing modified versions of staple crops is rightfully apparent. It is noteworthy that much of the conversation around GMOs is less overt, with their growth and manipulation occurring in stainless-steel tanks behind closed doors and under tightly controlled environments, rendering them essentially invisible to the average person. Thus, in the context of brewing, consumer transparency and ethical use of new products by way of genetic engineering requires a good foundational understating of the technical nomenclature involved at both the laboratory and regulatory levels.

To be transparent, the use of common and easily understandable terminology is key. The common use of "GMO" evokes imagery of modified organisms (plants, animals, microorganisms) altered through human-mediated genetic information insertion ("transgenic"; Table 1) despite the fact that the terminology encapsulates a large variety of genetic manipulation. It is important to note that under some regulatory bodies no legal definition of GMO exists in this parlance, possibly obfuscating labeling re-

quirements. Thus, a common ground is necessary to establish a regulatory definition that easily dictates what sort of modification has been performed, the extent of modification, how the modified organism has been used, and the total inclusion of a given organism within a product. Such topics are more difficult to cover in some cases (e.g. inclusion of single protein extract from genetically modified soya into a power bar versus a single raw Flavr Savr™ tomato). The two main technical terms that will be important to understand going forward are "transgenic" and "cisgenic," both of which are defined in Table 1. In brief, transgenic manipulation refers to the inclusion of genetic material from sexually incompatible organisms. Cisgenic manipulation refers to the inclusion of genetic material from sexually compatible organisms. The most stringent regulatory bodies may classify any

Table 1. Genetic engineering-related nomenclature, acronyms, and definitions[a]

Acronym (if applicable)	Nomenclature	Definition
General nomenclature		
BE	Bioengineered	A product or organism resulting from GE technology.
	Cisgenic modification	Where genetic material from a sexually compatible species is inserted into the genome of a host organism. This does not result in the expression of novel traits and generally does not impact the fitness of the organism itself.
	Domestication	A conceptual definition wherein an organism is altered through selective breeding and artificial selection to thrive in a manmade environment. This results in detectable and quantifiable changes to the organism's genome in the form of gene mutations, deletions, and copying.
GE	Genetically engineered or genetic engineering	Term used to describe an organism that has been modified using biotechnology. Often interchanged with "GMO."
GMO	Genetically modified organism	Vernacular term and acronym used to describe organisms that have undergone some form of heritable genetic modification. Typically associated with transgenic modification.
HGT	Horizontal gene transfer	The transfer and uptake of genetic material between two dissimilar species or across domains of life through natural means, but not through heritable methods such as sexual or asexual reproduction. Primarily occurs among microorganisms.
	Hybrid	The result of a rare mating occurrence wherein two dissimilar parents produce a living offspring. Hybrid mating typically occurs within the same phylogenic family but among different species. This also occurs at the unicellular level among sexually reproducing organisms such as yeasts.
LMO	Living modified organism	A living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology. Definition set by the CPB (see under Regulatory Nomenclature).
	Sexual mating	Among complex organisms such as plants and animals sexual mating is the act in which two compatible organisms create a viable offspring via the combination of two gametes (sperm and egg). Offspring are a combination of 50% of each parent's DNA. Sexual mating is a significant source of natural genetic diversity. Sexual reproduction may occur among certain species of unicellular organisms such as yeasts.
	Transgenic modification	Where genetic material from sexually incompatible species is inserted into the genome. This typically will result in expression of novel traits and may impact the fitness of the organism itself.
	Mutagenesis	Any process, physical, chemical, or radiological, that induces random heritable changes to an organism's DNA.
rDNA	Recombinant DNA or recombinant	A combination of DNA elements from different genes within a species, or DNA elements of different species, used to generate new genes.
Regulatory nomenclature		
CPB	Cartagena Protocol on Biosafety	The full name is The Cartagena Protocol on Biosafety to the Convention on Biological Diversity, an international treaty governing industry and commercial movements of LMOs resulting from modern biotechnology from one country to another.
EFSA	European Food Safety Authority	The European Union agency that provides independent scientific advice on existing and emerging risks facing the modern consumer food chain.
FDA	Food and Drug Administration	A U.S. regulatory body under the Department of Health and Human Services (HHS) that is responsible for protecting public health through regulatory control of food safety, among other responsibilities.
USDA	United States Department of Agriculture	The U.S. cabinet level executive agency responsible for developing and executing federal laws related to food and agricultural development, among other categories of regulation.
U.S. EPA	United States Environmental Protection Agency	An independent executive agency responsible for environmental protection tasks. In part, the U.S. EPA may only act pursuant to laws passed by the legislative branch of the U.S. federal government.
NBFDS	National Bioengineered Food Disclosure Standard	The U.S. standard requiring food manufacturers, importers, and other entities that label foods for retail sale to disclose information about BE food and BE food ingredients.

