

# iRNA-3typeA: Identifying Three Types of Modification at RNA's Adenosine Sites

Wei Chen,<sup>1,3,4</sup> Pengmian Feng,<sup>2</sup> Hui Yang,<sup>3</sup> Hui Ding,<sup>3</sup> Hao Lin,<sup>3,4</sup> and Kuo-Chen Chou<sup>3,4</sup>

<sup>1</sup>Department of Physics, School of Sciences, and Center for Genomics and Computational Biology, North China University of Science and Technology, Tangshan 063000, China; <sup>2</sup>Hebei Province Key Laboratory of Occupational Health and Safety for Coal Industry, School of Public Health, North China University of Science and Technology, Tangshan 063000, China; <sup>3</sup>Key Laboratory for Neuro-Information of Ministry of Education, School of Life Science and Technology, Center for Informational Biology, University of Electronic Science and Technology of China, Chengdu 610054, China; <sup>4</sup>Gordon Life Science Institute, Boston, MA 02478, USA

**RNA modifications are additions of chemical groups to nucleotides or their local structural changes. Knowledge about the occurrence sites of these modifications is essential for in-depth understanding of the biological functions and mechanisms and for treating some genomic diseases as well. With the avalanche of RNA sequences generated in the post-genomic age, many computational methods have been proposed for identifying various types of RNA modifications one by one. However, so far no method whatsoever has been developed for simultaneously identifying several different types of RNA modifications. To address such a challenge, we developed a predictor called “iRNA-3typeA,” by which we can simultaneously identify the occurrence sites of the following three most frequently observed modifications in RNA: (1) N<sup>1</sup>-methyladenosine (m<sup>1</sup>A), (2) N<sup>6</sup>-methyladenosine (m<sup>6</sup>A), and (3) adenosine to inosine (A-to-I). It has been shown via rigorous cross-validations for the RNA sequences from *Homo sapiens* and *Mus musculus* transcriptomes that the success rates achieved by the powerful new predictor are quite high. For the convenience of broad experimental scientists, a user-friendly web server for iRNA-3typeA has been established at <http://lin-group.cn/server/iRNA-3typeA/>. It is anticipated that iRNA-3typeA may become a useful high throughput tool for genome analysis.**

## INTRODUCTION

RNA modification means the addition of chemical groups to its constitutional nucleotides or structural changes therein.<sup>1</sup> So far, more than 100 types of RNA modifications have been observed in cellular RNAs of all living organisms.<sup>2</sup> Because they are involved in a series of crucial biological activities,<sup>3</sup> such as mRNA splicing, mRNA nuclear processing, mRNA export, and mRNA decay,<sup>3–6</sup> particularly linked with human diseases, RNA modifications have drawn great attention in the scientific community.

With the development of high-throughput experimental techniques,<sup>7–9</sup> lots of RNA modification data have been acquired; they are very helpful for revealing the novel functions of RNA modifications. As indicated in a recent review,<sup>10</sup> however, most of these methods are unable to discriminate among the different RNA modifications that may simultaneously occur in the same RNA molecule.

For example, the adenosine usually undergoes N<sup>1</sup>-methyladenosine (m<sup>1</sup>A), N<sup>6</sup>-methyladenosine (m<sup>6</sup>A), and adenosine to inosine (A-to-I or A→I) modifications<sup>7</sup> (Figure 1). Unfortunately, using the aforementioned techniques, one could not detect whether different types of RNA modifications might take place at the same time, let alone analyze their combinational biological functions.<sup>11</sup>

Therefore, it is urgently needed to develop computational methods to address this problem. As excellent complements to experimental techniques, computational methods have been developed to identify RNA modifications<sup>12–18</sup> via machine learning to train computational models based on the large data yielded from the high-throughput experiments. However, rarely are they able to simultaneously identify multiple RNA modifications.

