Predicting PRDM9 Binding Sites by a Convolutional Neural Network and Verification Using Genetic Recombination Map

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Abstract: PR domain-containing 9 (PRDM9) is a zinc-finger protein that binds to specific DNA motifs and induces the crossing-over between chromosomes, resulting in a high recombination rate around binding sites. Currently, the binding sites of PRDM9 are predicted with methods based on motif matching and Position-specific Weight Matrix (PWM). Meanwhile, the Convolutional Neural Network (CNN) has shown superior performance in recent studies to identify protein-binding regions in general, and it is expected to perform well in PRDM9 binding site prediction. In this study, we compared the performance of PWM and CNN for predicting PRDM9 binding sites with not only test data but also the correlation between the prediction score for a fragment and the local recombination rate to evaluate the performance without overfitting effects. Approximately 170,000 genomic DNA fragments of the human genome containing the Chromatin Immuno-Precipitation with high-throughput sequencing (ChIP-seq) peak of PRDM9 were used for constructing PWM and CNN. We found that CNN outperformed PWM in terms of area under the ROC curve and other metrics. Furthermore, the prediction scores of CNN correlated more strongly with the local recombination rate than PWM. We discuss that the superior performance of CNN would be in part due to the ability of CNN to capture the feature of surrounding sequences of actual PRDM9-binding sites.

Keywords: binding site prediction, CNN, PRDM9, genetic recombination

1. Introduction

Genetic recombination is a fundamental and important process in eukaryotes, and studies are underway to elucidate the mechanism of recombination hotspots, where recombination frequently occurs in early meiosis. PR domain-containing 9 (PRDM9) is a zinc finger protein that induces chromosomal recombination in mammalian germ cells. It binds to the DNA sequence in a sequence-specific manner with its zinc finger domain and localizes in a narrow (1–2 kb) hotspot [1], [2], [3], [4]. PRDM9 tri-methylates the surrounding histones at H3K4 and H3K36 with its PR/SET domain [5], [6], [7], [8]. Topoisomerase sporulation-specific 11 (SPO11) is followingly recruited, which forms a double-strand break, and crossing-over occurs at the site [8], [9].

PRDM9 is highly polymorphic [10]; there are three major alleles, A, B, and C alleles, which consist of 90%, 5%, and 1.5% of alleles in the non-African population, respectively [2], [4].

The binding sites of PRDM9 can be identified by Chromatin Immuno-Precipitation with high-throughput sequencing (ChIP-seq) experiments [8]. However, it is impossible to determine all the binding sites using a limited number of experimental conditions [11], which hampers the comprehensive understanding of the DNA-protein binding mechanisms. Previous studies showed that the canonical 13-mer binding motif of PRDM9 (CCNCC-NTNNCCNC), which is enriched in hotspots, is insufficient to explain all the hotspots [1], [2], [12], [13].

There are two approaches for exploring the factors that determine hotspots. One is to investigate features of hotspots without using PRDM9 binding information. In recent years, important features of recombination hotspots were captured by neural networks [14], [15]. However, these have not yet been able to fully explain the features. Another approach is to investigate the role of PRDM9 binding for producing recombination hotspots. The relationship among PRDM9 binding, chromatin state, and recombination was explored in many studies using mice and primates [16], [17], [18], [19], [20], [21].

In the latter approach, to predict the binding sites of PRDM9, simple motif string searches and Position-specific Weight Matrix (PWM) have been used in previous studies. PWM represents the DNA motifs to which a protein binds [22]. It is created on the basis of the frequency of nucleotides at each position or the binding properties predicted from the amino acid sequence of the zinc finger [23], [24]. Meanwhile, Convolutional Neural Network (CNN), which can learn complex features from large amounts of DNA sequence data, has been reported to predict protein-binding sites more accurately than other methods including PWM [25]. Although CNN could be a superior prediction method than PWM for PRDM9 binding site prediction, there has been no comparison of PRDM9 binding prediction between PWM and CNN.

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In general, when evaluating a model, the prediction accuracy is verified using test data obtained in advance, apart from the training data used to tune the model parameters [26]. However, as overfitting to noise is expected [25], the evaluation would not be independent of the original data. Therefore, it is difficult to use only one experimental data to evaluate the generalized performance of models. To amend the effect of overfitting to one type of training data, combining a validation method using test data and a method that does not require test data is desirable.

