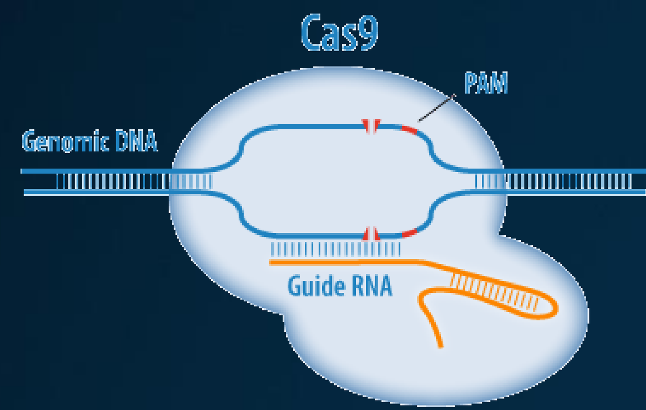
You may or may not have heard about CRISPR Cas-9. It's the latest technology in gene editing and was thrust into the limelight in March 2015. Since then it has gained much attention in the news and media. It has enabled the possibility of editing human genomes, bacteria, viruses, plants and other animals triggering many an ethical debate. For decades we have been engineering food and animals to suit our needs e.g. vegetables with longer shelf life and more aesthetic appeal. However, with CRISPR, the costs has been cut by 99% and the time taken is down to a few weeks rather than a year. CRISPR technology really is a revolutionary technology and has the potential to change humanity forever.

FOCUSING ON CRISPR

Targeting genomes specifically and modifying them to a high accuracy had been difficult to accomplish quickly and cheaply, until recently. The CRISPR-Cas9 system is an RNA guided nuclease system that can be used to manipulate and edit DNA. It is increasingly becoming the favoured method of targeted genome editing due to its simplicity, low cost and speed. With this technology we could see world hunger reduced, treat various diseases and genetically modify human embryos.

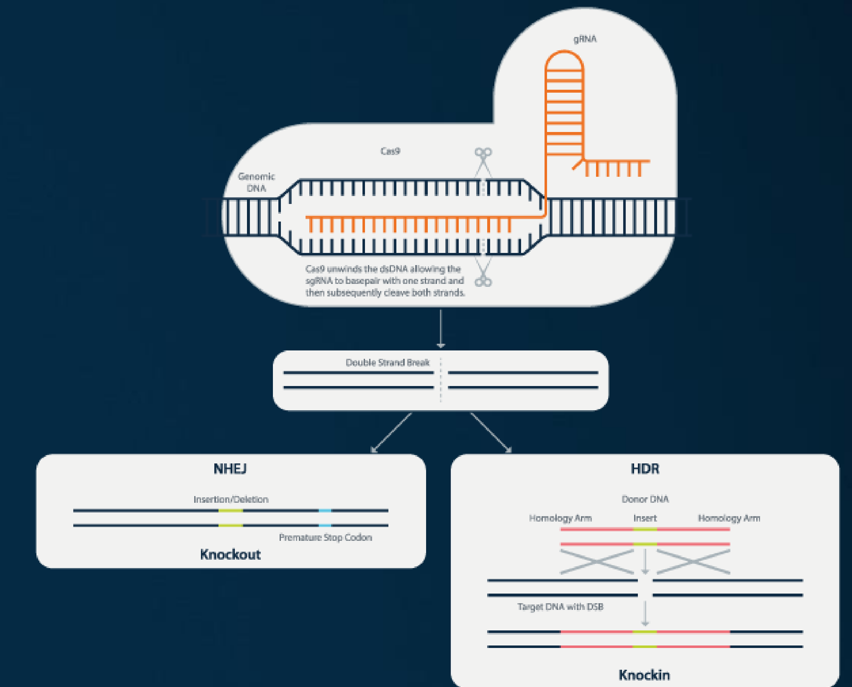
CRISPR evolved as a defence mechanism in bacteria against viruses known as bacteriophages. The system uses CRISPR (Clustered regularly interspaced short palindromic repeats) RNA as a guide to find the target DNA. The CRISPR RNA (crRNA) is made as a copy of the virus DNA. CrRNA is then used by the Cas proteins to make cuts in the DNA matching the crRNA. This cuts out viral DNA and deactivates the virus.



