im6A-TS-CNN: Identifying the N⁶-Methyladenine Site in Multiple Tissues by Using the Convolutional Neural Network

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N⁶-methyladenosine (m⁶A) is the most abundant post-transcriptional modification and involves a series of important biological processes. Therefore, accurate detection of the m⁶A site is very important for revealing its biological functions and impacts on diseases. Although both experimental and computational methods have been proposed for identifying m⁶A sites, few of them are able to detect m⁶A sites in different tissues. With the consideration of the spatial specificity of m⁶A modification, it is necessary to develop methods able to detect the m⁶A site in different tissues. In this work, by using the convolutional neural network (CNN), we proposed a new method, called im6A-TS-CNN, that can identify m⁶A sites in brain, liver, kidney, heart, and testis of Homo sapiens, Mus musculus, and Rattus norvegicus. In im6A-TS-CNN, the samples were encoded by using the one-hot encoding scheme. The results from both a 5-fold cross-validation test and independent dataset test demonstrate that im6A-TS-CNN is better than the existing method for the same purpose. The command-line version of im6A-TS-CNN is illustrated in Figure 1.

INTRODUCTION

As a common and abundant of RNA post-transcriptional modification (PTM), N⁶-methyladenosine (m⁶A) modification plays an important role in almost all processes of cell cycles, such as affecting translation efficiency, cell development, cell viability, etc. m⁶A is catalyzed by a methyltransferase complex containing METTL3, METTL14, and WTAP. As a kind of dynamic PTM, m⁶A can be catalyzed by a methyltransferase complex containing METTL3, erased by the demethylases FTO and ALKBH5. Recently, more studies have revealed that m⁶A is closely correlated with diseases, such as obesity, thyroid tumor, prostate cancer, zika virus, and acute myelogenous leukemia. However, our knowledge about the functions of m⁶A modifications is still unintelligible. To deepen our understanding on the functions of m⁶A, the key step is to know the precise position of m⁶A in transcriptomes.

There are two main ways to identify m⁶A sites. One way is using experimental methods, such as Methylated RNA Immunoprecipitation (MeRIP), m⁶A sequencing (m⁶A-seq), photo-crosslinking-assisted (PA)-m⁶A-seq, and m⁶A-crosslinking immunoprecipitation (CLIP). These experimental methods laid important foundations for the detection of m⁶A modification sites. Accordingly, some bioinformatics tools that are able to detect m⁶A sites directly from the reads generated by the experiments were proposed. However, as the amount of sequencing data increases, we need to find an effective and efficient way to detect m⁶A in the transcriptome. Accordingly, sequence information-based computational methods were proposed to identify m⁶A sites. These methods can be queried in a recent review. With the research on the spatial specificity of gene expression, it has been found that the location of the m⁶A site is distinct in different tissues and species. Therefore, Dao et al. proposed a tool, called iRNA-m6A that can identify m⁶A modification sites in different tissues in human, mouse, and rat by using the algorithm of SVM, based on the data of Zhang et al. This method greatly improves the accuracy of predicting the m⁶A site. However, the performance for predicting the m⁶A site still has great potential to be improved.

In recent years, the deep learning algorithms made great contributions to bioinformatics. A large number of computational methods based on deep-learning algorithm, such as Gene2Vec, BERMP, DeepM6ASeq, and iPseU-CNN have been proposed. Inspired by these successful applications of the deep-learning algorithm in identifying RNA modifications, in the present work, we proposed a convolutional neural network (CNN)-based method, called im6A-TS-CNN, to identify m⁶A sites in different tissues from human, mouse, and rat. Results from a 5-fold cross-validation test and independent dataset test demonstrated that the performance of im6A-TS-CNN is better than or comparable with that of the existing method for the same aim. Moreover, the universality of im6A-TS-CNN was also demonstrated by a cross-species validation test. The framework of im6A-TS-CNN is illustrated in Figure 1.