(Continued on next page)

[a] Definitions are derived through regulatory and academic literature within the context of this review. The table is split into three sections: general nomenclatures, regulatory nomenclature, and biotech nomenclature.

Table 1. *(Continued from previous page)*

Acronym (if applicable)	Nomenclature	Definition
Biotech nomenclature		
CRISPR	Clustered regularly interspaced short palindromic repeats	Specifically structured regions of DNA interspaced with unique DNA sequences (spacers) associated with exogenous DNA inserts (e.g., viral infections).
Cas	CRISPR-associated protein	RNA-guided DNA nuclease evolved for cutting specific segments of DNA spacers from prokaryotic genomes.
crRNA	CRISPR RNA	The mature RNA transcript of a DNA spacer. These combine with Cas to form a Cas-crRNA complex that can recognize the targeted DNA spacer.
DNA	Deoxyribonucleic acid	A double-stranded polymer composed of the nucleic acids adenine, thymine, cytosine, and guanine and sugar. It is the primary macromolecule responsible for hereditary instructions for the development of all known life on Earth.
DSB	Double strand break	The action in which both strands of a DNA molecule are broken. Gene editing methods induce targeted double strand breaks within an organism's genome.
RNA	Ribonucleic acid	A single-stranded polymer similar in function and composition to DNA, with uracil replacing thymine. In prokaryotic and eukaryotic organisms, DNA is transcribed by RNA polymerases into various types of RNA, which function to facilitate gene expression and protein synthesis. RNA is the primary means of genetic information storage in some viruses.
HR	Homologous recombination	Within the context of genetic modification, HR is a repair method used to fix DSB via double-stranded break repair. This method is highly accurate but may also play a role in naturally occurring genetic diversity in sexually reproducing organisms.
PAM	Proto-spacer adjacent motif	An essential component in the CRISPR system used to identify non-native DNA inserts. Acts as a potential binding site for the Cas-crRNA protein complex.
sgRNA	Single-guide ribonucleic acid	A version of the naturally occurring two-piece guide RNA complex engineered into a single, continuous sequence. The simplified single-guide RNA is used to direct the Cas9 protein to bind and cleave a particular DNA sequence for genome editing.
tracrRNA	Trans-activating CRISPR RNA	A more complex version of sgRNA. It works in conjunction with crRNA for locating and matching viral spacer DNA.
PCR	Polymerase chain reaction	A method for generating many copies of a DNA sequence using a purified DNA polymerase.
TALEN	Transcription activator-like effector nuclease	A restriction enzyme used to cleave DNA highly specific restriction sites—typically denoted by a repeating nucleic acid motif. Specifically engineered TALEN are an important tool in genetic engineering.
ZFN	Zinc finger nucleases	Artificially engineered restriction enzyme that makes use of bound zinc–protein moieties to target specific DNA restriction sites.

use of recombinant DNA (rDNA; Table 1) technology as "GMO," whereas the United States and Canada consider the change to an organism's DNA and, more specifically, refer to transgenic modifications as "GMO."

A nonbinding definition, as set forth by the World Health Organization, defines a GMO as an organism altered through a process that cannot happen through natural means (32). Other definitions adjust the language to be more specific, such as terminology established though the Cartagena Protocol on Biosafety (CPB), to provide groundwork on which sovereign nations may implement standardized regulatory control among ratifying entities. The CPB establishes defined nomenclature as "living modified organisms" (LMOs), meaning "any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology" (28). At the federal level within the United States, the U.S. Department of Agriculture (USDA) provides a legal definition of "biotechnology" for use in place of "GMO," which acts as a legal umbrella term to include those organisms that have undergone direct genetic manipulation or engineering (1,2,5,29,30,31).

