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# CRISPR-CAS9: A Revolutionary Tool

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## Abstract

As we all know that DNA is responsible for the physical traits of the living organisms, we can change their characteristics by editing their DNA. This can be possible by addition or removal of specific genetic materials from the DNA. The technology used for this process is called genome editing and CRISPR-CAS is the most popular genome editing tool now-a-days. By CRISPR-CAS we can target a specific sequence of a DNA and add or remove genetic materials from that specific part according to our need. CRISPR-CAS has a guide RNA which is homologous to the DNA in which we want to change the genetic sequence, and a protein body which has two scissors to cut the targeted DNA strand. By the help of CRISPR-CAS crop improvement can be possible. It is also helpful for the treatment of many diseases.

## Introduction

Genome editing is a technology by which we can change the physical appearance of living organisms by changing the genetic materials of their DNA. By this technology we can cut the DNA strand at a desirable spot and replace other desirable sequences at that spot. We can use this technology for the prevention and treatment of human diseases. We can also use this technology on some bacteria to change their genetic sequence and to make them suitable for us. This technology can be also used on the genomic sequence of crops to accelerate their growth and to make them more sustainable to pests.

For this technology some genome editing tools are used. These tools are the most important thing for these types of operations of genome editing. One of the examples of the genome editing tools is CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat). Now-a-days, scientists, who are doing research in genome editing, use CRISPR as the tool as it is very simple and faster with more accuracy than other known genome editing tools. CRISPR-CAS9 is basically the CRISPR associated with protein 9.

## Working Principles

CRISPR-CAS9 has two parts. One is customizable RNA and the other one is a DNA cutting protein (CAS9). The customizable RNA are homologous to the sequence of the DNA that we want to edit. This RNA is also known as the guide RNA. The CAS9 protein is like a postman. This protein has 2 scissors which are its nuclease domain. This CAS9 protein carries the RNA to the target DNA. When the sequence of the RNA is matched with the sequence of target DNA the scissors of the cas9 protein cuts the DNA strand. As DNA is very essential for cells, the cellular systems now activate to repair the cut part of the DNA. There are two repair mechanisms in

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the cell. One is NHEJ and the other one is HDR.

NHEJ is a predominant type of mechanism found in the case of eukaryotes cells. In this nucleotide are randomly inserted and removed in the DNA. By this the gene sequence is altered and not able to produce the same protein as previous. By this the gene becomes nonfunctional.

HDR (Homology Directed Repair) is very less efficient in eukaryotes. It copies the sequence of sister chromatin to repair the broken DNA perfectly by a donor template. This type of mechanism is very rare.

By using CRISPR-CAS9 any desirable gene can be knocked out. By using CRISPR-CAS9 we can target multiple genes at a single time. For this we must design multiple guide RNA.

As by using CAS9 we can break the two strands of DNA, we can also engineer the CAS9 in such a way that it has only one scissor instead of two. This engineered CAS9 is known as Nickase CAS9. This Nickase CAS9 will break only one strand of the targeted DNA. CAS9 has two important properties. One is binding to the exact position where the guide RNA directs (target sequence) and the other one is cutting the target sequence.

