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CRISPR-Cas9 off-target predictions using CNN and double CNN: Comparative analysis

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Abstract

CRISPR-Cas9, a revolutionary gene-editing technology, faces significant challenges due to off-target effects. These unintended edits can have detrimental consequences, necessitating accurate prediction methods. This research explores the efficacy of Convolutional Neural Networks (CNNs) and Double CNNs in predicting off-target sites, comparing their performance against traditional feed-forward neural networks (FNNs).

CRISPR-Cas9 has emerged as a transformative tool for precise gene editing, yet its off-target effects remain a critical concern, potentially causing unintended genetic modifications. Accurate prediction of these off-target sites is essential to enhance the safety and efficacy of CRISPR-Cas9 applications. This study investigates the use of deep learning models, specifically a four-layer feed-forward neural network (FNN), Convolutional Neural Networks (CNNs), and Double CNNs, to predict CRISPR-Cas9 off-target effects. By encoding DNA and guide RNA sequences into numerical vectors, these models can detect subtle mismatches and patterns indicative of off-target activity. The performance of each model is evaluated using the Area Under the Curve (AUC) metric. Results show that CNNs and Double CNNs significantly outperform the FNN, with the Double CNN model achieving the highest AUC score of 0.98. These findings highlight the potential of deep learning approaches to improve the precision of CRISPR-Cas9 off-target predictions, paving the way for safer genetic editing practices. Data Availability at https://github.com.

Keywords: CRISPR-Cas9; Gene Editing; Off-Targets; Convolutional Neural Network; Feed-forward neural networks

1. Introduction

CRISPR-Cas9, short for Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9, has revolutionized gene editing by enabling precise modifications to DNA sequences. This technology, which utilizes a guide RNA (gRNA) to direct the Cas9 enzyme to a specific DNA target, offers unprecedented control over genetic alterations. Despite its precision, a significant challenge remains: off-target effects. These unintended modifications occur when the gRNA binds to sequences similar to the target, leading to erroneous cuts by the Cas9 enzyme. Off-target effects can disrupt gene function, potentially triggering harmful consequences, such as the activation of oncogenes or the suppression of essential genes.

Current methods for predicting off-target effects predominantly rely on statistical models, which often lack the accuracy needed for reliable predictions. Deep learning, with its capacity to uncover intricate patterns in large datasets, presents a promising alternative. This study explores the efficacy of deep learning models—specifically a four-layer feed-forward neural network (FNN), Convolutional Neural Networks (CNNs), and Double CNNs—in predicting CRISPR-Cas9 off-target effects.

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The FNN serves as a baseline model, providing a point of comparison for the more advanced CNN and Double CNN architectures. CNNs are particularly well-suited for analyzing sequential data due to their ability to capture spatial hierarchies through convolutional layers. Double CNNs enhance this capability by processing the gRNA and DNA sequences in parallel, potentially offering even greater predictive power[1].

By converting gRNA and DNA sequences into numerical vectors, these models can identify mismatches and predict off-target sites with higher accuracy. The performance of each model is assessed using the Area Under the Curve (AUC) metric, a robust measure of classification accuracy. This research aims to demonstrate that deep learning models, especially CNNs and Double CNNs, can significantly improve the prediction of CRISPR-Cas9 off-target effects, thereby advancing the safety and effectiveness of gene-editing technologies.[5]

CRISPR-Cas9 stands for Clustered Regularly Interspaced Short Palindromic Repeats and associated Cas9 protein. It provides a precise method for DNA editing, leveraging a guide RNA (gRNA) to target specific DNA sequences. Despite its precision, CRISPR-Cas9 often binds to sequences with minor mismatches, causing off-target effects. These effects can disrupt gene function, leading to unintended and potentially harmful biological outcomes. Current prediction methods, primarily statistical models, lack accuracy and consistency. This study aims to improve off-target prediction using deep learning models, specifically CNNs and Double CNNs.

CRISPR-Cas9, a groundbreaking gene-editing technology, has its roots in the adaptive immune system of bacteria. Initially discovered as a defense mechanism against viral infections, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) allows bacteria to "remember" and cut viral DNA. This system consists of repetitive DNA sequences (CRISPR arrays) and associated Cas (CRISPR-associated) proteins, such as Cas9. When a bacterium encounters a virus, it incorporates snippets of the viral DNA into its CRISPR array, creating a genetic record. Upon subsequent infections, the bacterium transcribes these snippets into guide RNA (gRNA), which, in conjunction with the Cas9 protein, targets and cleaves the viral DNA, neutralizing the threat.