Received 5 June 2020; accepted 28 July 2020; https://doi.org/10.1016/j.omtn.2020.07.034.
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RESULTS AND DISCUSSION

Model Performance
In this article, the Keras in TensorFlow 2.0 under Python 3.6 was used to perform the predictions. The results from a 5-fold cross-validation test and independent dataset test of the proposed method for identifying the tissue-specific m^6A modification sites in the human, mouse, and rat were shown in Table 1. With the comparison of results from a 5-fold cross-validation test and independent test, it was found that the proposed method is stable for identifying the m^6A sites.

To measure objectively the performance of the proposed method, the receiver operating characteristic (ROC) curves from a 5-fold cross-validation test and independent test were plotted in Figure 2 as well. It was found that most of the areas under the ROC curve (AUCs) are higher than 0.8 in both the 5-fold cross-validation test and independent test, demonstrating the reliability of the proposed method for identifying tissue-specific m^6A sites.

Comparison with Existing Method
To further testify the superiority of im6A-TS-CNN, we compared its performance with that of Zhang et al.'s iRNA-m6A model, based on both the 5-fold cross-validation test and independent test. The comparative results in terms of AUC are shown in Table 2. Except for the identification of the m^6A sites from the brain of mouse and rat, im6A-TS-CNN outperforms iRNA-m6A for the identification of m^6A sites in the other tissues in the human, mouse, and rat. These results demonstrate that im6A-TS-CNN is a powerful tool for identifying tissue-specific m^6A sites.

Cross-Species and Cross-Tissue Validation
Since the datasets are from different species and tissues, it is interesting to test whether the model, trained based on the data from a specific tissue in a species, is able to identify m^6A from other tissues and species. Accordingly, the cross-species and cross-tissue validation was performed. The AUCs of im6A-TS-CNN for identifying m^6A sites from other species and tissues are shown in Figure 3. As shown in Figure 3, it can be concluded that im6A-TS-CNN is also effective for the cross-species and cross-tissue identification of m^6A sites, demonstrating the universality of the proposed method.

Conclusions
In this article, we proposed a CNN-based method, called im6A-TS-CNN, for identifying m^6A in the brain, liver, kidney, heart, and testis from the human, mouse, and rat. The results from a 5-fold cross-validation test and independent test demonstrate that im6A-TS-CNN is better than the existing method for identifying tissue-specific m^6A. For the convenience of the scientific community, the command-line version of im6A-TS-CNN, together with its source code and user manual, is provided at https://github.com/liukeweiaway/DeepM6A_cnn. In addition, the high-, normal-, and low-threshold options were provided to control the false-positive rate. The corresponding performance with different options was listed in Table S1. Taken together, we hope that the im6A-TS-CNN will become a useful tool for identifying m^6A sites.

MATERIALS AND METHODS

Datasets
A high-quality dataset is very important for the construction of a computational model. In 2019, Zhang et al. developed a high-throughput, antibody-independent m^6A detection method based on the m^6A-sensitive RNA endoribonuclease to identify the m^6A site in different tissues, namely the brain, liver, kidney, heart, and testis from the human, mouse, and rat. Based on these data, Dao et al. built a high-quality benchmark dataset that can be used to train a computational method for identifying m^6A sites, which contains both m^6A site- and non-m^6A site-containing sequences with the length of 41 nt. The CD-HIT program was used to make sure that the sequence similarity of the dataset was less than 80%. The detailed information of this dataset is provided in Table 3.

One-Hot Encoding
One-hot encoding is a common and effective method. According to such a scheme, in an RNA segment, A is represented as (1,0,0,0), U as...
(0,1,0,0), C as (0,0,1,0), and G as (0,0,0,1). Therefore, an RNA sequence of length \( l \) can be converted into a 4-\( l \) dimensional vector.

