Machine learning applications in RNA modification sites prediction

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Abstract

Ribonucleic acid (RNA) modifications are post-transcriptional chemical composition changes that have a fundamental role in regulating the main aspect of RNA function. Recently, large datasets have become available thanks to the recent development in deep sequencing and large-scale profiling. This availability of transcriptomic datasets has led to increased use of machine learning based approaches in epitranscriptomics, particularly in identifying RNA modifications. In this review, we comprehensively explore machine learning based approaches used for the prediction of 11 RNA modification types, namely, m^1A, m^2A, m^4C, 5hmC, ψ, 2r – O – Me, ac^4C, m^1G, A to I, m^2G, and D. This review covers the life cycle of machine learning methods to predict RNA modification sites including available benchmark datasets, feature extraction, and classification algorithms. We compare available methods in terms of datasets, target species, approach, and accuracy for each RNA modification type. Finally, we discuss the advantages and limitations of the reviewed approaches and suggest future perspectives.

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1. Introduction

RNA modification refers to post-transcriptional modifications of RNA in eukaryotes and prokaryotes. Currently, more than 150 types of RNA modifications have been discovered. The most dominant is RNA methylation, which is often referred to as epitranscriptome. Common modification types include, \( N^6 \)-methyladenosine (\( m^6A \)), pseudouridine (\( \psi \)), \( N^4 \)-methyladenosine (\( m^4A \)), 5-hydroxymethylcytosine (\( 5hmC \)), 5-methylcytosine (\( m^5C \)), and 2'-O-methylation of ribose (\( 2'-O-Me \)). Other less common types, such as \( N^4 \)-acetylcytidine (\( ac^4C \)), 7-methylguanosine (\( m^7G \)), \( N^6 \)-methylguanosine (\( m^6G \)), dihydrouridine (\( D \)), and adenosine-to-inosine (A-to-I) also exist (Fig. 1) [1].

\( m^6A \) modification occurs at a different stage of mRNA metabolism, including ncRNA processing and CircRNA translation [2]. This modification occurs at the nitrogen-6 position of the adenine base (A). There is convincing evidence that \( m^6A \) methylation plays a significant role in several pathological and physiological immune responses, including the homeostasis and differentiation of T cells, inflammation, and the development of interferon type [3]. \( m^6A \) is another methylation type that affects adenine. Most of what we know about \( m^6A \) originated from the analysis of tRNAs and rRNAs since its presence in mRNAs was only recently discovered. A methyl group is attached to the nitrogen atom at the first position of the adenine base in \( m^6A \) reaction. Research has shown that \( m^6A \) is related to respiratory chain malfunctioning and neurodevelopmental regression [4]. Another modification affecting adenine is A-to-I modification, the primary type of RNA modification in mammals. Depending on the environments in which RNA modification events occur, this deamination of adenine could influence gene regulation, expression, and functions concerning exchanges in the amino acid sequence and maturation [5].

Modifications that affect the cytosine base of RNA include \( m^5C \), \( ac^4C \), and \( 5hmC \). Recent research suggests that \( m^5C \) methyla-
tion influences the nuclear export efficiency of mRNA by impacting the function of the nuclear factor ALYREF/THOC4 [6]. It is also possible that \( m^5C \) has an impact on protein translation. It is established that \( m^5C \) is reversible, and TET-mediated oxidation of \( m^5C \) produces another form of RNA modification known as 5-hydroxymethylcytosine (\( 5hmC \)) [7]. Recently, \( ac^4C \) modification was found in yeast and identified in human mRNA. It is the only acetylation modification found on cytidine in eukaryotic mRNA and of all the modifications that have regulatory potential, \( ac^4C \) is retained in all areas of life [8].

At the uridine (U) level, several modifications can occur, such as \( \psi \). It was the first type of RNA modification to be discovered, and it remains the most common one. It is a C5-glycoside isomer of uridine and the only C–C bond connecting a nucleobase to a sugar known among nucleic acids. Although it was discovered in the 1950s, neither the enzymatic mechanism of its formation nor its function has been fully described. \( 2'-0-Me \) is a type of modification that alters ribose [9] and one of the most abundant types of RNA modification in various cellular RNAs. This modification occurs when the methyl group (\(-CH3\)) is attached to the 2'-hydroxyl (\(-OH\)) of the ribose moiety, which can modify any ribonucleotide (Am, Cm, Gm, Um) [10]. The \( 2'-0-Me \) can protect RNAs from nuclease attacks, increase their hydrophobicity and affect their reactions with proteins or various RNAs [11].

\( m^7G \) is a positively charged RNA modification that occurs at the guanine (G) nucleotide. \( m^7G \) is among the most conserved modifications in tRNA, rRNA, and mRNA 5'cap [12]. It is an adjusted purine nucleoside that plays an essential role in regulating RNA function and cell viability. Even with its propagation within internal mRNA regions at the transcriptional level, its regulation remains largely unresolved. Dihydrouridine (\( D \)) is another typical RNA post-transcriptional modification in eukaryotes, bacteria, and some archaea. Because of this change, individual nucleotide bases can have more conformational versatility, and cancerous tissues have higher levels of this post-transcription modification [13].

![Fig. 1. Chemical structures of popular RNA modifications.](image-url)
RNA modification can be detected using several RNA biochemistry approaches, including different types of chromatography, such as TLC, HPLC, and LC-MS/MS [14]. It can also be detected based on the chemical properties of RNA modification, including RTL-P (Reverse Transcription at low [dNTP] with PCR) approach based on reverse transcription at low dNTP concentration, as well as a PCR approach [15]. There are also deep sequencing approaches for RNA modification identification. These approaches utilize next-generation sequencing (NGS) and computational techniques to identify RNA modification [16].

Even though they are time and labor-consuming, biochemical and deep sequencing approaches for identifying RNA modification sites yield effective results. Due to the increase in sequencing data generated in the post-genomic era, computational algorithms have surfaced to perform in silico prediction of RNA modification sites. In 2013, the first machine learning-based approach was introduced to detect m^A sites. Later, multiple computational models were introduced to identify various RNA modifications. Machine learning approaches extract features from known benchmark datasets and build models that can predict modification sites. In this review, we explore machine learning approaches that predict the most common RNA modification sites. First, we introduce benchmark datasets used to predict post-transcriptional modifications. We then combine the most common statistical features used in machine learning approaches and summarize the feature selection methods used to identify modification sites. Finally, we review existing machine learning tools for each modification type in terms of methodology, performance, and accessibility.

### 2. Benchmark datasets

Perhaps the most important requirement when applying machine learning to modification sites prediction is the accessibility to curated data in order to achieve the best results. Most available benchmark datasets in the field are acquired from Gene Expression Omnibus (GEO) [17]. GEO is a database that contains experimental studies for gene expression and RNA modification profiling. Recently multiple databases were built for post-transcriptional modifications such as MODOMICS [18]. This database hosts a collection of modified RNA sequences, where modification records are highlighted. RMBase [2] is another popular RNA-modified database based on high throughput genome-wide data from 18 independent studies. RMBase V2.0 [1] is a 2017 update with more enhanced known RNA modification sites. These databases are used for constructing small benchmark datasets for machine learning based approaches for different modifications. Table 1 summarizes the most popular databases used in constructing RNA modification benchmark datasets. In order to build machine learning models, training datasets use experimentally identified RNA modifications as positive samples, while negative samples are randomly selected from non-modified samples. However, some of the negative samples used in training can be unidentified sites and introduce noise to the models.

