



# iDNA6mA (5-step rule): Identification of DNA N<sup>6</sup>-methyladenine sites in the rice genome by intelligent computational model via Chou's 5-step rule



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## ABSTRACT

DNA methylation is an elementary epigenetic process. The N<sup>6</sup>-methyladenine is related to a large kind of biological processes i.e., transcription, DNA replication, and repair. In genome, the N<sup>6</sup>-methyladenine (6 mA) site distribution is non-random; therefore, precise discrimination of 6 mA is necessary to understand its biological functions. Through biochemical experiments, the N<sup>6</sup>-methyladenine produced a positive outcome, still, these wet lab processes are very time consuming and high pricy. In view of this, it is of high priority to introduce a powerful, accurate, and fast computational model to identify N<sup>6</sup>-methyladenine sites. In this connection, we propose an intelligent computational model called iDNA6mA (5-step rule) using deep learning approach to identify N<sup>6</sup>-methyladenine sites from DNA sequences in the rice genome. Existing methods used handcrafted features to identify N<sup>6</sup>-methyladenine sites; however, the proposed computational model automatically extracts the key features from DNA input sequences via the proposed convolution neural network (CNN) model. The intelligent computational model iDNA6mA (5-step rule) obtained 86.64% of accuracy, 86.70% of sensitivity, 86.59% of specificity, 0.732 of MCC, and 0.931 of auROC. The results demonstrate that the proposed intelligent computational model achieved better performance in terms of all evaluation parameters than existing techniques. It is observed that iDNA6mA (5-step rule) model will become a useful tool in the fields of computational biology, bioinformatics, and for the academic research on N<sup>6</sup>-methyladenine sites prediction. A user-friendly webserver has been established and freely accessible at <https://home.jbnu.ac.kr/NSCL/iDNA6mA.htm>.

## 1. Introduction

DNA methylation is an elementary epigenetic process. In eukaryotes [1], the high recognized DNA modification is 5-methylcytosine (5 mC) sites [2] while in prokaryotes; the most pervasive DNA modification is N<sup>6</sup>-methyladenine (6 mA) sites [3]. The abundance and presence of 6 mA in eukaryotes have been reported in early discussion [1,4]. Specific unicellular eukaryotes, green algae, and ciliates consist of both 5 mC and 6 mA in their genomes, however the biological importance of these modifications, for living beings had remained mostly uncharacterized [5]. Most recently, DNA N<sup>6</sup>-methyladenine (6 mA) as a non-canonical DNA modification has been reported in three kingdoms of life [6]. The modification of N<sup>6</sup>-methyladenine is nearly concerned along with a number of biological processes i.e., transcription [7], DNA replication [8] and repair [9]. To decipher the complete biological functions of 6 mA, it

is imperative to identify its location throughout the whole genome.

A number of experimental procedures like single-molecule real-time sequencing (SMRT-seq) [10], methylated DNA Immunoprecipitation sequencing (MeDIP-seq) [11], and capillary electrophoresis and laser-induced fluorescence (CE-LIF) [12] have been reported. A group of Chinese scientists [13] has recently explored the 6 mA profile of rice genome by mass spectrometry analysis and immunoprecipitation followed by sequencing (IP-seq). However, along with productive data information, the experimental procedure also impedes the genome-wide prediction of N<sup>6</sup>-methyladenine. Therefore, the development of a computational model using the pre-existed experimental data to identify the N<sup>6</sup>-methyladenine site will cope with this issue and provide ease for future studies. Although the biochemical exploratory techniques can give some information related to the 6 mA sites, it is high pricy and labor-intensive. Thus, it is a huge challenge to establish fast and precise