The present study was devoted to developing a bioinformatics tool that can identify the RNA modification types for m<sup>1</sup>A, m<sup>6</sup>A, and A→I that may simultaneously occur on adenosine in both *Homo sapiens* and *Mus musculus* transcriptomes.

As shown in a series of recent publications,<sup>19–31</sup> in developing a bioinformatics tool, complying with the five-step rules yields the following advantages:<sup>32</sup> (1) clearer in logic deduction, (2) better illumination in stimulating other relevant tools, and (3) more usefulness in practical application.

In view of this, we elaborate the following procedures required in the five-step rules: (1) benchmark dataset, (2) sample formulation, (3) operative machine, (4) cross-validation, and (5) web server, and they are embedded into the rubrics according to the journal's format.

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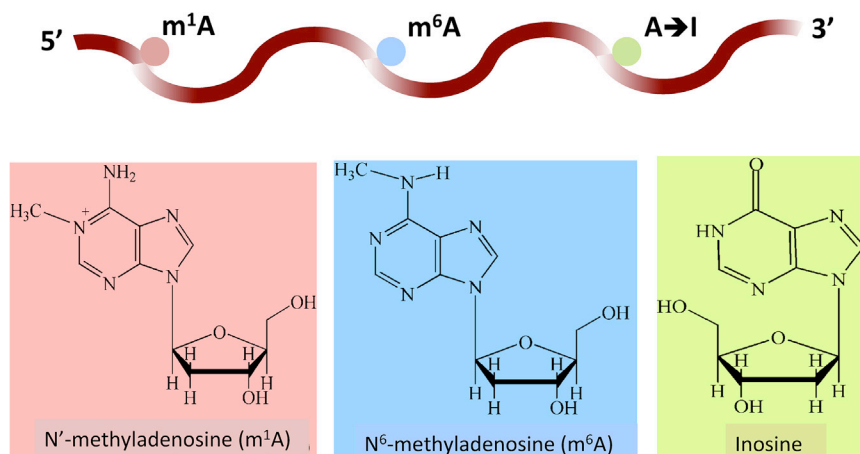
**Correspondence:** Wei Chen, Department of Physics, School of Sciences, and Center for Genomics and Computational Biology, North China University of Science and Technology, Tangshan 063000, China.

**E-mail:** [chenweiimu@gmail.com](mailto:chenweiimu@gmail.com)

**Correspondence:** Hao Lin, Key Laboratory for Neuro-Information of Ministry of Education, School of Life Science and Technology, Center for Informational Biology, University of Electronic Science and Technology of China, Chengdu 610054, China.

**E-mail:** [hlin@uestc.edu.cn](mailto:hlin@uestc.edu.cn)





**Figure 1. The Three Common Types of Modifications in RNA**

(1) N<sup>1</sup>-methyladenosine (m<sup>1</sup>A), (2) N<sup>6</sup>-methyladenosine (m<sup>6</sup>A), and (3) adenosine to inosine (A-to-I).

## RESULTS AND DISCUSSION

### Performance Report

Listed in Table 1 are the jackknife test results obtained by the proposed predictor on the benchmark datasets (Supplemental Information S1 and Supplemental Information S2 available at <http://lin-group.cn/server/iRNA3typeA/data.htm>) for *H. sapiens* and *M. musculus*, respectively. As we can see from the table, the rates for both overall accuracy (Acc) and stability (MCC) are quite high for all the three different types of modifications investigated, indicating that the predictor is not only high in overall success rate but also quite stable. Therefore, the potential is quite high for iRNA-type3A to become a high-throughput tool in both basic research and drug development.

It is instructive to point out that, although the current predictor is limited in identifying m<sup>1</sup>A, m<sup>6</sup>A, and A→I sites for the RNA sequences from *H. sapiens* and *M. musculus*, with more experimental data available for other types of modifications and other species in future, we can easily to extend our model to cover more different types of modifications and more different species. Therefore, the current predictor is just a good start; it will be subjected to updates with the aim to continuously enhance its power and coverage scope.

