WHISTLE: a high-accuracy map of the human \(N^6\)-methyladenosine (m\(^6\)A) epitranscriptome predicted using a machine learning approach

Kunqi Chen\(^1,2,\dagger\), Zhen Wei\(^1,2,\dagger\), Qing Zhang\(^1,\dagger\), Xiangyu Wu\(^1,2,\dagger\), Rong Rong\(^1,3,4\), Zhiliang Lu\(^1,3,4\), Jionglong Su\(^3,5\), João Pedro de Magalhães\(^2\), Daniel J. Rigden\(^4\) and Jia Meng\(^1,3,4,\ast\)

\(^1\)Department of Biological Sciences, Xi’an Jiaotong-Liverpool University, Suzhou, Jiangsu 215123, China, \(^2\)Institute of Ageing & Chronic Disease, University of Liverpool, L7 8TX Liverpool, UK, \(^3\)Research Center for Precision Medicine, Xi’an Jiaotong-Liverpool University, Suzhou, Jiangsu 215123, China, \(^4\)Institute of Integrative Biology, University of Liverpool, L7 8TX Liverpool, UK and \(^5\)Department of Mathematical Sciences, Xi’an Jiaotong-Liverpool University, Suzhou, Jiangsu 215123, China

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ABSTRACT

\(N^6\)-methyladenosine (m\(^6\)A) is the most prevalent post-transcriptional modification in eukaryotes, and plays a pivotal role in various biological processes, such as splicing, RNA degradation and RNA–protein interaction. We report here a prediction framework WHISTLE for transcriptome-wide m\(^6\)A RNA-methylation site prediction. When tested on six independent datasets, our approach, which integrated 35 additional genomic features besides the conventional sequence features, achieved a major improvement in the accuracy of m\(^6\)A site prediction (average AUC: 0.948 and 0.880 under the full transcript or mature messenger RNA models, respectively) compared to the state-of-the-art computational approaches MethyRNA (AUC: 0.790 and 0.732) and SRAMP (AUC: 0.761 and 0.706). It also outperformed the existing epitranscriptome databases MeT-DB (AUC: 0.798 and 0.744) and RMBase (AUC: 0.786 and 0.736), which were built upon hundreds of epitranscriptome high-throughput sequencing samples. To probe the putative biological processes impacted by changes in an individual m\(^6\)A site, a network-based approach was implemented according to the ‘guilt-by-association’ principle by integrating RNA methylation profiles, gene expression profiles and protein–protein interaction data. Finally, the WHISTLE web server was built to facilitate the query of our high-accuracy map of the human m\(^6\)A epitranscriptome, and the server is freely available at: www.xjtlu.edu.cn/biologicalsciences/whistle and http://whistle-epitranscriptome.com.

INTRODUCTION

Large scale analysis has revealed the abundance of RNA modifications in the human epitranscriptome (1). With the recent advances in the exploration of RNA epigenetics, more than 150 types of RNA modifications have been identified (2). Among them, the most prevalent non-cap modification marker present on eukaryotic messenger RNA (mRNA) and long non-coding RNA, \(N^6\)-methyladenosine (m\(^6\)A), (3) has emerged as an abundant and dynamically regulated modification (4). m\(^6\)A was detected within poly-A RNA for the first time in 1974 (5), and has since been characterized in various eukaryotic species. In the past five decades, various studies have demonstrated the biological significance of m\(^6\)A methylation, which includes roles in the circadian clock (6), regulation of mRNA translation (7), heat shock response (8), microRNA (miRNA) processing (9), DNA damage response (10), RNA–protein interaction (11) and regulation of RNA stability (12). Consequently, the accurate identification of m\(^6\)A locations is critical for the study and understanding of the downstream effects of RNA modification in biology.

