A brief history of gene editing

In the late 20^{th} century a meditaerranean microbe was discovered in an estuary on the coast of Spain with a peculiar genomic architecture: palindromic loci (palindromes are the same forward and backward [like "racecar" or "oozy rat in a sanitary zoo"]) were repeated and spaced out by variable areas. Spanish microbial geneticist Francisco Mojica and colleagues decided to name these loci "CRISPR", an acronym for "Clustered, Regularly-Interspaced Short Palindromic Repeats". While the scientists knew they had stumbled upon something important (unlike eukaryotic genomes, microbial genomes are small and cannot afford large loci lacking functional roles), they had no idea of the impact this discovery would have across all of biology.

A decade later, French food scientists at a Danish dairy company (Phillippe Horvath and Rodolphe Barrangou) discovered that the CRISPR spacer regions (i.e., the sequence spacing out the palindromic regions) matched the sequences of viral genomes. They realized that these loci made up a critical component of the microbial immune system. Check out this short interview to hear Dr. Barrangou talk about this discovery: `https://www.youtube.com/watch?v=7qS2fjeVzsk`. Shortly before this breakthrough, many CRISPR-associated proteins (Cas proteins) were described, including those that had the ability to cut DNA (the "genetic scissors", one of which is Cas9).

Researchers led by Dutch scientist John van der Oost and French scientist Emannuelle Charpentier found that transcribed regions of the CRISPR loci could lead Cas9 to targeted DNA regions, facilitating the cutting of the target DNA. The two critical transcripts are called "crRNA" and "tracrRNA", and they are combined into a single molecule ("sgRNA").

Scientists from all over the world recognized the potential power in using this system as a gene editing tool. CRISPR-Cas9 could be used to target and cut a target region of DNA, and the repair mechanisms contained within the cell could be used to either knock out a gene or insert a sequence to alter its function. Many individuals and institutes raced to prove the ability to harness CRISPR for gene editing of animal DNA. Jennifer Doudna (Berkeley, U.S.A.), Feng Zhang (Broad Institute, U.S.A.), George Church (Harvard, U.S.A.), Jin-Soo Kim (Seoul, South Korea), and J. Keith Joung (Harvard, U.S.A.) all published experiments where they used CRISPR-Cas9 to edit animal DNA (including human cell culture) in 2012 and 2013. Their work led to aggressive patent disputes that carried up until 2022: Zhang was granted the patent in the U.S.A., Doudna+Charpentier (who had joined forces) in Europe and elsewhere. Doudna and Charpentier were also awarded the Nobel Prize for Chemistry in 2020.

As may be expected, this new biotechnology has not been without controversy. The potential modification of heritable (i.e., germ line) and non-heritable (i.e., somatic) DNA presents ethical concerns regarding (1) inadvertent damage to generations of human health, (2) delineations of what qualifies as an editable trait [e.g., disease vs cosmetic and where the line is drawn], and (3) accessibility and whether present gaps in quality of life between classes will increase in size. Because of this, in 2015 scientists jointly published an open letter imploring geneticists to not edit the genomes of human embryos until ethical concerns had been thoroughly address. Despite their urgings, two Chinese research groups went forward. One (led by Puping Liang later in 2015) published an article on the editing of human embryonic genomes, and another (led by Jiankui He in 2018) announced the birth of the first CRISPR-manipulated humans, three individuals with artificial mutations in the *CCR5* gene aimed to make them more resistant to HIV infection. Although the manuscript for this second study remains unpublished (essentially being blacklisted from all journals), the rippling effects from the leaked science impacted the world as much as, if not than, a peer-reviewed publication. He was sentenced to three years in

prison.

However, gene editing in somatic cells (i.e., non-heritable gene editing) is being implemented to treat genetic diseases in individuals, such as cancer, Alzheimer's, and sickle cell anemia, among others. In fact, Gene editing as a therapy for genetic disease has existed since before the discovery of CRISPR. Other techniques, including adeno-associated viruses, homing endonucleases, zinc fingers nucleass, and transcription activator-like effector nucleases were used throughout the late 20th century for gene editing. However, their precision, consistency, scalability, and affordability make each of these approaches less effective means of gene editing compared to CRISPR-CasX. We have only begun to understand how variation in the genome influences disease phenotypes, and CRISPR-based therapy will continue to grow as more discoveries are made. A future where every person's genome is sequenced to scan for disease-causing variants that can be treated via gene editing is no longer science fiction- it is just science future. And not even the distant future, the field of "precision medicine", which is individualized medicine that focuses on patients' genetics rather than just treating their symptoms, is already exploding. Precision medicine and other genomic applications will be examined in a future unit.

