



CRISPR-Cas9: An Innovation in Genetic Engineering

Gloriani Sánchez Marrero | Nya Perry | ENGR 0012 | University of Pittsburgh

What is Genetic Engineering?

Genetic engineering is a subsection of bioengineering focused on deliberately modifying an organism's genome. To do such a thing there is a need for genome editing tools, which allows for these manipulation of genomes. There are several different types of genome editing tool but recently the CRISPR-Cas9 system has made a significant impact in the field of genetic engineering.

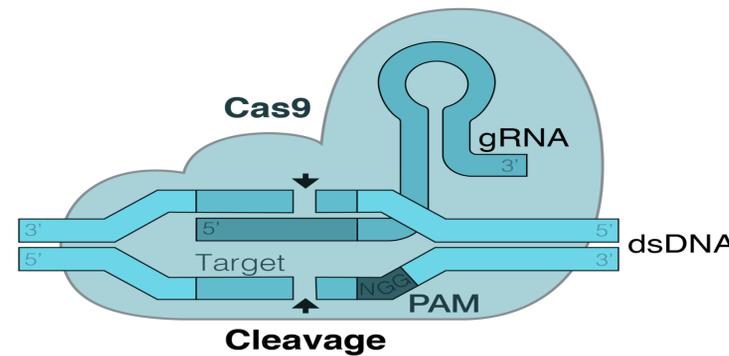
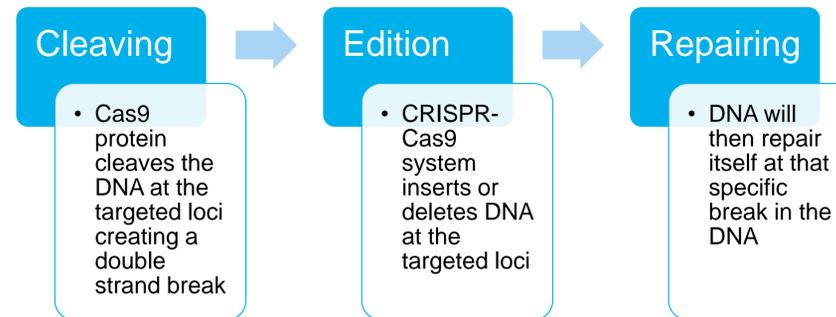
History and Development

- CRISPR-Cas9 was found in bacteria. It acts like an adaptive immune system in bacteria.
- Jennifer Doudna and her team discovered the use of CRISPR-Cas9 system as a genome editing tool.
- CRISPR-Cas9 is programmable, allowing for targeted breaks in the genome and inducing a repair by the cell.
- One important thing about CRISPR-Cas9 is its ability work on embryos.
- During Doudna's TED talk in 2012, she discussed possible ethical issues that arised with CRISPR-Cas9 and suggested a global pause on research involving CRISPR-Cas9 modifying embryos.

Genome Editing Tools

ZFNs	TALENs	CRISPR Cas9
<ul style="list-style-type: none"> • Double strand breaks at specific sites • Difficult to assemble • Low rate of off target mutation 	<ul style="list-style-type: none"> • Double strand breaks at specific sites • Used for gene knockout and to input mutations • Easier to design than ZFNs • Large size disadvantage 	<ul style="list-style-type: none"> • Can be recoded using smaller DNA segments than ZFNs and TALENS • Can simultaneously target multiple sites • Cas9 protein larger than TALENS and ZFNs

Mechanism

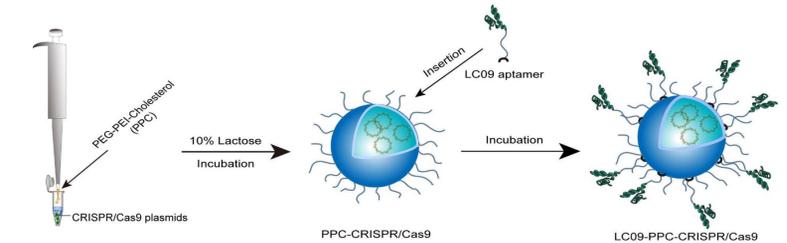


Linking *nonagouti* gene with Domestication

- Use of CRISPR in wild mice to remove the *nonagouti* gene.
- Wild mice were used because of their genetic diversity.
- Easy to identify the modified (knockout) mice based off coat color.
 - The mice without the *nonagouti* gene had a black coat color
- Behavioral test were conducted to test the domestication of the mice.
- The stay-on-hand test showed there was a significant correlation between the amount of time on the hand and the genotype of the mouse.
 - The knockout mice stayed on the hand longer than the wild mice.
- CRISPR performed with high efficiency to knock out the *nonagouti* gene
- The researchers who conducted this study believe that it is the first study to experimentally show that link between domestication and the *nonagouti* gene.

Application on Osteosarcoma

- Osteosarcoma is the most common pediatric cancer and it usually starts in the bones. The cancer cells are usually found in the ends of long bones, where new bone tissue is being created.
- In approximately 15-20% of the children the cancer will have already propagated by the time it is diagnosed.
- Current treatment only include surgery and chemotherapy.
- A recent study, utilized CRISPR-Cas9 to help destroy tumor cells.
- CRISPR-Cas9 was to target the VEGFA, a growth factor that was found around the cancer cells. The PPC capsuled CRISPR and the LC09 aptamer distributed it in the correct region.
- CRISPR-Cas9 effectively inhibited VEGFA, leaving the cancer cells with a growth factor that helped get nutrients and oxygen.



Ethics

- Main reasons for the arise of ethical issues surrounding CRISPR-Cas9:
- **Safety**
 - CRISPR-Cas9 has not been researched enough to deem it safe for public use. Off-targeting and cell mosaics occurrence has not been totally minimized. Without doing this, research involving editing embryos cannot start.
 - **Population disequilibrium**
 - Using CRISPR-Cas9 for genetic enhancement is not ethical. Further discussions on the governance of the technology need to be done before it is available to the public.

Sustainability

- Sustainability can be defined as having a technology that is capable of long-term use without ever being detrimental to the **individual** and/or the **society**.
- If we only take into account the individual, CRISPR-Cas9 is a sustainable technology because once the edition is made it is not organically reversed.
- In terms of the society, CRISPR-Cas9 is not a sustainable technology because it will favor those genetically altered, specifically those genetically enhanced. The government will need to take the necessary precautions for this not to happen.