### 3. Feature extraction

Feature extraction is an essential step in machine learning. In most cases, prediction models are as good as the features they use to distinguish between prediction classes. There are several features used to predict modification sites in RNA sequences. For example, K-nucleotide frequencies (KNF) [28] represent the frequencies of all possible k-nucleotides in each sequence. Position-specific dinucleotide sequence profile (PSDSP) [28] measures the difference of dinucleotide profiles between positive and negative samples. Other composition-based features such as the composition of k-spaced nucleic acid pairs (CKSNAP) and Ksnf [29,24] rely on the frequencies of nucleotide pairs separated by k-residues. Dinucleotide-based auto-cross covariance (DACCC) [30] incorporates information about the overall order of RNA sequences, while Enhanced nucleic acid composition (ENAC) [29] calculates the nucleic acid composition based on a fixed-length window.

Sequence and genome derived features are used by several algorithms, including binary variables that indicate whether modification sites are overlapped with the topological regions on the major RNA transcript or not, relative position on the region, the region length in bp, clustering information of modification sites from training data, scores related to evolutionary conservation, RNA secondary structure prediction score such as the minimum free energy (MFE), RNA binding protein annotation from MeTDB database, and gene characteristics such as annotation [12,31]. In addition to the features mentioned above, several more common features are further detailed in the following sections.

### Table 1

| Database            | Available RNA modification dataset | Link                                           |
|---------------------|-----------------------------------|------------------------------------------------|
| RMBase V2 [1]       | m^A (4128 H. sapiens, 3320 M. musculus, 2122 S. cerevisiae) | http://rna.sysu.edu.cn/rmbase/                 |
| MODOMICS [18]       | m^A, m^C, 2r – 0 – Me, m^A, m^3C, and other Types | http://modomics.genesilico.pl                  |
| DARNED [19]         | A-to-t and other types             | http://darned.uc.ie/                          |
| RNAMDB [2]          | M1Gm, m^A, tm5SU, tm5s2U, ac6A, and other types | Not available                                 |
| m^A-Atlas [20]      | m^A, m^C, 20Me, m^A, m^3C and other Types | http://www.xjtlu.edu.cn/biologicalsciences/atlas |
| REDPiportal [21]    | A-to-t                             | http://srv00.recas.ba.infn.it/atlas            |
| RADAR [22]          | A-to-t (1 379 403 human, 8108 M. musculus, 2698 fly) | Not available                                 |
| Met-D8 V2.0 [23]    | m^A                                | Not available                                 |
| REPiC [24]          | m^A                                | https://repiomod.uchicago.edu/repic/           |
| CVm6A [25]          | m^A                                | http://gb.whu.edu.cn:8080/CVm6A               |
| ConsRM [26]         | m^A                                | http://180.208.58.19/conservation/             |
| Gene-Expression Omnibus (GEO) [17] | Available as experimental data | https://www.ncbi.nlm.nih.gov/geo/           |
| m7GHub [27]         | m^7G                                | Not Available                                 |

**Note:** Table 1 lists various databases used in post-transcriptional RNA modifications. It summarizes the most popular databases used in constructing RNA modification benchmark datasets. These databases are used for building machine learning models to predict RNA modification sites. The table includes information on the types of modifications detected by each database and links to their respective websites for further exploration.
3.1. Nucleotide chemical property

Nucleic acids have common and distinctive properties in terms of the ring structure, hydrogen bond, and functional group. For example, adenine and guanine have a similar double ring structure, while cytosine and uracil contain a single ring. In addition, when forming a secondary structure, cytosine and guanine are strongly hydrogen-bonded, whereas adenine and uracil are weakly hydrogen-bonded. Chemical functionality allows us to divide nucleotides into two groups. Cytosine and adenine belong to the amino group, while guanine and uracil belong to the keto group. Given these different chemical properties, we can generally divide nucleotides into three distinct groups. Membership of each nucleotide in these groups allows the encoding of RNA sequences using a tuple (a,b,c) for each base. Therefore, A is represented as (1,1,1), C is represented as (0,1,0), G is represented as (1,0,0) and U is represented as (0,0,1) [32].

3.2. One-hot encoding

One-hot encoding techniques are often used to represent DNA and RNA sequences to transform categorical data to numerical form [33]. In particular, Adenine is encoded as (1,0,0,0), Uracil as (0,0,1,0), Cytosine as (0,1,0,0), and Guanine as (1,0,0,1). A matrix of 4 by n is then used to represent an RNA sequence of length n.

3.3. PseEIIP

Electron–Ion Interaction Pseudopotentials (EIIP) represent the energy of delocalized electrons in nucleotides. They have been used as a composition measure that has been effective in several bioinformatics algorithms [34,35]. Using the EIIP technique, every nucleotide in an RNA sequence is encoded using the distribution of free electron energies. The adenine, cytosine, guanine, and uracil values are 0.1260, 0.1340, 0.0806, and 0.1335, respectively. In order to apply pseudo-EIIP (PseEIIP) to trinucleotides, the mean EIIP value for each nucleotide is used as the composition measure.

\[ \text{PseEIIP} = [\text{EIIP}_{\text{AAA}}, \text{EIIP}_{\text{AAC}}, \text{EIIP}_{\text{ACG}}, \ldots, \text{EIIP}_{\text{TGG}}, \ldots, \text{EIIP}_{\text{TTT}}] \]

3.4. K-mer content

K-mer content is a common feature extracted from nucleotide sequences and used in several prediction algorithms. The composition measure represents frequencies of all sub-sequences of length k (k = 1, 2, 3...) in a given sequence. For example, 1-mer contains single nucleotide frequencies, 2-mer represents dinucleotide frequencies, and 3-mer represents the frequency of triple nucleotides. K-mer content is used in conjunction with the position to enhance sensitivity to the position-specific sequence slope around RNA modification sites. This measure is often referred to as a position weight matrix (PWM) [33].

3.5. PseDNC and PseKNC measures

Another alternative to k-mer content is to formulate a dynamic coding scheme that simultaneously represents small local sequence patterns and global data arrangement. Due to the success of pseudo amino acid composition (PseAAC) in protein and peptide-related problems, another approach called pseudo nucleotide composition (PseDNC) was introduced to encode modification in RNA sequences. The discrete measure describes the physical and chemical properties of oligonucleotides with the sequence. This type of pseudo-synthesis can retain much of the sequence-order information, especially the global or long-range sequence-order information. Compared to one-hot or k-mer encoding, both PseDNC and PseKNC associate short sequence order information with RNA physical–chemical properties. Type 2 PseKNC reflects both the local and the global sequence information of the nucleotide sequence.

4. Machine Learning

Machine Learning is a branch of artificial intelligence that involves computer self-learning to perform tasks automatically and is divided into three types: supervised learning, unsupervised learning, and reinforced learning. Supervised learning includes mapping an input to an output based on a set of known data. The output is either a class in the case of classification or a value in regression. Unsupervised learning involves studying the class itself by building models that are capable of describing the data and the relationships found in the data without the use of labels. Unsupervised learning includes dividing data into groups in the case of clustering and summarizing data distribution in density estimation. Reinforcement learning involves learning actions rather than class, and the input is mapped to actions based on feedback. The learning, in this case, is action-oriented, where the most rewarding actions are retained. Hybrid learning approaches combine supervised and unsupervised learning. When the training does not contain enough labeled data, semi-supervised learning builds models that make advantage of available unlabeled data. Self-learning is another hybrid approach that treats unsupervised problems as a supervised one. In this case, models are trained from related data and then applied to the original unlabeled data for prediction. Multi-instance learning is a supervised learning approach that uses unsupervised concepts in learning. This hybrid approach groups unlabeled data into labeled groups using unsupervised learning and uses the resulting labeled data in a supervised way. In our review, we are concerned with supervised learning and particularly classification. The prediction algorithms we studied use benchmark data with known RNA modification sites to predict whether novel RNA sequences have RNA modifications. Several pipelines exist to train a machine learning model and conduct a classification experiment. Fig. 2 shows an overview of the steps involved in performing supervised learning.