**Convolutional Neural Network**

In recent years, Convolutional Neural Network (CNN) has been widely used to solve biological problems.\(^{22,27,28}\) The structure of the CNN is shown in Figure 1. It contains a convolutional layer with 200 filters in which the kernel size is 6. After convolution operation, a max-pooling layer with the size of 4 was added. The convolution layer is mathematically represented and computed as the following:

\[
\text{Conv}(R)_f = \text{ReLU} \left( \sum_{j=0}^{S-1} \sum_{n=0}^{N-1} W^f_{n,j} R_{i,n} \right),
\]

(Equation 1)

where \( R \) represents the RNA segment, \( f \) denotes the index of the kernel, and \( j \) denotes the index of the output position. In Equation 1, each filter \( W^f \) is an \( S \times N \) weight matrix, where \( S \) is the filter size, and

![Figure 1.](image)

**Figure 1. The Performance of im6A-TS-CNN for Identifying m^6^A Sites**

| 5-Fold Cross Validation |  |  |  |  | Independent Test |  |  |  |  |
|-------------------------|---|---|---|---|-----------------|---|---|---|---|
| Sn (%)                  | Sp (%) | Acc (%) | MCC | AUC | Sn (%)                  | Sp (%) | Acc (%) | MCC | AUC |
| h_b                     | 75.35  | 69.71  | 72.53 | 0.4523 | 75.17  | 70.20  | 72.69 | 0.4543 | 0.8029 | 0.8056 |
| h_k                     | 81.70  | 78.25  | 79.98 | 0.6006 | 79.95  | 78.53  | 79.24 | 0.5848 | 0.8781 | 0.8727 |
| h_l                     | 80.18  | 79.69  | 79.94 | 0.5992 | 84.81  | 75.02  | 79.92 | 0.6012 | 0.8805 |
| m_b                     | 81.50  | 75.85  | 78.67 | 0.5749 | 86.22  | 70.74  | 78.48 | 0.5765 | 0.8722 |
| m_h                     | 78.37  | 67.60  | 72.99 | 0.4633 | 75.82  | 71.36  | 73.59 | 0.4723 | 0.8161 |
| m_k                     | 79.91  | 81.00  | 80.46 | 0.6094 | 80.52  | 81.00  | 80.76 | 0.6151 | 0.8855 |
| m_l                     | 72.39  | 70.24  | 71.32 | 0.4288 | 75.56  | 67.58  | 71.57 | 0.4328 | 0.7927 |
| m_t                     | 75.21  | 75.61  | 75.41 | 0.5909 | 83.45  | 68.87  | 76.16 | 0.5288 | 0.8467 |
| r_b                     | 79.04  | 74.23  | 76.64 | 0.5379 | 78.05  | 75.84  | 76.95 | 0.5391 | 0.8516 |
| r_k                     | 84.15  | 80.77  | 82.46 | 0.6500 | 84.85  | 80.59  | 82.72 | 0.6550 | 0.9077 |
| r_l                     | 81.56  | 79.63  | 80.59 | 0.6126 | 84.51  | 75.94  | 80.22 | 0.6067 | 0.8847 |

h, m and r before the hyphen stand for human, mouse, and rat, respectively; after the hyphen stand for brain, heart, kidney, liver, and testis, respectively.

**Table 1. The Performance of im6A-TS-CNN for Identifying m^6^A Sites**

![Figure 2.](image)

**Figure 2. The ROC Curves for Identifying m^6^A in Different Tissues in the Three Species under the 5-Fold Cross-Validation Test and Independent Dataset Test**

The value of AUC is given in the right corner of each graph.
N is the input channels. The rectified linear function (ReLU) is expressed as the following:

\[
\text{ReLU}(z) = \begin{cases} 
  z & \text{if } z \geq 0 \\
  0 & \text{if } z < 0 
\end{cases} \quad \text{(Equation 2)}
\]

In order to prevent overfitting, we choose to lose some parameters and set the dropout rate of 0.16. The results were output to a fully connected layer containing 164 neural units and then compressed to 32 neural units. Finally, the softmax function was used to predict whether the RNA segment contains m₆A sites or not and is expressed as the following:

\[
\text{Softmax}(x) = \frac{e^x}{\sum e^x} \quad \text{(Equation 3)}
\]