The Cas-9 protein is extremely accurate and precise – it acts as a DNA surgeon. The CRISPR system can also be programmed. This is what makes this technology stand out above all the others. All that you have to do is give the system a copy of the DNA you want to modify and put it in a living cell. This copy is given as a single guide RNA (sgRNA) which attaches to the target DNA. The target DNA is next to a PAM (protospacer adjacent motif) sequence which is bound by the protein in order to start cutting the DNA.

The Cas-9 protein has two sub-units known as nuclease domains. They act as DNA scissors to cut it specifically at the point specified by the sgRNA and PAM. They are known as the HNH like and the RuvC like domains. The HNH like domain cuts the strand that attaches to the sgRNA and the Ruv-C like domain cuts the other strand. A mutation of either or both of these nuclease domains can alter Cas9 activity and be advantageous in different situations.

Once the DNA is cut, host DNA repair mechanisms will attempt to repair the broken DNA. There are two main repair mechanisms; non-homologous end joining (NHEJ) and homology directed repair (HDR). The NHEJ repair mechanism is very error prone and frequently generates indel mutations, and ultimately premature stop codons. This method is used mostly when stopping a gene from working is preferred. On the other hand, if gene editing is preferred, HDR is used. In the presence of template DNA the sequence that is desired can be incorporated into the newly repaired DNA, allowing accurate gene editing.



Uses of CRISPR Cas-9 Technology

One of the many advantages of CRISPR is that it is easily accessible to anyone with a lab due to its simplicity. The technology can be modified to achieve different results. For example we can modify the Cas-9 protein so that only one of the scissor-like nuclease domains is active. This means that the protein only cuts DNA at one point. If we have two sgRNA's we can remove much larger sequences and genes. We can also inactivate both nuclease domains, meaning that the protein binds to DNA but doesn't cut it. This occupies the DNA effectively inactivating it – this is known as a repressor. These modifications are very important when it comes to studying DNA sequences and genes.

Much of the media attention has been on the DNA editing capacity of CRISPR however. Many studies have been conducted on a variety of species; from wheat crop to humans. A few examples are listed below:

- Modifying wheat plants so that they are resistant to powdery mildew, a common fungus that reduces yield by 20%
- Removing over 50% of HIV virus from live rats
- Removing lymphoblastic leukaemia and other cancers from humans
- Removing genetically inherited diseases from human embryos
- Make glow in the dark clownfish

However, these studies are only the beginning, and the technology is only in its early stages. CRIPSR technology will get better and become more refined.

Ethics regarding CRISPR

The application and potential applications of CRISPR have brought up some ethical and societal worries much like any gene editing technique. Many articles and other media outlets have stated the concerns they have with CRISPR. They feel that once we start editing human embryos this could lead to "designer babies" where traits such as eye colour and height are all pre-determined – the slippery slope argument. However, laws can be put in place to stop this happening and guidance councils already have their own regulations that have to be adhered to e.g. reproductive cloning is forbidden as well as modification of inheritable genes. There is also an argument for having the best interests of the people involved and how their quality of life will be affected. A few question to think about are:

- Is it acceptable that we can intervene with what is natural and if so at what point do we stop?
- When it comes to genetically engineering in those with a disability of future disability, does intervening mean that those individuals are less human than those who are not disabled?

Genome Editing Glossary

- DNA – The chemical made up of nucleotides that carries genetic information
- RNA – A single stranded chemical with a slightly different make up to DNA. It also carries genetic material
- Nucleotide – the building blocks of nucleic acids such as DNA
- Cas – CRISPR associated genes
- Cas-9 – A Cas protein that has two nuclease domains
- sgRNA – The RNA that guides the Cas-9 protein to the target host sequence
- crRNA – CRISPR RNA
- Nuclease - An enzyme that cleaves the chains of nucleotides
- Indel – An insertion or deletion of a sequence in the DNA
- Codon – A group of 3 nucleotides that code for a specific protein
- Stop Codon – A codon that signals to cell machinery to stop protein synthesis
- PAM – Protospacer adjacent motif
- Gene Knock-out – When a mutation/genetic engineering method which results in a gene being inactivated or "knocked out"
- Gene Knock-in – When a mutation/genetic engineering method that results in a gene substitution or insertion and it's activation
- Slippery slope – an argument in a relatively small first step leads to a chain of related events resulting in a much larger, significant (usually negative) effect