Despite the differences in officially recognized terminology, the common thread among the aforementioned examples is the phrase "through the use of modern biotechnology," which is clearly expanded to mean those recombination events that cannot occur in nature (e.g., Article 3 § i of the CPB). This distinction is important as there exists a set of common breeding methods that have been in widespread use since before the rapid advancements in modern biotechnology made during the bio-computing revolution of the early 2000s. These methods include artificial selection and forced evolution, ionizing radiation-induced mutagenesis, and forced hybridization within a related taxonomic group and are more closely related to cisgenic manipulation. While ostensibly genetic modification in and of itself, these methods avoid GMO classification and potential taboo by virtue of the fact they can (however unlikely) occur in nature (25). It is understandable, therefore, that these methods are often grandfathered into "classical" biotechnology methods rather than "modern" biotechnology methods and, thus, lack any specific labeling requirements. As a result, when dealing specifically with GMOs/LMOs, labeling will almost always be in reference to transgenic (Table 1) products, i.e., those organisms wherein taxonomically disparate genetic material is introduced into a new host genome. Keeping these original definitions in mind, the aim of this review is to shed light on the oft-overlooked world of genetically modified unicellular microorganisms, elucidate the differences that occur at the microscopic level, and examine how they impact modifications expressed at the macroscopic level.

Genetic Engineering Methods in Yeast

Genetically modified *S. cerevisiae* strains have been employed not only for the advancement and application in food and beverage science, but for the fundamental exploration of biological principles. To highlight a few of these advancements, Nobel Prize-winning studies elucidating the genes and mechanisms of fundamental cellular processes, including the cell cycle, transcrip-

tion, telomeres, membrane transport, protein sorting, and autophagy, all relied on the ease of yeast genetic screens and genetic manipulation (3). As academic scientists fueled these discoveries, breakthroughs in molecular biology and sequencing technology followed hand in hand. In 1996, *S. cerevisiae* was the first eukaryotic genome sequenced, and more recently, a large-scale sequencing effort has provided a species-wide look into the level of genomic diversity found across 1,011 industrial yeast and natural *S. cerevisiae* isolates (12,22). In fact, whole genome sequences for a number of ale (>150) and lager (>30) yeast strains are publicly available through National Center for Biotechnology Information (NCBI). The result of such a rich field of yeast genetics and cellular biology is a wide breadth of understanding of the brewing yeast genome.

Many of these discoveries relied on simple approaches to manipulate the yeast genome. The two major methods of introducing foreign DNA into yeast are transient and stable DNA transformations (Fig. 1). Transient DNA transformations do not directly insert into the yeast genomic DNA, but instead express genes from a yeast shuttling vector or plasmid DNA. Stable transformations result in targeted integration of the introduced DNA into the yeast genome and rely on homologous recombination, a robust and accurate pathway for DNA repair in *S. cerevisiae.* Briefly, a double-stranded DNA element that contains a region of homology at each end (donor DNA) to DNA sequences in the yeast genome is introduced through genetic transformation. This process briefly permeabilizes the cell and allows for uptake of the donor DNA. Once the double-stranded DNA is in the cell, the homologous ends of the donor DNA are used to repair spontaneous breaks in the yeast genome that naturally occur at a very low frequency. Because these events are so rare, there must be a way to select for cells that have incorporated the donor DNA. To do so, the donor DNA element will contain either a drug resistance marker or disrupt an endogenous yeast gene that results in a selectable phenotype. For any food-based or industrial application, there must be confirmation that no drug resistance marker is in the final product; thus, these applications must allow for the removal of the drug resistance marker, employ other selection methods, or make use of new gene editing tools that enhance the efficiency of stable DNA transformations.

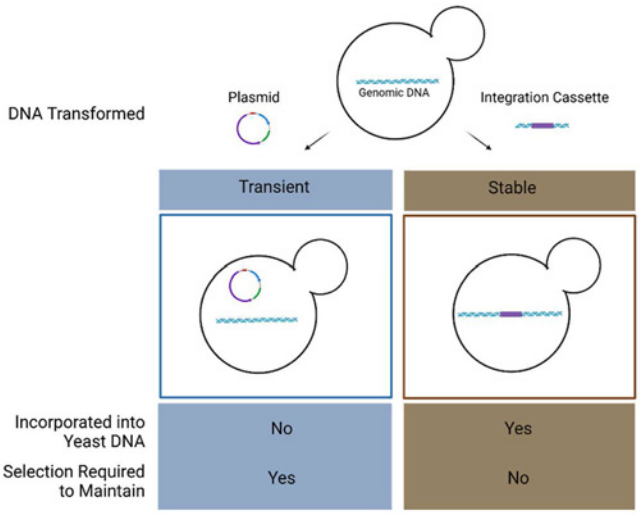

Figure 1. DNA transformations in *Saccharomyces cerevisiae.* Transient DNA transformations introduce a DNA plasmid that is not incorporated into the yeast genome but is maintained through selective pressure. Stable DNA transformations introduce a donor DNA sequence that is incorporated into the genome through homologous recombination and once incorporated does not require selective pressure.

