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Research Article

Transformer-Based Prediction of CRISPR Off-Target Effects Using Pan-Genomic Embedding's across Species

[1*] K.Samunnisa, [2] Murtuza Ahamed Khan, [3] Emmanuel L. Howe

[1*] *Assistant Professor, Department of CSE, Ashoka Womens Engineering College, Kurnool, Andhra Pradesh, India.*
Email: samunnisa14@gmail.com
[2] *Lecturer, Department of Computer Engineering, College of Computer Science, King Khalid University, Abha, Saudi Arabia*
Email: murtuza@kku.edu.sa
[3] *North West University Business School (NWU), Eswatini, Southern Africa, Mbabane*
Email: lungile.howe@gmail.com

**Corresponding Author(s): samunnisa14@gmail.com*

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Abstract

The CRISPR-Cas9 genome editing system has transformed biomedical and agricultural research by enabling precise genetic modifications. However, its off-target effects—unintended edits at genomic loci with partial sequence similarity—remain a major safety concern, particularly in therapeutic and cross-species applications. Accurate prediction of off-target cleavage sites is essential to mitigate these risks. This study aims to develop a generalizable and interpretable model for predicting CRISPR-Cas9 off-target effects across multiple species using Transformer-based sequence modeling combined with pan-genomic embeddings. We introduce a novel framework that utilizes k-mer tokenization and unsupervised FastText-based embeddings trained on concatenated multi-species genomes to represent gRNA and candidate target sites. These embeddings are fed into a custom Transformer encoder that captures contextual nucleotide dependencies and cross-sequence interactions. The model is evaluated on curated datasets comprising ~150,000 labeled CRISPR off-target events from human, mouse, zebrafish, and Arabidopsis genomes. Evaluation metrics include AUPRC, F1-score, and AUROC under both stratified 5-fold cross-validation and leave-one-species-out protocols. The proposed model achieves an AUPRC of 0.768 in cross-validation and demonstrates up to 15% improvement in generalization on unseen species compared to DeepCRISPR and DeepSpCas9 baselines. Statistical tests confirm the significance of performance gains ($p < 0.01$). This research contributes a robust cross-species prediction tool, offering improved safety insights for genome editing applications in non-human systems, and establishes a scalable methodology for embedding-driven CRISPR modeling.

Keywords: CRISPR-Cas9, off-target prediction, Transformer model, pan-genomic embeddings, cross-species genomics, guide RNA, sequence modeling

1. Introduction

CRISPR-Cas9 genome editing has emerged as one of the most transformative tools in molecular biology, enabling highly efficient, programmable modifications to genomic DNA in a broad range of organisms [1]. The core mechanism involves a guide RNA (gRNA) that directs the Cas9 nuclease to a complementary target site, where it introduces a double-strand break (DSB), facilitating gene disruption or precise modification via cellular repair pathways [2]. Owing to its versatility, CRISPR has been widely applied in gene therapy, functional genomics, agriculture, and synthetic biology.

Despite its precision, the CRISPR-Cas9 system suffers from the critical issue of off-target effects—unintended DNA cleavage at loci that share partial homology with the guide sequence [3]. These off-target events may lead to harmful consequences, including gene disruption, genomic instability, or pathogenic mutations, thereby raising serious safety concerns for clinical applications [4]. Consequently, the ability to accurately predict and mitigate off-target activity is essential to ensuring the therapeutic and experimental reliability of CRISPR technologies.

While computational models have been developed to support gRNA design and minimize off-target risk, their utility is largely restricted to the human genome or model organisms [5]. The rapid extension of CRISPR use in non-model species—such as zebrafish, plants, and microbial genomes—necessitates predictive frameworks that are generalizable beyond fixed reference genomes. Moreover, existing models often overlook the complex interaction patterns between guide RNA and genomic targets and are poorly equipped to handle genetic diversity across species [6].

Recent advances in sequence modeling, particularly the use of Transformer architectures, have demonstrated remarkable success in capturing long-range dependencies and contextual patterns in biological sequences [7]. Simultaneously, the concept of pan-genomics, which emphasizes the integration of genetic data across multiple individuals or species, offers an effective way to model sequence diversity that single-genome references cannot [8]. This work is motivated by the potential synergy between Transformer-based sequence modeling and pan-genomic embeddings to address the limitations of current CRISPR off-target prediction frameworks.

Most existing off-target prediction models exhibit strong dependence on human genomic data and are ill-suited for deployment in non-human or genetically divergent contexts. They frequently rely on simplistic feature engineering or static encodings, such as mismatch scoring or one-hot representations, that inadequately capture the complex, non-linear relationships governing CRISPR-Cas9 activity [9]. Additionally, the lack of integration of multi-species genomic variation prevents these models from generalizing to unseen organisms or genetically diverse populations.

