CRISPR Technique

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The CRISPR/Cas 9 technique is one of a number of gene-editing tools. Many favour the CRISPR/Cas9 technique because of its high degree of flexibility and accuracy in cutting and pasting DNA. One of the reasons for its popularity is that it makes it possible to carry out genetic engineering on an unprecedented scale at a very low cost. How it differs from previous genetic engineering techniques is that it allows for the introduction or removal of more than one gene at a time. This makes it possible to manipulate many different genes in a cell line, plant or animal very quickly, reducing the process from taking a number of years to a matter of weeks. It is also different in that it is not species-specific, so can be used on organisms previously resistant to genetic engineering.

The term "CRISPR" stands for "clusters of regularly interspaced short palindromic repeats" and its importance was recognised with the awarding of the Nobel Prize in Chemistry to Jennifer Doudna and Emmanuel Charpentier on 7th October 2020. What is missed in the awarding of the Prize is the significant role that many others, including Virginijus Siksnys, played in helping to bring about the development of gene editing. A palindrome, like the word "racecar," reads the same forward as it does backward; similarly, in a palindromic sequence, bases on one side of the DNA ladder match those on the opposing side when you read them in opposite directions.

For example, a super simple palindromic sequence might look like this:

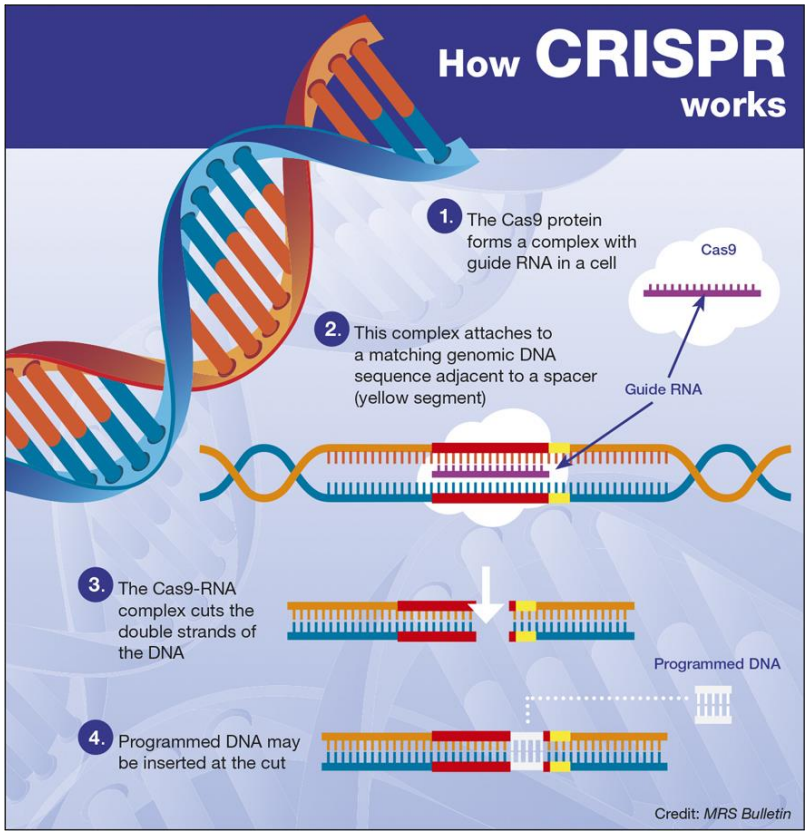
- Side 1 - GATC
- Side 2 - CTAG

CRISPR-Cas9 was adapted from a naturally occurring genome editing system that bacteria use as an immune defense. When infected with viruses, bacteria capture small pieces of the viruses' DNA and insert them into their own DNA in a particular pattern to create segments known as CRISPR arrays. The CRISPR arrays allow the bacteria to "remember" the viruses (or closely related ones). If the viruses attack again, the bacteria produce RNA segments from the CRISPR arrays that recognize and attach to specific regions of the viruses' DNA. The bacteria then use Cas9 or a similar enzyme to cut the DNA apart, which disables the virus.

Researchers adapted this immune defense system to edit DNA. They create a small piece of RNA with a short "guide" sequence that attaches (binds) to a specific target sequence in a cell's DNA, much like the RNA segments bacteria produce from the CRISPR array. This guide RNA also attaches to the Cas9 enzyme. When introduced into cells, the guide RNA recognizes the intended DNA sequence, and the Cas9 enzyme cuts the DNA at the targeted location, mirroring the process in bacteria. Although Cas9 is the enzyme that is used most often, other enzymes (for example Cpf1) can also be used. Once the DNA is cut, researchers use the cell's own DNA repair machinery to add or delete pieces of genetic material, or to make changes to the DNA by replacing an existing segment with a customized DNA sequence.

Genome editing is of great interest in the prevention and treatment of human diseases. Currently, genome editing is used in cells and animal models in research labs to understand diseases. Scientists are still working to determine whether this approach is safe and effective for use in people. It is being explored in research and clinical trials for a wide

variety of diseases, including single-gene disorders such as cystic fibrosis, hemophilia, and sickle cell disease. It also holds promise for the treatment and prevention of more complex diseases, such as cancer, heart disease, mental illness, and human immunodeficiency virus (HIV) infection.



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