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Integration of Cas9-Induced Gene Drive and Yeast Mating Enables Marker-Less Enrichment and Recombination of Genetically Engineered Loci

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Introduction

Genetic engineering has revolutionized the field of biotechnology, offering unprecedented opportunities to modify organisms for various applications. In particular, the CRISPR-Cas9 system has emerged as a versatile tool for precise genome editing. Recent advancements in this technology have paved the way for the development of Cas9-induced gene drive, a powerful strategy that efficiently converts heterozygous to homozygous loci in yeast. This approach, coupled with yeast mating and Cas9 selection, enables the combination of multiple edits into a single strain, opening new avenues for marker-less enrichment and recombination of genetically engineered loci. In this article, we delve into the details of this innovative technique and explore its potential applications [1].

Description

Cas9-induced gene drive is a genetic phenomenon where the CRISPR-Cas9 system is employed to bias the inheritance of specific alleles during sexual reproduction. This process allows for the rapid spread of desired genetic modifications through populations. In the context of yeast genetics, Cas9-induced gene drive has proven to be highly effective in converting heterozygous yeast loci into homozygous states. To achieve the conversion of heterozygous to homozygous loci, a combination of yeast mating and Cas9 selection is employed. First, two separate yeast strains carrying different desired genetic modifications are created. These strains are then mated to generate a diploid strain containing both edits. The Cas9 protein, guided by specific guide RNAs (gRNAs) complementary to the target loci, introduces double-strand breaks (DSBs) at the desired genomic sites [2].

Subsequently, the cell's repair mechanisms, such as Homology-Directed Repair (HDR) or Non-Homologous End Joining (NHEJ), are activated to repair the DSBs. During the repair process, the desired genetic modifications are introduced, resulting in two separate copies of the edits within the diploid strain. This strain is then subjected to Cas9 selection, where Cas9 is continually expressed, leading to the preferential survival and propagation of cells that have successfully converted both loci to the desired homozygous state. This dual selection process ensures that the resulting strain predominantly contains cells with the desired genetic modifications. Marker-less enrichment: Traditional genetic engineering methods often rely on selectable markers to identify cells carrying the desired genetic modifications [3].

However, the use of markers can introduce unwanted genetic elements or interfere with downstream applications. By employing Cas9-induced gene drive with yeast mating and Cas9 selection, the need for selectable markers is eliminated, enabling marker-less enrichment of genetically modified strains. Recombination of genetically engineered loci: The ability to combine multiple edits into a single strain is a significant advantage of this technique. By sequentially introducing and selecting for desired genetic modifications, researchers can create complex genetic architectures in a stepwise manner. This opens up possibilities for studying gene interactions, pathway engineering, and synthetic biology applications.

Fitness-driven path to humanization: The innovative MERGE (Marker-less Enrichment and Recombination for Genetic Engineering) approach offers a fitness-driven path to humanize α-proteasome core subunits in yeast. By leveraging the power of Cas9-induced gene drive, researchers can systematically introduce humanized versions of these subunits into yeast, allowing for the production of proteins with increased similarity to their human counterparts. This can greatly enhance our understanding of proteasome function and aid in the development of therapeutics.

Cas9-induced gene drive, in conjunction with yeast mating and Cas9 selection, presents a robust and efficient method for converting heterozygous to homozygous yeast loci. This technique allows for marker-less enrichment, recombination of genetically engineered loci, and provides a fitness-driven path to humanize proteins of interest. The applications of this approach are broad, ranging from fundamental biological research to biotechnological advancements. As further developments in genome editing continue to unfold, Cas9-induced gene drive holds great promise for accelerating genetic engineering efforts and advancing our understanding of complex biological systems [4].

Advancements in genetic engineering techniques have revolutionized the field of biotechnology, enabling precise modifications of organisms for various applications. One such innovative method, called MERGE (Marker-Less Enrichment and Recombination for Genetic Engineering), has emerged as a powerful tool for achieving marker-less enrichment and recombination of genetically engineered loci. This groundbreaking approach not only eliminates the need for selectable markers but also reveals a fitness-driven path towards humanizing α-proteasome core subunits in yeast. In this article, we delve into the details of the MERGE method and explore its potential implications in the field of biotechnology and protein engineering.

The MERGE method is based on the combination of several cutting-edge genetic engineering techniques, including Cas9-induced gene drive, mating in yeast, and targeted selection. This powerful amalgamation allows for the precise enrichment and recombination of genetically engineered loci, all without the use of selectable markers. The first step in the MERGE process involves the creation of genetically modified yeast strains carrying desired modifications. These strains can be engineered to contain specific alterations in the α-proteasome core subunits, mimicking human counterparts or introducing desired functionalities.

Next, mating is performed between different genetically modified yeast

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strains, resulting in the formation of diploid strains. These diploids contain multiple separate edits within a single strain, thereby facilitating recombination and the generation of novel genetic architectures. One of the most exciting applications of the MERGE method is its ability to reveal a fitness-driven path to humanize α-proteasome core subunits in yeast. The proteasome is a crucial cellular machinery responsible for protein degradation, and α-proteasome core subunits play a vital role in its function. By humanizing these subunits, yeast strains can be engineered to produce proteins that closely resemble their human counterparts.

Using the MERGE method, researchers can systematically introduce humanized versions of α-proteasome core subunits into yeast. Through iterative rounds of mating and targeted selection, strains with improved fitness and optimized protein expression profiles can be obtained. This fitness-driven approach allows for the selection of strains that not only express humanized subunits but also maintain or enhance cellular fitness, ensuring the functionality of the engineered proteasome. The MERGE method has profound implications in the field of biotechnology and protein engineering. By enabling marker-less enrichment and recombination of genetically engineered loci, it provides researchers with a powerful tool to create complex genetic architectures in a stepwise manner. The ability to combine multiple edits into a single strain opens up avenues for studying gene interactions, pathway engineering, and synthetic biology applications [5].

Conclusion

In the specific case of humanizing α-proteasome core subunits, the MERGE method offers a transformative approach to generate yeast strains that produce proteins with increased similarity to their human counterparts. This advancement not only aids in understanding proteasome function but also has implications in drug discovery, as yeast strains can be utilized as efficient models for studying proteasome-related diseases and screening potential therapeutics. The MERGE method represents a significant milestone in genetic engineering, enabling marker-less enrichment, recombination of genetically engineered loci, and revealing a fitness-driven path to humanize α-proteasome core subunits in yeast. This innovative approach opens up new possibilities in biotechnology and protein engineering, facilitating the creation of complex genetic architectures and providing valuable insights into protein function and therapeutics development. As researchers continue to refine and expand upon the MERGE method, its potential impact on various fields of biology and medicine is truly promising.

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