In this study, we compared the performance of the PWM and CNN in terms of area under the ROC curve (AUC) and other metrics using test data. We also examined the correlation between the predicted binding score for a genomic fragment and the recombination rate of the region to test whether CNN outperforms PWM, which can avoid overfitting effects. We observed a stronger correlation between the prediction scores and local recombination rates in CNN than in PWM. In addition, we revealed that the DNA fragments with high prediction scores in CNN had higher rates in CNN than in PWM. In addition, we revealed that the relation between the prediction scores and local recombination rates in CNN is independent of the original data. Therefore, it is difficult to use only one experimental data to evaluate the generalized performance of models. To amend the effect of overfitting to one type of training data, combining a validation method using test data and a method that does not require test data is desirable.

2. Materials and Methods

2.1 Materials

We retrieved data of ChIP-seq experiments performed by Altemose et al. (2017) to obtain DNA-PRDM9 binding sequence data [8] (https://www.ncbi.nlm.nih.gov/geo/, GSE99407). The experiments obtained 170,198 nonoverlapping fragments by transfecting HEK293T cell line with N-terminal YFP-tagged human PRDM9 of B-allele. Each ChIP-seq peak was placed at the center of a 301-bp region. We assigned around 10% of the peak regions (17,009) to positive test data and the remaining 90% (153,189) to positive training data for CNN. For negative data, non-overlapping 100-bp-length sequences that are at least 1,000 bp away from any ChIP-seq peaks were extracted from the human reference genomes (hg19).

We used a recombination map estimated by pyrho [21] considering the population demography using genomic polymorphism data. In this study, we used the estimations based on the demography of the CEU (Utah residents with northern and western European ancestry) population.

Overview of the workflow for validation with test data is represented in Fig. 1.

2.2 Preparation for Test Data

To verify the prediction accuracy of CNN and PWM, we created PRDM9-binding test data. Positive test data were generated by cutting out 100-bp-length DNA fragments containing 31 bp around the peaks (ChIP-seq core region) at random positions within the fragments. Negative test data were randomly sampled so that the positive data constituted 2% of total test data, based on the ratio of the ChIP-seq-positive to -negative regions, yielding 833,441 negative test regions.

2.3 PWM Used in the Study and its Scoring

We used PWMs obtained from the same ChIP-seq data and constructed using the Bayesian de novo motif-finding algorithm [27] (Additional file of Altemose et al. [8]). In total, 17 PWMs were obtained.

For scoring, the log-likelihood ratio score $S$, which is obtained based on the occurrence probability of a motif considering the nucleotide frequency in the background sequence, was calculated. The nucleotide frequencies of the background sequences were calculated for each chromosome and strand. The score $S_{i,j}$ is calculated by summing up the log-likelihood values for all sites of the $j$-th PWM, starting from the $k$-th site of the $i$-th fragment for both strands [28].

Next, $S_{i,ja}$ was summarized to the score for the $i$-th fragment and the $j$-th PWM, $S_{i,j}$, expressed as follows:

$$S_{i,j} = \max_{k} S_{i,ja}$$

There are two ways to summarize the score for the $i$-th fragment. One is to accept the maximum score among $N$ PWMs, $S_{\max,i}$, expressed as follows:

$$S_{\max,i} = \max_{1 \leq j \leq N} S_{i,j}$$

The other method is to sum the scores of all $N$ PWMs, $S_{\sum,i}$, expressed as follows:

$$S_{\sum,i} = \sum_{j=1}^{N} 2^{S_{i,j}}$$

We determined how many PWMs should be used for the prediction. All PWMs were ranked by the probability of hitting within 100 bp of ChIP-seq peaks. We added PWMs one by one from the one with the maximum score and evaluated the performance in terms of AUC using $S_{\max,i}$ and $S_{\sum,i}$. As a result, AUC was maximized using up to the 15th PWM with $S_{\sum,i}$, and the condition was adopted as the final PWM scoring (Table 1).

2.4 CNN and its Scoring

We modified the equivariant Bayesian convolutional network (EBCN) [14] written in Python using deep learning libraries: TensorFlow-GPU (Version 1.13.1) [29] and Keras-GPU (Version 2.3.1). The model employs Monte Carlo (MC) dropout [30], in which the dropout is performed during not only training but also
prediction, and produces the average of the repeated output predictions. In addition, the model is internally adjusted so that the output for an input forward sequence is equal to the output of its reverse complement sequence. These features make the accuracy less dependent on the amount of training data, the internal representations simple [31], and inconsistency in the model accuracy from training to training small. It also has an advantage for understanding features CNN detects (i.e., finding motifs), and we adopt this model. The input is a DNA fragment of constant length, which is converted into a matrix of \(4 \times \text{input length}\) by one-hot vectorizing the nucleotides (Table 2). Then, the matrix undergoes multiple convolutional and pooling layers, and scores (0–1) for PRDM9 binding through a dense layer with softmax function are output.