This natural mechanism has been adapted for precise gene editing in various organisms. In the CRISPR-Cas9 system, the gRNA is designed to match a specific DNA sequence within the genome of the target organism. The Cas9 protein then introduces a double-strand break at this site, allowing for targeted modifications through subsequent DNA repair processes. This capability has revolutionized genetic research, enabling precise modifications for therapeutic, agricultural, and fundamental research applications.[6]

Despite its precision, CRISPR-Cas9 is not without limitations. One of the primary challenges is the occurrence of off-target effects, where the gRNA-Cas9 complex binds to and cleaves DNA sequences that are similar, but not

identical, to the intended target. These off-target effects can result in unintended genetic alterations, potentially leading to harmful consequences such as the activation of oncogenes or disruption of essential genes. This issue underscores the critical need for accurate prediction and minimization of off-target effects to ensure the safety and efficacy of CRISPR-Cas9 applications.

1.1. Traditional Prediction Methods

Traditional approaches to predicting off-target effects rely on statistical models and heuristic algorithms. These methods typically assess sequence similarity and use various scoring schemes to predict potential off-target sites. However, their accuracy is limited due to the complex nature of DNA-protein interactions and the vast diversity of genomic sequences.[7] These models often fail to capture the subtle nuances and contextual dependencies that influence off-target activity, leading to inconsistent and sometimes unreliable predictions.

1.2. Deep Learning for Off-Target Prediction

Deep learning, a subset of machine learning, has emerged as a powerful tool for analyzing complex datasets and uncovering hidden patterns. Neural networks, particularly Convolutional Neural Networks (CNNs), have demonstrated exceptional performance in tasks such as image and speech recognition, where they can learn hierarchical features from raw input data. In the context of CRISPR-Cas9 off-target prediction, deep learning models offer several advantages over traditional methods:

- Feature Extraction: CNNs can automatically extract relevant features from input sequences, capturing both local and global patterns that may influence off-target effects.[8]
- Handling Large Datasets: Deep learning models are well-suited for handling large and complex datasets, making them ideal for genomic data analysis.

- Improved Accuracy: By learning directly from data, deep learning models can achieve higher predictive accuracy compared to traditional heuristic-based approaches.

1.3. Research Focus

This study focuses on developing and comparing three types of neural network models to predict CRISPR-Cas9 off-target effects: a four-layer feed-forward neural network (FNN), a standard Convolutional Neural Network (CNN), and a Double CNN.[2] Each model processes the input sequences differently to capture potential off-target sites effectively.

- Four-Layer Feed-Forward Neural Network (FNN): This model serves as a baseline and includes an input layer, four hidden layers with varying numbers of neurons, and an output layer. The FNN is designed to test the performance of traditional neural network architecture in off-target prediction.[9]
- Convolutional Neural Network (CNN): The CNN model includes convolutional layers that perform feature extraction, followed by pooling layers that reduce dimensionality, and fully connected layers that make the final prediction. CNNs are particularly effective for sequential data, making them well-suited for analyzing DNA sequences.
- Double CNN: This model consists of two parallel CNNs that process the guide RNA (gRNA) and target DNA sequences separately. The features extracted by both CNNs are then combined and processed through additional layers to make the final prediction. The Double CNN aims to capture the interactions between gRNA and target DNA more comprehensively.

1.4. Data Preparation

The dataset comprises gRNA sequences and their corresponding DNA targets, annotated with information on mismatches and off-target effects. Each gRNA sequence includes 23 base pairs, incorporating the Protospacer Adjacent Motif (PAM) sequence. The sequences are encoded into numerical vectors to be compatible with the neural network models. For example, adenine (A) is encoded as [1, 0, 0, 0], guanine (G) as [0, 1, 0, 0], cytosine (C) as [0, 0, 1, 0], and thymine (T) as [0, 0, 0, 1]. Mismatches between gRNA and DNA are represented by combining the vectors of mismatched base pairs.

The performance of each model is evaluated using the Area Under the Curve (AUC) metric, which provides a robust measure of classification accuracy. A higher AUC score indicates better predictive performance. This study aims to demonstrate that CNN and Double CNN models can significantly outperform the traditional FNN, thereby offering a more reliable method for predicting CRISPR-Cas9 off-target effects.