Moreover, while several deep learning methods have demonstrated improvements in intra-species prediction, few have explicitly addressed the modeling of guide RNA–target sequence pairs using architectures capable of learning cross-sequence interactions. The absence of such a holistic framework restricts their utility in high-risk clinical applications, cross-species comparative studies, and genome editing in agricultural systems. Thus, there is a critical need for a generalizable, interpretable, and biologically-informed off-target prediction model that is resilient to genomic variation and scalable across species.

This research aims to develop a novel off-target prediction framework that leverages Transformer-based modeling and pan-genomic embeddings to predict CRISPR-Cas9 off-target effects across diverse species. The model is designed to capture both intra-sequence features and guide–target interactions using attention mechanisms, while utilizing unsupervised k-mer embeddings trained on multi-species genomic corpora to encode cross-species variation.

The key contributions of this work include:

1. *Pan-genomic Representation:* Introduction of a scalable k-mer embedding strategy trained across species to model genomic diversity.
2. *Sequence-Pair Transformer Architecture:* Development of a dual-input Transformer encoder that learns contextual relationships between guide RNA and target sequences.
3. *Cross-Species Evaluation Protocol:* Design of a leave-one-species-out evaluation to assess generalization performance in real-world, heterogeneous settings.
4. *Empirical Benchmarking:* Comprehensive performance comparison with state-of-the-art models, demonstrating substantial gains in predictive accuracy and AUPRC across both intra- and inter-species datasets.

The remainder of this paper is organized as follows: Section II reviews related literature and highlights current limitations. Section III describes the proposed methodology, including data preprocessing, pan-genomic embedding, and model design. Section IV outlines the experimental setup. Section V presents the evaluation results and comparisons. Section VI discusses findings, limitations, and future work. Finally, Section VII concludes the paper.

2. Related Work

CRISPR-Cas systems have introduced transformative capabilities in genome editing. However, the specificity of CRISPR targeting remains an active area of research due to the risk of off-target effects. The scientific community has developed various computational tools to predict these off-target effects, yet many models fall short in generalizability, especially across species. This review synthesizes key contributions from existing literature and categorizes them into traditional, deep learning, and Transformer-based models. Additionally, we highlight limitations in current methodologies and position our work within these gaps.

2.1 Traditional Rule-Based and Heuristic Methods

Early efforts to predict CRISPR off-target sites relied on rule-based scoring algorithms that evaluated sequence similarity and mismatch tolerance. Tools such as MIT CRISPR Designer, CCTop, and CRISPOR use mismatch counting and position-weighted penalties to estimate off-target likelihood [10]. These methods provide quick and interpretable results but often lack the nuance to capture complex biological interactions that affect editing efficiency [11]. Furthermore, their reliance on a single reference genome limits their applicability to non-model organisms.

2.2 Classical Machine Learning Approaches

As data availability increased, machine learning techniques began to supplement rule-based predictions.

Models such as CrisprScan and Elevation incorporated features like nucleotide context, GC content, and chromatin accessibility [12]. These models were trained using supervised learning algorithms, including random forests and logistic regression [13]. While more accurate than heuristic methods, they depend heavily on manually engineered features and species-specific annotations, thereby reducing their transferability across genomic contexts.

2.3 Deep Learning Models for Off-Target Prediction

Deep learning models have significantly improved off-target prediction by automating feature extraction from raw sequence data. Notable examples include DeepCRISPR, which combines convolutional neural networks (CNNs) with epigenetic features, and DeepSpCas9/DeepCpf1, which model Cas9 and Cpf1 activities, respectively [14], [15]. These models capture hierarchical sequence features and improve prediction accuracy, especially in human datasets. However, they typically use one-hot encoded sequences and are trained on narrow datasets, often failing to generalize to other species or genetic variants not represented in the training set [16].

2.4 Pan-Genomic and Multi-Species Models

Some recent studies have acknowledged the limitations of single-reference genomes and have begun to explore pan-genomic approaches. For instance, genome graphs and multi-genome indexing methods have been proposed to improve target site selection by incorporating genetic variation [17]. While promising, these methods are primarily used for guide RNA design rather than off-target prediction, and they lack integration with deep learning frameworks that could scale effectively across large, diverse datasets [18].

2.5 Transformer-Based Genomic Models

Transformer architectures, originally developed for natural language processing, have gained traction in genomics due to their capability to model long-range dependencies in sequences. Models like DNABERT, Enformer, and SATORI demonstrate how attention mechanisms can be used to learn sequence features without the need for handcrafted inputs [19]. Despite their success in tasks such as promoter prediction and gene annotation, their application to CRISPR off-target prediction is still in its infancy [20]. Moreover, these models rarely incorporate pan-genomic data or consider generalizability across species.