### 2.5 Creating Training Data for CNN and the Network Optimization

Positive training data were created using 90% of the ChIP-seq-positive region using the same method for generating the positive test data (Fig. 1). To construct a robust CNN, we performed data augmentation for ChIP-seq-positive data. We trained the model using three-fold augmented positive fragments with randomly shifting the peak three times for each fragment. ChIP-seq-negative data were prepared to match the number of positive augmented data. The prediction without the augmentation showed smaller AUC than the prediction with the augmentation (data not shown).

We employed the EBCN structure (recombination topology) developed by Brown and Lunter (2019) [14] and further optimized the network structure and hyperparameters. We tuned the number of filters in each convolutional layer, kernel size of the first convolutional layer, number of internal convolutional layers, kernel size of inner convolutional layers, pool size, learning rate, coefficient of the L2 normalization term, dropout rate, batch size, optimizer, and activation function of the convolutional layer (Table 3). In particular, batch size was optimized from among 32, 64, 128, 256, 512, 1024, and 2048, optimizer was from among SGD [32], Momentum [33], and Adam [34], and activation function was from among ELU [35], ReLU [36], SELU [37], and LReLU [38]. The hyperparameter auto-optimization library, Optuna, was employed [39].

Approximately 10% of the training data were used for the hyperparameter optimization. The epoch was set to 20, and the trials were conducted 100 times. After the hyperparameter search, if the network structure conflicts with equivariance, we trimmed at most 1 bp of the input sequence (Tables 4, 5).

### 3. Results

#### 3.1 CNN Outperformed PWM in Prediction Accuracy Using Test Data

We trained CNN after the hyperparameter optimization. The training was repeated five times, 50 epochs for each, and the model with the highest accuracy was adopted as the final model. We compared the AUC of CNN and PWM, using the test data. The AUC of PWM and CNN were 0.8629 and 0.9167, respectively (Fig. 2). To evaluate using other statistics, the optimal threshold was decided using the ROC curve. The point whose
coordinates were closest to (0, 1) was used for the threshold [40]. Accuracies of CNN and PWM were 0.8399 and 0.7911, respectively. In Table 6, we also show sensitivity and specificity.

### 3.2 CNN Outperformed PWM in Prediction Accuracy Validated Using Recombination Map

To evaluate prediction power using recombination rate, the entire autosomal genome was divided into fragments of 100-bp length from the start position, and fragments without ambiguous nucleotide (N) were selected. For each fragment, the average recombination rate (cM/bp) was calculated from the recombination map. We predicted the PRDM9 binding score for each fragment using CNN and PWM. The correlation coefficient ($\rho$) between the prediction score and recombination rate was statistically significant for CNN ($\rho = 0.180$, $p$-value $< 1.0 \times 10^{-47}$) and for PWM ($\rho = 0.138$, $p$-value $< 1.0 \times 10^{-47}$), but the correlation coefficient was higher in CNN than in PWM.

For the visualization shown in Fig. 3, the scored fragments were sorted in descending order by prediction score, and fragments were grouped into 10 bins (10% of data for each bin). The mean and standard error of recombination rate in each bin was calculated and shown in Fig. 3. For CNN, the average recombination rate was high in bins with high prediction scores and low in bins with low prediction scores (Fig. 3 B). To observe the correlation within the group of high scores, we grouped the top 10% of fragments into 1% bins and examined the average recombination rate. Again, CNN showed a stronger correlation than PWM (Fig. 3 A).

### 3.3 Can PWM and CNN Detect Potential Recombination Hotspots without ChIP-seq Peaks?

To examine whether PWM and CNN detect potential recombination hotspots missed by the ChIP-seq experiments, we plot the prediction score and average recombination rate in each bin for ChIP-seq-negative fragments (Fig. 4). Although the correlation became somewhat weaker, we observed a statistically significant correlation between prediction scores and recombination rates. The correlation coefficient between the prediction score and the recombination rate ($\rho$) was 0.129 ($p$-value $< 1.0 \times 10^{-47}$) in PWM and 0.169 ($p$-value $< 1.0 \times 10^{-47}$) in CNN. The results indicate that PWM and CNN properly capture sequence features of recombination hotspots.