4.1. Feature Selection

Feature selection is a crucial step in machine learning as it has a direct role in the performance of prediction models. During feature selection, redundant and noisy features are illuminated, reducing overfitting and increasing the calculation speed. Feature selection is used to reduce the feature space by choosing the most relevant and discriminant features. For example, BFS + LF [36] is an incremental feature selection method. Features that improve classification using a logistic function (LF) are kept while those that decrease the accuracy are removed. In addition, strategies such as principal component analysis (PCA) [37], n-Grams, minimal-redundance maximum-Relevance (mRMR) [38] are widely used in order to select a subset of the features. Studies show that applying feature selection algorithms produce better performance than using the extracted features directly or applying a multi-layer machine learning approach [39,40]. However, feature selection should be performed on a different dataset than the training to avoid biases in the performance analysis during testing. If the data is not enough, existing algorithms can help avoid feature selection bias [41,42].

4.2. Machine Learning Algorithms

Prediction algorithms learn a target function that best maps input variables to an output variable. The function is referred to
as the model, and the input variables are called features. If the output variable is a discrete value, then the prediction is called classification, while regression refers to prediction models that output continuous values. Support vector machines [43], XGboost [44], Random Forest [45], Convolutional neural network [46], and Recurrent Neural Network [47] are among the most commonly used machine learning algorithms for predicting RNA methylation sites.

Support vector machines (SVM) is a widespread algorithm for dealing with small sample sizes [43]. It is considered the most popular machine learning algorithm and the most commonly used in computational biology. The key idea behind SVM is to find the best separating hyperplane from data transformed into a high-dimensional feature space [48]. Another equally popular machine learning classifier is random forest (RF) [45] which is based on bagging. During training, features are randomly selected to create various decision trees. Each individual tree finds a sub-optimal solution. The average of predictions from all trees gives a better estimate of the true output. Likewise, eXtreme gradient boosting (XGboost) [10] is a boosting algorithm that uses tree models to classify data. XGboost’s processing speed was increased by parallel computing. Furthermore, XGboost is extremely customizable, enabling users to identify their own optimization objectives and assessment criteria. Convolutional neural network (CNN) is a popular deep learning model initially introduced to classify images. Recent studies have demonstrated the utility of CNN in biological problems [46,33]. CNN automatically extracts the most suitable features from the input sequences and structures algorithms into layers to create an artificial neural network that can learn and make intelligent decisions on its own. Meanwhile, Recurrent Neural Network (RNN) [47] is a deep architecture that can recall context information, making it ideal for analyzing biological sequences. GRU, a simplified version of RNN, helps predict modification sites. CNN and RNN networks are often coupled with a strategy called transfer learning in order to overcome the problem of small data. In transfer learning, the training and testing datasets are not required to be independent and their distributions do not have to be identical. This allows the extrapolation of features learned from one domain to generate a pre-trained model that is further trained using a small training data from another closer domain.

4.3. Performance metrics

Several metrics are used to measure the performance of machine learning algorithms. In this review, we report the most common measures among the reviewed papers. The Area Under Curve score (AUROC) measures the area under the Receiver Operating Characteristic (ROC) curve, which plots the false positive rate vs. the true positive rate. Similarly, the AUPRC score measures the

Fig. 2. Supervised learning workflow.
Table 2  
Performance of m^6A modification sites prediction tools. NS refers to the number of samples and WS represents the word size.