### Comparison with Other Classifiers

The proposed predictor iRNA-3typeA is the first predictor ever constructed for identifying the three types of RNA modifications (m<sup>1</sup>A; m<sup>6</sup>A; A→I) simultaneously. It is not possible to show its power via a conventional comparison since there is no other predictor whatsoever that can do the same. Nevertheless, below we can carry out a special comparison to further demonstrate its superiority.

As mentioned above, the operative machine used for iRNA-3typeA is a support vector machine (SVM) classifier. What would happen if we use other classifiers instead? Listed in Table 2 are the results when the SVM classifier was substituted with the other classifiers, respectively.

From the table, we can see the following: (1) the SVM classifier is better than J48 Tree in all the metrics rates. (2) Although the SVM classifier is a little bit lower than the BayesNet classifier and Naive Bayes classifier in identifying the m<sup>6</sup>A sites for *H. sapiens*, its accuracies in identifying all the other types of modifications for both *H. sapiens* and *M. musculus* are significantly higher than those of BayesNet and Naive Bayes. All these results have further indicated

that the SVM classifier is indeed a correct choice for the iRNA-3typeA predictor.

### Web Server and User Guide

The last step of the five-step rules<sup>32</sup> is about the web server. It is indeed important because user-friendly and publicly accessible web servers represent the future direction for developing practically more useful predictors.<sup>33</sup> Actually, it has been demonstrated by a series of recent publications (see, e.g., Cheng et al.,<sup>25,34–36</sup> Liu et al.,<sup>28</sup> Lin et al.,<sup>37</sup> Jia et al.,<sup>38,39</sup> and Cheng and Xiao<sup>40</sup>) that a new prediction method with its web server available would significantly enhance its impacts.<sup>41,42</sup> In view of this, the web server for iRNA-3typeA has been established. Furthermore, to maximize the convenience of broad experimental scientists, a step-by-step guide is given below:

Step 1. Open the iRNA-3typeA web server at <http://lin-group.cn/server/iRNA-3typeA>; you will see the top page of the web server as shown in Figure 2A.

Step 2. Either type or copy/paste the query RNA sequences (in FASTA format) into the input box. Example sequences can be found by clicking on the Example button.

Step 3. Click the open circle (*H. sapiens* and *M. musculus*) to choose the species concerned, followed by clicking the Submit button. For example, if using the query RNA sequences in the Example window as the input and choosing *H. sapiens*, after submission you will see the predicted results summarized in a table (Figure 2B), clearly indicating (1) the adenosine at position 21 of sequence #1 has the potential to be of the site for m<sup>1</sup>A or A-to-I editing modification. (2) The adenosine at position 21 of sequence #2 has the potential to be of m<sup>6</sup>A modification only. All these predicted results are fully consistent with experimental observations.

## MATERIALS AND METHODS

### Benchmark Datasets

The benchmark datasets for m<sup>1</sup>A, m<sup>6</sup>A, and A-to-I editing sites in *H. sapiens* and *M. musculus* genomes were derived from the previous

**Table 1. The Success Rates Achieved by iRNA-3typeA via Jackknife Tests on the Benchmark Datasets for *H. sapiens* and *M. musculus*, Respectively**

Species	Type of Modification	Sn (%)	Sp (%)	Acc (%)	MCC
<i>H. sapiens</i>	m <sup>1</sup> A <sup>a</sup>	98.38	99.89	99.13	0.98
	m <sup>6</sup> A <sup>b</sup>	81.68	99.11	90.38	0.82
	A → I <sup>c</sup>	86.18	95.23	90.71	0.82
<i>M. musculus</i>	m <sup>1</sup> A <sup>d</sup>	97.46	100.00	98.73	0.97
	m <sup>6</sup> A <sup>e</sup>	77.79	100.00	88.39	0.80
	A → I <sup>f</sup>	96.75	100.00	98.38	0.96

<sup>a</sup>The parameters used for SVM are  $C=8$  and  $\gamma=0.0078125$ .