CRISPR visuals

CRISPR in its natural state

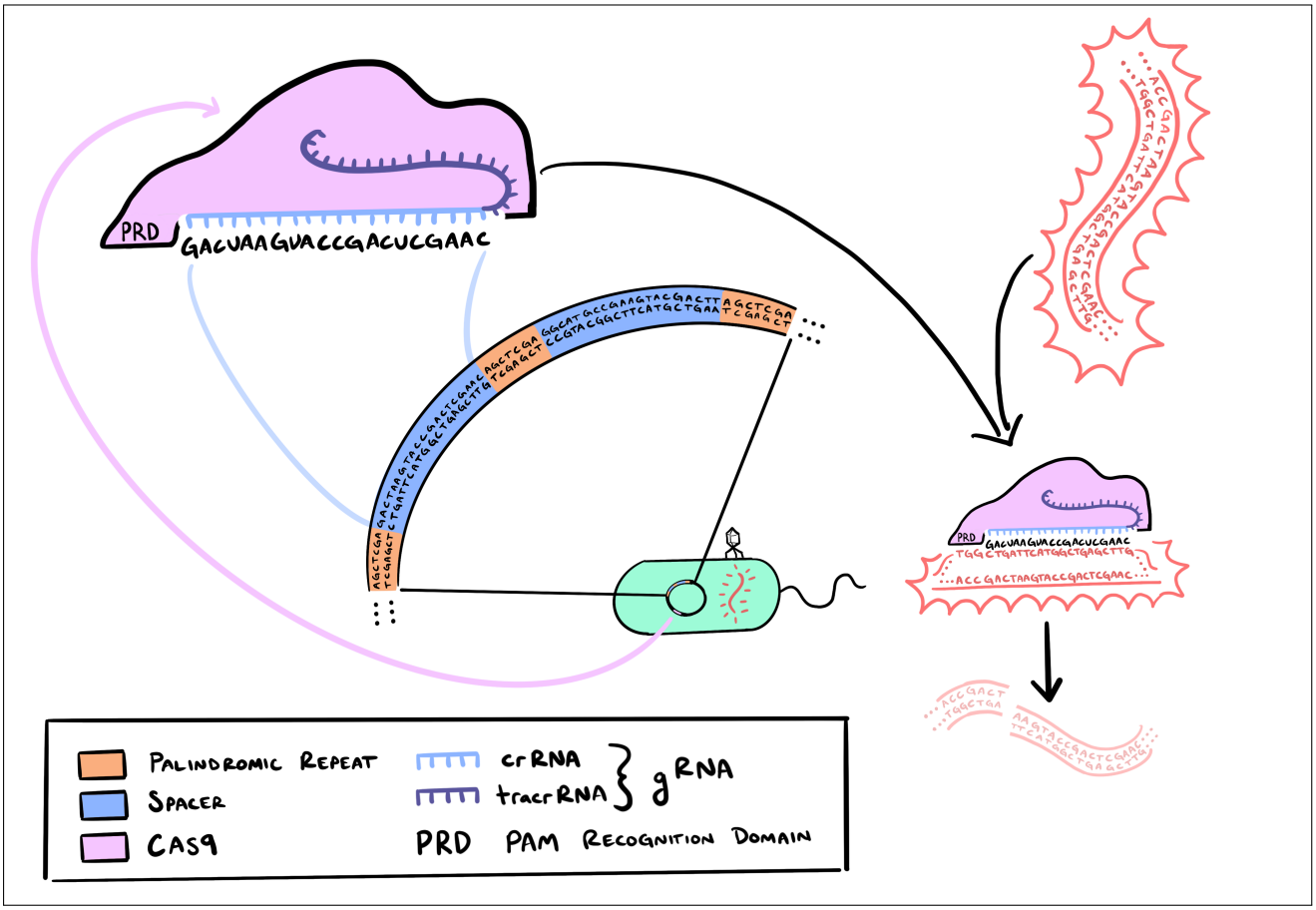

Figure 1: Visual depiction of how CRISPR-Cas9 works in the bacterial immune system to target an invading virus whose species has infected the bacterium before. The red DNA is the viral DNA. While the gene for the tracrRNA isn't shown in the bacterial genome, it is also encoded within the bacterial genome.