| Predictor          | ML-Algorithm | Features                                      | Testing                                      | Species                     | NS   | WS   | Sn   | Sp   | ACC  | ROC  | PRC  | Link                                           |
|--------------------|--------------|-----------------------------------------------|----------------------------------------------|-----------------------------|------|------|------|------|------|------|------|------------------------------------------------|
| iN6-Methyl         | CNN and Chou’s 5-step rules                      | PseKNC                                        | 10-fold cross validation                     | S. cerevisiae              | 2614 | 51 bp| 76.15%| 74.62%| 75.38%| -    | -    | -                                             |
|                    |              |                                               |                                              | H. sapiens                  | 2260 | 41 bp| 82.14%| 100.0%| 91.11%| -    | -    | -                                             |
|                    |              |                                               |                                              | M. musculus                 | 1450 | 41 bp| 78.87%| 100.0%| 89.51%| -    | -    | -                                             |
| Gene2vec           | CNN          | Gene2vec                                      | Independent test                            | H. sapiens and M. musculus | 57516| 1001 bp| -      | -    | -    | 84.1%| 98.0%| https://github.com/jingcheng-du/Gene2vec       |
| DeepM6ASeq         | CNN          | One-hot                                       | Independent test                            | H. sapiens and M. musculus | 22012| 101 bp| -      | -    | -    | 76.4%| 84.4%| 83.1%| https://github.com/rreybeyb/DeepM6ASeq         |
| Pm6A-CNN           | CNN          | One-hot, NCP                                  | 10-fold cross validation                     | H. sapiens                  | 2260 | 41 bp| 88.6% | 98.6% | 93.6% | 96.0%| -    | https://home.jhu.edu/NSCL/Pm6ACNN.htm         |
|                    |              |                                               |                                              | M. musculus                 | 1450 | 41 bp| 90.4% | 97.2% | 93.8% | 97.0%| -    | -                                             |
|                    |              |                                               |                                              | S. cerevisiae               | 2614 | 51 bp| 84.6% | 85.5% | 85.0% | 92.0%| -    | -                                             |
|                    |              |                                               |                                              | A. thaliana                 | 4200 | 101 bp| 92.3% | 92.6% | 92.5% | 97.0%| -    | -                                             |
| M6APrede-EN [53]   | Ensemble SVM | PS (k-mer) NP, PCPs, RFHC-GACs                | 10-fold cross validation                     | S. cerevisiae              | 2614 | 51 bp| 70.55%| 60.63%| 65.59%| -    | -    | http://lin-group.cn/server/M6APrede-EN        |
| iRNA-Methyl [48]   | SVM          | PseDNC                                        | 10-fold cross validation                     | H. sapiens                  | 1130 | 41 bp| 81.68%| 99.11%| 90.38%| -    | -    | -                                             |
|                    |              |                                               |                                              | M. musculus                 | 725  | 41 bp| 77.79%| 100.0%| 88.39%| -    | -    | methyRNA                                      |
|                    |              |                                               |                                              | S. cerevisiae               | 2614 | 51 bp| 88.21%| 98.05%| 92.69%| -    | -    | -                                             |
| M6AMRFs [54]       | XGBoost      | NCP, ANF                                      | 10-fold cross validation                     | H. sapiens                  | 2260 | 41 bp| 82.04%| 100.0%| 91.02%| -    | -    | -                                             |
|                    |              |                                               |                                              | M. musculus                 | 1450 | 41 bp| 82.81%| 75.84%| 79.33%| -    | -    | -                                             |
|                    |              |                                               |                                              | S. cerevisiae               | 2614 | 51 bp| 75.21%| 73.30%| 74.25%| -    | -    | -                                             |
|                    |              |                                               | 10-fold cross validation                     | A. thaliana                 | 2000 | 101 bp| 80.67%| 81.43%| 81.05%| -    | -    | -                                             |
| MethylRNA [51]     | SVM          | NCP, ANF                                      | 10-fold cross validation                     | H. sapiens                  | 1130 | 41 bp| 81.68%| 99.11%| 90.38%| -    | -    | http://lin-group.cn/server/MethylRNA          |
| M6A-word2vec [60]  | CNN          | Word2vec, Distributed features encoding       | 10-fold cross validation                     | S. cerevisiae              | 2614 | 51 bp| 85.89%| 95%    | 90.50%| -    | -    | -                                             |
|                    |              |                                               |                                              | M. musculus                 | 788  | 41 bp| 85.89%| 95%    | 90.50%| -    | -    | -                                             |
| WHISTLE [12]       | SVM          | Sequence and genome derived features          | Independent test                            | H. sapiens                  | 30358| 41 bp| -      | -    | -    | 89.5%| -    | https://whistle-epitranscriptome.com           |
|                    |              |                                               |                                              | Mature RNA                  | 30358| 41 bp| -      | -    | -    | -    | -    | -                                             |
|                    |              |                                               |                                              | Full transcript             | 73940|      | -      | -    | -    | 78.4%| 34.2%| http://www.cuilab.cn/sramp/                    |
| SRAMP [61]         | Random forest| Binary, KNN, Spectrum, RP in transcript       | Independent test                            | H. sapiens                  | 73940|      | -      | -    | -    | 78.4%| 34.2%| -                                             |
|                    |              |                                               |                                              | S. cerevisiae              | -    |      | -      | -    | -    | 63.3%| 25.3%| -                                             |
| EDLm6APred [59]    | CNN          | One-hot, word2vec, word embedding             | Independent test                            | H. sapiens                  | -    | 1001 bp| 67.50%| 78.43%| 86.6% | -    | -    | -                                             |
|                    |              |                                               |                                              | M. musculus                 | -    | 1001 bp| 66.39%| 77.34%| 85.8% | -    | -    | -                                             |
|                    |              |                                               |                                              | S. cerevisiae               | -    | 1001 bp| 71.28%| 78.62%| 86.0% | -    | -    | -                                             |
| m6A Reader [52]    | SVM          | Sequence and genome derived features          | Independent test                            | H. sapiens                  | 30358| 41 bp| -      | -    | -    | 89.3%| -    | -                                             |
|                    |              |                                               |                                              | Mature RNA                  | 30358| 41 bp| -      | -    | -    | -    | -    | -                                             |
|                    |              |                                               |                                              | Full transcript             | 32188| 41 bp| -      | -    | -    | 98.1%| -    | -                                             |
| RNAMethPre [31]    | SVM          | K-mer, RP in mRNA, MFE score                  | Independent test                            | H. sapiens                  | 9849 | 101 bp| -      | -    | -    | 84.0%| 55.6%| -                                             |
|                    |              |                                               |                                              | M. musculus                 | 7580 | 101 bp| -      | -    | -    | 89.3%| 67.0%| -                                             |
| M6ATH [50]         | SVM          | NCP, DN                                       | Jackknife                                    | A. thaliana                 | 788  | 25 bp| 68.78%| 100%   | 84.39%| 84.6%| 87.0%| http://lin-group.cn/server/M6ATH              |
| RFAthM6A [62]      | Random Forest| NCP, DN                                       | 5-fold cross validation                      | A. thaliana                 | 2200 | 101 bp| -      | -    | -    | 93.0%| -    | -                                             |
area under the precision-recall (PR) curve, a plot showing the trade-off between precision and recall. In addition to AUROC and AUPRC, we also report the sensitivity (Sn), specificity (Sp), and overall accuracy (Acc) as indicated below:

\[
\begin{align*}
Sn &= \frac{TP}{TP + FN} \\
Sp &= \frac{TN}{TN + FP} \\
Acc &= \frac{TP + TN}{TP + TN + FP + FN}
\end{align*}
\]

where TP, TN, FP, and FN represent true positive, true negative, false positive, and false negative, respectively.

5. Machine learning approaches for RNA modification sites prediction

5.1. N6-methyladenosine

Among all types of RNA modification, the m\(^6\)A site has the most significant number of datasets due to its high presence and early discovery in various types of RNAs. Therefore, several computational methods have been used for m\(^6\)A site classification. iRNA-Methyl [48] was the first published algorithm with an available server developed using an SVM model and PseDNC as features. An updated version called M6Apred [49] was introduced to predict m\(^6\)A sites in the S. cerevisiae genome. Similar to iRNA-Methyl, M6Apred uses SVM for classification. However, features are extracted from RNA fragments based on the physicochemical properties of nucleotides. M6Apred showed intermediate results with an accuracy of 79.21%. A year later, RNA secondary structure and compositional based features were combined using SVM to produce RNA MethPred [31], and resulted in ROC score of 84% for \(H.\) sapiens and 89.3% for \(M.\) musculus. Simultaneously, Chen et al. created M6ATH [50] to predict m\(^6\)A in \(A.\) thaliana using SVM with sequence and genome derived features reaching an accuracy of 84.39%. Later, Chen et al. developed another SVM based tool called MethyRNA [51], which predicts m\(^6\)A in \(H.\) sapiens and \(M.\) musculus. MethyRNA uses the physicochemical properties of nucleotides (NCP and ANF). Using jackknife cross-validation test, MethyRNA produced an accuracy of 90.38% for \(H.\) sapiens, and 88.39% for \(M.\) musculus. Few years later, Chen et al. and Zhen et al. added more sequence and genome derived features to build SVM models for their tools WHISTLE [12] and m6A Reader [52], respectively.

In addition to SVM, ensemble classifiers are also used to predict m\(^6\)A sites. M6Apred-EL [53] used three SVM classifiers and three features, namely RFHC- GACs, PCPs, and PS[1-mer] NPs, achieving an accuracy of 80.83%. In addition, an eXtreme gradient boosting algorithm called M6AMRFS [54] was used with nucleotide chemical properties for four distinct species, namely \(S.\) cerevisiae, \(A.\) thaliana, \(M.\) musculus, and \(H.\) sapiens, achieving an accuracy of 74.25%, 81.05%, 79.33, and 91.02%, respectively. In addition, a random forest based model called SRAMP gave an accuracy of 78.4% on an independent dataset of combined \(H.\) sapiens and \(M.\) musculus sequences.

Recently, multiple deep learning-based approaches have been introduced to predict m\(^6\)A, such as Gene2vec [55], iN6-Methyl [5-steps] [56], DeepM6ASeq [57], Pm6A-CNN [58], EDLm6Apred [59], and m6A-word2vec [60]. Pm6A-CNN [58], a CNN-based algorithm, achieved an accuracy of 93.6%, 93.8%, 85.0%, and 92.5% on \(H.\) sapiens, \(M.\) musculus, \(S.\) cerevisiae, and \(A.\) thaliana, respectively. m6A-word2vec [60] is another deep learning based approach that transforms raw RNA sequences into a binary vector before applying a custom CNN architecture, achieving an accuracy of 83.17%. In addition, Gene2Vec [55] used word integration with CNN, achieving a low false positive rate and an ROC score of 84.1%. Table 2 gives an overall summary of available m\(^6\)A site prediction tools.