To identify the precise location of m\(^6\)A sites on mRNA, the first whole-transcriptome m\(^6\)A profiling technique m\(^6\)A-seq (or MeRIP-seq) was introduced in 2012 (13,14), in which the m\(^6\)A containing RNA fragments is immunoprecipitated, purified and then subjected to further analysis. This technique applies high-throughput sequencing to...
the IP sample enriched with m^6^A-containing mRNA fragments. In contrast to the input control samples, it typically results in the detection of m^6^A containing peaks with around 100 nt resolution using MACS, the exomePeak R/Bioconductor Package and other peak callers (15,16). The precise location of the m^6^A sites may be further narrowed down to base-resolution by searching for the m^6^A motif RRACH within the peaks detected with m^6^A signal. Most existing epitranscriptome databases, such as, MeT-
DB and RMBase, rely on this very simple strategy (17,18). A major limitation of this method is that it cannot differentiate between a randomly-occurring RRACH motif and a real m^6^A-containing motif located nearby, i.e. all the RRACH motifs located within an m^6^A peak will be reported as holding an m^6^A site, including the chance occurrences, resulting in false positive predictions. Since m^6^A-seq is currently the most widely used approach for profiling the transcriptome-wide m^6^A, and a very large number of the m^6^A-seq samples have been acquired in different studies, the m^6^A site information extracted from the m^6^A-seq using the motif search strategy essentially dominates the existing epitranscriptome databases. For this reason, it is not surprising that both MeT-
DB (426 544 sites) and RMBase (477 452 sites) report a very large number of transcriptome m^6^A sites, many of which may be false positive due to a chance RRACH motif located close to a real m^6^A site (or within an m^6^A peak).

Besides the m^6^A-seq technique, single-based resolution techniques such as the miCLIP (19) and m^6^A-CLIP (20) were also developed. However, these experiments are usually more laborious to perform but still offer limited coverage of the m^6^A epitranscriptome, since the reported RNA-methylation sites are still restricted to the transcripts more readily expressed under a specific cell/tissue condition. Although base-resolution profiling techniques have not been very widely applied in biological studies due to their expense and difficulties, they provide the ground truth of m^6^A site information that is necessary for computational prediction. To date, a large number of RNA-methylation site prediction methods and web servers have been developed based on the information extracted from base-resolution techniques since 2015, including the pseudo nucleotide composition based approach iRNA-Methyl by Chen et al. (21) and physical-chemical properties-based approach pRNAm-PC (22). Subsequently, Zhou et al. employed SRAMP, a random forest machine learning framework, to predict mammalian m^6^A sites using sequence features (23). Many other site predictors have been developed for m^6^A and other RNA modification, such as MethyRNA (24), RNA MethPre (25), RAM-NPPS (26), Target M6^A (27), AthMethPre (28), iRNA-PseColl (29), M6APred-EL (30), iMethyl-STTNC (31), iRNA-PseDNC (32), etc. (33–39). These methods have been recently reviewed (40). These site predictors usually take the transcript sequence as the input and report a number of possible m^6^A sites as the output, making them very convenient to use. However, to our knowledge, they are exclusively based on the sequence-derived information—even when the secondary structure or other high level features (41) are used, the information is still directly extracted from sequence without considering other potentially useful genomic features, such as, conservation, transcript type and gene annotation. Although the sequence information probably plays a central role, other genomic features may also be helpful in the prediction of m^6^A sites and thus should be incorporated in the analysis. Additionally, although potentially feasible, none of these approaches have been applied transcriptome-wide to reconstruct the entire m^6^A epitranscriptome, thus limiting their usage in large-scale or high-throughput analysis.

In this project, we proposed a prediction framework, WHISTLE, which stands for whole-transcriptome m^6^A site prediction from multiple genomic features. The framework extracted a comprehensive set of domain knowledge based on various genomic features, and integrated them with conventional sequence-derived features for reconstructing a high-accuracy map of the m^6^A epitranscriptome. The ‘guilt-by-association’ principle was then applied to further annotate the functional relevance of each individual RNA-methylation site by integrating gene expression profiles, RNA methylation profiles and PPI networks.

MATERIALS AND METHODS

Training and testing data for m^6^A site prediction

The data used for training and benchmarking in m^6^A site prediction includes six single-base resolution m^6^A experiment obtained from five cell types (see Table 1). The base-resolution m^6^A sites in each experiment were downloaded directly from Gene Expression Omnibus (GEO). The two samples (MOLM13 mi-CLIP sample and A549 m^6^A-CLIP) reported based on the human genome assembly hg18 were lifted using UCSC liftOver tool (https://genome.ucsc.edu/cgi-bin/hgLiftOver). A total of 20 516 and 17 383 m^6^A sites out of the original 23 480 and 19 683 sites were lifted to hg19, respectively. Both samples have very large number of (>17000) positive sites that can be used for training and testing after liftOver, and the majority (four out of six) base-resolution samples are based on hg19 and thus do not require extra processing step.