The processes for generating targeted DNA integrations described above are inefficient and become even less efficient in more genetically complex industrial strains. So, how does the process become more efficient? By introducing targeted DNA breaks to specific sites in the genome. Herein lies the beginning of the new era of gene editing—the permanent modification of DNA at a specific site of the genome using targeted nucleases (1). Several different gene editing systems that are commonly used include zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats (CRISPR-Cas) system. These nucleases target a specific site in the genome and act as molecular scissors to perform very specific cuts in the DNA. From there, the cellular DNA repair pathways can repair the site using homologous DNA in the genome (homologous recombination) or nonspecifically join (nonhomologous end joining) the DNA double strand break—often resulting in the disruption of a gene target. Alternatively, donor DNA can be provided for homology-directed repair and, similar to a word processor, result in an insertion, deletion, or single nucleotide change to the targeted DNA sequence. Interestingly, regulation around the use of these gene editing tools differs considerably. Some countries regulate the use of the tool, whereby any gene-edited organism is considered genetically modified, while others consider the result of the gene editing event—for example, cisgenic/transgenic, whether a DNA repair template was provided (10).

The most recent and publicly lauded gene editing technology, CRISPR/Cas, requires three components: a Cas enzyme, a single guide RNA (sgRNA), and a donor DNA template with homology to the targeted region (Fig. 2). In *S. cerevisiae*, a yeast shuttling vector that encodes both the Cas enzyme and sgRNA often is transiently transformed and removed after the editing event is confirmed. To target the Cas enzyme to a specific region in the yeast genome, an sgRNA needs to be designed for each new DNA target. Bioinformatic tools are used to score each potential sgRNA within a given target sequence and determine the likelihood of any off-target sites throughout the rest of the genome. The resulting sgRNA sequence contains a 20-nt targeting sequence (crRNA) and the Cas nuclease-recruiting sequence (tracrRNA). The 3′ end of the targeting sequence in the genome must contain a protospacer adjacent motif (PAM) sequence specific to the Cas enzyme used (ex. spCas9 PAM is 5′-NGG-3′). The major benefit of this method is that the Cas enzyme cuts the specific site targeted by the sgRNA, and the yeast homologous recombination repairs the double strand break with the targeted donor DNA template, which is no longer recognized by the sgRNA and, therefore, is no longer be targeted by the CRISPR/Cas system. The efficiency of CRISPR/Cas9 allows for the editing of each chromosome allele in diploid or polyploid organisms and can even be used to target multiple sites in the genome at once with the co-expression of multiple sgRNAs. Also, in contrast to insertions made with traditional homologous recombination, screening can be performed without additional selection methods (ex. auxotrophy or drug resistance) and can be performed on individual transformants by expected phenotype, or through PCR primers that detect the specific change, insertion, of deletion in the genome. After the editing event is confirmed, whole genome sequencing is used to compare the edited strain to the parental strain and verify there were no off-target effects of CRISPR. In summary, the process of gene editing with CRISPR/Cas has the advantages of targeting specific sites within the genome, introducing DNA changes from single nucleotide substitutions to insertion and deletions,