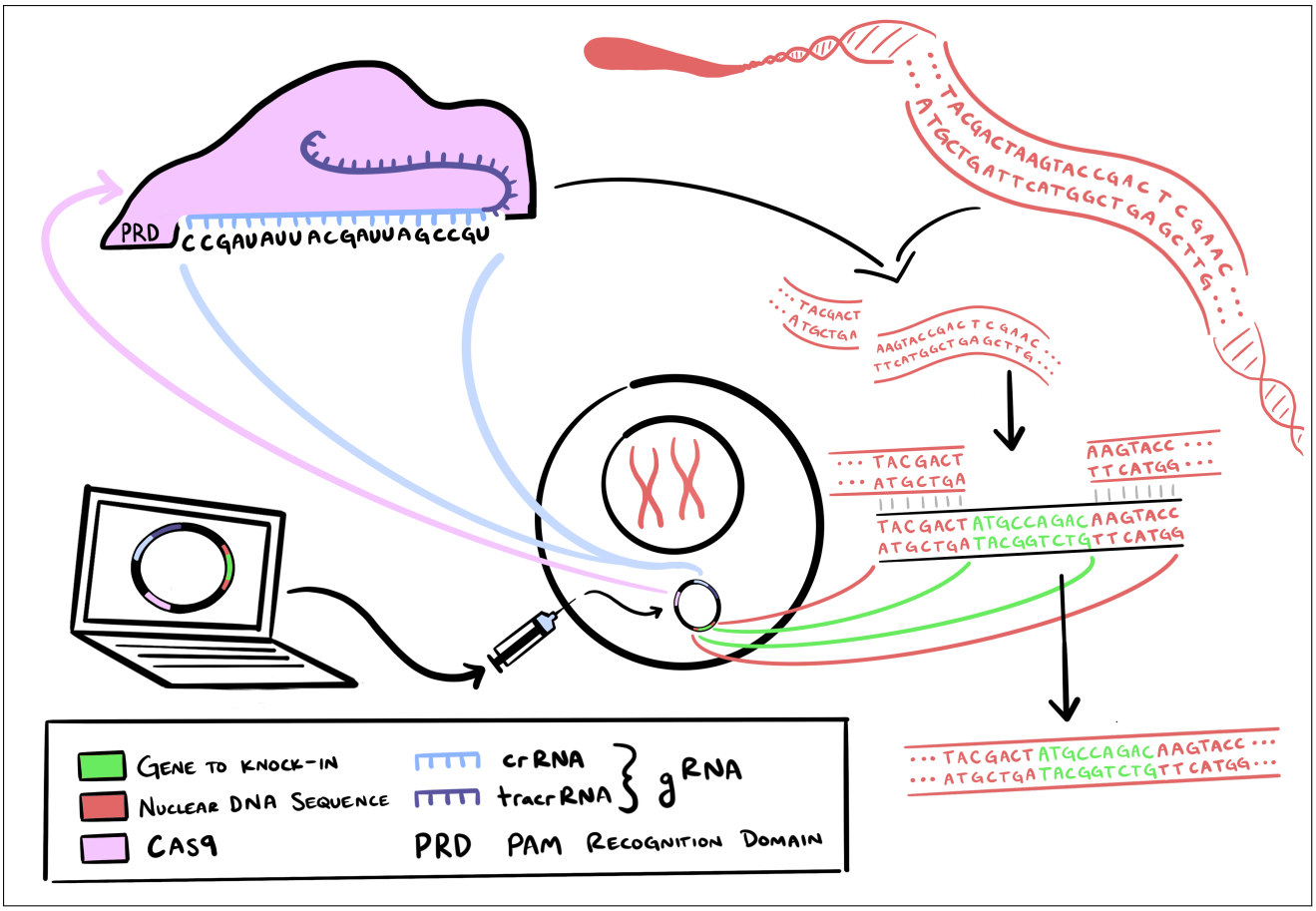

Figure 2: Visual depiction of how CRISPR-Cas9 can be used as a biotechnology tool for gene editing. A vector designed by a scientist containing crRNA, tracrRNA, and Cas9 genes along with a gene to be inserted (which is surrounded by homologous sequence of the target region) is inserted into the cell. CRISPR-Cas9 performs the excision ("cutting") and the gene is inserted into the target region using homologous directed repair.