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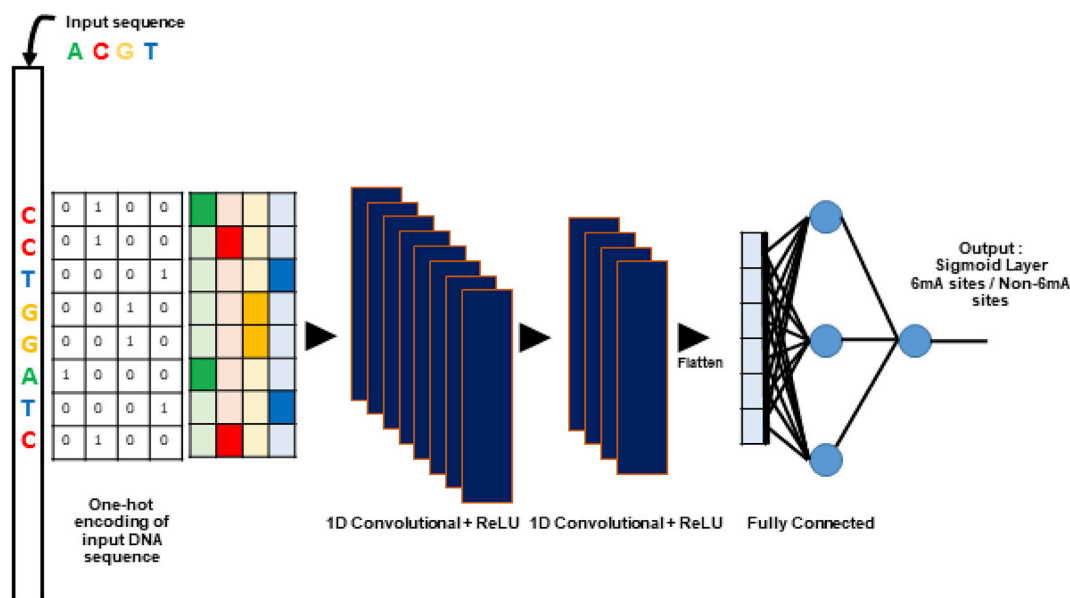


Fig. 1. The architecture of iDNA6mA (5-step rule) model.

computational model to identifying the 6 mA sites.

The ubiquitous and complex post-translational modifications (PTMs) play a key roles in different biological processes, as well as in protein localization and trafficking, protein folding, cell signaling, transcriptional regulation, cell-cell interactions, apoptosis, and regulating cellular dynamic and plasticity [14–40]. The irregularity of the PTMs are nearly related or connected with harmful diseases i.e., Alzheimer's, Parkinson's and cancers. Accordingly, researchers introduced a variety of predictors and techniques for discrimination of PTM sites using protein and RNA/DNA samples [41–45]. Recently, Chen et al. [46] introduced a predictor using machine learning to predict the  $N^6$ -methyladenine sites in the rice genome based on support vector machine (SVM) and nucleotide frequency and nucleotide chemical properties as features extraction techniques. The existing computational models need domain knowledge to hand design the input feature spaces. The second step of the guideline of Chou's 5-step rules [17,44,47–59] for developing a useful prediction model is to extract feature space from the RNA/DNA sequences. The computational model may automatically capture the important features of 6 mA sites from input samples. This concept achieved by deep learning to extract the features from multiple levels of abstraction. Deep learning has generated very successful results in natural language processing [60, 61], speech recognition [62–64], and image recognition [65–67]. Currently, various predictors have been proposed based on deep learning such as iDeepS [68], branch point selection [69], alternative splicing sites prediction [70], and iRNA-PseKNC(2methyl) prediction model [71].

In this connection, we propose iDNA6mA (5-step rule) model for the identification of DNA  $N^6$ -methyladenine sites that is based on the deep learning approach. We observe that our proposed novel deep learning method has superior prediction outcomes compared to the existing machine learning method [46]. In addition, a user-friendly webserver has been established and freely accessible at <https://home.jbnu.ac.kr/NSCL/iDNA6mA.htm>.

## 2. Materials and methods

### 2.1. Benchmark dataset

In accordance to the guideline of Chou's 5-step rules for constructing a useful prediction model [34,72–79], the first step is to select/construct a reliable benchmark dataset for training and testing the prediction system effectively. Therefore, we have selected and downloaded a valid benchmark dataset (<http://lin-group.cn/server/i6mAPred/data>), which was

constructed by Chen et al. [46], to show the efficiency of the proposed prediction model. The length of all samples is 41-bp long with the 6 mA site in the center. Thus, the benchmark dataset can be formulated as below:

$$S = S^+ \cup S^- \quad (1)$$

The benchmark dataset  $S$  consists of 1760 samples; where  $S^+$  represents the positive subset and contains 880 6 mA sites and  $S^-$  is the negative subset and contains 880 non-6mA sites. The  $\cup$  is the union in the set theory. The dataset is divided as 70% training, 10% validation and 20% testing.

