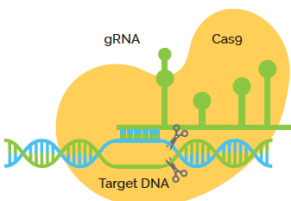


CRISPR-CAS9

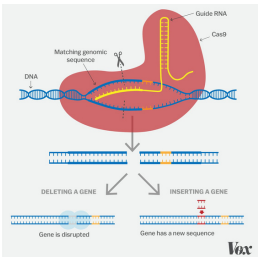
WHAT IS CRISPR-CAS9?

CRISPR-Cas9 is a gene-editing tool used to easily find and edit specific DNA sequences. A CRISPR is a specialized region of the genome and stands for clusters of regularly interspaced short palindromic repeats. These CRISPRs include nucleotide repeats, that are spaced throughout the region, and spacers comprised of pieces of DNA that are found inside the repeats. In bacteria, these spacers are made up of genomes of viruses that have previously infected the bacteria. When the same virus attacks again, part of the CRISPR is transcribed into crRNA, or CRISPR RNA, consisting of a nucleotide repeat and a spacer (What Is CRISPR?, 2020) . This allows the bacteria to remember the virus so that it can better defend itself from the attack. Cas9 is the protein associated with the CRISPRs. The Cas9 protein is an enzyme that works like a pair of “molecular scissors” to cut foreign DNA. It binds to crRNA and trans-activating crRNA, or tracrRNA, and is guided to its target site, where it cuts both strands of DNA. To ensure that Cas9 only cuts at its target, PAMs, or protospacer adjacent motifs, sit next to the target site to signal to the Cas9 where it should cut.



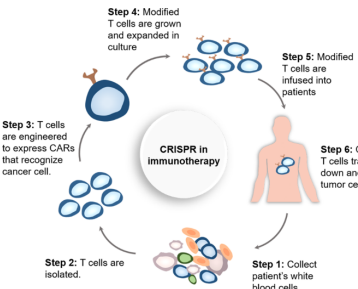
HOW IS IT USED?

The CRISPR-Cas9 mechanism can be used to edit genomes by changing the nucleotide sequence of crRNA to get it to bind with a target made of complementary DNA or by combining crRNA and tracrRNA to crate a guide RNA (What Is CRISPR?, 2020). The guide RNA signals to the CAS9 to cut the DNA, then the cell’s natural repair mechanisms turn on to repair the DNA using existing DNA as a template. This means that scientists can provide their own DNA sequence that will be ligated with the rest of the DNA, thereby editing the genome.



MEDICAL APPLICATIONS

Researchers around the world have been testing the ability of CRISPR-Cas9 to treat diseases in humans. In 2016, the first in-human clinical trial of CRISPR-Cas9 was carried out in patients with advanced metastatic non-small-cell lung cancer (Lu et al., 2020). Chinese researchers, led by oncologist Dr. You Lu, removed immune cells from the patients’ blood and used CRISPR-Cas9 to disable the gene that codes for the protein PD-1 (Cyranoski, 2016). Although it keeps T cells from killing other healthy cells, PD-1 also inhibits T cells from killing cancer cells (National Cancer Institute, n.d.). Dr. Lu’s group then cultivated the edited cells and injected them back into the patient. Researchers hope that the edited cells will attack the cancer cells in the patients (Cyranoski, 2016). Dr. Lu’s team reported that the CRISPR-Cas9 edited cells were detectable in peripheral blood after injection, and they concluded that the clinical application of CRISPR-Cas9 in T cells is “generally safe and feasible” (Lu et al., 2020). Since 2016, other clinical trials have launched to test CRISPR-Cas9’s ability to treat various diseases, including human immunodeficiency virus (HIV) and sickle cell anemia. However, there has not been enough data collected to determine the efficacy of CRISPR-Cas9 therapies (Ledford, 2020).



