ers GENOMICS

Your connection to CRISPR/Cas9

BASE EDITING

AN EVOLUTION OF CRISPR/CAS9

OVERVIEW

The CRISPR/Cas9 system is a revolutionary technology that has transformed the field of genetics and genetic engineering. This technology has evolved into an even more precise and controlled method for making specific changes to the DNA sequence in what is known as base editing.

Traditional CRISPR/Cas9 systems introduce double-stranded breaks into the DNA sequence, which are then repaired by the cellular machinery through non-homologous end joining or homology directed repair. This process can result in unintended effects, such as insertions, deletions, or chromosomal rearrangements, which pose some risk as a treatment of human genetic diseases. In response to these limitations, researchers have used the framework of CRISPR/Cas9 to develop new technologies that allow for the direct conversion of one nucleotide into another (i.e., a C-G base pair is converted into a T-A base pair), without the need for double-stranded breaks. This is achieved through the use of the CRISPR/Cas9 complex (which has been neutralized of its cleavage capability) to bring a tethered specialized enzyme to a specific basepair location where the enzyme can convert that basepair into a different nucleotide.

APPLICATIONS:

In recent years, base editing has become a promising tool for the study of genetic diseases caused by specific point mutations in the DNA. It has the potential to explore and correct diseases such as sickle cell anaemia, cystic fibrosis, and Huntington's disease in cell models and as a direct therapeutic.

Some examples of how base editing can be applied are:

- Studying Gene Function: Base editing can be used to study the function of specific genes by introducing well-defined mutations into the DNA sequence. This can help researchers to understand the role of individual genes in a variety of biological processes and diseases.

- Understanding Genetic Variation: The advent of genome sequencing has identified a vast number of single nucleotide polymorphisms (or SNPs) which are variations in the genes between individuals. Base editing can be used to study the impact of these specific differences through mimicking the wide variety of naturally occurring genetic variants against a known genetic background. This can help researchers to understand the effects of genetic variation on disease risk and biological processes.

- Modelling Genetic Diseases: Base editing can be used to create disease models by introducing specific mutations into the DNA sequence that are known to cause specific genetic diseases. By using identically matched cells that differ at only this single basepair location, researchers can confidently study the underlying mechanisms of these diseases and develop new treatments.

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- Synthetic Biology: Base editing can be used in synthetic biology to engineer cells and organisms with specific functions, such as the production of useful chemicals or the degradation of environmental pollutants. This can help to develop new sustainable technologies for energy, agriculture, and environmental protection.

- Development of Biomedical Research Tools: Base editing can also be used to develop new biomedical research tools by introducing specific mutations into the DNA sequence of research organisms, such as mice or zebrafish, that result in the creation of new research models. These models can be used to study the underlying mechanisms of diseases, to test new treatments, and to develop new technologies.

MECHANISMS & DEAMINASE:

One base editing system consists of a cleavage-inactive Cas9 protein linked to a deaminase protein, complexed with an sgRNA to direct the complex to bind to a specific DNA sequence. Deaminases are enzymes that remove an amino group from a specific nucleotide in a DNA sequence, thus converting it into a different nucleotide. So once localized to a specific location, the deaminase makes contact and induces the base conversion. This type of base editing is particularly useful for the correction of specific point mutations that cause genetic diseases. The mechanism of deamination is dependent on the specific deaminase being used, but in general, it involves the formation of a transient intermediate between the deaminase and the nucleotide via the transfer of an electron or a proton from the deaminase to the nitrogen atom of the nucleotide. The resulting modified nucleotide can then be recognized and repaired by the cellular machinery, resulting in the conversion of the original nucleotide into a different nucleotide.

The most widely used deaminase, cytidine deaminase, can convert a cytosine nucleotide into a uracil nucleotide, which is then recognized and repaired by the cellular machinery as a thymine nucleotide. This allows researchers to convert a C-G base pair into a T-A base pair, thus introducing a highly specific change in the DNA sequence.