### 2.2. The proposed model

Fig. 1 illustrates the framework of iDNA6mA (5-step rule) model that is based on convolutional neural networks (CNN). The CNN is a frequently and widely employed method by various researchers [69,70, 80] in the field of bioinformatics. During training the convolutional neural network, automatically learns the primary features from the input samples. The iDNA6mA (5-step rule) model takes a single input of a DNA sequence  $s = \{s_1 s_2 s_3 \dots s_n\}$ , where  $s_i \in \{A, C, G, T\}$  and  $n = 41$ , and produces a real-valued prediction output. We first employ one-hot encoding for the sequences and feed them into CNN to identify 6 mA sites. The length of vector equals to the length of the input sample (e.g. here the length is 41) and 4-channel are A, C, G, and T and represented as (1, 0, 0, 0), (0, 1, 0, 0), (0, 0, 1, 0), and (0, 0, 0, 1), respectively.

Generally, one processing step in convolution neural network named a layer, that is represented by convolution layer, pooling layer, ReLU layer, normalization layer, dropout layer, fully connected layer, etc. Different hyper-parameters have been tuned during learning such as filter size, number of convolution layers and so forth. Table 1 shows the tuned hyper-parameters in the proposed CNN model (see Table 2).

The best hyper-parameters have been chosen on the bases of high

Table 1

The hyper parameters to be tuned in CNN.

Hyper Parameter	Range
No. of convolution layers	[1,2]
Filter size	[2–5,7]
The number of the filters	[2,4,6,8,10,12]
Dropout probability	[0.1, 0.2, 0.3, 0.4]

**Table 2**  
The summary of iDNA6mA (5-step rule) model.

Model Layers	Output Shape
Sample Input	(41,4)
Conv1D(8,5,1)	(41,8)
Conv1D(4,3,1)	(41,4)
Dropout(0.25)	164
Dense(1)	1

success rate of auROC, MCC, accuracy, sensitivity, and specificity. The convolution layer can be numerically expressed as below:

$$\text{Conv}(R)_{jk} = \text{ReLU} \left( \sum_{fs=0}^{FS-1} \sum_{f=0}^{F-1} W_{fsf}^k R_{j+fs,f} \right) \quad (2)$$

In Equation (2), R represents the input DNA/RNA sample, fs and f represent the filter size and the number of the input channels, respectively, j denotes the index of the output position, and k denotes the index of the filters. ReLU denotes the rectified linear function and numerically represented below:

$$\text{ReLU}(t) = \max(0, t) \quad (3)$$

Where t is the input to the neuron and “max” is an operand that returns the maximum of its inputs. The sigmoid layer is a nonlinear activation function and outputs in the range [0, 1]. It is used for predicting whether the input sequence is an N<sup>6</sup>-methyladenine site or not. This function can be mathematically expressed as below:

$$\text{Sigmoid}(t) = \frac{1}{1 + e^{-t}} \quad (4)$$

Where t is the input to the neuron.

In this paper, Keras framework [81] is used for building the proposed model iDNA6mA (5-step rule). The number of batches is set to 32 and the number of epochs is set to 50. Learning rate is set to 0.001 and optimizer is Adam.

