GENOME ENGINEERING USING CRISPR CAS9 TECHNOLOGY

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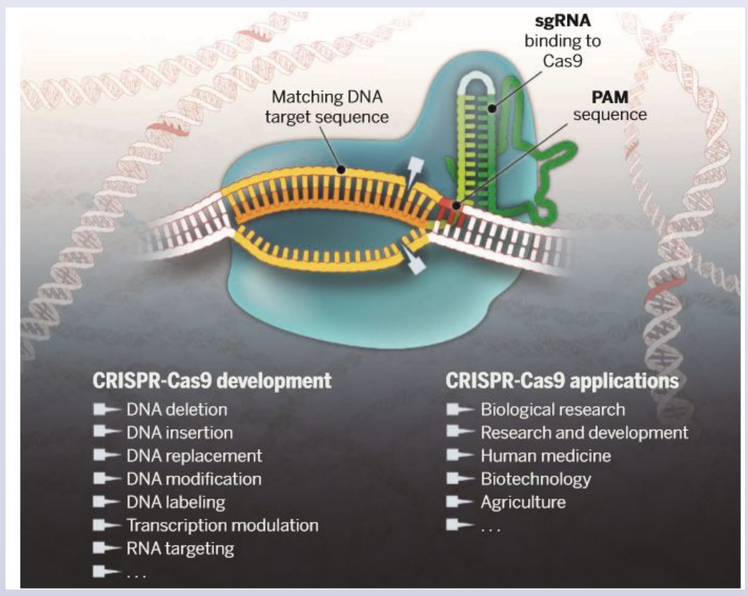
ABSTRACT

This project mainly focuses on using CRISPR-Cas9 technology in order to modify genes, specifically slicing certain amino acid sequences by using CRISPR-Cas9. The main purpose of this project is to analyze the effects of CRISPR-Cas9 on the expression of specific genes and proteins, in which E. coli competent cells are primarily used. The term "competent" refers to the cells' ability to have other genes inserted in them, hence they are "transformed". After the targeted cells have been genetically transformed using CRISPR-Cas9, those cells are analyzed with various techniques such as gel electrophoresis and flow cytometry to observe whether they have been correctly modified. Genetic modification in this project refers to plasmid insertion, hence gel electrophoresis can indicate whether the plasmids have been properly inserted or not by creating specific bands of DNA.

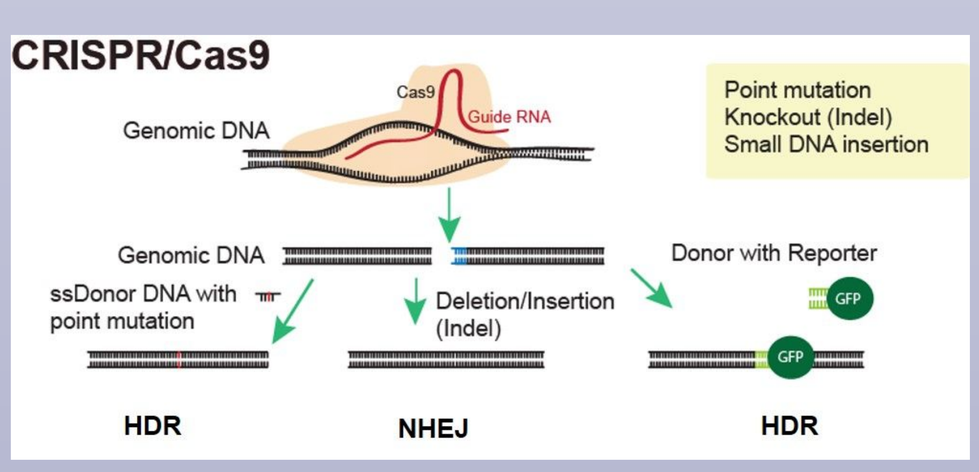


OBJECTIVES

To learn how to knock out the genes with the following methods:

- Plasmid isolation
- Culture growth
- Ligation
- Digestion
- Sonication
- Protein Purification
- qPCR/PCR
- Surface plasmon resonance

CRISPR-CAS9 TECHNOLOGY



CRISPR is short for Clustered Regularly Interspaced Short Palindromic Repeats, which refers to nucleotides, and Cas9 is the enzyme that cuts the DNA as a double stranded break. A strand of gRNA (guide RNA) is also used in order to lead Cas9 to the desired nucleotide strand for it to slice the desired strand as the gRNA will be the complementary of the targeted sequence. gRNA has 20 nucleotides that determine its target sequence in DNA. Also a protospacer adjacent motif (PAM) sequence is required for binding of Cas9 to the DNA. For example the commonly used *S. pyogenes* Cas9 requires 3'-NGG as PAM sequence and Cas9 cuts DNA 3 base pairs before this sequence. While constructing gRNA, the target sequence should be selected regarding a common exon sequence of all the transcript variants of that gene and a PAM sequence at the end. There is two major DNA damage repair mechanisms that locus will undergo after DSB. Either the ends of DNA ligate which is called non-homologous end joining (NHEJ), or a donor DNA may assist gene correction by recombination which is called homology directed repair (HDR). If a DSB ligates through NHEJ usually have some insertions and deletions (INDEL) of base pairs which is useful to knock out a gene because it creates frameshift mutations and pre-mature stop codons.