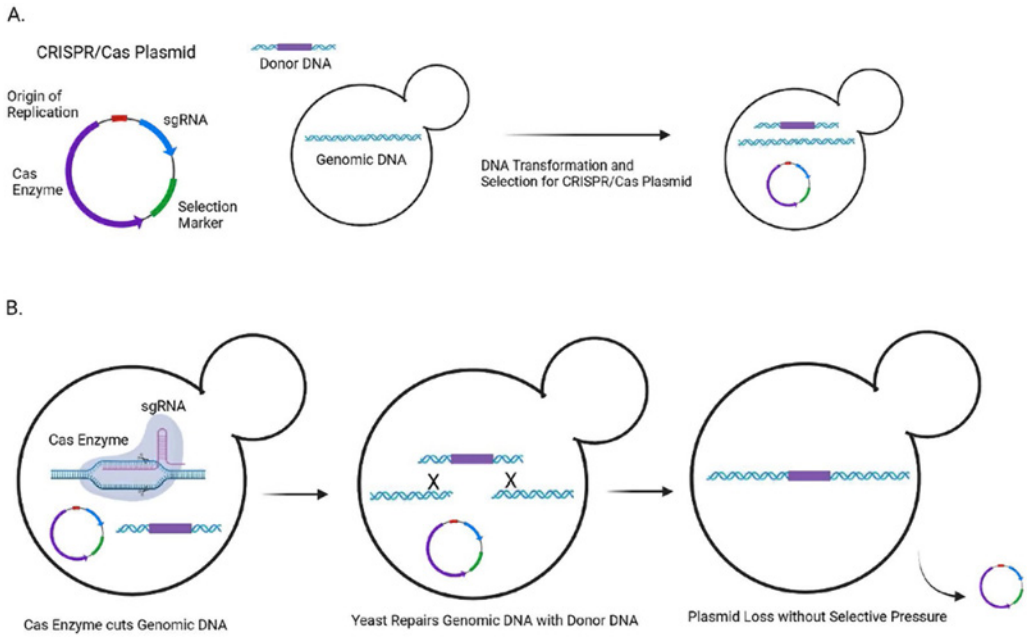

Figure 2. CRISPR/Cas Editing in *Saccharomyces cerevisiae.* **A,** Transient transformation of CRISPR/Cas components. The CRISPR/Cas plasmid includes the small guide RNA sequence, the Cas enzyme, and a selection marker and origin of replication for maintaining the plasmid. The donor DNA sequence is cotransformed. **B,** CRISPR/Cas targeting and repair with donor DNA. The Cas9 enzyme and sgRNA are expressed, and the sgRNA directs the Cas enzyme to the specific site of homology in the yeast genome. The Cas enzyme cleaves the yeast genomic DNA and then the yeast endogenous homologous recombination repair pathway incorporates the donor DNA. The CRISPR/Cas plasmid is lost once the selective pressure is removed.

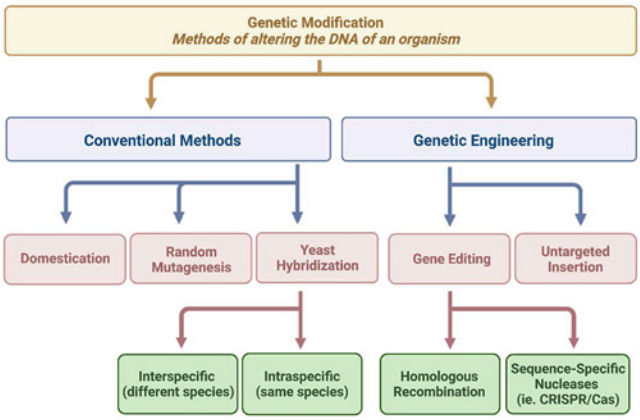

Figure 3. Common methods for genetically modifying yeast brewing strains. Genetic modification refers to methods that alter the DNA of an organism. Conventional methods include domestication, random mutagenesis, and yeast hybridization—all of which occur in nature but can also be heavily influenced by humans and used as directed breeding approaches. Genetic engineering includes gene editing methods, such as homologous recombination and the use of sequence-specific nucleases (i.e., CRISPR/Cas), to accelerate breeding approaches and make very targeted changes to the yeast genome.

and the resulting edits to the genome can truly be synonymous with those that occur in nature through traditional breeding.

Terminology

The terminology used to describe processes of genetic modification and the resulting organisms can get a little confusing. To break it down a bit further, we will put these into four categories: the process or technique of generating an organism, the type of modification, the resulting organism, and regulatory terminology. For the technique or process of generating an organism, common terms are genetically modified, genetically engineered, bioengineered, and genetic editing. Genetic modification is a broad term that encompasses any change to an organism's DNA. Thus, genetic modification can refer to random mutagenesis, breeding, domestication, or recombinant DNA techniques (Fig. 3). In contrast to the common use of the term "GMO" as a broad term referring to transgenic (described further below) organisms, scientific terminology is more specific to the method that was used to introduce the genetic change or the type of change that was introduced. For example, more specific terms like genetically engineered or genetic editing describe the technique that was employed. "Genetically engineered" can broadly refer to use of recombinant DNA techniques and the insertion of DNA, and "genetic editing" refers to specific use of homologous recombination, ZNFs, TALENs, and CRISPR-Cas nucleases for targeted DNA changes.

For the type of modification, cisgenic and transgenic refer to whether the genetically engineered organism could be achieved through conventional breeding approaches or not. Several examples of modifications are included in Figure 4 to help distinguish between the terms cisgenic, intergenic, and transgenic. Cisgenic organisms incorporate DNA from a sexually compatible organism. An example of this would be the introduction of a naturally occurring loss or gain of function mutation in a particular gene from one *S. cerevisiae* to another or the introduction of a gene from a closely related *Saccharomyces* species that could otherwise be achieved through conventional breeding methods. Intergenic is similar to cisgenic in that the introduced DNA is from a sexually compatible organism, but this DNA is combined with cisgenic regulatory sequences (ex. promoter or terminator) that alter the expression of the inserted DNA. Transgenic organisms incorporate DNA from a sexually incompatible organism.