By leveraging the power of deep learning, specifically CNNs and Double CNNs, this research aims to enhance the accuracy of CRISPR-Cas9 off-target predictions. Improved prediction models will contribute to safer and more effective gene-editing practices, ultimately advancing the field of genetic research and its applications.[10]

2. Methodology

2.1. Materials and Methods

2.1.1. Sequence Encoding

In this study, we utilize a one-hot encoding scheme to represent the single-guide RNA (sgRNA) and target DNA sequences for the CRISPR-Cas9 system. This encoding method allows us to transform nucleotide sequences into numerical vectors suitable for neural network input.

2.1.2. One-Hot Encoding

Each nucleotide (A, G, C, T) in the sgRNA and target DNA sequences is encoded as a one-hot vector:

This encoding results in a 4x23 matrix for each sgRNA-DNA sequence pair, where 23 represents the length of the sequence including the 3-bp PAM (Protospacer Adjacent Motif) adjacent to the 20 bases of the target sequence.[2]

Table 1 One hot vector defining nucleotide

Nucleotide	One-Hot Vector
Adenine (A)	[1, 0, 0, 0]
Guanine (G)	[0, 1, 0, 0]
Cytosine (C)	[0, 0, 1, 0]
Thymine (T)	[0, 0, 0, 1]

Encoding Mismatches

To capture the mismatch information between sgRNA and target DNA, we create a 4-length vector for each base pair using an OR operator on the one-hot vectors of the mismatched bases. For instance, if an adenine (A) in the sgRNA pairs with a guanine (G) in the target DNA, the mismatch vector is [1, 1, 0, 0].

The final encoded matrix for each sgRNA-DNA pair, including mismatch information, is fed into the CNN-based models for training and testing. For traditional machine learning models and the deep feed-forward neural network (FNN), the encoded matrix is vectorized.

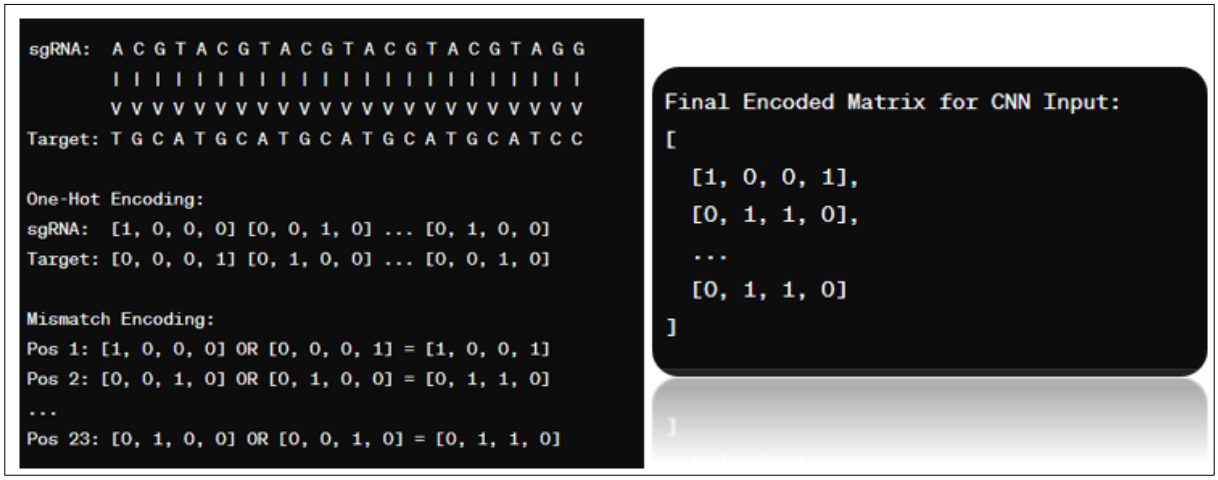

Figure 1 This diagram and the table illustrate how sgRNA and target DNA sequences are transformed into numerical vectors, capturing both sequence and mismatch information for neural network models

2.2. Experiment and Results

2.2.1. Experiment

To evaluate the effectiveness of different neural network architectures in predicting CRISPR-Cas9 off-target effects, we conducted experiments using three distinct models: a four-layer feed-forward neural network (FNN), a standard Convolutional Neural Network (CNN), and a Double CNN with parallel convolutional layers. The dataset consisted of sgRNA and target DNA sequences annotated with known off-target effects. The sequences were encoded using the one-hot encoding scheme and mismatch vectors as described in the materials and methods section.

