Research Experiences for Teachers

In-Depth Exploration of CRISPR Technology and Recent Discoveries in Gene Editing Tools: Silencing the lacZ Gene in E. Coli using Bacteria's Natural Prokaryotic Defense Mechanisms

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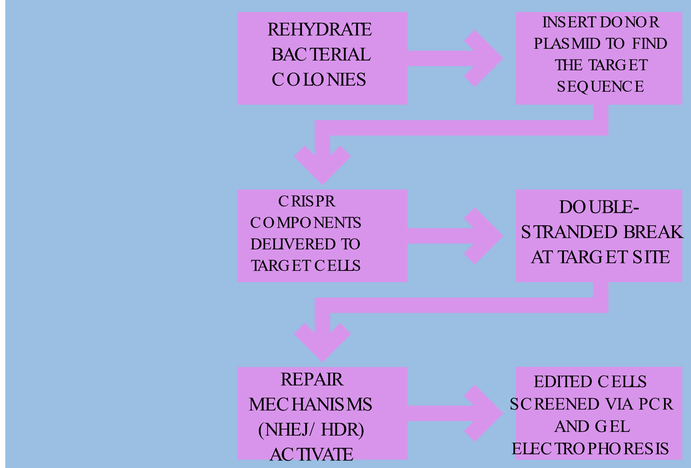
ABSTRACT

A shrinking global breadbasket threatens future generations with the hunger pains of potential starvation and malnutrition. Genetic engineers and molecular biologists are using a revolutionary genetic technology to manipulate and edit the genomes of various species of the plantae and animalia kingdoms fostering gene expression of more favorable and sustainable traits. Clustered regularly interspaced short palindromic repeats (CRISPR) DNA sequences were discovered in archaea and bacteria in 2002 during exploratory bioinformatic analyses. Genes discovered in close proximity to these CRISPR sequences were named as CRISPR associated genes ("Cas" genes). The CRISPR Cas system is a genome editing technology that allows for the insertion of beneficial genes of one species to another while "knocking out" or minimizing less desirable traits. The research conducted this summer sought to identify the processes and methodology involved in gene editing of a common gene under study as well as exploring new developments in gene editing technologies.

INTRODUCTION

The two-part technological system, known as CRISPR Cas9, uses a Cas-associated protein (Cas9) and a single guide ribonucleic acid (sgRNA), also known as a CRISPR RNA (crRNA) molecule, to make an intentional molecular cut of the DNA strand thereby activating the cell's natural repair system, either by homology directed repair (HDR) or non-homologous end joining (NHEJ). Once the CRISPR Cas complex (Cas9 enzyme and sgRNA) locates and cleaves the target sequence, the desirable genetic code is supplanted in place of the "knocked-out" gene. Genomic editing technologies like the CRISPR/Cas system rely on exploiting the natural defense mechanisms of bacterial and archaeal cell physiology. Subsequently, the rise of new bioinformatics and AI-assisted omics applications have uncovered areas for research on the use of newly discovered Cas proteins and other programmable RNA insertion sequences (IS) like seekRNA that attempts to maximize guide RNA programmability and minimize off-target results. Gene modification technologies may lead to improved human/plant health and genetic wellness.

METHODOLOGY



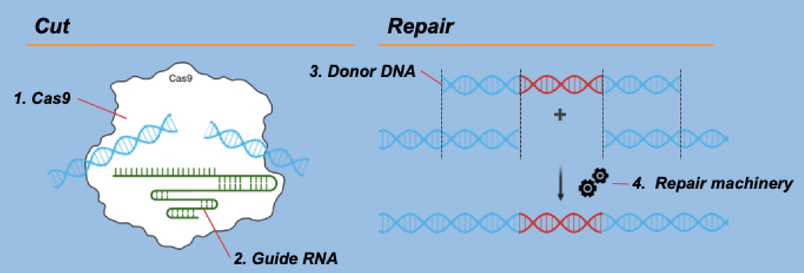


Figure 1: Diagram and CRISPR system components courtesy of Bio-Rad Laboratories, Inc.

RESULTS

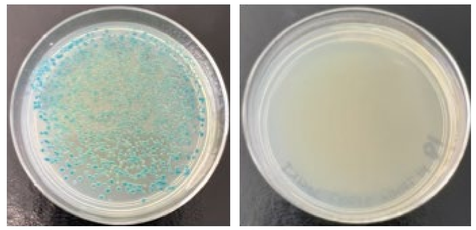


Figure 2: Plate A, grown without arabinose and containing the donor plasmid (pLZDonor), shows blue colonies due to the hydrolysis of X-gal by β-gal, indicating no double-strand breaks or repair necessity. Plate B lacked arabinose and contained the pLZDonorGuide plasmid with a single-guide RNA for Cas9 to make double-stranded DNA cuts. Without arabinose, cells could not use their repair mechanism, resulting in no colonies.