AGRICULTURAL APPLICATIONS

With this application in agriculture, it has become one of the most rapid emerging technologies in bioscience. But, mostly, CRISPR-Cas9 has become a user-friendly tool to develop a non-transgenic genome to edit crop plants to adapt with changing climate and ensure to be approved by food security. CRISPR-Cas9 has developed a sustainable and profound agricultural system in a way of improving yield, abiotic stress tolerance, enhancing resistance to diseases and pests, and a modification of plants for product quality. For example, CRISPR has affected mushrooms to prevent browning, rice to improve yielding, and citrus fruits to prevent greening. Haque et al. (2018) has explained that crop plants were modified by plant breeding methods in the past, but with these techniques, it has fallen out due to time, compatibility with the species, and the genetic variation of plants. However, with the technology of CRISPR-Cas9 that is always improving, scientists believe CRISPR-Cas9 will show a “great promise for quickly addressing emerging challenges in agriculture” (Haque et al., 2018).

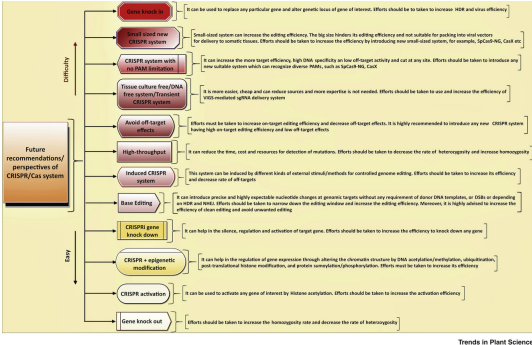
Crop	Target gene(s)	Target trait(s)/stress	References
Avocado	PAI, and LOR	Anthracnose disease resistance	Shi et al., 2017
Avocado	PaNPR2 and PaNPR4	Phytophthora cinchonum resistance	Starker et al., 2015
Banana	MaSWEET1a, MaSWEET1b, MaSWEET1c, MaSWEET1d, MaSWEET1e, MaSWEET1f, MaSWEET1g, MaSWEET1h, MaSWEET1i, MaSWEET1j, MaSWEET1k, MaSWEET1l, MaSWEET1m, MaSWEET1n, MaSWEET1o, MaSWEET1p, MaSWEET1q, MaSWEET1r, MaSWEET1s, MaSWEET1t, MaSWEET1u, MaSWEET1v, MaSWEET1w, MaSWEET1x, MaSWEET1y, MaSWEET1z, MaSWEET1aa, MaSWEET1ab, MaSWEET1ac, MaSWEET1ad, MaSWEET1ae, MaSWEET1af, MaSWEET1ag, MaSWEET1ah, MaSWEET1ai, MaSWEET1aj, MaSWEET1ak, MaSWEET1al, MaSWEET1am, MaSWEET1an, MaSWEET1ao, MaSWEET1ap, MaSWEET1aq, MaSWEET1ar, MaSWEET1as, MaSWEET1at, MaSWEET1au, MaSWEET1av, MaSWEET1aw, MaSWEET1ax, MaSWEET1ay, MaSWEET1az, MaSWEET1ba, MaSWEET1bb, MaSWEET1bc, MaSWEET1bd, MaSWEET1be, MaSWEET1bf, MaSWEET1bg, MaSWEET1bh, MaSWEET1bi, MaSWEET1bj, MaSWEET1bk, MaSWEET1bl, MaSWEET1bm, MaSWEET1bn, MaSWEET1bo, MaSWEET1bp, MaSWEET1bq, MaSWEET1br, MaSWEET1bs, MaSWEET1bt, MaSWEET1bu, MaSWEET1bv, MaSWEET1bw, MaSWEET1bx, MaSWEET1by, MaSWEET1bz, MaSWEET1ca, MaSWEET1cb, MaSWEET1cc, MaSWEET1cd, MaSWEET1ce, MaSWEET1cf, MaSWEET1cg, 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Banana	MaDGL1	Fusarium oxysporum f. sp. cubense (Foc) resistance	Wai et al., 2017b
Banana	Hpa1, Ptp1	Xanthomonas campestris pv. muscaneorum resistance	Tijunshi et al., 2010, 2014; Narasimhan et al., 2012
Banana	MaAPR1 and MaAPL3	Abiotic stresses (cold and salt) and Fusarium Oxysporum f. sp. cubense (Foc) Tropical Race 4 (TR4) resistance	Miao et al., 2017b
Cassava	RDM1	Xanthomonas axonopodis pv. manihoti (Xam) strain-specific resistance to XamC0158	Diaz Tello et al., 2018
Cassava	MeWRVY20, MeVIGL1898h (MeVIGL18), MeVIGL1898i, MeVIGL1898j, MeVIGL1898k, MeVIGL1898l, MeVIGL1898m, MeVIGL1898n, MeVIGL1898o, MeVIGL1898p, MeVIGL1898q, MeVIGL1898r, MeVIGL1898s, MeVIGL1898t, MeVIGL1898u, MeVIGL1898v, MeVIGL1898w, MeVIGL1898x, MeVIGL1898y, MeVIGL1898z, MeVIGL1898aa, MeVIGL1898ab, MeVIGL1898ac, MeVIGL1898ad, MeVIGL1898ae, MeVIGL1898af, MeVIGL1898ag, MeVIGL1898ah, MeVIGL1898ai, MeVIGL1898aj, MeVIGL1898ak, MeVIGL1898al, MeVIGL1898am, MeVIGL1898an, MeVIGL1898ao, MeVIGL1898ap, MeVIGL1898aq, MeVIGL1898ar, MeVIGL1898as, MeVIGL1898at, MeVIGL1898au, MeVIGL1898av, MeVIGL1898aw, MeVIGL1898ax, MeVIGL1898ay, MeVIGL1898az, MeVIGL1898ba, MeVIGL1898bb, MeVIGL1898bc, MeVIGL1898bd, MeVIGL1898be, MeVIGL1898bf, MeVIGL1898bg, MeVIGL1898bh, MeVIGL1898bi, MeVIGL1898bj, MeVIGL1898bk, MeVIGL1898bl, MeVIGL1898bm, MeVIGL1898bn, MeVIGL1898bo, MeVIGL1898bp, MeVIGL1898bq, MeVIGL1898br, MeVIGL1898bs, MeVIGL1898bt, MeVIGL1898bu, MeVIGL1898bv, MeVIGL1898bw, MeVIGL1898bx, MeVIGL1898by, 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ETHICS OF CRISPR-CAS9