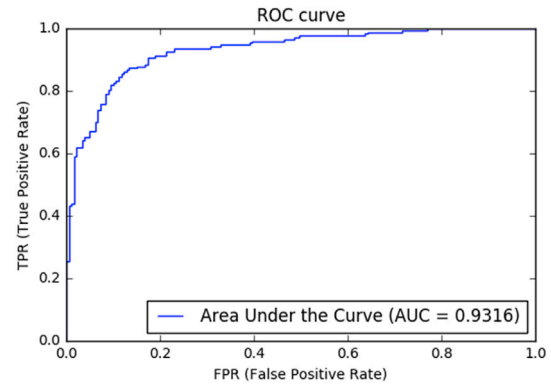
### 3. Results and discussion

#### 3.1. Performance evaluation

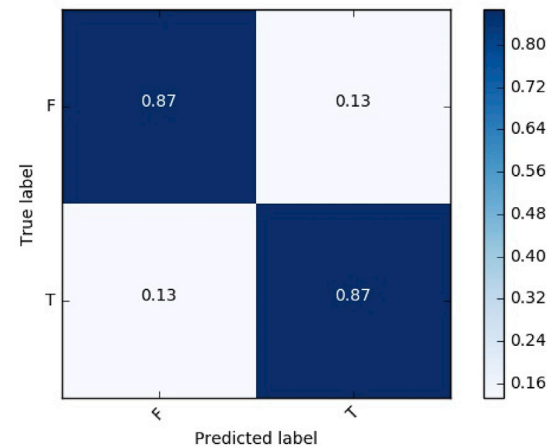
In order to measure the success rate of the prediction system, the following four metrics are used [75,82–89].

$$\left\{ \begin{array}{l} \text{MCC} = \frac{1 - \left( \frac{E_+^+ + E_-^-}{E_-^+ + E_+^-} \right)}{\sqrt{\left( \frac{E_-^+ + E_+^-}{E_-^+ + E_+^-} + 1 \right) \left( \frac{E_+^+ + E_-^-}{E_+^+ + E_-^-} + 1 \right)}} \quad -1 \leq \text{MCC} \leq 1 \\ \text{Accuracy} = 1 - \left( \frac{E_-^+ + E_+^-}{E_+^+ + E_-^-} \right) \quad 0 \leq \text{Acc} \leq 1 \\ \text{Sensitivity} = 1 - \left( \frac{E_+^-}{E_+^+} \right) \quad 0 \leq \text{Sn} \leq 1 \\ \text{Specificity} = 1 - \left( \frac{E_-^+}{E_-^-} \right) \quad 0 \leq \text{Sp} \leq 1 \end{array} \right. \quad (5)$$

The significance and details of these evaluation metrics can be found in Refs. [87–90].  $E_+^+$  denotes the size of the positive dataset samples or N<sup>6</sup>-methyladenine sites; while  $E_-^-$  denotes the size of the negative dataset samples or non- N<sup>6</sup>-methyladenine sites;  $E_+^-$  is the number of non-N<sup>6</sup>-methyladenine sites sample that are predicted incorrectly to be N<sup>6</sup>-methyladenine sites while  $E_-^+$  is the number of N<sup>6</sup>-methyladenine sites samples that are predicted incorrectly to be of non- N<sup>6</sup>-methyladenine sites. MCC reflects the performance of proposed prediction model on imbalance dataset, here the ratio of negative and positive sequences is



**Fig. 2.** The auROC curve of the intelligent computational model iDNA6mA (5-step rule).



**Fig. 3.** The visualization of the confusion matrix of the intelligent computational model iDNA6mA (5-step rule).

the same. The ROC curve is used to calculate the success rate of proposed intelligent computational model. The auROC (area under the ROC curve) is the most important indicator to measure the prediction quality of a binary classifier.

#### 3.2. Results and Discussion

In this section, we discuss the success rate of the proposed prediction system iDNA6mA (5-step rule) using one benchmark dataset. The proposed predictor obtains 86.64% of accuracy, 86.59% of specificity, 86.70% of sensitivity, 0.732 of MCC, and 0.931 of auROC. The detailed outcomes of the proposed predictor are shown belows:

$$\left\{ \begin{array}{l} \text{Acc} = 86.64\% \\ \text{Sen} = 86.70\% \\ \text{Sp} = 86.59\% \\ \text{MCC} = 0.732 \end{array} \right.$$

Additionally, Fig. 2 shows the performance of the auROC curve of the iDNA6mA (5-step rule) model and the visualization representation of the confusion matrix is shown in Fig. 3.

**Table 3**

The performance comparison between iDNA6mA (5-step rule) and other with Existing Method.