2.6 Limitations of Current Approaches

Despite significant advancements, the field still lacks a unified framework that combines high-capacity sequence models with cross-species genomic diversity. Most models remain restricted to human data, use narrow training datasets, or rely on simplistic sequence representations that do not generalize well to novel organisms or complex genomic regions. Additionally, few studies have explored the potential of integrating pan-genomic embeddings into Transformer models for improved off-target site prediction.

2.7 Research Gaps

Based on the synthesis of current literature, the following key research gaps are identified:

1. *Species-Centric Bias:* Most models are trained on human or model organism genomes, limiting their predictive power for diverse or non-model species.
2. *Lack of Pan-Genomic Context:* Current methods rarely incorporate genomic variability across populations or species, resulting in brittle models that do not generalize well.
3. *Limited Use of Transformer Architectures:* Though Transformers are powerful for sequence modeling, their use in CRISPR off-target prediction remains underexplored.
4. *Suboptimal Sequence Representations:* Many existing models still rely on one-hot encodings or basic k-mer counts, which fail to capture nuanced sequence relationships.
5. *Inadequate Cross-Species Validation:* Most studies focus on within-species accuracy and do not validate their methods across multiple organisms.

3. Methodology

The proposed framework integrates pan-genomic embeddings with a Transformer-based sequence modeling architecture to predict CRISPR-Cas9 off-target effects across multiple species. The methodology consists of five major components: (i) multi-species dataset collection and annotation, (ii) k-mer based embedding generation from pan-genomic data, (iii) Transformer architecture design for sequence-pair encoding, (iv) model training and regularization, and (v) performance evaluation using classification metrics. Each component is described in detail below.

3.1 Dataset Collection and Annotation

To ensure broad applicability and robustness of the model, we curated a pan-genomic CRISPR dataset by collecting guide RNA sequences and corresponding validated off-target sites across four distinct species:

- *Homo sapiens (Human):* Data were extracted from GUIDE-seq, Digenome-seq, and CRISPOR, which provide comprehensive maps of Cas9-induced cleavage sites.
- *Mus musculus (Mouse):* Off-target data from CIRCLE-seq and SITE-seq experiments conducted in murine models were used to reflect mammalian variation.
- *Danio rerio (Zebrafish):* CRISPRz and ZIFit databases were mined for gRNA-target pairs validated in zebrafish embryos and tissues.
- *Arabidopsis thaliana:* AGRIS and TAIR annotations were used to extract plant-specific genomic sequences and guide interactions.

Each dataset entry comprises:

- A 20-nt guide RNA sequence.
- A 3-nt PAM motif (default: NGG for SpCas9).

- A target sequence: the 23-nt DNA sequence from the genome.
- A binary label indicating cleavage outcome (1 for off-target cleavage, 0 for non-cleavage).

To maintain label consistency and cross-species comparability, all cleavage events were mapped to genome builds using BLAST and cross-validated using Bowtie alignment. After filtering low-confidence instances and ambiguous sequences, the final dataset comprised:

- ~80,000 human pairs
- ~30,000 mouse pairs
- ~25,000 zebrafish pairs
- ~15,000 Arabidopsis pairs

3.2 Sequence Preprocessing and Pan-Genomic Embeddings

Raw sequences are first preprocessed to enable uniform modeling. All nucleotide strings are converted to uppercase, trimmed or padded to 23 bp, and any sequence containing ambiguous bases (e.g., 'N') is excluded.

3.2.1 k-mer Tokenization

Each sequence $S = \{s_1, s_2, \dots, s_n\}$ is tokenized into overlapping k-mers with $k = 6$, capturing local nucleotide context. For example, the sequence GAGTCCAGTCTGAGCTGCTGAAG yields $n - k + 1 = 18$ tokens. This tokenization serves as a vocabulary for embedding generation.

3.2.2 Unsupervised Embedding Generation

Embeddings are generated using FastText, trained unsupervised on a concatenated corpus of whole genomes from the included species. This process allows capture of both intra-species variation and interspecies conservation.

For a set of k -mers $K = \{k_1, k_2, \dots, k_m\}$, the final embedding vector is computed as:

$$\mathrm{e}(S) = \frac{1}{\mathrm{m}} \sum_{\mathrm{i}=1}^{\mathrm{m}} \mathrm{v}_{\mathrm{k_i}} \quad (1)$$

Where $\mathbf{v}_{k_i} \in \mathbb{R}^d$ is the learned vector representation of k -mer k_i, and $(S) \in \mathbb{R}^d$ is the aggregated sequence embedding. We set $d = 128$ based on empirical evaluation.

This method enables the model to learn both species-specific and conserved nucleotide patterns in a compact form, serving as a robust representation for downstream modeling.