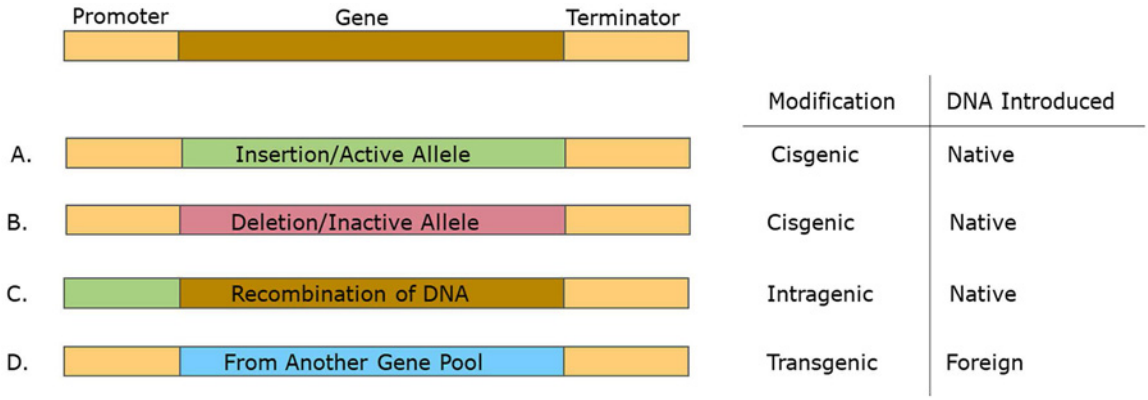

Figure 4. Examples of cisgenic, intergenic, and transgenic modifications. Cisgenic modifications include the insertion or deletion of DNA or another allele of a gene (active or inactive) from a sexually compatible organism (**A–D**). Intergenic modifications are cisgenic changes but involve recombination of regulatory sequences (ex. promoter and terminator) that results in changes in expression of the introduced DNA (**C**). Transgenic modifications include the introduction of DNA from sexually incompatible organisms (**D**).

There are several examples of transgenic brewing strains that express a bacterial, fungal, or plant enzyme to confer a new industrial phenotype (26).

The resulting organism can be referred to as a GMP, genetically engineered microbe, genetically engineered yeast, or bioengineered—all of which can accurately describe genetically engineered brewing strains depending on the context of their use. The terms GMO and bioengineered specifically refer to transgenic organisms.

Finally, regulatory terminology is highly influenced by the governing body. In the United States, the Food and Drug Administration (FDA) and USDA have made a more recent transition from referring to genetically engineered organisms as GMO to bioengineered. The definition of bioengineered is an organism that has been generated through the use of recombinant DNA (rDNA) methods and has introduced DNA that could not be achieved through conventional breeding (transgenic). Internationally, GM or GMO remain the more widely used regulatory terms.

Regulatory

Genetically engineered organisms have been part of the consumer industry since 1982 when the FDA approved the use of a bacterium that produced pharmaceutical-grade human insulin, a revolutionary development that improved production and costs. Anticipating how these technologies could revolutionize various industries, in 1986, the U.S. government developed the Coordinated Framework for the Regulation of Biotechnology (updated further in 1992 and 2017), which established an organized effort for regulating GMOs between the FDA, U.S. Environmental Protection Agency (EPA), and the USDA, laying the groundwork for safety standards and proper assessment of modified organisms. Calgene's Flavr Savr™ tomato was the first food product approved for commercial production by the USDA in 1992 and was approved for consumers in 1994 by the FDA. Since then, a wave of genetically modified agricultural products has been accepted into the food, animal feed, and processing industries, many of which have improved cultivation and crop yield due to traits such as herbicide or pesticide resistance. Those traits have been engineered into the primary global crops of corn, soy, cotton, and canola. According to a 2019 ISAAA (International Service for the Acquisition of Agri-biotech Applications) report, biotech crops have increased 112-fold since the mid-1990s, with an accumulated 6.7 billion acres of commercialization by 29 countries and importation by 43 additional countries (15).

