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CRISPR Technology

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Welcome to the world of CRISPR Technology

The clustered regularly interspaced short palindromic repeats known as CRISPR together with Cas9 protein is a genome editing technology. Scientist can edit any part of the genome by using this technique.

History

CRISPR technique was first discovered by Japanese scientists who were doing research on

bacteria. They noticed unique palindromic repeat sequences in the bacterial DNA. After decades, scientists studied these repeat sequences and find out that they were short segments of viral DNA which the bacteria use to recognise invading viruses. It was first observed in bacteria Streptococcus pyogenes as a defence system against invading bacteriophage. In 2012, Jennifer Duodna and Emmanuelle Charpentier published a research paper demonstrating that CRISPR together with a protein Cas9 could be used as gene editing tool. We can precisely cut any specific segment of DNA in the test rube by using this technique. Following this discovery, research into CRISPR accelerated rapidly.

Components

There are several key components of CRISPR technique that work together to generate a precise cut in the genome of an organism

CRISPR RNA (CrRNA)

A small RNA molecule that contains sequence commentary to the target DNA sequences.

Cas9

An enzyme that acts as a molecular scissor. It binds with target DNA sequence specified by CrRNA and cut both strands of DNA at that location.

TracrRNA

Another small RNA molecule that forms complex with CrRNA and serve as a scaffold for binding of cas9 protein and for directing the cas9 protein to the target sequence.

SgRNA

In some Crispr systems, both crRNA and TracrRNA combine to form a single guide RNA. This guide RNA not only bind with the target DNA sequence but also guide the cas9 to the target site.

Mechanism

First gRNA forms complex with Cas9 endonuclease. Then this complex attacks on the target DNA sequence containing the protospacer adjacent motif (PAM) at 5'-3' strand and Cas9 produces double stranded break (DSB) in the DNA. After damage in the DNA, cell activates the DNA repair mechanism and damaged DNA can be repaired by two methods, one is homology directed DNA repair (HDR) mechanism and second is Non-homology end joining (NHEJ) repair mechanism. In homology directed repair mechanism, cell uses a template DNA as a reference to repair the damaged DNA. We can edit DNA according to our interest by providing a template DNA to the cell itself. While non-homology end joining is an error prone DNA repair mechanism that directly ligate the broken ends of DNA without using template DNA as a reference.

Application

This technique can be used to treat genetic diseases in human by replacing faulty gene with

healthy gene. By using this technique, scientists can create animal models with specific mutations. These models help researchers to understand disease mechanism and to test potential therapies and to develop new treatments for wide range of genetic disorders.