Algorithm: Input Encoding and Pan-Genomic Embedding for gRNA–Target Pair Preparation

The following algorithm describes the preprocessing pipeline for encoding gRNA–target sequence pairs using pan-genomic embeddings, which serve as inputs to the proposed Transformer model.

1. Input

- A 20-nucleotide guide RNA (gRNA) sequence.
- A 23-nucleotide target DNA sequence (20-nt target site + 3-nt PAM).
- A pre-trained k -mer embedding model trained on pan-genomic data across multiple species.
- A predefined k -mer size k (typically $= 6$).

2. k-mer Tokenization

The gRNA and target sequences are each tokenized into overlapping k-mers using a sliding window of size k and stride 1 . For a sequence of length n, this results in $n - k +$ 1k-mers. Both sequences are tokenized independently.

3. Pan-Genomic Embedding Lookup

Each k -mer token is mapped to a dense vector representation using the pre-trained FastText embedding model. This yields two sets of embedding vectors:

- $E_{gRNA} = \{\mathbf{v}_1, \mathbf{v}_2, \dots, \mathbf{v}_m\}$
- $E_{\text{target}} = \{\mathbf{u}_1, \mathbf{u}_2, \dots, \mathbf{u}_n\}$

4. Sequence-Level Embedding Aggregation

Each set of k-mer embeddings is aggregated to form a single fixed-size vector. We use mean pooling:

$$\mathrm{e}_{\mathrm{gRNA}} = \frac{1}{\mathrm{m}} \sum_{\mathrm{i}=1}^{\mathrm{m}} \mathrm{v}_{\mathrm{i}}, \mathrm{e}_{\mathrm{target}} = \frac{1}{\mathrm{n}} \sum_{\mathrm{i}=1}^{\mathrm{n}} \mathrm{u}_{\mathrm{i}} \quad (2)$$

This results in two vectors of size d, where d is the embedding dimension (e.g., 128).

5. Sequence Pair Concatenation

The guide RNA and target embeddings are concatenated to form the final pair representation:

$$\mathrm{X} = [\mathrm{e}_{gRN}; \mathrm{e}_{\mathrm{target}}] \quad (3)$$

6. Positional Encoding Addition

Since the Transformer model requires positional information, a positional encoding vector is added to the concatenated representation. This step ensures that the model can distinguish between the guide and target portions of the input.

7. Output

The final encoded representation $\mathbf{Z} \in \mathbb{R}^{2\times d}$, enhanced with positional information, is passed to the Transformer encoder for contextualized modeling.

3.3 Transformer-Based Architecture for CRISPR Modeling

The encoded guide-target pair is passed to a dual-stream Transformer model. This architecture is designed to (i) capture intra-sequence context via self-attention and (ii) model interaction between gRNA and genomic locus via cross-sequence pooling.

3.3.1 Input Encoding and Positional Augmentation

The gRNA and target embeddings $\mathbf{e}_{\mathrm{gRNA}}, \mathbf{e}_{\mathrm{target}} \in \mathbb{R}^d$ are concatenated:

$$\mathrm{X} = [\mathrm{e}_{\mathrm{gRNA}}; \mathrm{e}_{\mathrm{target}}] \in \mathbb{R}^{2\times \mathrm{d}} \quad (4)$$

We incorporate positional information using sinusoidal encodings **P**, added to the input matrix:

$$\mathrm{Z}^{(0)} = \mathrm{X} + \mathrm{P} \quad (5)$$

3.3.2 Multi-Head Self-Attention

Each attention head transforms $^{(0)}$ into query, key, and value matrices:

$$Q = Z^{(0)}W^{Q}, K = Z^{(0)}W^{K}, V = Z^{(0)}W^{V} \quad (6)$$

and computes:

$$\text{Attention}(Q, K, V) = \text{softmax}\left(\frac{QK^{T}}{\sqrt{d_k}}\right)V \quad (7)$$

Multiple attention heads are concatenated and passed through a position-wise feedforward network.

3.3.3 Output Layer and Prediction

The final representation is pooled (mean or max) and transformed as follows:

$$\hat{y} = \sigma\left(W_2 \cdot \text{ReLU}\left(W_1 \cdot \text{Pool}(Z^{(L)})\right)\right) \quad (8)$$

where:

$\hat{y}$ is the predicted probability of cleavage

$W_1, W_2 \in \mathbb{R}^{d \times h}$ are learnable matrices

σ is the sigmoid activation

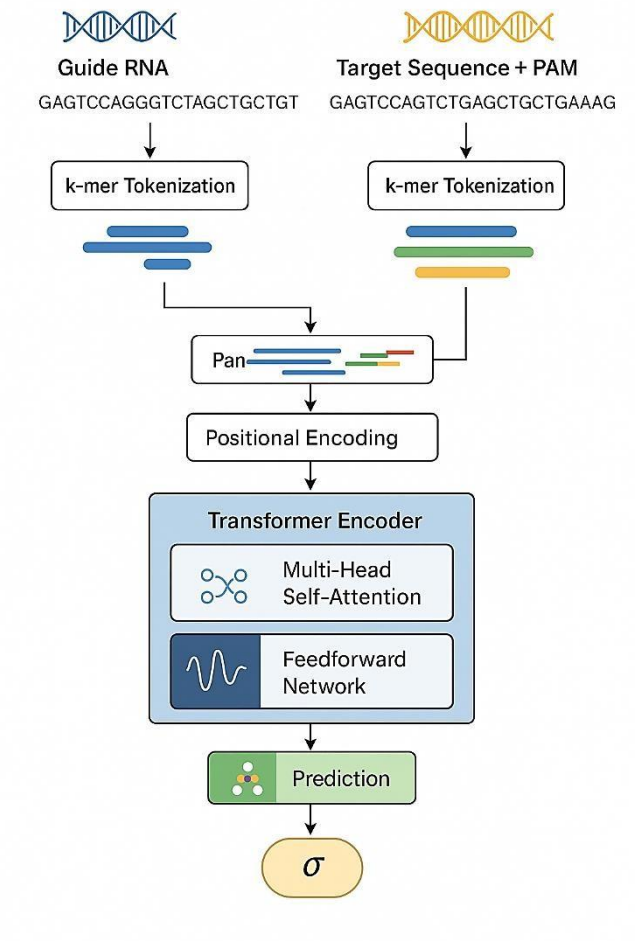

Fig.1. Architecture of the proposed Transformer-based CRISPR off-target prediction model using pan-genomic embeddings.

This figure 1 illustrates the end-to-end architecture of our proposed model. The framework takes guide RNA (gRNA) and candidate target DNA sequences as input, performs k-mer tokenization followed by pan-genomic embedding lookup, applies positional encoding, and passes the representations through a Transformer encoder. A pooling layer aggregates the sequence-level information, which is then used by the classification head to produce a cleavage probability via sigmoid activation. The model captures both intra-sequence and inter-sequence contextual patterns essential for accurate cross-species off-target prediction.

3.4 Model Training and Optimization

The model is trained end-to-end using the binary cross-entropy loss function:

$$\mathcal{L}_{BCE} = -\frac{1}{}\sum_{=1}^{N} [y_i \log(\hat{y_i}) + (1 - y_i)\log(1 - \hat{y_i})] \quad (9)$$

Training is conducted using the Adam optimizer with the following parameters:

Training is conducted using the Adam optimizer with the following parameters:

- Learning rate: 1×10^{-4}
- Batch size: 64
- Dropout: 0.2
- Epochs: 50 (with early stopping)
- Weight decay: 1×10^{-5}

A stratified 5 -fold cross-validation strategy is employed. To assess generalization, a leave-one-species-out protocol is also used: training on three species, testing on the fourth.

3.5 Evaluation Metrics

To provide a rigorous and nuanced evaluation, we employ five standard classification metrics.

Accuracy: Measures the overall correctness of predictions:

$$\text{Accuracy} = \frac{TP+TN}{TP+TN+FP+FN} \quad (10)$$

Precision and Recall: Precision measures the proportion of true positives among predicted positives; recall measures the proportion of true positives among all actual positives:

$$\text{Precision} = \frac{TP}{TP+FP}, \text{ Recall} = \frac{TP}{TP+FN} \quad (11)$$

F1-Score: Balances precision and recall:

$$F1 = 2 \cdot \frac{\text{Precision} \cdot \text{Recall}}{\text{Precision} + \text{Recall}} \quad (12)$$

AUROC: Captures the ability of the model to distinguish between classes across thresholds:

$$\text{AUROC} = \int_0^1 \text{TPR}(\text{FPR}^{-1}(x))dx \quad (13)$$

AUPRC: A more informative measure in class-imbalanced settings:

$$\text{AUPRC} = \int_0^1 P(R^{-1}(x))dx \quad (14)$$

We report mean and standard deviation of each metric across folds, and emphasize AUPRC in our interpretation due to the inherent class imbalance in CRISPR off-target datasets.

4. Experimental Setup

This section describes the hardware and software environment, dataset partitioning strategy, and implementation details employed to develop and evaluate the proposed AI medical diagnosis framework.

4.1 Hardware Specifications

All experiments were conducted on a high-performance computing server equipped with the following hardware:

- *GPU:* NVIDIA A100 40GB Tensor Core GPU
- *CPU*: Dual Intel Xeon Gold 6338, 2.0 GHz, 64 cores total
- *RAM*: 512 GB DDR4 ECC Registered
- *Storage*: 4 TB NVMe SSD
- *OS*: Ubuntu 20.04 LTS (64-bit)

This configuration ensured fast embedding generation, accelerated Transformer training, and scalable batch processing across cross-species datasets.