Some considerations have been made to international frameworks regarding the oversight of GMP, as the World Trade Organization and the CPB have attempted to provide appropriate risk assessments in the production, handling, labeling, and use of GMOs. However, diverse socioeconomic and political views have made universal consistency challenging. As a result, many countries have adapted their own laws and requirements regarding the assessment of new GMO products and subsequent labeling. The United States relies on the FDA for proper risk assessment of novel products targeting the pharmaceutical, food, animal feed, and cosmetic industries, working closely with the EPA and USDA when necessary. The FDA sets the safety standards for GM foods, feeds, and ingredients, assessing the risks for human, plant, and animal health, as well as environmental safety, throughout production, processing, storage, and application of products.

The FDA primarily uses the GRAS (Generally Recognized as Safe) review process, which is the designation for food additives that are considered safe under their intended use and, therefore, are exempt from premarket review and approval under the Federal Food, Drug, and Cosmetic Act. The GRAS conclusion may be "self-affirmed" by the manufacturer, reviewed by a scientific panel, and/or submitted as a GRAS notification to the FDA. In all instances, the GRAS conclusion is based on scientific procedures and requires the same quantity and quality of scientific evidence as would be required to obtain approval of any food additive. A thorough evaluation of the GMO is documented in a dossier that provides extensive descriptions of how the product was developed, how it will be used, how it will be manufactured, a history of safe use, and dietary exposures of any byproducts or coproducts from both manufacturing and industrial processing. The dossier should include (but not be limited to) scientific data regarding the toxicity and allergenicity of the organism and its resulting products, confirmation of the absence of antibiotic genes (if used during the process), inactivation studies, and confirmed genetic stability. It is important to note that under the GRAS qualification, for foods and food ingredients, the product is considered FDA compliant if the FDA does not have concerns about the reviewed documentation, but it is not considered "FDA Approved," as that designation is reserved for product health claims.

It is only recently that GM-containing food products require labeling in the United States, as the National Bioengineered Food Disclosure Standard (NBFDS) has provided a national mandate to food manufacturers, importers, and other entities that label foods for retail to disclose information about bioengineered foods and ingredients by 2022. However, as outlined in the NBFDS, "distilled spirits, wines, or malt beverages as defined by the Federal Alcohol Administration Act (FAA Act) are foods under the Federal Food, Drug, and Cosmetic Act (FDCA), but are not subject to the NBFDS because they are subject to the labeling provisions of the Federal Alcohol Administration (FAA) Act rather than the labeling requirements of the FDCA," indicating that categorial beverages that use bioengineered ingredients do not have to label their products. However, it is important to note that this exemption is subject to change as the labeling laws start to take effect, and the standard also indicates that "alcoholic beverages not subject to the labeling provisions of the FAA Act, such as wines with less than 7% alcohol by volume and beers brewed without malted barley and hops, would be subject to the NBFDS."

Despite the evolving regulatory jurisdictions, bioengineered products continue to enter the consumer marketplace as cost and sustainability needs grow. Just as new GM agricultural products generated debate in the early 1990s, the increased prevalence of GM animal and microbial consumer-based products is rapidly growing, as meat alternatives, probiotics, feed additives, and brewing yeast have inevitably sparked similar conversations. Yet, thanks to the decades of safe use of GM agricultural products, the regulatory process has established a stringent set of guidelines to reduce health and environmental risks associated with any new product, and as is the goal of these regulatory governances, it is similarly important that the consumer continue to hold producers and stakeholders to high standards and accountability to ensure health and environmental safety are top priorities when introducing new products.

Impact on the Brewing Industry and Preparation for the Future

As previously discussed, genetic engineering in the brewing industry is not a new concept, but it is one that revolves around the use of scientific terminology. A 1978 article on yeast genetics in the brewing industry spells this out as "many scientists believe that scientists had better sound scientific—the more scientific the better. They are only too delighted when their jargon renders their field incomprehensible to outsiders, enabling them to cultivate the impression that only they—the experts—can understand the deep mysteries involved. This does not have to be the case: Science does not have to be incomprehensible" (26).

The fact that the brewing industry was discussing yeast genetics as far back as the 1950s (17) demonstrates that, like many new technologies, there is a gap between "discovery" and "practice." The first field trials for transgenic corn were seen in 1991, about 11 years after the first commercially available genetically engineered product (insulin) was produced (4). The first reported genetically engineered yeast strain (*S. cerevisiae*) occurred in 1978, with the 1980s and 1990s seeing a flurry of scientific articles on research on engineered brewing yeasts and barley (21).