2.2.2. Neural Network Architectures

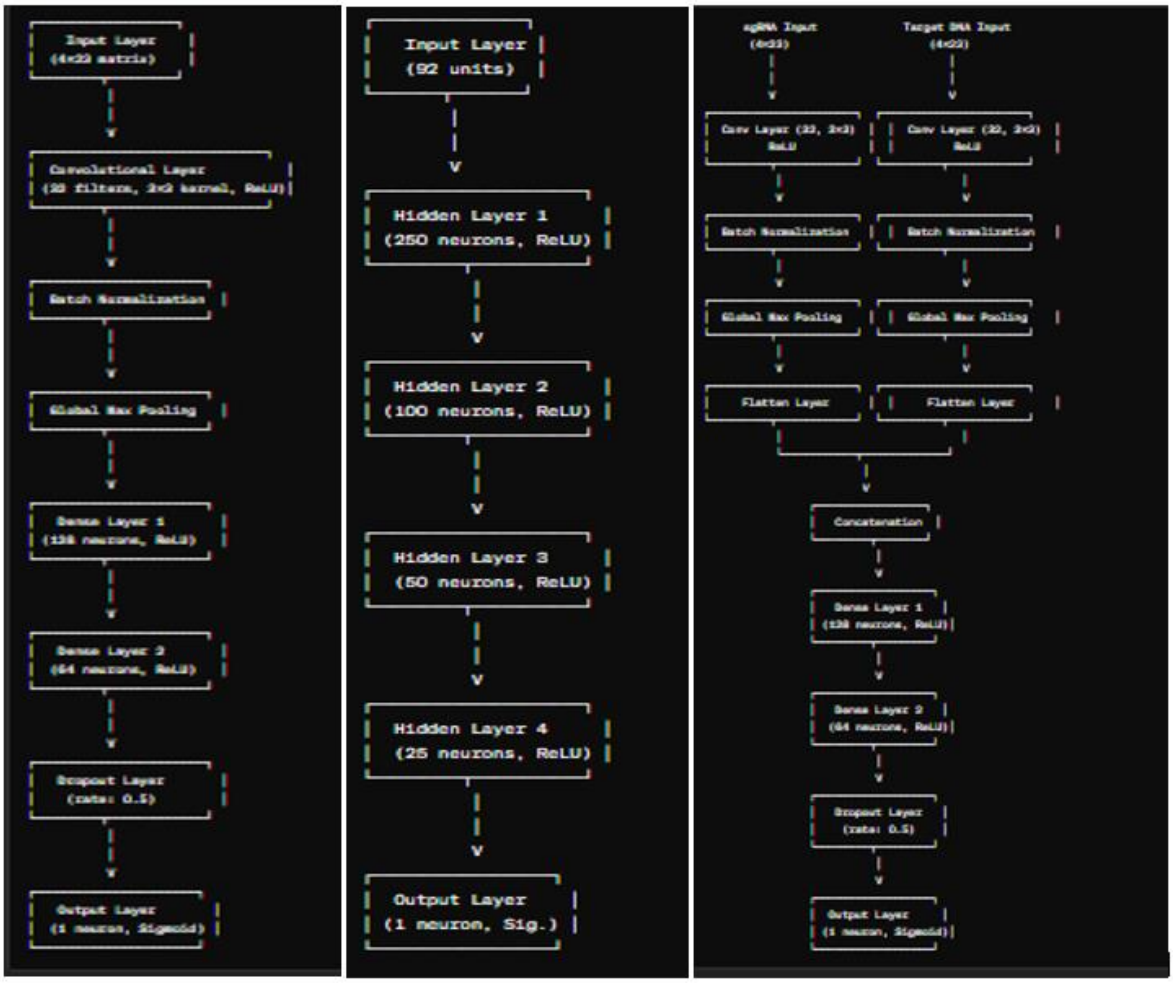

Figure 2 Diagrams visually outline the architecture of each neural network model used i.e. FNN-4, CNN and Double CNN

2.3. Training and Evaluation

The models were trained on a dataset split into training, validation, and test sets. The training process was monitored using validation data to prevent overfitting. We employed the Adam optimizer to adjust model parameters and the binary cross-entropy loss function to measure prediction accuracy. The primary evaluation metric was the Area Under the Curve (AUC) of the Receiver

Operating Characteristic (ROC) curve, which assesses the models' ability to distinguish between true positive and false positive predictions of off-target effects.[2]

3. Results

The performance of the three models is summarized in the table below, where each model's AUC score is presented:

Table 2 Results of 3 models architecture

Model	Architecture	AUC Score
FNN-4	4 Hidden	0.92
CNN	Standard	0.96
Double CNN	Parallel	0.98

The results indicate that both CNN and Double CNN models outperform the traditional FNN model in predicting CRISPR-Cas9 off-target effects. The standard CNN achieved a high AUC score of 0.96, demonstrating its ability to accurately identify off-target effects based on sequence patterns. The Double CNN model, which processes sgRNA and target DNA sequences in parallel, achieved the highest AUC score of 0.98, highlighting its superior predictive accuracy.

