AMRITA VISHWA VIDYAPEETHAM
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CANCER THERAPY USING CRISPR – CAS9

SUBJECT:

24AIM112 Molecular biology and basic cellular physiology

24AIM115 Ethics, innovative research businesses & IPR

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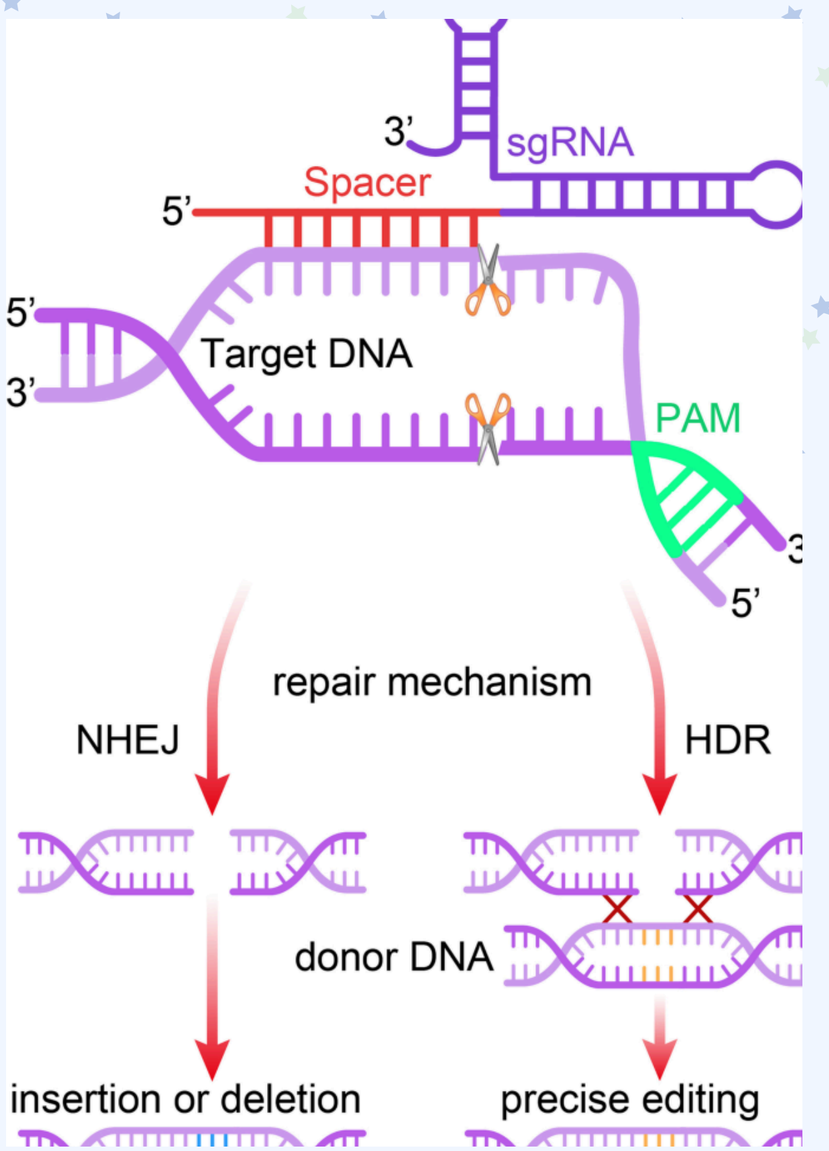
INTRODUCTION

- **CRISPR-Cas9** is a revolutionary gene-editing technology that enables precise modifications of DNA. It consists of two main components: a **guide RNA (gRNA)** that directs the Cas9 enzyme to specific DNA sequences, and the **Cas9 protein** that creates targeted cuts in the genome.

- In cancer therapy, CRISPR-Cas9 offers significant potential by allowing researchers to directly target genes involved in tumor growth and drug resistance. This technology can correct mutations in tumor suppressor genes, knock out oncogenes, and enhance immune responses against cancer cells.

OBJECTIVE:

- **To Understand CRISPR-Cas9 Function:** Gain insights into how CRISPR-Cas9 operates and its applications in cancer treatment.
- **To Develop a Predictive Model:** Create a model for off-target effect prediction in CRISPR-Cas9.
- **Address Ethical Considerations:** Explore ethical implications of using gene editing technologies in cancer therapy.
- **Contribute to Cancer Treatment:** Enhance the safety and efficacy of CRISPR therapies for personalized cancer treatment.