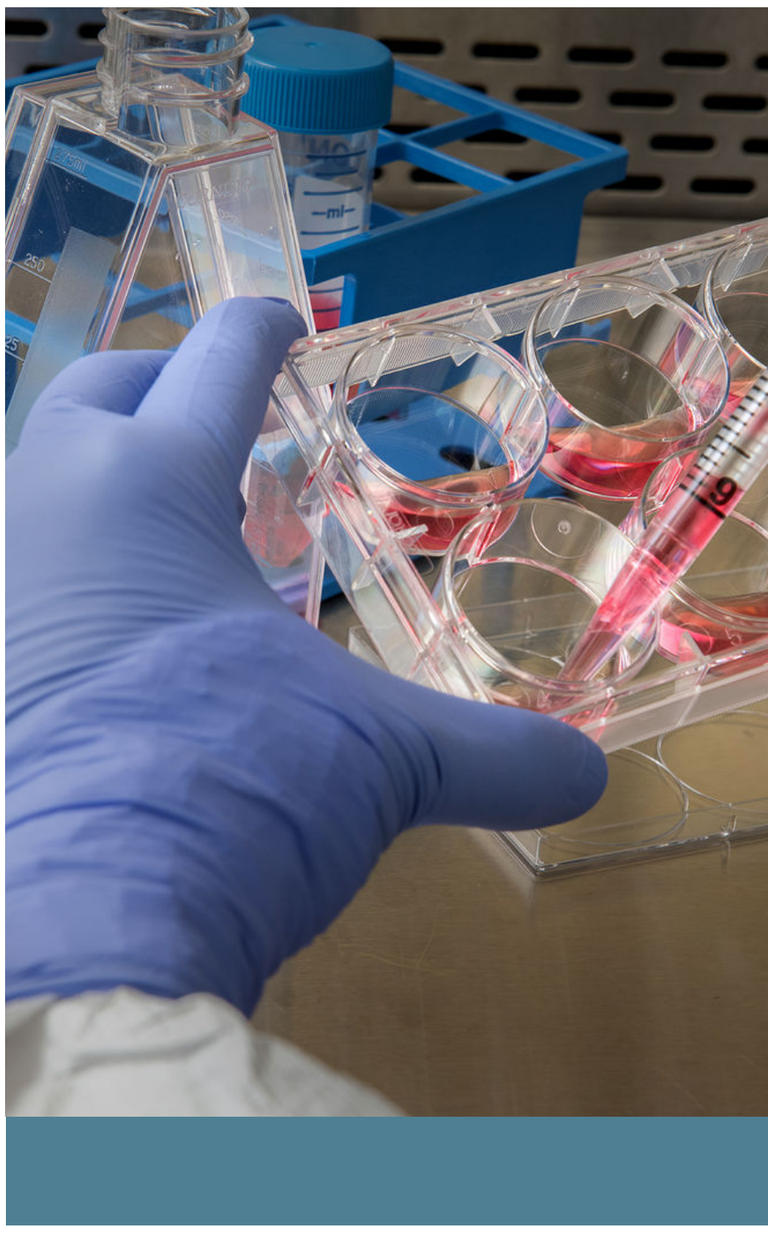
Adenine deaminase can convert an adenine nucleotide into an inosine nucleotide, while the guanine deaminase can convert a guanine nucleotide into a xanthine nucleotide. These enzymes have different specificities and efficiencies and can be used to introduce different types of changes in the DNA sequence.

The use of deaminases for base editing has certain advantages over traditional CRISPR/Cas9 systems. Base editing is much more precise and does not introduce double-stranded breaks into the DNA sequence. This reduces the risk of unintended effects, such as insertions, deletions, or chromosomal rearrangements, which are associated with the introduction of a double-strand break. Additionally, due to the constraints placed on the targeting of a base editing complex (it must bind the intended sequence first and THEN the specific basepair target for the deaminase must be in the precisely correct location) base editing has lower off-target potential compared to traditional CRISPR/Cas9 systems where the initial binding event itself triggers cleavage. This can be an important safety consideration for the treatment of certain genetic diseases.

Deaminases allow researchers to change specific codons in a DNA sequence, which in turn can result in different amino acids being produced during translation. This opens up the possibility of correcting mutations that cause genetic diseases, as well as altering the expression of specific genes.

DISEASE MODELLING:

Another important aspect of base editing is its use in introducing specific disease related mutations (such as human cancer associated mutations) into animal and cell models, which can be used to study the effects of these mutations on various physiological processes. Researchers are using base editing to introduce mutations into cells and animal models to study cancer progression and to test new drug candidates. This enables researchers to better understand the underlying mechanisms of these diseases, and to begin to develop new treatments. (Kim Younggwang et al.,2022)



LIMITATIONS:

For all its value in precise editing of single basepairs, base editing is still restricted to single base changes at a time. The ability to affect more than a single basepair would require multiple rounds of editing. For changes which require changes to more than a single basepair, approaches involving double-strand breaks and subsequent repair remain the most viable. Additionally... insertions, deletions, and rearrangements of DNA are not possible using the base editing approach.

CONCLUSION:

Base editing is a rapidly evolving field of genetic engineering that has the potential to revolutionize the treatment of certain genetic diseases. It is a highly precise tool that enables researchers to make very specific changes to DNA sequences, which can have significant impacts on the study of these changes across all organisms. It is an exciting time to be working in this field, and there is much potential for future breakthroughs in the years to come.

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