The first commercial bioengineered yeasts were available as early as 1994, as a brewing yeast engineered with the STA2 glucoamylase gene and copper resistance was approved in the United Kingdom, followed by approval in Japan (2001) of a self-cloned (cisgenic) sake yeast for increased ethyl caproate production to increase the "green apple" aroma. The wine strain ML01 was approved in the United States (2003) and Canada (2006) with introduction of the *Schizosaccharomyces pombe* malate transport gene and the malolactic gene from *Oenococcus oeni* to eliminate or reduce the malate to lactate conversion time. In the same year, the ECMo01 wine strain with overexpression of the native DUR1,2 urea amidolyase gene, targeting the reduction of the carcinogen ethyl carbamate, was approved in the United States and Canada. Despite the commercial and regulatory precedence of these bioengineered microbes, none of the strains ever gained commercial traction. This was primarily due to limited perceived consumer acceptance, as well as limited use in their respective industries.

The positives of genetic engineering are not without rigorous debate on innovation and safety concerns. Fortunately, we now have more than 30 years of extensive research since the advent of these initial GMOs, making the agricultural products, in particular, one of the most meticulously evaluated and tested products in human history. The National Academies of Sciences, Engineering & Medicine released a decisive report in 2017 with more than 20 scientists reviewing over 20 plus years and more than 900 studies/publications assessing the scientific basis of GE crops and associated human health and safety, finding "no substantiated evidence of a difference in risks to human health between current commercially available GMO crops and conventionally bred crops" (19). Extensive health and safety research on GE products and the elucidation of terminology around these technologies have improved consumer education and helped consumers overall to make their own informed decisions (4). Consumer acceptance is also aligning with the sustainability points raised by GE, as many countries and regions (including the European Union) appear to be gaining increased acceptance of GE foods in general (8,26).

Today, the paradigm is changing. A wider general acceptance and understanding of GE technology, combined with rapid adoption of specialized genetically modified brewing yeasts, have opened up discussion and visibility of research in other brewing ingredient sectors. Genetic engineering has been a tool also used by barley scientists to study disease resistance and expand the limited genomic base of barley for crop improvements (18). This research illustrates how genetic engineering can be considered a tool to address the challenges of a changing climate, with cereal researchers using technologies such as CRISPR to "focus on the mitigation of climate change effects, pathogen resistance, abiotic stress tolerance, improved yields and nutritional quality" (26).

The world, and indeed the whole of the brewing industry, is now catching up to the environmental reality that agriculture producers have been facing for the past several decades. With the rapid increase in the global population and the rising challenges of climate change, biotechnology offers significant advantages that may be crucial to sustainability. While disease- and drought-resistant cereal crops show overt connections to environmental remediation efforts, genetically engineered yeasts also provide tangible sustainable benefits to brewers, including improvements in product quality, production efficiencies, and access to new avenues of product innovation (24).

Final Thoughts

As Dunn et al. (6) noted, the brewing industry has historically been the impetus for scientific progress globally. Genetically engineered yeasts are another tool in the brewer's ever-expanding toolbox that can aid in improved production demands, reduce costs, and perhaps improve sustainability demands of the current period. A yeast that expresses pineapple aroma will not forever replace a pineapple, but for those breweries in the Cots-

wolds, where pineapples are not grown, these yeasts offer a sustainable opportunity for our industry to reduce transport, labor, and the food costs of using a nonindigenous fruit or hops, and, thus, cut their carbon footprint among others. In 1990 Charles Lieberman took on the task of addressing the persistent challenges the brewing industry faced (notably effective CO_2 utilization and yeast management). Not neglecting the role of genetic engineering, he wrote of the yeast as the "perfect employee" (16).

THE BREWERS' YEAST

Those microscopic beings
We fondly call our yeast,
When in brewery wort they're pitched,
Enjoy a luscious feast.
It's odd when they are finished
There're few of them deceased;
What's even more amazing—
Their numbers have increased.

Though trillions of these beings,
We simply term them "yeast,"
Which seems as wrong as saying
A "goose" when we mean "geese."
We think of them as animal,
A vegetable or beast—
Confusing bits of knowledge
Most difficult to piece.

Sex, too, is disconcerting
About our precious yeast;
Should we call the young 'uns "nephews"
Or settle for just "niece"?

Nutritious little buddies,
They tend toward the obese;
But to we careful brewers
They are our "Golden Fleece."

Symmetric tiny ovals,
They hardly show a crease.
No dorsal, ventral, head or tail;
They don't know west from east.
Like cultures and philosophies
In Rome or Athens, Greece,
They're solids and have substance,
But known by gas release.

Strains and many characters,
As found in "War and Peace,"
We save the more desirable
And discard those liked least.
Though motives may be selfish,
We'll keep them well policed,
'Cause if they'd give up working
Our jobs would also cease.

—C. E. Lieberman

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