Literature Review

Paper Title	Authors & Year	Key Focus	Findings on Off-Target Effects	Proposed Solutions
Recent advances and applications of CRISPR-Cas9 in cancer immunotherapy	Liu et al., 2023	Highlights CRISPR-Cas9's role in enhancing cancer immunotherapy by modifying CAR-T and NK cells.	Notes off-target effects can cause unwanted genetic changes, impacting immune response.	Improving gRNA specificity and using high-fidelity Cas9 variants can reduce off-target effects.
CRISPR in cancer biology and therapy	Katti et al., 2022	Reviews CRISPR's impact on cancer research, including gene function, tumor modeling, and targeted therapies.	Unintended DNA breaks may cause mutations and genomic instability.	Engineered Cas9 enzymes like Cas12 and Cas13, along with base/prime editing, can enhance precision.

Off-target effects in CRISPR/Cas9 gene editing	Guo et al., 2023	Examines mechanisms and challenges of CRISPR-Cas9 off-target effects.	Off-target mutations in CRISPR can cause harmful genetic changes, reducing therapy safety.	Computational tools like DeepCRISPR and experimental methods like GUIDE-seq can improve accuracy.
A Novel Anti-Cancer Therapy: CRISPR/Cas9 Gene Editing	Zhang et al., 2022	CRISPR/Cas9 can be used to inhibit tumor migration and invasion.	Off-target mutations in tumor suppressor genes could accelerate cancer progression.	Thorough screening of sgRNA sequences and using paired nickases can reduce off-target activity.
CRISPR/Cas9 therapeutics: progress and prospects	Li et al., 2023	Reviews the advancements in CRISPR/Cas9-based therapies and their clinical applications.	CRISPR/dCas9 systems have a lower risk of off-target effects compared to active Cas9 nucleases.	CRISPR/dCas9 systems for gene regulation offer a safer alternative to genome editing.

Computational Aspect : SVM Algorithm

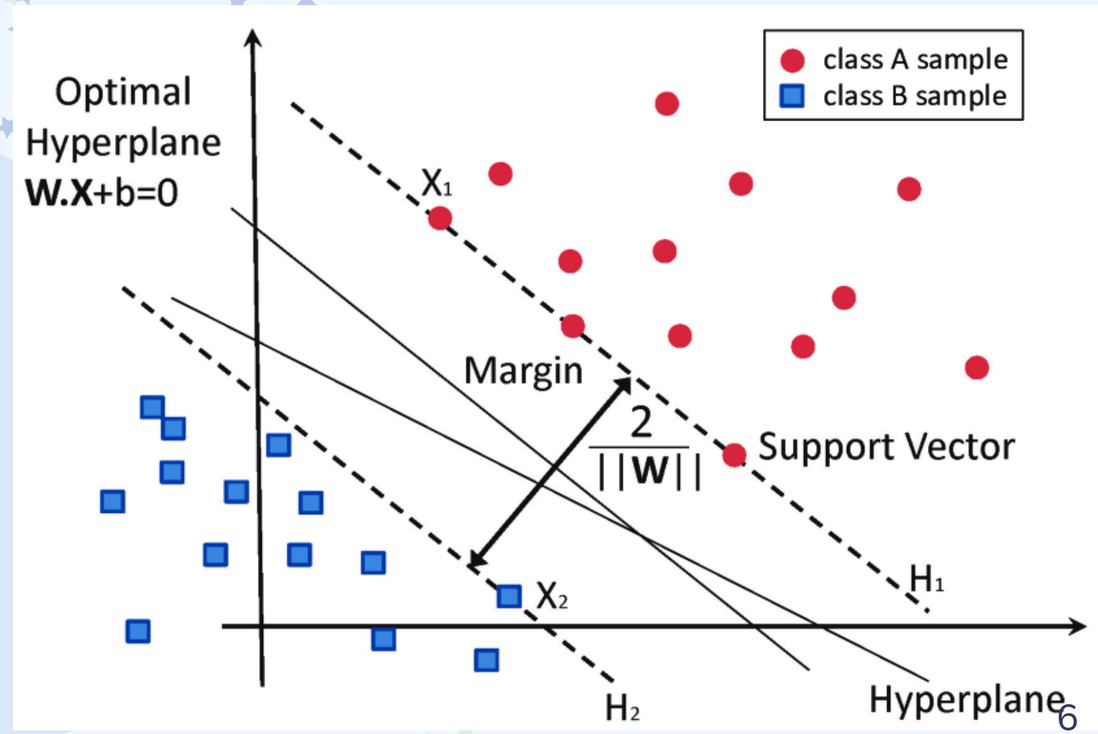
- Support Vector Machine (SVM) is a supervised learning algorithm used to classify data points into distinct categories.
- SVM constructs a hyperplane that separates on-target from off-target sites in high-dimensional feature space. The goal is to maximize the margin between these classes.