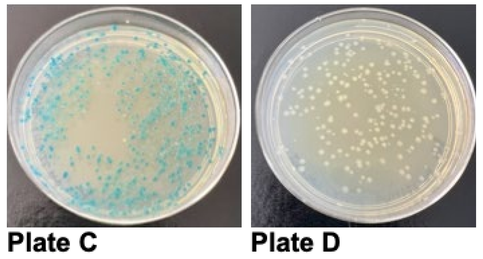


Figure 3: Plate C, grown with arabinose and containing the pLZDonor plasmid, showed blue colonies due to β-gal production from X-gal hydrolysis, indicating no double-stranded breaks and no need for repair. Unlike Plate A, Plate C had an arabinose-inducible promoter for potential repair of double-stranded breaks. Plate D, containing the pLZDonorGuide plasmid and grown with arabinose, showed white colonies due to successful knockout of the lacZ gene by the single-guide RNA and repair of DNA breaks facilitated by arabinose. The absence of blue pigment confirms the knockout.

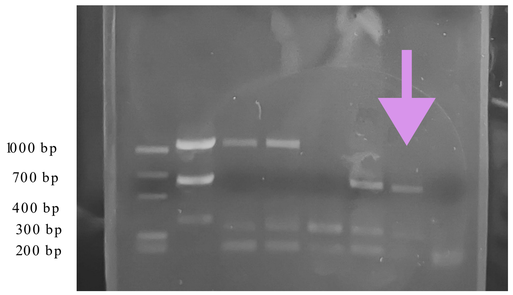


Figure 4: Gel electrophoresis results under UV light with knocked lacZ gene highlighted.

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Cas systems / Features	Class 2, type II	Class 2, type V-A	Class 2, type V-B	Class 2, type V-J	Class 1, type I
Cas protein requirement	Cas9	Cas12a (Cpf1)	Cas12b (C2c1)	Cas12j (CasΦ)	Cascade complex + Cas3
Cas protein size	1000–1400 aa	1100–1300 aa	1100–1500 aa	700–800 aa	–
RNA requirement	crRNA + tracrRNA	crRNA	crRNA + tracrRNA	crRNA	crRNA
PAM	3' PAM, G–C rich	5' PAM, A–T rich	5' PAM, A–T rich	5' PAM, A–T rich	5' PAM, A–T rich
Nuclease domain	HNH + RuvC	RuvC	RuvC	RuvC	HD
Cleavage pattern	Blunt end	Staggered end	Staggered end	Staggered end	Various
Predominant editing events	1 bp or small InDels	Multi-nucleotide deletion	4–14-bp deletion	8–10-bp deletion	100-bp–100-kb deletion
Multiplexing capability	High	High	Unknown	Unknown	Unknown
Current applications	Knock-out, knock-in, base editing, prime editing, epigenetic editing, gene regulation...	Knock-out, knock-in, gene regulation...	Knock-out, knock-in, gene regulation...	Knock-out, epigenetic editing, gene regulation...	Knock-out, gene regulation...
Applicable in promoter editing	Currently the main tool, adapt almost all tasks without efficiency consideration	An equally effective tool with clear advantages, especially in non-coding regions like promotors	Could be promising promoter editing tools and worth further exploration	Could be promising promoter editing tools and worth further exploration	A multi-component editor with limited application, but has relative advantages in large-scale editing

Figure 5: Chart of alternate CRISPR-associated (Cas) protein systems, highlighting their most prominent features.

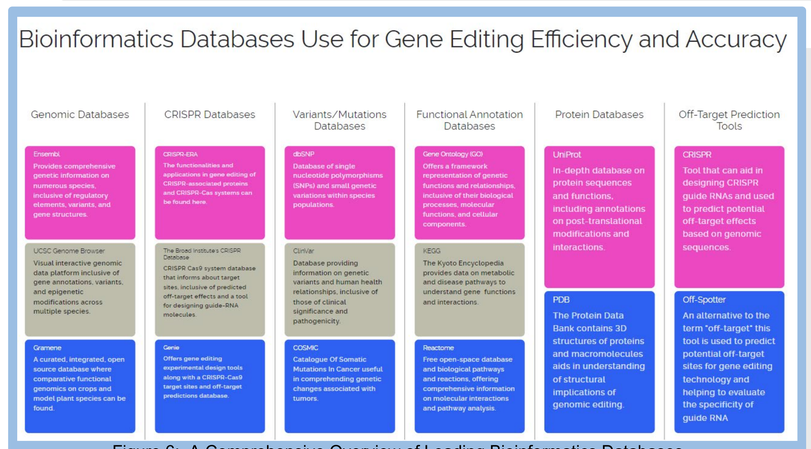

Figure 6: A Comprehensive Overview of Leading Bioinformatics Databases

CONCLUSION

1. CRISPR Effectiveness: CRISPR/Cas9 with pLZDonorGuide successfully knocked out the lacZ gene, as shown by white colonies on Plate D.
2. Repair Activation: HDR repair was effective with arabinose (Plate D) but not without it (Plate B), demonstrating that repair mechanisms are crucial for CRISPR/Cas9 gene editing.
3. Single-Guide RNA: The single-guide RNA effectively directed Cas9 to lacZ, causing a DSB and gene knockout. When HDR repair mechanisms were active, the cell survived.
4. Arabinose Role: Arabinose is essential for activating repair pathways and achieving successful gene editing with CRISPR/Cas9, without it the cell would undergo apoptosis due to the DSB.

REFERENCES

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