### 3.4 CNN Captures Sequence Features Surrounding PRDM9-Binding Site

The results shown in Sections 3.1 and 3.2 indicated that CNN
outperformed PWM in predicting PRDM9 binding, and the result presented in Section 3.3 suggested the possibility of detecting PRDM9 binding sites undetected by ChIP-seq experiments. However, the high recombination rate of fragments with high prediction scores did not necessarily mean that the fragments contain actual PRDM9 binding sites. There are two possible reasons. One possibility is that CNN finds the potential binding sites missed in the ChIP-seq experiments due to differences in the conditions of PRDM9 binding between actual germ cells and cultured cells, or it detects binding sites of other alleles rather than B-allele used for the experiments. Another possibility is that CNN detects the recombination hotspots using surrounding features of the PRDM9 binding site rather than the binding motif.

To test the latter possibility, we examined the distances from ChIP-seq-negative/CNN-negative fragments to the nearest ChIP-seq peaks for autosomal regions. To define CNN-positive fragments, we used the optimal threshold used for the evaluation of accuracy. We obtained 4,118,963 CNN-positive/ChIP-seq-negative fragments, and 22,062,475 CNN-negative/ChIP-seq-negative fragments. We randomly extracted equal numbers of CNN-positive and -negative fragments without overlap (411,886 fragments each) from the ChIP-seq-negative fragments and examined the distance to the nearest ChIP-seq peak (Fig. 5). The results showed that the CNN-positive DNA fragments were significantly closer to ChIP-seq peaks than the CNN-negative fragments (p-value < 1.0 × 10^{-47}, Mann–Whitney U test).

For comparison, we conducted the same procedure using PWM-positive fragments. We obtained 5,579,323 PWM-positive/ChIP-seq-negative fragments and 20,602,115 PWM-negative/ChIP-seq-negative fragments. We randomly extracted 557,922 PWM-positive fragments without overlap. The CNN-positive fragments were still significantly closer to ChIP-seq peaks than the PWM-positive fragments (p-value < 1.0 × 10^{-47}, Mann–Whitney U test) (Fig. 6).

4. Discussion

4.1 Advantages of Validation Using Recombination Map

In this study, we compared the performance of CNN and PWM in terms of the correlation between prediction scores and recombination rates as well as the evaluation using test data. In these two methods, CNN consistently outperformed PWM, and the method using recombination map was confirmed its usefulness for validation. The method allows us to evaluate models without the influence of overfitting to the ChIP-seq data and verify the prediction accuracy even for ChIP-seq-negative data. This validation method is not limited to PWM or CNN but can be applied to a variety of prediction models.

4.2 ChIP-seq-positive Fragments Are GC-rich

The results shown in Fig. 5 indicated that CNN potentially captures the surrounding sequence features as well as directly recognizing binding sites of PRDM9. We hypothesized that this could be in part due to the cytosine-rich nature of the PWM motif and the change in GC content caused by the biased gene conversion that occurs after recombination repair [41]. We found that the GC contents of ChIP-seq-positive fragments in test data were significantly skewed toward high GC% (Table 7) (p-value < 1.0 × 10^{-47}, Chi-squared test). This high GC content can be one explanation for the features around the motifs that CNN recognized.

4.3 How Much Canonical Binding Motif Has Importance when CNN Predicts?

We further conducted an analysis using the 13-mer motif to examine how much importance the CNN places on the binding site motif of the PRDM9 when scoring a fragment. First, we classified sequences that were erroneously determined as positive by CNN and PWM (i.e., false-positive fragments), after excluding the overlap. We counted the number of occurrences of the 13-mer motif in each group (Table 8). The results showed there was a significantly higher proportion of 13-mer motifs in the PWM-false-positive fragments than that in the CNN-false-
positive fragments ($p$-value $= 4.71 \times 10^{-47}$, Chi-squared test). We performed a similar analysis for false-negative fragments; however, there was no significant difference between the CNN-false-negative and PWM-false-negative fragments ($p$-value $= 0.176$, Chi-squared test). These results indicate that PWM is prone to output false positives in response to the presence of the motifs more than CNN.