Input :

- sgRNA and target DNA sequences.
- GC content.
- Mismatch positions and types (e.g., single nucleotide mismatches).
- PAM sequence information.

Output :

- on-target or off-target.



Datasets

- **CRISPOR Dataset :** It includes information on sgRNA-target pairs, their predicted off-target sites with Mismatch count.
- **GUIDE-seq Dataset :** This contains experimental data that identifies actual off-target sites resulting from CRISPR-Cas9 gene editing.

sgRNA Sequence	Target Gene	Predicted Off-Target Sites	Off-Target Sequence	Mismatch Count	Off-Target Effect	Cancer Type
AGCTGTCAGTAC	EGFR	chr13:3289140	AGCTGTCAGTAC	0	No Effect	Lung Cancer
CAGTTCGATGAC	BRCA1	chr12:2512345	CAGTTCGATGAC	0	No Effect	Colon Cancer
CAGTTCGATGAC	BRCA1	chr13:3289140	GTCATGACTGTC	1	Mutation	Lung Cancer
GTCATGACTGTC	TP53	chr7:5528472	TCGAGTCGATGC	0	No Effect	Breast Cancer
TCGAGTCGATGC	BRCA1	chr13:3289140	GTCATGACTGTC	1	Mutation	Colon Cancer
TCGAGTCGATGC	EGFR	chr12:2512345	CAGTTCGATGAC	1	Mutation	Colon Cancer
AGCTGTCAGTAC	KRAS	chr17:7665581	GTCATGACTGTC	0	No Effect	Breast Cancer
CAGTTCGATGAC	TP53	chr13:3289140	CAGTTCGATGAC	3	Mutation	Breast Cancer
GTCATGACTGTC	BRCA1	chr12:2512345	AGCTGTCAGTAC	0	No Effect	Lung Cancer
AGCTGTCAGTAC	EGFR	chr17:7665581	CAGTTCGATGAC	0	No Effect	Lung Cancer
TCGAGTCGATGC	BRCA1	chr7:5528472	GTCATGACTGTC	0	No Effect	Lung Cancer
TCGAGTCGATGC	TP53	chr13:3289140	GTCATGACTGTC	1	Mutation	Lung Cancer
TCGAGTCGATGC	EGFR	chr13:3289140	CAGTTCGATGAC	3	Mutation	Breast Cancer
CAGTTCGATGAC	BRCA1	chr13:3289140	TCGAGTCGATGC	3	Mutation	Lung Cancer

Ethical Considerations

- **Risk of Off-Target Effects:** The potential for off-target effects raises significant ethical concerns, unintended edits could lead to harmful mutations.

- **Bioterrorism Risks:** The dual-use nature of CRISPR technology poses risks for misuse in bioterrorism, necessitating strict regulatory oversight.

- **Potential for Germline Editing:** Germline editing involves modifications that are passed down to future generations, raising concerns human evolution and genetic diversity.

- **Equity in Access:** Ensuring equitable access to CRISPR therapies is essential to prevent disparities in treatment availability.

Intellectual Property Rights (IPR)

- **Patent holders:**

  Key Holders: Broad Institute, UC Berkeley, and CRISPR biotech companies.
  Cancer Therapy Patents: Cover CRISPR in immunotherapy and precision medicine.
  Off-Target Editing: Patents for high-fidelity Cas9 variants (eSpCas9, Cas12, Cas13).

- **Ethical & Regulatory Compliance:**

  Regulations: Follow FDA (USA), EMA (Europe), and CDSCO (India) for clinical trials.
  Editing Types: Somatic editing is regulated; germline editing is restricted.
  Patient Rights: WHO and UNESCO emphasize transparency and informed consent.

Key Patents :

- **U.S. Patent No. 10,000,772:Holders: The Regents of the University of California, University of Vienna, and Emmanuelle Charpentier.**

  Description: Covers methods for using optimized guide RNA formats in CRISPR/Cas9 genome editing, applicable in eukaryotic cells (including human cells).
  Significance: This foundational patent is pivotal for many applications in gene editing, including therapeutic uses in humans and animals.

- **Broad Institute Patents:**

  Description: The Broad Institute holds patents for using CRISPR-Cas9 in mammalian cells, which cover methods for editing eukaryotic genomes.
  Significance: These patents have led to significant commercial applications and have been at the center of ongoing patent disputes with the University of California 34.

References

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Thank You