When building the model, the stochastic gradient descent (SGD) was used as the optimizer with a learning rate of 0.001, and the categorical

|      | 5-Fold Cross Validation (AUC) | Independent Test (AUC) |
|------|------------------------------|------------------------|
|      | m6A-TS-CNN | iRNA-m₆A | Difference | m6A-TS-CNN | iRNA-m₆A | Difference |
| h_b  | 0.8029     | 0.7756   | 0.0273*   | 0.8056     | 0.7845   | 0.0211*   |
| h_k  | 0.8781     | 0.8634   | 0.0147*   | 0.8727     | 0.8565   | 0.0162*   |
| h_l  | 0.8811     | 0.8738   | 0.0073*   | 0.8805     | 0.8681   | 0.0124*   |
| m_b  | 0.8705     | 0.8731   | -0.0026   | 0.8722     | 0.8613   | 0.0109*   |
| m_h  | 0.8115     | 0.7948   | 0.0167*   | 0.8161     | 0.7878   | 0.0283*   |
| m_l  | 0.7953     | 0.7743   | 0.0210*   | 0.7927     | 0.762    | 0.0307*   |
| m_t  | 0.8380     | 0.8156   | 0.0224*   | 0.8467     | 0.8182   | 0.0285*   |
| r_b  | 0.8469     | 0.8282   | 0.0187*   | 0.8516     | 0.8968   | -0.0452   |
| r_h  | 0.9017     | 0.8877   | 0.0140*   | 0.9077     | 0.8761   | 0.0316*   |
| r_l  | 0.8830     | 0.8766   | 0.0064*   | 0.8847     | 0.8265   | 0.0582*   |

h, m and r before the hyphen stand for human, mouse and rat; b, h, k, l, t after the hyphen stand for brain, liver, kidney, heart and testis, respectively. *indicates the performance of im6A-TS-CNN is better than iRNA-m₆A for identifying m₆A sites.

Figure 3. Heatmap Showing the AUC Values of Cross-Species and Cross-Tissue Validation
The abscissa represents the independent dataset, and the ordinate represents the model.
cross entropy was used as the loss function. In the training process, a total of 2,000 epochs were carried out by using the early stopping method with the patience of 50 and min_delta of 0.001.

**Evaluation Metrics**

In order to evaluate the model, we use the sensitivity (Sn), specificity (Sp), accuracy (Acc), and Matthews correlation coefficient (MCC), which are defined as the following,\(^{26,29,30}\) to perform the evaluation of the following model:

\[
\begin{align*}
Sn &= 1 - \frac{N^-}{N^+} \\
Sp &= 1 - \frac{N^+}{N^-} \\
Acc &= 1 \frac{N^+ + N^-}{N^+ + N^-} \\
Mcc &= \frac{1 - \frac{N^+ + N^-}{N^+ + N^-}}{\sqrt{\left(1 + \frac{N^+ - N^-}{N^+}\right) \left(1 + \frac{N^+ - N^-}{N^-}\right)}}
\end{align*}
\]

where \(N^\prime\) is the total number of the RNA sequence containing modification site, \(N^+_\prime\) is the number of false-negative samples, \(N^-\) is the total number of the RNA sequence that did not contain any modification site, and \(N^+\) is the number of false-positive samples.

In addition, we also used the ROC curve\(^{31}\) and the area under the ROC curve (AUC) to evaluate the proposed model.

**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.omtn.2020.07.034.

**AUTHOR CONTRIBUTIONS**

W.C. conceived and designed the study. K.L. conducted the experiments and implemented the algorithms. W.C., L.C., P.D., and K.L. performed the analysis and wrote the paper. All authors read and approved the final manuscript.

**CONFLICTS OF INTEREST**

The authors declare no competing interests.

**ACKNOWLEDGMENTS**

The authors would like to express their gratitude to Prof. Quan Zou for his help in optimizing the parameters of CNN. This work was supported by the National Nature Scientific Foundation of China (31771471) and Natural Science Foundation for Distinguished Young Scholar of Hebei Province (number C2017209244).

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