Serving as an example, the use of CRISPR in humans, specifically in minors by parents/guardians for non-health related reasons presents the issue of consent in gene-editing, one of many debated issues regarding this technology. Another commonly discussed ethical topic is that those with access to this technology who decide to use this to edit "phenotypic characteristics" (such as improved performance for athletes) may be given unfair advantages that some demographics may not have equal access to. (Ayanoğlu et al., 2020) Aside from that, in editing embryos or animals, the side effects are unpredictable which may lead to difficult to resolve consequences/unwanted mutations. As mentioned by Ayanoğlu et al. (2020), there is argument that in editing animals for the purpose of organ transplants, its use may not be ethical in objectifying animals to serve for human use. Though there are presented benefits to this technology, there are many unknown risks and gray areas in regards to ethics of actually putting this technology to use.

OUTLOOK OF CRISPR-CAS9

CRISPR-Cas9 technology is rapidly evolving to alter the way we go about genetics. Although researchers are pushing for advancements in this technology, there are still some limitations to the CRISPR-Cas9 system. CRISPR is not able to edit large protein sequences, it has limited PAM sites, introduces multiple and random mutations in the genome, it has low HDR efficiency, it is hard to commercialize transgenic crops that express CRISPR-Cas9 in certain places, and it needs a specific transformation system to create mutant plants. Researchers are pushing the bounds of the CRISPR-Cas9 technology to further its efficiency and accuracy in gene editing in order to decrease the amount of limitations this technology currently poses (Current and Future Prospects for CRISPR, 2015). Future recommendations and perspectives of the CRISPR-Cas9 systems are gene knock out, CRISPR activation, CRISPR + epigenetic modification, CRISPRi gene knock down, Base editing, induced CRISPR system, high-throughput, to avoid off-target effects, tissue culture free/DNA free/transient CRISPR system, CRISPR system with no PAM limitation, small sized new CRISPR system, and gene knock in (CRISPR/Cas System, 2019).



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