5.2. 2’-O-Methylation

In 2015, Chen et al. [10] were the first to use nucleotide composition and chemical properties to detect 2’-O-methylation sites, with an accuracy of 95.58%. Two years later, iRNA-2OM [63] was introduced for \(H.\) sapiens. The Features used in iRNA-2OM were filtered using mRMR, and the best 32 features were selected. SVM was used to predict methylation sites, which improved the results to 97.95% accuracy. iRNA-2methyl is another method that integrated PseKNC composition with a set of sequence coupled variables. The model was generated by combining 12 simple random forest classifiers into four ensemble predictors, achieving 93% accuracy [45]. Another tool called NmSEER V2.0 [28] uses a new random forest approach with an introduced combination of one-hot encoding, PSDSP, and KNF; they achieved an ROC of 86.2% [28]. Deep learning approaches include Deep-2-O-Me [64] and iRNA- PsKNC(2methyl) [46]. The latter gave better results with an accuracy of 98.27%. It used the 5-step rules of Chou and CNN to detect 2’-O-Methylation sites considering the raw genome sequence data. Deep-2-O-Me employed word2vec for encoding and a larger dataset but its accuracy was 85.36%. Table 3 gives an overall summary of some popular 2OMe site prediction tools.

5.3. N7-methylguanosine

Using the immuno–histochemical m7G-methylated RNA sequencing method (MeRIP-seq), Zhang et al. [65] created a benchmark dataset of m\(^7\)G sites in \(H.\) sapiens and \(M.\) musculus, in addition, produced the location of 801 m\(^7\)G in Human Hela and HepG2 [65]. In late 2019, Wei Chen et al. built an m\(^7\)G predictor called iRNA-M7G [12]. It focused on combining sequence and structure-based features to extract the best features, followed by an SVM model.

### Table 3

| Predictor                | ML Algorithm | Features                          | Testing  | NS  | WS  | Sn  | Sp  | ACC | ROC | PRC | Link                  |
|--------------------------|--------------|-----------------------------------|----------|-----|-----|-----|-----|-----|-----|-----|-----------------------|
| Chen et al. [10]         | SVM          | NCP, ANF                          | Jackknife| 294 | 41  | 92.52| 98.64| 95.58| -  | -  | -                     |
| iRNA-2OM [63]            | SVM          | NCP, ANF Type 2 PseKNC one-hot    | 5-fold cross validation Independent test | 294 | 41  | 97.27| 98.63| 97.95| 99.5% | -  | -  | http://lin-group.cn/ server/iRNA-2OM/ www.manut.net/ nmseer-v2/ |
| NmSEER V2.0 [28]         | Random forest| PseKNC                            | Jackknife| 147 | -   | -   | -   | 93.00 | -  | -  | -                     |
| iRNA-2methyl [45]        | Random forest| One-hot, MMI 5-fold cross validation Independent test | 294 | 41  | 96.29| 100.0| 98.27| -   | -  | -  | -                     |
| iRNA-PsKNC (methyl) [46] | CNN          | PseKNC                            | Jackknife| 147 | -   | -   | -   | 93.00 | -  | -  | -                     |
| Deep-2-O-Me [64]         | CNN          | word2vec                          | 20160 | 25  | -   | 85.36| 92.0%| 93.0% | -  | -  | -                     |
reaching an accuracy of 89.81%. M7g_model [66] is another algorithm that used feature selection methods such as mRMR and Relief [66] and produces an accuracy of 94.67%. In addition, m7GHub [27] is an online platform for decoding location and regulating the internal N7-methylguanosine in mRNA. It used SVM and m7GFinder [66], a feature extraction approach based on likelihood ratio (LR), reaching an accuracy of 76% on an independent dataset obtained using three approaches (m7G-Seq, m7G-MeRIP-Seq, and m7G-miCLIP-Seq) [67]. m7GPredictor [68] is another SVM based approach that combined NP, K-mer, PseDNC, Ksnpf,

### Table 4

| Predictor          | ML-Algorithm | Features                                                                 | Testing                        | NS   | WS  | Sn     | Sp     | ACC  | ROC  | Link                                                                 |
|--------------------|--------------|---------------------------------------------------------------------------|--------------------------------|------|-----|--------|--------|------|-------|----------------------------------------------------------------------|
| iRNA-m7G [12]     | SVM          | NPF, SSC, PseDNC, m7G-Seq                                                 | 10-fold cross validation       | 1482 | 41  | 88.66% | 90.96% | 89.81% | 94.6% | http://lin-group.cn/server/iRNA-m7G/                                  |
| XG-m7G [29]       | XGBoost      | NPF, SSC, PseDNC, m7G-Seq                                                 | 10-fold cross validation       | 1482 | 41  | 91.48% | 90.96% | 91.22% | 97.2% | -                                                                  |
| m7G model [66]    | SVM          | One-hot, NCP, K-mer, PseKNC                                               | 10-fold cross validation       | 1482 | 41  | 95.11% | 93.74% | 94.67% | 98.2% | https://github.com/MapFM/m7g_model                                    |
| m7GHub [27]       | SVM          | Sequence and genome derived features. NP, K-mer, PseDNC, Ksnpf, PseKNC    | Independent test               | -    | 30  | 84.20% | 71.00% | 76.00% | 85.5% | -                                                                  |
| m7GPredictor [68] | SVM          | NP, K-mer, PseDNC, Ksnpf, PseKNC                                          | Independent test               | 300  | 50  | 84.00% | 88.00% | 86.00% | 93.3% | https://github.com/NWAFU-LiuLab/m7Gpredictor                         |