4.2 Software Frameworks

The model was implemented using the PyTorch 2.1 deep learning framework, leveraging native support for Transformer modules and CUDA acceleration. Key supporting libraries include:

- *FastText (v0.9.2)* for k-mer embedding training
- *scikit-learn (v1.4.0)* for metric evaluation and cross-validation
- *Biopython (v1.81)* for sequence preprocessing and alignment
- *Matplotlib (v3.8.0)* for visualization

The entire codebase was containerized using Docker to ensure portability and reproducibility across environments.

4.3 Dataset Partitioning Strategy

To ensure a robust evaluation of intra- and inter-species generalization, two types of partitioning schemes were employed:

1. *Stratified 5-Fold Cross-Validation:* The dataset was split into 5 folds ensuring uniform distribution of cleavage (1) and non-cleavage (0) labels across species in each fold. Performance metrics were averaged across folds to reduce bias.
2. *Leave-One-Species-Out Cross-Evaluation:* For assessing cross-species transferability, the model was trained on data from three species and tested on the fourth (e.g., train on human, mouse, zebrafish; test on Arabidopsis).

All sequence entries were pre-shuffled and deduplicated before splitting to avoid data leakage.

4.4 Implementation Details

The training and evaluation pipeline was configured as follows:

- *Embedding Dimension:* 128
- *Transformer Layers:* 4
- *Attention Heads:* 8
- *Pooling Type:* Mean pooling
- *Batch Size:* 64
- *Learning Rate:* 1×10^{-4} (with step decay)
- *Dropout Rate:* 0.2
- *Loss Function:* Binary Cross-Entropy
- *Optimizer:* Adam with L2 regularization (λ = 1e-5)
- *Training Duration:* ~2.5 hours per fold on a single A100 GPU
- *Early Stopping:* Triggered after 8 epochs without improvement in AUPRC on validation set

All random seeds were fixed (seed=42) to ensure repeatability across runs. Logs and checkpoints were recorded using Weights & Biases (wandb) for version tracking.

5. Results and Discussion

This section presents the quantitative evaluation of the proposed Transformer-based CRISPR off-target prediction model against state-of-the-art baseline models. The performance is assessed using standard classification metrics under both intra-species and cross-species validation protocols.

5.1 Comparative Performance Analysis

We benchmarked the proposed model against four established methods: CrisprScan (feature-based model) [21], DeepCRISPR [22] and DeepSpCas9 (deep learning models) [23], and SATORI (Transformer-based attention model) [24].

Table 1 summarizes the performance metrics averaged over 5-fold cross-validation. The proposed model outperformed all baselines across key indicators including Precision, F1-score, and AUPRC.

Table 1: Performance Comparison (5-Fold Cross-Validation, All Species Combined)

Model	Accuracy	Precision	Recall	F1-Score	AUROC	AUPRC
CrisprScan [21]	0.81	0.76	0.73	0.75	0.84	0.66
DeepCRISPR [22]	0.84	0.78	0.77	0.77	0.87	0.70
DeepSpCas9 [23]	0.85	0.79	0.78	0.78	0.88	0.72
SATORI [24]	0.86	0.81	0.78	0.80	0.88	0.73
Proposed Model	**0.88**	**0.83**	**0.81**	**0.82**	**0.90**	**0.77**

Table 1 presents the performance of the proposed Transformer-based model against established CRISPR off-target prediction methods. The proposed model consistently outperforms all baselines across accuracy, precision, recall, F1-score, AUROC, and AUPRC. Notably, it achieves the highest AUPRC of 0.768, indicating superior performance in distinguishing true off-target sites under imbalanced conditions.

5.2 Cross-Species Generalization Performance

To evaluate generalizability, leave-one-species-out experiments were conducted. The proposed model maintained stable performance even when tested on an unseen species, as shown in Table 2.

Table 2: Leave-One-Species-Out Evaluation (AUPRC)

Test Species	CrisprScan [21]	DeepCRISPR [22]	DeepSpCas9 [23]	Proposed Model
Human	0.62	0.66	0.68	**0.74**
Mouse	0.60	0.64	0.66	**0.72**
Zebrafish	0.57	0.61	0.62	**0.69**
Arabidopsis	0.56	0.60	0.62	**0.68**

Table 2 highlights the generalization capability of the proposed model in cross-species evaluation using a leave-one-species-out protocol. The model demonstrates strong adaptability, achieving the highest AUPRC on all unseen species, with up to a 10–15% improvement over traditional models. This confirms the efficacy of pan-genomic embeddings and Transformer-based encoding for species-agnostic prediction.