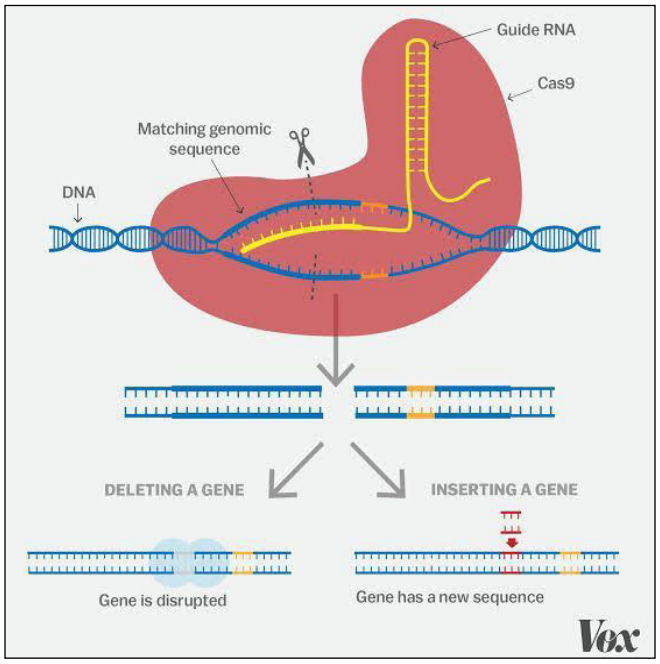



Figure 1: Working principle of CRISPR-CAS9

## Applications of CRISPR-CAS9

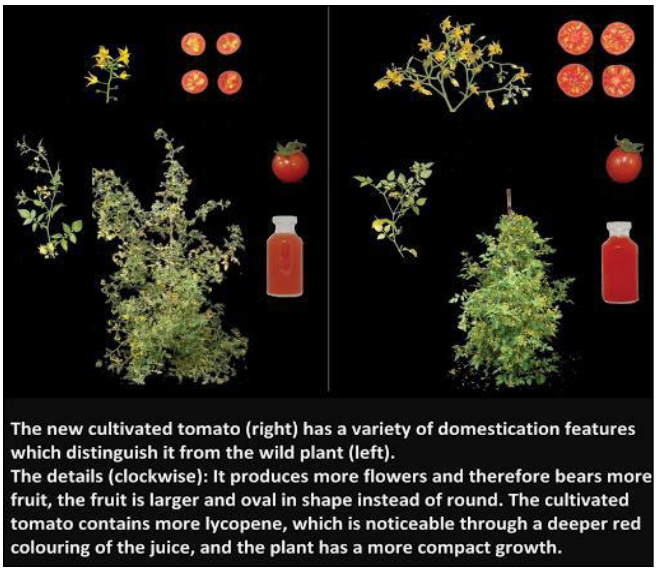
***Wild Berries***

We can domesticate the wild plants by targeting specific genes. For example, wild berries are very bushy in nature and the number of fruits is very low. By domesticating the wild berry with the help of CRISPR-CAS9 we can make them less bushy in nature and with a greater number of fruits.

Figure 2: Application of CRISPR-CAS9 on Wild berries

***Wild Tomato***

Another example of domestication of wild fruits is *Solanum pimpinellifolium* (Wild tomato). These fruits test well but the production number is very low naturally and sizes of these fruits are very small. So now, by using CRISPR-CAS9, scientists can increase their size and production number.



The new cultivated tomato (right) has a variety of domestication features which distinguish it from the wild plant (left).
The details (clockwise): It produces more flowers and therefore bears more fruit, the fruit is larger and oval in shape instead of round. The cultivated tomato contains more lycopene, which is noticeable through a deeper red colouring of the juice, and the plant has a more compact growth.

Figure 3: Application of CRISPR-CAS on *Solanum pimpinellifolium*

***Mushrooms***

Mushrooms are generally turned into brown color if we keep it for a longer time after it is cut. But now scientists can knock out the polyphenol oxidase (PPO) gene that causes the browning of mushrooms, by the application of CRISPR-CAS9. By this now the mushrooms will not have turned into brown color as rapidly as before.

***Waxy Corn***

Waxy corn is a type of corn which has a sticky texture due to the presence of a large amount of amylopectin. By the application of CRISPR-CAS9,

Figure 4: Application of CRISPR-CAS on Mushrooms

scientists are now able to increase the amount of amylopectin and stop the production of amylose to make waxy corn from the natural yellow dent corn.

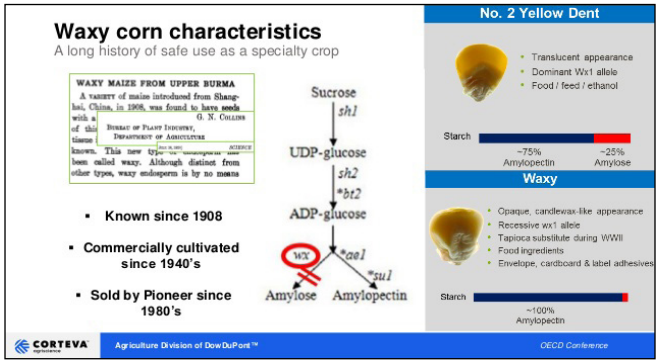



Figure 5: Application of CRISPR-CAS on Corn

## Conclusion

CRISPR-CAS is such a genome editing tool by which many kinds of things can be done related to human disease control and crop improvement. It is very simple and efficient which makes it easy to work with. This technique is still in a development phase hence there are more possible ways to use this technique for human welfare.

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