<sup>b</sup>The parameters used for SVM are  $C=128$  and  $\gamma=3.05158e-5$ .

<sup>c</sup>The parameters used for SVM are  $C=8$  and  $\gamma=0.0078125$ .

<sup>d</sup>The parameters used for SVM are  $C=2$  and  $\gamma=0.0078125$ .

<sup>e</sup>The parameters used for SVM are  $C=32$  and  $\gamma=0.00012207$ .

<sup>f</sup>The parameters used for SVM are  $C=512$  and  $\gamma=0.000488281$ .

works.<sup>12,14,43</sup> Listed in Table 3 are the numbers of positive and negative samples for each of the benchmark datasets. It has been found by similar approaches<sup>12,14</sup> that the optimal length of the sequence samples in the benchmark datasets are 41nt, with the modified sites (m<sup>1</sup>A, m<sup>6</sup>A, or A → I editing site) at the center. For readers' convenience, the benchmark dataset thus obtained for *H. sapiens* is given in Supplemental Information S1, while that for *M. musculus* given in Supplemental Information S2; both can be downloaded from the link at <http://lin-group.cn/server/iRNA3typeA/data.htm>.

### Sample Formulation

An RNA sample with 41 nt is usually sequentially formulated by

$$\mathbf{R} = N_1 N_2 N_3 \cdots N_i \cdots N_{41}, \quad (\text{Equation 1})$$

where

$$N_i \in \{A(\text{adenine}), C(\text{cytosine}), G(\text{guanine}), U(\text{uracil})\} \quad (\text{Equation 2})$$

denotes the nucleotide at the  $i$ -th sequence position, and  $\in$  is the symbol in the set theory meaning "member of."

To enable the existing machine-learning algorithms handle the RNA sample,<sup>41</sup> the first thing we need to do is to convert its sequential formulation into a vector. But a vector in a discrete framework might totally miss all the sequence-order information or pattern feature. To deal with this problem, the PseAAC (pseudo amino acid composition) was introduced.<sup>44</sup> Ever since the concept of PseAAC was proposed, it has been swiftly penetrated into many biomedicine and drug development areas<sup>45,46</sup> and nearly all the areas of computational proteomics (see, e.g., Esmaili et al.,<sup>47</sup> Mohabatkar et al.,<sup>48</sup> Nanni et al.,<sup>49</sup> Pacharawongsakda and Theeramunkong,<sup>50</sup> Mondal and Pai,<sup>51</sup> Ahman et al.,<sup>52</sup> Kabir and Hayat,<sup>53</sup> Yu et al.,<sup>54</sup> Zhang and Duan,<sup>55</sup> Muthu Krishnan,<sup>56</sup> and a long list of references cited in two review papers<sup>42,57</sup>). Encouraged by the successes of using PseAAC to deal with protein/peptide sequences, this idea has been

**Table 2. The Comparative Results of the Proposed Predictor When Its Operating Algorithm<sup>32</sup> Was Replaced from SVM to Other Classifiers**