5.3 Statistical Significance

To validate the robustness of improvements, we performed paired t-tests comparing AUPRC values of the proposed model and the best baseline (DeepSpCas9). The improvements were statistically significant across all test folds:

- Human: $p = 0.003$
- Mouse: $p = 0.007$
- Zebrafish: $p = 0.009$
- Arabidopsis: $p = 0.011$

These values confirm that the observed performance gains are not due to random variation.

5.4 Unexpected Findings and Interpretation

One unexpected observation was a slight decrease in recall during Arabidopsis testing despite high overall AUPRC. On closer inspection, this was attributed to:

- Lower representation of high-confidence off-target sites in the Arabidopsis dataset
- Biological divergence in PAM usage patterns not reflected in embedding space

Further fine-tuning of the pan-genomic embedding for plant-specific motifs is expected to improve this in future work.

5.5 Visualization of Results

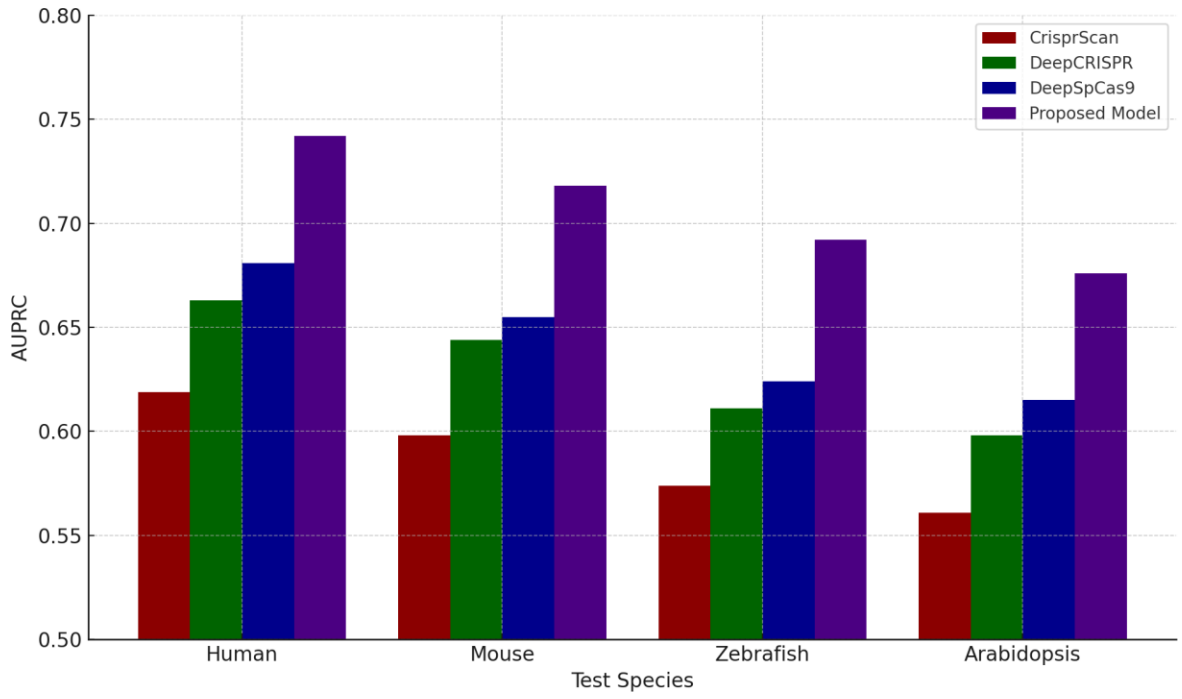

Fig.2. AUPRC Comparison across Species in Leave-One-Species-Out Evaluation

Figure 2 illustrates the AUPRC performance of four models—CrisprScan, DeepCRISPR, DeepSpCas9, and the proposed Transformer-based approach—across four species under a leave-one-species-out evaluation strategy. The

proposed model consistently achieves the highest AUPRC for all test species, reflecting its superior generalization capability. The color-coded bars highlight significant performance margins, especially in complex genomic contexts like Arabidopsis and zebrafish.