METHODS

The process of using CRISPR involves the following: plasmid design on CLC workbench, gRNA design with Ensembl and Crispor websites, digestion, ligation, transformation and purification of plasmid DNA. CLC workbench is a computer program used for bioinformatics, hence certain plasmids or nucleotides can be designed and analyzed via CLC. gRNA, as mentioned previously, is the guide RNA sequence that guides Cas9 to the desired DNA strand to make the double stranded cut. Digestion refers to the double stranded break, which creates sticky ends in DNA sequences, whereas ligation connects two pieces of DNA in order to eliminate sticky ends. Restriction enzymes, which are responsible for cutting the DNA, slice the DNA in a zigzag, which leaves a few bases unpaired. These unpaired bases are referred to as "sticky ends", which are again fused together via ligation. Transformation is the insertion of plasmid DNA into bacteria, primarily E. coli, hence making the bacteria competent. Lastly, plasmid purification is separating plasmid DNA from genomic DNA, which needs to be done in order to analyze the genes that were inserted since genomic DNA is unchanged. Transfection is the next step, which involves the analysis of CRISPR-mutant cells. Gel electrophoresis and flow cytometry are generally used for the analysis of these cells. Gel electrophoresis produces several bands of DNA which show whether or not DNA was cut and a new plasmid was inserted using CRISPR.

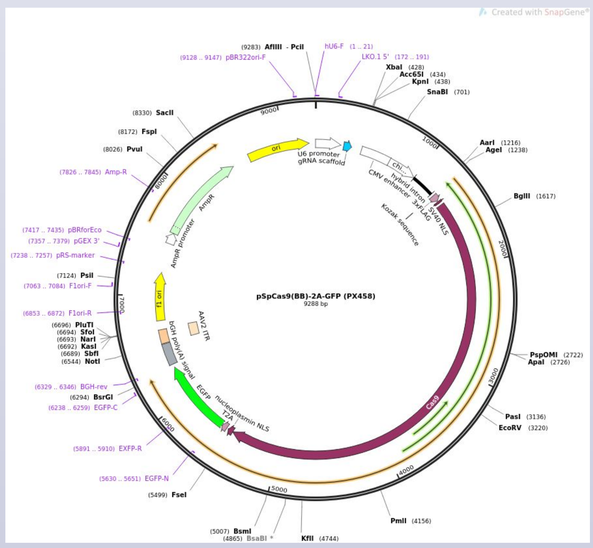


Figure 1. CRISPR plasmid

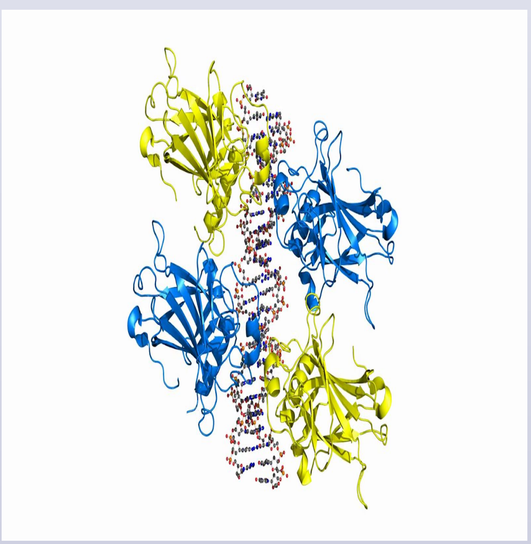


Figure 2. p53 tetramerization

CONCLUSION

In conclusion, several molecules such as p53 and GFP were used in order to understand the methodology behind CRISPR-Cas9 genome engineering and its impacts on gene and protein expression.The transformation part of the project involved inserting antibiotic resistance genes in bacteria and culturing them in that antibiotic overnight to observe whether or not the plasmid containing the resistance gene was correctly inserted. Moreover, a plasmid containing GFP was also inserted in bacterial cells via transformation and its insertion was tested by flow cytometry. The cut sites in DNA were observed by gel electrophoresis as it formed specific bands of DNA on the gel, indicating the size of DNA and hence whether the plasmid was inserted or not. Gel electrophoresis was used more for indication purposes whereas flow cytometry directly showed if the genes in the inserted plasmid were actually expressed in the cells. The results of these experiments illustrate the impacts of genetic engineering by CRISPR-Cas9 and how the expression of genes or proteins that were inserted in lab can be observed with various techniques such as flow cytometry. This implies that CRISPR-Cas9 is a powerful tool for cancer therapy and while it can lead to certain consequences in the genotypes and phenotypes of organisms, it is an intricate process in which every detail of the procedure holds great importance. The ability of transformed E. coli cells to colonize in media containing antibiotics indicates that CRISPR-Cas9 is a very significant and powerful gene editing tool that can alter the way genes are expressed and can even express new genes in organisms. This suggests that it can be used for research on tumors and cancer therapy, maybe potentially taking a step towards finding a solution. Future research ideas may include using CRISPR on not only bacterial cells but eukaryotic cells as well to compare the impacts on both types of organisms. For research on cancer therapy, on the basis of p53 in particular, more molecules can be analyzed for potential cancer causing symptoms and the effects of CRISPR on those molecules similar to p53 can be investigated.

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