Classifier	Species	Modification Type	Sn (%)	Sp (%)	Acc (%)	MCC
BayesNet <sup>a</sup>	<i>H. sapiens</i>	m <sup>1</sup> A	98.81	98.85	98.83	0.98
		m <sup>6</sup> A	82.04	100.00	91.02	0.83
		A → I	88.50	89.57	89.03	0.78
	<i>M. musculus</i>	m <sup>1</sup> A	97.18	98.78	97.98	0.96
		m <sup>6</sup> A	77.79	100.00	88.90	0.80
		A → I	96.51	99.88	98.20	0.96
Naive Bayes <sup>a</sup>	<i>H. sapiens</i>	m <sup>1</sup> A	98.16	98.30	98.23	0.96
		m <sup>6</sup> A	82.04	99.73	90.88	0.83
		A → I	89.40	87.04	88.22	0.76
	<i>M. musculus</i>	m <sup>1</sup> A	96.43	97.75	97.09	0.94
		m <sup>6</sup> A	77.79	98.62	88.22	0.78
		A → I	95.91	97.95	96.93	0.94
J48 Tree <sup>a</sup>	<i>H. sapiens</i>	m <sup>1</sup> A	98.77	99.40	99.09	0.98
		m <sup>6</sup> A	82.48	84.35	83.41	0.67
		A → I	88.18	89.04	88.60	0.77
	<i>M. musculus</i>	m <sup>1</sup> A	96.71	98.68	97.70	0.95
		m <sup>6</sup> A	83.03	82.21	82.62	0.65
		A → I	96.27	99.04	97.65	0.95
SVM <sup>b</sup>	<i>H. sapiens</i>	m <sup>1</sup> A	98.46	99.89	99.18	0.98
		m <sup>6</sup> A	80.44	100.00	90.23	0.82
		A → I	86.73	95.40	91.07	0.82
	<i>M. musculus</i>	m <sup>1</sup> A	97.46	100.00	98.73	0.97
		m <sup>6</sup> A	77.79	100.00	88.90	0.80
		A → I	97.35	100.00	98.67	0.97

All the rates below are obtained by the 10-fold cross-validations on the same benchmark datasets (Supplemental Information S1 and Supplemental Information S2 available at <http://lin-group.cn/server/iRNA3typeA/data.htm>).

<sup>a</sup>Taken from the WEKA package.<sup>91</sup>

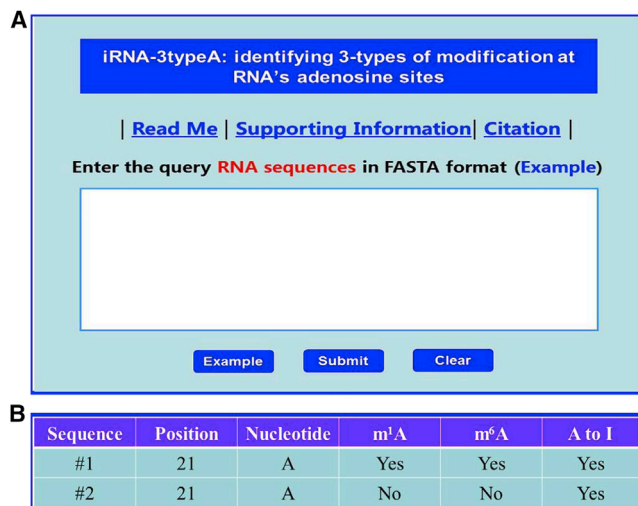
<sup>b</sup>Proposed in this paper.

extended to deal with DNA/RNA sequences<sup>21,28,37,58–60</sup> in computational genomics via PseKNC (pseudo K-tuple nucleotide composition).<sup>61,62</sup> According to Chen et al.,<sup>63</sup> the general form of PseKNC can be formulated as

$$\mathbf{R} = [\phi_1 \phi_2 \cdots \phi_u \cdots \phi_\Gamma]^T, \quad (\text{Equation 3})$$

where  $\mathbf{T}$  is the transposing operator, the subscript  $\Gamma$  is an integer, and its value and the components  $\phi_u (u=1, 2, \dots)$  will depend on how to extract the desired features and properties from the RNA sequence (cf. Equation 1). In this study, their definitions are described below.

The four bases (A, C, G, and U) of RNA have different chemical properties and structures.<sup>64,65</sup> Therefore, based on their different chemical properties and structures,<sup>64,65</sup> A, C, G, and U can be represented by (1, 1, 1), (0, 0, 1), (1, 0, 0), and (0, 1, 0), respectively.<sup>20,27</sup> For instance, the RNA sequence with six nucleotides "GUGCAG" can be expressed



**Figure 2. The Semi-screenshot for the Top Page of the iRNA-3typeA Web Server and the Prediction Result of the Two Example Query Sequences**  
The Semi-screenshot for the top page of the iRNA-3typeA Web Server (top panel) and the Prediction Result of the two example query sequences (bottom panel).

by the vector of  $(3 \times 6) = 18$  components; i.e.,  $[1, 0, 0, 0, 1, 0, 1, 0, 0, 0, 0, 1, 1, 1, 1, 1, 0, 0]$ .