### Table 5

| Predictor            | ML-Algorithm | Features         | Testing                   | Species     | NS   | WS  | Sn     | Sp     | ACC  | ROC  | PRC  | Link                                                                 |
|----------------------|--------------|------------------|---------------------------|--------------|------|-----|--------|--------|------|-------|-------|----------------------------------------------------------------------|
| iRNAm5C-PseDNC [69] | Random Forest| PseDNC           | Jackknife                 | H. sapiens, M. musculus | 1900 | 41  | 69.86% | 99.86% | 92.37% | -    | -    | http://www.jci-bioinfo.cn/iRNAm5C-PseDNC                             |
| PEA-m5C [70]         | Random Forest| Binary encoding, k-mer, PseDNC, KNF, KSNPFs. and pseDNC PseKNC         | 10-fold cross validation   | A. thaliana | 158  | 43  | 86.0%  | 90.0%  | 88.0%  | 93.9% | 94.5% | https://github.com/cma2015/PEA-m5C                                   |
| RNAm5CPred [72]      | SVM          | KNF, KSNPFs, and pseDNC PseKNC                                          | 10-fold cross validation   | H. sapiens  | 240  | 41  | 90.83% | 94.17% | 92.5%  | 95.7% | -    | -                                                                  |
| iRNA-PseColl [71]    | SVM          | PseDNC           | Jackknife                 | H. sapiens  | 240  | 41  | 75.83% | 79.17% | 77.50% | -    | -    | http://lin-group.cn/server/iRNA-PseColl/                             |
| M5C–HPCR [57]        | Ensemble of SVM | PseDNC, HPCR      | Jackknife                 | H. sapiens  | 240  | 41  | 90.83% | 95%    | 92.92% | 96.2% | -    | -                                                                  |
| IRNAm5C_NB [79]      | SVM          | BPB, k-mer, ENAC, EII, EII, PseKNC                                      | Jackknife                 | H. sapiens  | 240  | 41  | 82.81% | 81.11% | 82.20% | 91%   | 91%   | -                                                                  |
| m5C-PseDNC [74]      | SVM          | PseDNC, KNF, KSNPFs, and pseDNC PseKNC                                  | 10-fold cross validation   | A. thaliana | 12578| 41  | 68.1%  | 75.5%  | 71.8%  | -    | -    | -                                                                  |
| iRNA-m5C_SVM [73]    | SVM          | KNF, KSNPFs, and pseDNC PseKNC                                          | 10-fold cross validation   | A. thaliana | 10578| 41  | 79.40% | 80.90% | 80.15% | 88%   | -    | -                                                                  |
| m5CPred-SVM [75]     | SVM          | PseDNC, KNF, KSNPFs, and pseDNC PseKNC                                  | 10-fold cross validation   | A. thaliana | 2000 | 41  | 75.4%  | 79.9%  | 79.9%  | 77.5% | -    | 85.8% | -                                                                  |
| iRNA5hmC [76]        | SVM          | K-mer              | 5-fold cross validation   | D. melanogaster | 1324 | 41  | 67.67% | 63.29% | 65.48% | -    | -    | http://server.malab.cn/iRNA5hmC/                                    |
| iRNA5hmC-PS [77]     | LR           | Pse-Mono           | 5-fold cross validation   | D. melanogaster | 1192 | 41  | 80%    | 79.5%  | 78.3%  | -    | -    | http://nsclbio.jhnu.ac.kr/IRhmmCNN/                                 |
| iRhms5CNN [78]       | CNN          | One-hot, NCP       | 5-fold cross validation   | D. melanogaster | 1324 | 41  | 82%    | 80%    | 81%    | -    | -    | http://nsclbio.jhnu.ac.kr/IRhms5CNN/                                |
Table 6  
Performance of ψ site prediction tools. NS represents the number of samples and WS is to the window size.

| Predictor       | ML-Algorithm | Features             | Testing          | Species     | NS   | WS   | Sn    | Sp    | ACC   | ROC  | PRC  | Link                                      |
|-----------------|--------------|----------------------|------------------|-------------|------|------|-------|-------|-------|------|------|--------------------------------------------|
| iPseU-NCP [82]  | Random Forest| NCP                  | 5-fold cross validation | *H. sapiens* | 990  | 31 bp| 58.79%| 65.05%| 62.92%| -    | -    | https://github.com/ngphubinh/iPseU-NCP    |
| iRNA-PseU [43]  | SVM          | NCP                  | Jackknife        | *H. sapiens*| 990  | 31 bp| 61.01%| 59.80%| 60.40%| -    | -    |                                            |
| iRNA-PseU [43]  | SVM          | NCP                  | Jackknife        | *S. cerevisiae*| 628  | 21 bp| 64.65%| 64.33%| 64.49%| -    | -    |                                            |
| iRNA-PseU [43]  | SVM          | NCP                  | Jackknife        | *M. musculus*| 944  | 21 bp| 73.31%| 64.83%| 69.07%| -    | -    |                                            |
| iPseU-CNN [86]  | CNN          | n-gram               | 5-fold cross validation | *H. sapiens*| 990  | 31 bp| 61.01%| 59.80%| 60.40%| -    | -    |                                            |
| iPseU-CNN [86]  | CNN          | n-gram               | 5-fold cross validation | *S. cerevisiae*| 628  | 21 bp| 64.65%| 64.33%| 64.49%| -    | -    |                                            |
| iPseU-CNN [86]  | CNN          | n-gram               | 5-fold cross validation | *M. musculus*| 944  | 21 bp| 73.31%| 64.83%| 69.07%| -    | -    |                                            |
| EnsemPseU [84]  | SVM, RF      | K-mer                | 10-fold cross validation | *H. sapiens*| 990  | 31 bp| 63.46%| 60.09%| 66.28%| 70.0%| -    | https://github.com/biyue1026/EnsemPseU    |
| PSI-MOUSE [85]   | SVM          | Genome and sequence derived features | 5-fold cross validation | *S. cerevisiae*| 628  | 21 bp| 74.12%| 77.64%| 71.94%| -    | -    |                                            |

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and PseKNC features to reach an accuracy of 86%. XG-m7G [29] is a technique that uses the XGBoost algorithm along with multiple features (ND, NCP, ENAC, KSNAP), followed by SHAP (Shapley additive interpretations) to predict the modification sites. XG-m7G showed promising results in terms of true positive rate with an accuracy of 91.22%. Table 4 gives an overall summary of popular m7G site prediction tools.

5.4. 5-Methylcytosine and 5-Hydroxymethylcytosine

Computational techniques advantageously complement traditional sequential methods and are a suitable alternative for further research on m5C RNA modification. In mammalian genomes, iRNAm5C-PseDNC [69] is one of the few available algorithms that predict m5C sites. iRNAm5C-PseDNC uses PseDNC features and Random Forest, reaching an accuracy of 92.37%. PEA-m5C [70] is another Random Forest based algorithm trained on a dataset with a highly unbalanced positive/negative ratio, making it robust while excluding false positives but indifferent to true positives, resulting in an accuracy of 88%.

Several algorithms produce performances that sacrifice sensitivity for specificity and vice versa. RNAI-PseColl [71] showed a specificity of 79.17%, meaning that the algorithm could predict most m5C sites but simultaneously resulted in a large number of false positives. M5C-HPCR [57] balanced the true and false positive rates, producing a sensitivity of 90.83% and a specificity of 95.00%. RNAm5CPred [72] produced an accuracy of 92.5% due to its different feature extraction approaches. RNAm5CPred proposed three different types of features: KNFs (K-nucleotide frequencies), pseDNC (pseudo-dinucleotide composition), KSNPFs (K-spaced nucleotide pair frequencies) where K random nucleotides separate a nucleotide pair.

The majority of m5C predictors use SVM algorithm. For example, pM5CS-Comp-mRMR [73], m5C-PseDNC [74], iRNA-m5C_SVM [73] and m5CPred-SVM [75], used different datasets and feature extraction approaches.

Lui et al. developed a prediction method called iRNA5hmC [76]. In this method, an SVM classifier used k-mers as features, producing an accuracy of 65.48%. Similarly, another SVM-based predictor called iRNA5hmC-Ps [77] was established. It proposed a new feature extraction method called Position-Specific Gapped k-mer and used Position-Specific k-mer to retain most of the characteristic information of RNA sequences. This approach achieved an accuracy of 73.8%. iRhm5CNN is another tool based on CNN for 5hmC site prediction that uses primary RNA sequences [78]. The CNN architecture derives the most relevant information from the primary RNA-seq representations, resulting in an accuracy of 81%. Table 5 gives an overall summary of some popular m5C site prediction tools.