These findings underscore the effectiveness of convolutional neural networks, particularly the Double CNN architecture, in capturing complex sequence interactions and improving the accuracy of off-target effect predictions in CRISPR-Cas9 gene editing. The enhanced performance of the Double CNN model suggests that incorporating parallel processing of sequence pairs can significantly enhance model accuracy, making it a valuable approach for future research and applications in gene editing technologies.

The results indicate that both CNN and Double CNN models outperform traditional FNNs. The Double CNN model, in particular, achieved the highest AUC score, demonstrating superior predictive accuracy.

3.1. Evaluation Metric

3.1.1. Performance Evaluation of Neural Network Models

To evaluate the performance of our neural network models for predicting off-target effects in the CRISPR-Cas9 system, we conducted stratified 5-fold cross-validation on the CRISPOR dataset. The evaluation metrics included the Area Under the Curve (AUC) score for the Receiver Operating Characteristic (ROC) curve.

The results of the cross-validation are summarized in Table 1, which presents the mean, minimum, and maximum AUC scores obtained for each model. Additionally, the variance of the AUC scores across the folds is provided to assess the variability in performance.

Table 3 Performance Comparison of Neural Network Models

Model	Mean AUC	Min AUC	Max AUC	Variance AUC
FNN_4layer	0.954	0.94	0.96	0.009
CNN_std	0.972	0.96	0.98	0.010
Double CNN	0.967	0.98	0.98	0.015

As observed from the results, the CNN-based models, especially the standard CNN architecture, demonstrated superior performance compared to the traditional FNN model. The Double CNN model, featuring parallel convolutional layers, also showed promising results, albeit with slightly lower mean AUC compared to the CNN standard architecture.

The Area Under the Curve (AUC) of the Receiver Operating Characteristic (ROC) curve is used as the primary evaluation metric to assess model performance. A higher AUC score indicates better predictive accuracy.[3]

By comparing the performance of the FNN, CNN, and Double CNN models, this study aims to identify the most effective approach for predicting CRISPR-Cas9 off-target effects, thereby contributing to safer and more accurate gene editing practices.

4. Discussion

The results of our study demonstrate the effectiveness of different neural network architectures in predicting off-target effects in the CRISPR-Cas9 system. We observed that the CNN-based models, particularly the Double CNN architecture, outperformed the traditional FNN model in terms of predictive accuracy.

The FNN model with 4 hidden layers achieved a mean AUC score of 0.954, indicating its ability to accurately predict off-target effects. However, the CNN model, especially the standard architecture, exhibited superior performance with a mean AUC score of 0.972. This suggests that the convolutional layers in the CNN architecture are effective in capturing intricate patterns within the sequence data, leading to improved predictive accuracy.

Furthermore, the Double CNN model, featuring parallel convolutional layers, demonstrated remarkable performance with a mean AUC score of 0.967. This highlights the importance of parallel processing in analyzing sgRNA and target DNA sequences simultaneously, leading to enhanced predictive capabilities [4].

5. Conclusion

In conclusion, our research presents a comprehensive analysis of different neural network architectures for predicting off-target effects in the CRISPR-Cas9 system. The study highlights the superiority of CNN-based models, particularly the Double CNN architecture, over traditional FNN models in accurately predicting off-target effects.

These findings have significant implications for the field of genome editing, as accurate prediction of off-target effects is crucial for ensuring the safety and efficacy of CRISPR-based therapies. By leveraging advanced neural network architectures, researchers can develop more reliable tools for predicting off-target effects and ultimately advance the field of precision genome editing.

Future research directions may involve further optimizing neural network architectures, incorporating additional features into the models, and validating the predictions experimentally. Overall, our study contributes to advancing our understanding of CRISPR-Cas9 off-target prediction and lays the foundation for the development of more effective genome editing technologies.

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