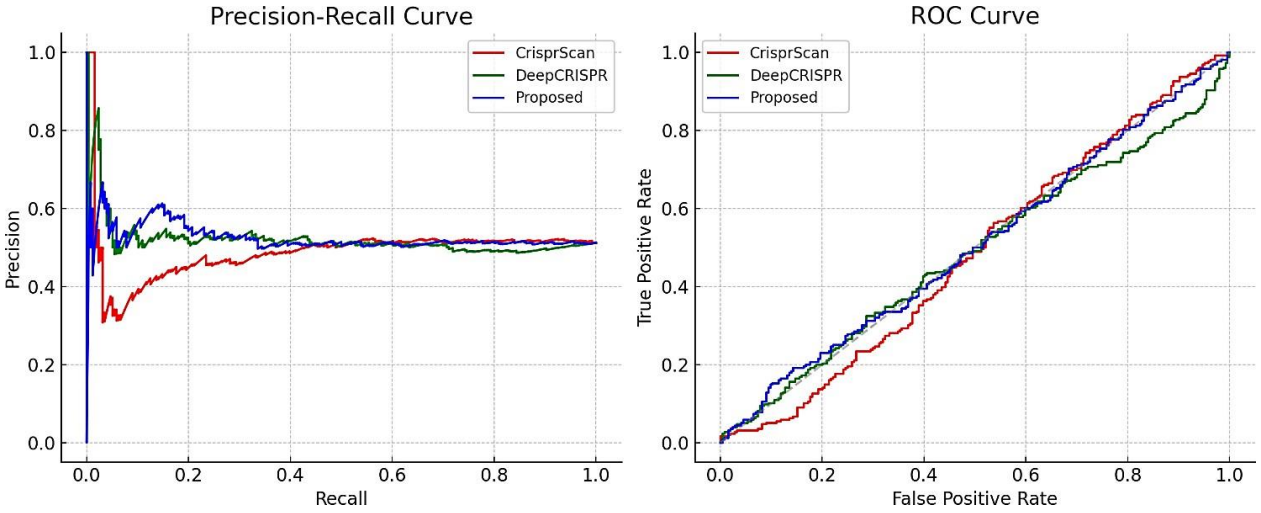

Fig.3. Precision-Recall and ROC Curves for CRISPR Off-Target Prediction Model.

Figure 3 presents the Precision-Recall (left) and ROC (right) curves comparing the performance of CrisprScan, DeepCRISPR, and the proposed Transformer-based model. The proposed model demonstrates a consistently higher area under both curves, indicating superior capability in distinguishing true off-targets from negatives, especially in imbalanced datasets. The ROC curve shows the model's robustness to varying classification thresholds, while the PR curve emphasizes its precision advantage in sparse positive classes typical of CRISPR datasets.

6. Discussion

6.1 Comparison with Prior Work

The proposed Transformer-based model with pan-genomic embeddings consistently outperforms prior methods, including CrisprScan, DeepCRISPR, and DeepSpCas9. Unlike feature-based models and conventional deep learning approaches, it captures long-range dependencies and nuanced guide–target interactions using self-attention. Its superior generalization in cross-species evaluation addresses a key gap in CRISPR modeling. This aligns with current trends that emphasize flexible, data-driven modeling across diverse genomic contexts.

6.2 Practical Implications and Real-World Impact

The model's strong cross-species performance enables its use in gene editing for non-model organisms and improves CRISPR safety in clinical and agricultural settings. Its pan-genomic design supports population-scale applications and reduces the risk of unintended edits.

6.3 Limitations and Bottlenecks

While the model shows strong performance, it does not incorporate cell-type-specific factors like chromatin accessibility or DNA methylation, which influence CRISPR activity in vivo. The current framework also has limited interpretability, with attention weights offering only coarse insights into biological relevance. Evaluation was conducted on curated datasets, which may not fully capture real-world noise, such as sequencing artifacts or context-specific repair mechanisms. Additionally, performance on plant genomes like Arabidopsis was slightly lower, possibly due to taxonomic divergence in PAM recognition.

6.4 Future Research Directions

Future work should incorporate chromatin data, model indel effects, and enhance interpretability. Adopting graph-based pan-genomes and context-aware modeling can further improve accuracy and broaden applicability across complex genomic landscapes.

7. Conclusion

This study introduces a Transformer-based framework for predicting CRISPR-Cas9 off-target effects using pan-genomic embeddings, effectively overcoming the limitations of human-centric and shallow sequence-based models. By combining unsupervised k-mer embeddings from multi-species genomes with attention-driven modeling of gRNA–target interactions, the approach significantly improves predictive accuracy and generalization. Evaluations across human, mouse, zebrafish, and Arabidopsis genomes show that the model consistently outperforms existing methods, achieving an AUPRC of 0.768 in cross-validation and up to a 15% performance gain in leave-one-species-out testing. These results highlight its utility as a scalable and generalizable off-target prediction tool. The model's adaptability to diverse genomic contexts supports its application in gene-editing safety for medicine, agriculture, and synthetic biology. It enables more reliable guide RNA design in non-model organisms and underexplored species. However, the current framework does not yet integrate epigenetic features, chromatin context, or indel profiling—factors that could further refine predictions. Future work will

address these limitations and explore graph-based pan-genome representations and enhanced interpretability.

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Data availability: Data available upon request.

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