In the beginning of the performance evaluation procedure, dataset 6 of the base-resolution data (Table 1) was used as the independent testing data, while the other five datasets were used as the training data. The positive training data (m^6^A sites) was determined as the m^6^A sites under RRACH consensus motifs that have been reproduced in at least two of the five training datasets. The negative training data (non-m^6^A sites) was randomly selected from the non-positive RRACH adenosines on the full transcripts containing the positive sites (see Figure 1). Initially, the number of randomly selected negative sites was ten times the number of positive sites. Later, the positive-negative ratio was balanced by randomly splitting the negative samples into 10 random subsets. Consequently, 10 training datasets, each with 1:1 positive-to-negative ratio, were constructed using different negative samples. The negative data was also generated similarly on testing data (Dataset 6), i.e. the negative data were randomly selected non-positive m^6^A sites from the m^6^A containing transcripts. The ratio of positive testing data to negative testing data was also kept as 1:10. The testing performances from the 10 independent sessions were averaged.
To exclude randomness of testing dataset from the m6A site prediction evaluation, we also applied dataset level leave-one-out validation over the six base-resolution datasets. In each round of the dataset level validation, one of the six base-resolution datasets was used as the independent testing data, while the remaining five datasets were used as the training data. The same rules of the training and testing data, while the remaining five datasets were used as the training data. The sequence-based information around the RRACH motif was encoded using the following formula:

\[ x_i = \begin{cases} \{1 \text{ if } s_i \in \{A,G\} \} & \text{if } i \neq 1,7,13,19,25,31,37,43,49,55 \\ 0 \text{ otherwise} & \text{if } i = 1,7,13,19,25,31,37,43,49,55 \end{cases} \]

\[ y_i = \begin{cases} 1 \text{ if } s_i \in \{A,C\} \\ 0 \text{ otherwise} \end{cases} \]

\[ z_i = \begin{cases} 1 \text{ if } s_i \in \{A,U\} \\ 0 \text{ otherwise} \end{cases} \]  

(1)

Therefore, the A, C, G, U can be encoded as a vector of three features (1,1,1), (0,1,0), (1,0,0) and (0,0,1), respectively. Additionally, a feature of the cumulative nucleotide frequency is calculated for each nucleotide position in the sequence. The density of the i-th nucleotide di is defined as the sum of all the instances of the i-th nucleotide before the i + 1 position. The nucleotide frequency fi is defined by the following formula: fi = di/i. Using the sequence ‘AUG-GACACU’ as an example, the cumulative frequency for adenine is 1.00 (1/1), 0.40 (2/5) and 0.43(3/7) at the first, fifth and seven position, respectively; while the frequency for uracil is 0.50 (1/2) and 0.11 (1/9) at the second and ninth respective position.

Features for m6A site prediction

Sequence-derived features. The sequence-based information around the RRACH motif was encoded using the same method of m6Apred (44) and MethyRNA (24), which have been shown to be quite effective and achieved good performance in human and yeast m6A site prediction. The sequence feature encodes the nucleotides sequence by the following formula:

\[ x_i = \begin{cases} \{1 \text{ if } s_i \in \{A,G\} \} & \text{if } i \neq 1,7,13,19,25,31,37,43,49,55 \\ 0 \text{ otherwise} & \text{if } i = 1,7,13,19,25,31,37,43,49,55 \end{cases} \]

\[ y_i = \begin{cases} 1 \text{ if } s_i \in \{A,C\} \\ 0 \text{ otherwise} \end{cases} \]

\[ z_i = \begin{cases} 1 \text{ if } s_i \in \{A,U\} \\ 0 \text{ otherwise} \end{cases} \]  

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Machine learning approach used for m6A site prediction

The Support Vector Machine (SVM) is one of the most widely used machine learning algorithms in computational biology. It was previously used for mammalian miRNA target prediction (50), protein kinase-specific phosphorylation sites prediction (51) and mammalian m6A modification sites prediction (24,25). In this project, we used an R language interface of LIBSVM (52) to construct the SVM-based m6A site predictors. Following previous approaches (21,22), the radial basis function was chosen as the kernel function, and the other parameters were set at the default. Random Forest is another popular machine learning algorithm applied in biology data, and one of the earliest mammalian m6A site predictor SRAMP was developed based on the Random Forest approach (23). In this project, we also use Random Forest from the R package randomforest (53) to compare the predictive performance using SVM.