5.5. Pseudouridine (ψ)

In 2015, Li et al. were the first to develop a ψ modification sites algorithm based on the SVM algorithm called PPUS [80]. Later in the year, PseU [81] was introduced and used SVM and multiple features, including NC, DC, pseDNC, position-specific nucleotide propensity (PSNP), and position-specific dinucleotide propensity (PSDP). One year later, Chen et al. established iRNA-PseU [43] using SVM and PseKNC features in S. cerevisiae, M. musculus, and H. sapiens. IPseU-NCP [82] was constructed in 2019 using Random Forest as a classifier and NCP as features, resulting in an accuracy of 62.92%, 69.59%, and 71.82% for H. sapiens, S. cerevisiae, and M. musculus, respectively. Similarly, an XGboost based predictor called XG-PseU [83] was published in 2020. Lui

Table 7

| Predictor | ML-Algorithm | Features Testing | Species | NS | Sn | Sp | ACC | RPC | PRC | Link |
|-----------|--------------|------------------|---------|----|----|----|-----|-----|-----|------|
| RAMPred   | SVM          | NCP, ANF         | H. sapiens | 12732 | 98.38% | 99.89% | 99.13% | 98% | 98% | http://lin-group.cn/server/RAMPred |
| ISGm1A    | Random Forest| NCP, ANF         | S. cerevisiae | 966 | 95.65% | 100% | 97.83% | 98% | 98% | http://lin-group.cn/server/RAMPred |

Table 8

| Predictor | ML-Algorithm | Features Testing | Species | NS | Sn | Sp | ACC | RPC | PRC | Link |
|-----------|--------------|------------------|---------|----|----|----|-----|-----|-----|------|
| D-pred    | Ensemble SVM | NCPC, PseKNC, NCP, ANF, SSC | S. cerevisiae | 136 | 41 bp | 76.47% | 89.71% | 83.09% | - | - |
| iRNAD     | SVM          | NCPC, PseKNC, NCP, ANF, SSC | H. sapiens, M. musculus, D. melanogaster, S. cerevisiae, and E. coli | 551 | 23 bp | 92.05 | 98.13 | 96.18 | 98.39 | http://lin-group.cn/server/iRNAD/ |
et al. improved the accuracy on *H. sapiens*, *S. cerevisiae*, and *M. musculus* datasets to 65.44%, 68.15%, and 72.03%, respectively. Recently, EnsemPseU [84] combined known classification algorithms such as SVM, NB, KNN, XGBoost, and RF into an ensemble predictor. This approach improved the state-of-the-art accuracy to 66.28% for *H. sapiens*, 74.16% for *S. cerevisiae*, and 73.84% for *M. musculus*. PSI-MOUSE [85] is an SVM based tool that combined genome and sequence derived features such as clustering information, secondary structure and density nucleotide. PSI-MOUSE performed the best on *M. musculus*, reaching an accuracy of 91.97% using 5-fold cross validation. iPseU-CNN [86] is a predictor that was introduced by Tahir et al. in 2019 based on a CNN architecture, allowing them to automatically extract features from *ψ* sites. MU-PseUDeep [87] is another CNN-based predictor that used a novel approach based on the secondary structure context of an mRNA sample as an input feature, resulting in an accuracy of 72.6% for *H. sapiens*, 76.8% for *S. cerevisiae*, and 76% for *M. musculus*. Table 6 gives an overall summary of popular *ψ* site prediction tools.

### 5.6. N4-acetylcytidine (ac4C)

PACES [89] was the first algorithm to predict ac4C modification sites in human mRNA. The authors created a reference dataset using data generated by Arango et al. [8]. Site-specific dinucleotide sequence profiles, K-nucleotide frequencies, and two random forest classifiers are included in PACES, which resulted in an ROC score of 88.5%. PACES results were further improved by Tahir et al., who used a model called XG-ac4C [34] based on the eXtreme Gradient Boosting (XGboost) as classifier and nucleotide chemical features (NCP), hot coding, nucleotide density (DN), Kmer, pseudo-electron–ionic interaction (PseEI), and pseudo-nucleotide triple interactions (PseEI) as features. This predictor improved the accuracy on *H. sapiens*, *S. cerevisiae*, and *M. musculus*. Table 6 gives an overall summary of popular *ψ* site prediction tools.

### Table 6

Performance of A-to-I site prediction tools. NS represents the number of samples and the window size is equal to 51 bp.

| Predictor | ML-Algorithm | Features | Testing | Species | NS  | Sn  | Sp  | ACC  | Link                                      |
|-----------|--------------|----------|---------|---------|-----|-----|-----|------|-------------------------------------------|
| iRNA-AI   | SVM          | NCP, ANF, PseKNC, DN | Independent set | *H. sapiens* | 6486 | 84.19% | 84.19% | 93.81% | http://lin-group.cn/server/iRNA-AI/        |
| PAI       | SVM          | PseDNc   | Jackknife | *D. melanogaster* | 244  | 85.60% | 73.11% | 79.51% | http://lin-group.cn/server/PAI            |
| EPAM-NC   | SVM          | PseKNC, l-mers, n-gapped-lmers | Jackknife | *D. melanogaster* | 244  | 85.60% | 73.11% | 79.51% | -                                         |
| PAI-SAE   | SVM          | DACC, PseDNc | Jackknife | *D. melanogaster* | 224  | 87.20% | 76.47% | 81.97% | -                                         |
| PRESa2i   | Hoefding tree | K-mer, Gapped k-mers, and other sequence based features | Independent set | *D. melanogaster* | 300  | 95.20% | 77.31% | 86.48% | http://brl.uiu.ac.bd/presa2i/               |

### Table 11

Summary of multi-modification type prediction tools. NS represents the number of samples and WS is to the window size.

| Predictor | ML-Algorithm | Features | Species | RNA modification | NS  | WS  | Link                                      |
|-----------|--------------|----------|---------|------------------|-----|-----|-------------------------------------------|
| iMRM [98] | XGboost      | k-tuple, one-hot, DBE, ND, NCP, DPCP | *H. sapiens* | m1A | 12732 | 41 | http://www.bioml.cn/XG_iRNA/home             |
|           |              |          |         | m5C | 240  | 41  |
|           |              |          | *S. cerevisiae* | m1A | 2260 | 41  |
|           |              |          |         | m5C | 990  | 21  |
|           |              |          |         | A-to-I | 6000 | 51  |
|           |              |          | *M. musculus* | m1A | 966  | 41  |
|           |              |          |         | m5C | 422  | 41  |
|           |              |          |         | m1A | 2614 | 51  |
|           |              |          |         | m5C | 627  | 31  |
| iRNA-3typeA [99] | SVM | PseKNC, PseAAC, NCP, one hot | *H. sapiens* | m1A | 12732 | 41 | http://lin-group.cn/server/iRNA-3typeA         |
|           |              |          |         | m5C | 240  | 41  |
|           |              |          | *M. musculus* | m1A | 2260 | 41  |
|           |              |          |         | A-to-I | 6000 | 51  |
| iRNA-PseColl [71] | SVM | PseKNC, PseAAC, DN | *H. sapiens* | m1A | 12732 | 41 | http://lin-group.cn/server/iRNA-PseColl/     |
|           |              |          |         | m5C | 240  | 41  |
|           |              |          | *S. cerevisiae* | m1A | 2260 | 41  |
|           |              |          |         | m5C | 990  | 21  |
|           |              |          |         | A-to-I | 6000 | 51  |
| DeepMRMP [47] | BGRU and transfer learning | One-hot | *H. sapiens* | m1A | 2574  | 41 | https://github.com/Chenyb939/DeepMRMP/     |
|           |              |          |         | m5C | 422  | 41  |
|           |              |          | *S. cerevisiae* | m1A | 2614 | 51  |
|           |              |          |         | m5C | 627  | 31  |
|           |              |          |         | A-to-I | 1662 | 41  |
|           |              |          | *M. musculus* | m1A | 194  | 41  |
|           |              |          |         | m5C | 1450 | 41  |
|           |              |          |         | m1A | 944  | 21  |

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For more information, refer to the original publication by A. El Allali, Z. Elhamraoui and R. Daoud in *Computational and Structural Biotechnology Journal*.
the previous algorithm’s ROC score to 91%. Table 7 gives an overall summary of some popular m4C site prediction tools.