Moreover, to incorporate into Equation 3 the sequence-coupled information<sup>66</sup> for the nucleotides around the modification sites, we adopt the lingering density as defined below

$$D_i = \frac{1}{\|L\|} \sum_{j=i}^{\ell} f(N_j), \quad (\text{Equation 4})$$

where  $D_i$  is the density of the nucleotide  $N_i$  at the site  $i$  of a RNA sequence,  $\|L\|$  the length of the sliding substring concerned;  $\ell$  denotes each of the site locations counted in the substring, and

$$f(N_j) = \begin{cases} 1, & \text{if } N_j = \text{the nucleotide concerned} \\ 0, & \text{otherwise} \end{cases} \quad (\text{Equation 5})$$

For example, the RNA sequence “GUGCAG” can be represented by the vector  $[1, 0.5, 0.66, 0.25, 0.2, 0.5]$ .

Thus, by using both nucleotide chemical properties and the lingering density (cf. Equation 4), each nucleotide can be defined by four variables. Accordingly, the RNA sequence of Equation 1 can be defined by a vector with  $(41 \times 6) = 164$  components; namely  $\Gamma = 164$  for Equation 3 now.

**Operative Machine**

In this study, the SVM was chosen as the operative machine. The SVM has been widely used in computational genomics and proteomics (see, e.g., Ehsan et al.,<sup>26</sup> Feng et al.,<sup>20,27,67–69</sup> Chen et al.,<sup>70–72</sup> Lin et al.,<sup>73</sup> Lai et al.,<sup>74</sup> Zhao et al.,<sup>75</sup> and Yang et al.<sup>76</sup>). The implementation of the SVM was conducted by using the LibSVM package 3.18

**Table 3. A Breakdown of the Benchmark Dataset**

Species	Attribute	Number of Samples		
		m <sup>1</sup> A	m <sup>6</sup> A	A → I
<i>H. sapiens</i>	positive	6,366	1,130	3,000
	negative	6,366	1,130	3,000
<i>M. musculus</i>	positive	1,064	725	831
	negative	1,064	725	831

available at <https://www.csie.ntu.edu.tw/~cjlin/libsvm/>. The radial basis kernel function (RBF) was used to obtain the classification hyperplane, and the grid search method was applied to optimize the regularization parameter  $C$  and kernel parameter  $\gamma$ .

The predictor obtained via the above procedures is called “iRNA-3typeA,” where “i” stands for “identify,” and “3typeA” means RNA’s “three types of modifications at adenosine sites.” Illustrated in Figure 3 is a flowchart to show the process of how the iRNA-3typeA predictor is working.

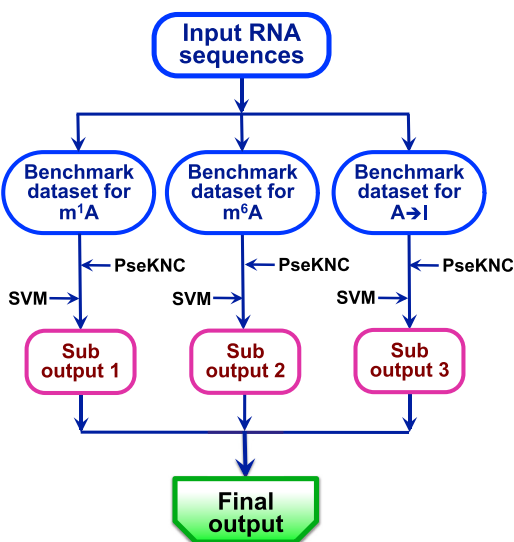
**Cross-Validation**

To evaluate the quality of a new predictor, we need to consider the following two problems. What metrics should be used to quantitatively display its performance? And what concrete procedure should be followed to derive the metrics’ values?