Genome-derived features. Most existing RNA modification site prediction algorithms use exclusively sequence-based features; however, such features alone may not fully capture the attributes of RNA modification topology. Hence, we generated 35 additional genomic features that may contribute to the prediction. Genomic Features 1–13 are dummy variable features indicating whether the adenosine sites shall fall within the transcript regions that satisfy certain topological properties. All the features in this category are generated by the GenomicFeatures R/Bioconductor package (45) using the transcript annotations hg19 TxDB package. To remove the ambiguity caused by transcript isoforms, only the primary (longest) transcripts of each gene were kept for the extraction of the transcript sub-regions. Genomic Features 14–16 are real valued features defining the relative position of the transcript regions (3′UTR, 5′UTR and whole transcript), i.e. the distance from the adenine to the 5′ end divided by the width of the region. The values are also set to zero for sites that do not belong to the region. Genomic features 17–19 represent the length of the transcript region containing the modification site. The values are also set to zero for sites that do not belong to the region. Features 20–22 capture the distance from the adenine sites to the 5′ end or 3′ end of the splicing junc- tions. Additionally, the distance to the nearest neighboring m6A sites in the training data is generated to measure the clustering effect of the m6A RNA modification sites. Features 23–26 represent the evolutionary conservation score of the adenosine sites and its flanking regions; two metrics of nucleotide conservation, Phast-Cons score (46) and the fitness consequence scores are used to measure the conservation level of the underlying nucleotide sequence. Features 27 and 28 represent the RNA secondary structures around the adenine site, the RNA secondary structures are predicted using RNAfold from the Vienna RNA package (47). Finally, features 29–35 are the properties of the genes or transcripts containing the m6A sites, such as being the miRNA target genes or housekeeping genes. The annotation of miRNA target sites are from miRanda (48) and TargetScan (49). Supplementary Table S1 contains more details about the genomic features we considered in the prediction.
Performance evaluation of m^6A site prediction

For both the SVM and random forest classifiers, a 5-fold cross-validation was employed on the training datasets for model selection purpose, and the final performance of the predictor was measured on the independent testing dataset. The receiver operating characteristic curve (sensitivity against 1-specificity) was used to measure the prediction performance under different decision thresholds, and the area under the curve (AUC) was calculated as the main performance evaluation metric.

When evaluating the accuracy of m^6A site information stored in existing epitranscriptome m^6A site databases MeT-DB Version 2 and RMBase Version 2, the reliability was determined by the number of experiments that support the existence of a specific m^6A site, based on which the AUC can be calculated. In addition, the sensitivity (Sn), specificity (Sp) and Matthews correlation coefficient (MCC) were calculated to measure the performance of predictor:

\[
Sn = \frac{TP}{TP + FN} \tag{2}
\]

\[
Sp = \frac{TN}{TN + FP} \tag{3}
\]

\[
MCC = \frac{TP \times TN - FP \times FN}{\sqrt{(TP + FN)(TP + FP)(TN + FN)(TN + FP)}} \tag{4}
\]

where, TP, TN, FP and FN represent true positive, true negative, false positive and false negative, respectively. When different methods were compared under AUC, they always use the same positive and negative gold standard dataset, and AUCs were always calculated in the same way. The AUCs of different methods reported in our manuscript are therefore strictly comparable.