### 4.4 The Features that CNN Detects and the Reason why CNN Outperformed PWM

To understand the features of the sequences captured by CNN more directly, we performed motif extraction, following the method by Brown and Lunter [14]. We performed a convolution operation to sampled 1% of all non-overlapping fragments without ambiguous nucleotide for each filter in the first layer of the trained EBCN and recorded the sequence (filter length) at the position where the output was maximum for each 100 bp DNA sequence. The DNA sequences that scored up to the top 0.1% were aligned and PWMs corresponding to each filter were created on the basis of the observed frequency of nucleotides at each position. To avoid zero probabilities, pseudocounts of 0.25 were added to correct the nucleotide frequencies at each position [42]. These PWMs should represent motifs that play an important role in prediction, but whether they under- or over-represent motifs in the ChIP-seq-positive fragments is not clear. Therefore, we measured AUC for each PWM with the test data. Because of the nature of EBCN structure, the weights of the first half of filters and the second half of filters are reverse-complementary. The analysis was therefore performed using the first 44 filters. The results are presented in Table 9. We found that most of the motifs recorded AUC above 0.5, which indicates they are more likely to appear in the ChIP-seq sequence (overrepresented motifs). On the other hand, eight motifs showed AUC below 0.5, which indicates they are less likely to occur in the ChIP-seq-positve fragments (underrepresented motifs). Some of the overrepresented motifs were similar to the 13-mer motif (examples are shown in Fig. 7). The underrepresented motifs were AT-rich (examples are shown in Fig. 8), which is consistent with the results shown in Table 7.

While PWM uses a scoring method that adds up the scores of sequences that appear frequently in ChIP-seq-positve fragments, CNN would subtract scores reacting to motifs that are less frequently appear in ChIP-seq-positve fragments, which can be one of the reasons why CNN outperformed PWM.

### 4.5 Drawbacks of Current Methods for PRDM9-binding Site Prediction

So far, we have compared CNN and PWM, and revealed CNN can be an alternative prediction method for PWM. However, the current prediction ability is restricted for several reasons. In this study, we used ChIP-seq data using PRDM9 B-allele. In the B-allele, there is no change in the zinc fingers recognizing the major 13-mer motif compared to the A allele, but there is a change from serine to threonine in the outside of the motif [2], [4], and the substitution was suggested a slightly altered binding property [2], [8], [10]. Therefore, ideally, a large dataset using other alleles should be included in the training. Sequences that do not bind specifically to the B-allele (or do not bind easily) can be captured using a larger dataset and a CNN with higher prediction performance can be obtained. We also should note that the prediction is based on only DNA sequence information in this study and the method does not take into account chromatin structure or 3D structure information. Such multimodal learnings will allow us to predict the binding site with more accuracy and know further features of PRDM9 binding sites.
eliminate the e
formed PWM in the validation using test data. In addition, to
and CNN created from the same ChIP-seq data. CNN outper-
Underrepresented AT-rich motifs. The heights of strings represent
Fig. 8
Overrepresented motifs aligned with the canonical motif. The
heights of strings represent Information Content. For ease of view-
ing, Filter ID 22 was depicted in its reverse-complement.

![Fig. 7](image)

**Fig. 7** Overrepresented motifs aligned with the canonical motif. The heights of strings represent Information Content.

13-mer

**CCNCCNTNCCNC**

![Fig. 8](image)

**Fig. 8** Underrepresented AT-rich motifs. The heights of strings represent Information Content.

5. Concluding Remarks

We evaluated PRDM9 binding site prediction methods, PWM and CNN created from the same ChIP-seq data. CNN outper-
formed PWM in the validation using test data. In addition, to
eliminate the effect of overfitting to a particular dataset, we devel-
oped a strategy to evaluate the accuracy of prediction by examin-
ing the correlation between prediction score and local recombina-
tion rate. Consequently, we found that CNN outperformed in both
methods. This validation method is applicable for not only PWM and
CNN but also a variety of models. Further, we examined the
genomic distance between the ChIP-seq peak and CNN-positive
fragments, nucleotide composition, and occurrence of the 13-mer
motif in fragments and motifs extracted from EBCN. The analysis
suggested that CNN recognized not only the binding motif of the
zinc finger but also the features of surrounding sequences such as
skewed GC content. In addition, a different scoring strategy of
CNN from PWM, where CNN detects underrepresented features
in ChIP-seq fragments, was confirmed. However, the ability of
CNN is currently restricted. With large flexibility, CNN can learn
other information such as other alleles' binding sites, chromatin
state, and 3D structure of DNA sequence for better prediction,
which will allow CNN to make better predictions.

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Appendix
The source codes and supplementary materials which include all PWMs extracted from EBCN are available on the GitHub repository (https://github.com/ibio-nakamura/PRDM9-public).

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