5.7. N1-Methyladenosine

To our knowledge, only two predictors have been introduced to identify N1-Methyladenosine sites, namely RAMPred and ISGm1A. RAMPred [90] was developed to predict m^1A modification sites in M. musculus, H. sapiens, and S. cerevisiae. It is based on PC, CP, and CFB features and uses the SVM classifier. In contrast, ISGm1A used a random forest algorithm and typical sequence properties, namely physical and chemical properties of nucleotides and cumulative frequencies, and 75 additional properties derived from genome annotations. Table 8 gives an overall summary of some popular m^1A site prediction tools.

5.8. Dihydrouridine (D)

An SVM-based classifier, namely D-pred [92], was implemented to detect D-modification sites in S. cerevisiae. The algorithm incorporates sequence-based heterogeneous features, resulting in an accuracy of 83.09%. Likewise, iRNAD [93] used the chemical properties of nucleotides and nucleotide density to encode RNA samples of five different species, namely H. sapiens, M. musculus, S. cerevisiae, E. coli, and D. melanogaster. The classification was done using SVM and reached an accuracy of 96.18%. Table 9 gives an overall summary of some popular D site prediction tools.

5.9. A-to-I modification

In 2016, Chen et al. introduced the first algorithm used to detect A-to-I modification sites in D. melanogaster called PAI [94]. The algorithm is based on SVM and PseDNC features and produced an accuracy of 79.51%. Another SVM-based algorithm called iRNA-AI [95] was proposed in the following year using a different dataset. iRNA-AI achieved promising results with an accuracy of 93.81%. Xia et al. improved the accuracy of PAI using an approach called PAI-SAE [30]. The algorithm used new hybrid features by incorporating DACC and PseDNC density, nucleotide density, and a backup autoencoder with the SVM algorithm. EPAI-NC [96] also used SVM with K-mer, reaching an accuracy of 93.90% on an independent test set. The Hoeffding tree, an incremental decision tree for sample identification, along with BFS + LF for feature selection, was used by PRESa2i [36]. This method achieved an accuracy of 86.48% in the independent dataset. Table 10 gives an overall summary of popular m^6A site prediction tools.

5.10. N2-methylguanosine

iRNA-m2G [97] is the only available predictor proposed for m^2G sites for eukaryotes. The models were trained using SVM, and RNA sequences were encoded using the chemical property of nucleotides and the accumulated replication of nucleotides. The results are promising, with an accuracy of 94.56% for H. sapiens, 100.00% for M. musculus, and 96.27% for S. cerevisiae. iRNA-m2G reached a sensitivity of 89.13% for H. sapiens, 100.00% for M. musculus, 92.53% for S. cerevisiae, and specificity of 100.00% for H. sapiens, M. musculus, and S. cerevisiae.

5.11. Simultaneous identification of more than one RNA modification

Numerous RNA modification data have been collected as a result of the advances in high-throughput sequencing techniques. However, most of these approaches cannot distinguish between different RNA modifications in the same RNA sequence. For example, Adenosine typically undergoes different RNA modification sites, namely m^1A, m^6A, m*w, m^5C, m^5mC, m^6A, and A-to-I modifications in H. sapiens, M. musculus, S. cerevisiae, and E. coli. Unfortunately, it is not easy to determine whether Adenosine modifications have occurred simultaneously using the methods reviewed so far. Consequently, the development of computational methods for solving this problem is critical. iRNA-PseColl [71] is the first platform developed to simultaneously identify three different RNA modification sites, namely m^1A, m^6A, and m^6C, based solely on the sequence information reaching an accuracy of 99.13% 90.38%, 77.50%, respectively. iMRM [98] is an XGboost-based prediction tool that can concurrently classify m^4A, m^4C, m^1A, ψ, and A-to-I modifications in H. sapiens, M. muscu-
lus, and S. cerevisiae using sequence information and nucleotide physicochemical properties. Similarly, Chen et al. developed an SVM-based predictor named iRNA-3type [99] that considered nucleotide chemical properties and lingering density. It can detect m\(^1\)A, m\(^2\)A, and A-to-I RNA modifications in both H. sapiens and M. musculus transcriptomes.

Another multi-type predictor is DeepMRMP [47] for m\(^1\)A, \(\psi\), m\(^3\)C. It is the first deep learning-based tool for multiple types of RNA modification site prediction. The model used large-scale m\(^1\)A data to pre-train a deep learning model and then employed a transfer-learning strategy to fine-tune its network parameters for the targeted types of RNA modifications, reaching an accuracy of 99.27\% for m\(^1\)A, 62.64\% for \(\psi\), and 66.32\% for m\(^3\)C. Table 11 summarizes the available multi-type predictors and Table 12 provides the performance measures for each tool and dataset.

6. Summary and outlook

The study of RNA modification has gained high interest as it reveals the importance of RNA modifications in regulating gene expression and disease pathogenesis. As epitranscriptome sequencing data increases, more RNA modification benchmark datasets become available. The recent availability of large datasets and the advances in computational biology through machine learning have transformed research in the area. As a result, these technologies have ultimately improved our understanding of the biological significance of RNA modifications. This paper reviewed and updated recent advances and emerging machine learning-based approaches to RNA modification prediction. Despite the rapid progress in this field, several limitations and problems exist and should be addressed.

Perhaps the most important observation is that most approaches across all modification types share common techniques, features, and classification algorithms. However, their reported accuracy varies within the same or between different modification types. Our review shows that the performance correlates with the benchmark dataset’s quality and size. For example, m7g-model [66] and m7GPredictor [24] tested several classifiers and features. When using SVM with the PseKNC feature, m7g-model reported specificity of 81.49\% on an independent dataset of 300 samples, while m7GPredictor reported a specificity of 88\% on the same independent dataset. m7GPredictor extracted 801 m7G sites from high-confident internal sites as a positive dataset and randomly selected 801 negative examples. Conversely, m7g-model created a positive dataset from 741 experimentally validated m7G sites and 741 random negative samples.

We also observe a trade-off between sensitivity and specificity among different approaches, and in several cases, the false positives and false negatives rates have an inverse causal relationship. For example, some algorithms have a higher false positive rate, such as DeepM6ASeq [57], while others have a higher false negative rate, such as Gene2vec [55]. Some tools attempt to circumvent this problem using imbalanced dataset. For example, DeepM6ASeq has a 16:10 positive-to-negative ratio, while for Gene2vec it is 1:1 for training and 1:1:0 for testing.

Therefore, the most critical step is to create a single comprehensive Benchmark dataset for each modification type. Current databases include small epitranscriptomics data with matching RNA modification profiles. In addition, the lack of a standard benchmark directly impacts the performance comparison between different approaches. In most cases, new approaches use custom-built datasets that make the comparison biased. Therefore, more enriched experimentally verified data is required to train better machine learning models and compare emerging tools fairly. These benchmark datasets should consider the confidence of the modification sites, the correct size of the sequences, the best positive-to-negative ratio, and the best way to select negative samples.

Most reviewed approaches use similar features and explore multiple features and classification models before concluding with the best combination. However, future algorithms should explore other biological-related features, such as structure-based features. In addition, features specific to a particular species or tissue could be introduced to facilitate the functional investigation of modifications in other species such as plants [70].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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