Methods	Accuracy	Specificity	Sensitivity	MCC	auROC
iDNA6mA (5-step rule)	86.64	86.59	86.70	0.73	0.931
6 mA-Pred [46]	83.13	83.30	82.95	0.66	0.886

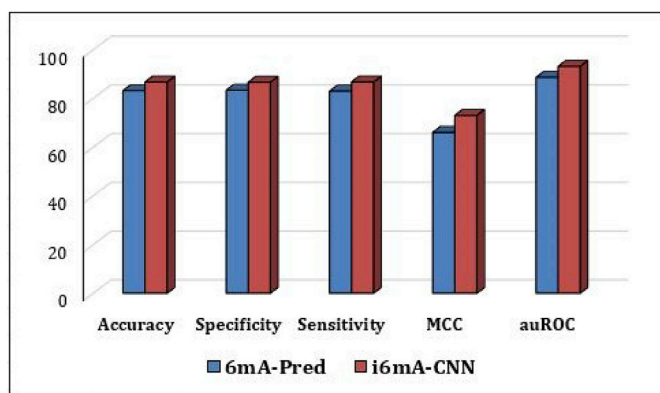


Fig. 4. Performance Comparison of proposed iDNA6mA (5-step rule) with existing method 6 mA-Pred.

The success rate of the iDNA6mA (5-step rule) computational model is compared with existing 6 mA-Pred model [46]. Table 3 shows the performance of the four evaluation metrics of the both models. It is evident that iDNA6mA (5-step rule) model outperforms the 6 mA-Pred model by 3.51% of accuracy, 3.29% of specificity, 3.75% of sensitivity, 7.00% of MCC, and 4.50% of auROC.

The graphical illustration of the experimental outcomes is presented in Fig. 4 in which the iDNA6mA (5-step rule) method obtains remarkable outcomes compared to the existing prediction system.

#### 4. Webserver

In order to make the proposed iDNA6mA (5-step rule) model accessible by other researchers, a user-friendly webserver has been constructed [91–97]. The developed webserver supports finding i6mA sites using either direct input in Fasta format as shown in Fig. 5 or uploading the sequences in on file in Fasta format as shown in Fig. 6. The webserver has been built using Python programming language with Flask library. It is made accessible at <https://home.jbnu.ac.kr/NSCL/iDNA6mA.htm>.

#### 5. Conclusions

We developed a novel and fully automatic deep learning based computational model namely iDNA6mA (5-step rule) to identify N<sup>6</sup>-methyladenine sites from DNA sequences only. We first employed one-hot encoding for the input DNA sequences and fed them into CNN to identify N<sup>6</sup>-methyladenine sites. The simulation outcomes showed the effectiveness of our proposed iDNA6mA (5-step rule) prediction model. It

##### Direct Input Fasta Format

[Example]

Input sequence: The input length is fixed at 41bp.

```
>seq1
AATTGGATAGGAGGAGCCGATGTAGCTGATTCTAGCAAGA
>seq2
GTATATAACTTTTCTTCAAGGCAGCAGGTGCTGCCTAA
>seq3
AACGGGTGGACGTCCACCGAATGATTAGAATCCCTCTCCA
>seq4
GAGCAATTAGGGATGAGTGACCGACCGGAAATCTTCTC
>seq5
CCCAGGCCGGGCCGCTTAAATCTGGCAGCTCTCATAGGTC
>seq6
AGGGACATAATCAGTTTCGAGGCAAAATTTGAATATATTT
>seq7
AAAAAAATGATGGAATGAGGTACCAACAGTGTGAGTAT
>seq8
GCAAAAGGGGTTGAGAAAAAGATGTACCAAGAAATCCAAGGG
```

Threshold: 0.5

Submit sequences

Fig. 5. Direct input sequences for i6mA sites identification using the proposed iDNA6mA (5-step rule) model.

##### Process Fasta File (Max 1000 sequences)

Threshold: 0.5

Please upload a text file containing sequences for 6mA identification

Browse... No file selected.

Upload and process the file

Figure 6. Processing FASTA file containing sequences for i6mA sites identification using the proposed iDNA6mA (5-step rule) model.

provided better prediction success rates in terms of all evaluation metrics (accuracy, specificity, sensitivity, MCC, and ROC) compared with the state-of-the-art technique. The proposed methodology can be potentially effective in pharmaceutical industry for drug innovation and design and in the area of the bioinformatics. Finally, a webserver has been built using Python programming language with Flask library and made accessible at <https://home.jbnu.ac.kr/NSCL/iDNA6mA.htm>. In addition, we have deposited the model with the best learnt weights on the github at <https://github.com/hilal-t/iDNA6mA>.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemolab.2019.04.007>.

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