- (1) A set of four metrics. In literature, the following four conventional metrics are generally used to evaluate a predictor’s quality:<sup>77</sup> (1) Acc, (2) MCC, (3) sensitivity (Sn), and (4) specificity (Sp). But the conventional expressions copied directly from math books are lacking in inductivity and hard to understand for most biological scientists. Fortunately, by using the symbols introduced by Chou in studying signal peptides,<sup>78</sup> the four metrics can be converted to a set of intuitive ones<sup>58,79</sup> as given below:

$$\begin{cases} \text{Sn} = 1 - \frac{N_-^+}{N^+} & 0 \leq \text{Sn} \leq 1 \\ \text{Sp} = 1 - \frac{N_+^-}{N^-} & 0 \leq \text{Sp} \leq 1 \\ \text{Acc} = 1 - \frac{N_-^+ + N_+^-}{N^+ + N^-} & 0 \leq \text{Acc} \leq 1 \\ \text{MCC} = \frac{1 - \left(\frac{N_-^+}{N^+} + \frac{N_+^-}{N^-}\right)}{\sqrt{\left(1 + \frac{N_-^-}{N^+}\right)\left(1 + \frac{N_+^+}{N^-}\right)}} & -1 \leq \text{MCC} \leq 1 \end{cases}, \quad (\text{Equation 6})$$

where  $N^+$  represents the total number of positive samples investigated, while  $N_+^+$  is the number of positive samples incorrectly predicted to be negative, and  $N^-$  represents the total number of negative



**Figure 3. A Flowchart to Show How the iRNA-3typeA Predictor Is Working**

samples investigated, while  $N_+$  the number of the negative samples incorrectly predicted to be positive. With the set of formulations in Equation 6, the meanings of Sn, Sp, Acc, and MCC have become much more intuitive and easier to understand, as discussed in a series of recent studies in various biological areas (see, e.g., Liu et al.,<sup>21,24,28,60</sup> Ehsan et al.,<sup>26</sup> Feng et al.,<sup>20,27</sup> Song et al.,<sup>31</sup> Lin et al.,<sup>37</sup> and Xu et al.<sup>80,81</sup>).

(2) Jackknife test. Now the next problem is how to test the values of these metrics in an objective way. As is well known, the independent dataset test, subsampling (or K-fold cross-validation) test, and jackknife test are the three cross-validation methods widely used for testing a prediction method.<sup>82</sup> Of the three test methods, however, the jackknife test is deemed the least arbitrary and most objective one.<sup>32</sup> Accordingly, the jackknife test has been widely recognized and increasingly adopted by investigators to examine the quality of various predictors (see, e.g., Ahmad et al.,<sup>52,83</sup> Lin et al.,<sup>84</sup> Tang et al.,<sup>85</sup> Tripathi and Pandey,<sup>86</sup> and Dao et al.<sup>87</sup>). In view of this, the jackknife test was also adopted in the current study to examine the proposed predictor. During the jackknife test, each sample in the benchmark dataset is in turn singled out as an independent test sample and all the rule-parameters are calculated without including the one being identified. One more advantage of using the jackknife test is that there is no need to artificially separate the benchmark dataset into two subsets, one for training the model and one for testing it. This is because the outcome obtained by the jackknife test is actually a combination from many different independent dataset tests.<sup>88–90</sup>

#### AUTHOR CONTRIBUTIONS

W.C. and H.L. designed the study; P.F., H.Y., and H.D. conducted the experiments; W.C., H.L., and K.-C.C. analyzed the results; W.C., H.L., and K.-C.C. wrote the paper.

#### CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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