Estimate the posterior probability of RNA methylation

The existing machine learning approaches usually report the probability of an m^A motif to be an actual methylation site under the assumption of equal prior probability, i.e. the prior probability of an m^A motif being an m^6A site is 0.5. However, it is known in practice that the number of m^6A sites is a lot smaller than the number of m^A motifs, so the number of RNA-methylation sites under a specific experimental condition is likely to be significantly over-estimated. To address this bias, a posterior probability of RNA methylation under a specific condition is calculated with: \( q_{M,i} = \frac{\pi_{M,i}}{\pi_{M,i} p_{M,i} + (1 - \pi_{M,i}) (1 - p_{M,i})} \), where, \( \pi_M \) is the prior probability that a transcriptome RRACH motif embraces a true m^6A site under a specific model, which is calculated empirically from the 6 base-resolution datasets (see Table 1) as the average number of m^6A sites under a condition divided by the number of occurrences of transcriptome RRACH motifs that are supported by at least one m^6A record in MeTDB for the mature mRNA model, or RMBase for the full transcript model. This is also the search space of our predicted m^6A epitranscriptome. \( p_{M,i} \) is the predicted probability (or likelihood) of the i-th site being a real m^6A site under a specific model \( M \), and \((1 - p_{M,i}) \) is the probability of the opposite being true. \( q_{M,i} \) is a posteriori probability of the i-th site being a real m^6A site under a specific condition. The posterior probability \( q_{M,i} \) is also reported in the WHISTLE database along with the probability \( p_{M,i} \).

RESULTS AND DISCUSSION

m^6A site prediction

The predictors on the full-transcript data were established first in which the true m^6A site and negative sites may be located in both exonic and intronic regions. Because experimental procedures, especially the polyA selection step, may induce bias toward mRNA, we also consider a mature mRNA model, under which, the goal is to predict only exonic m^6A sites, and thus only the exonic regions are considered.

We show in Supplementary Table S3 that, although the genome-derived features alone are already very effective for predicting m^6A sites, the best performance is achieved when the sequence features and genomic features are combined. Consequently, our m^6A site predictor was established based on both the genome-derived features and sequence-derived features.

Feature selection was performed to identify the most effective genomic features for m^6A site prediction. Here, datasets 1–5 were used as the training data, while dataset 6 was used as the independent testing data. The relative importance of each genome-derived feature in the prediction was firstly assessed with the Perturb method (54) using the R caret package. Next, the N most important features were retained in the prediction analysis, and the prediction performance was evaluated using a 5-fold cross-validation. As shown in Supplementary Figure S1A, the predictor performance under the full transcript model stops increasing after including the top 14 most important genomic features. The top three most critical genomic features under this model are long exon, miRNA target and conservation score. To achieve the most robust performance and to avoid potential overfitting, only the top 14 genomic features were used in the full transcript model for m^6A site prediction purpose in later analysis. Similarly, the top 19 genome-derived features with the highest importance were selected for the mature mRNA model (see Supplementary Figure S1B). The distance to known m^6A sites became the most important predictive feature, which demonstrated the clustering effect of m^6A modification, followed by long exon and conservation under the mature mRNA model.

The performance of the proposed m^6A predictors was then evaluated using independent datasets and compared

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Table 1. Base-resolution dataset used in m^6A site prediction

| ID | Cell | Note | Technique | Source |
|----|------|------|-----------|--------|
| 1  | HEK293 | abcam antibody | mi-CLIP | (19) |
| 2  | HEK293 | sysy antibody | | (19) |
| 3  | MOLM13 | | | (42) |
| 4  | A549 | | m^6A-CLIP | (20) |
| 5  | CD8T | | | (20) |
| 6  | HeLa | | | (43) |
Table 2. Performance evaluation of m6A site prediction methods

| Model          | Method    | A549   | CD8T   | Hela   | HEK293 (sysy) | HEK293 (abacm) | MOLM13 | Average AUC |
|----------------|-----------|--------|--------|--------|---------------|---------------|--------|-------------|
| Full Transcript| WHISTLE   | 0.965  | 0.930  | 0.953  | 0.936         | 0.968         | 0.933  | 0.948       |
|                | MethyRNA* | 0.807  | 0.800  | 0.741  | 0.848         | 0.778         | 0.765  | 0.790       |
|                | SRAMP     | 0.856  | 0.841  | 0.762  | 0.883         | 0.838         | 0.759  | 0.761       |
| Mature mRNA    | WHISTLE   | 0.903  | 0.904  | 0.894  | 0.936         | 0.818         | 0.823  | 0.880       |
|                | MethyRNA  | 0.751  | 0.734  | 0.676  | 0.848         | 0.698         | 0.686  | 0.732       |
|                | SRAMP     | 0.814  | 0.796  | 0.702  | 0.869         | 0.796         | 0.710  | 0.706       |

Note: *The MethyRNA approach uses sequence-derived features with SVM (24), which we reproduced faithfully with the same training data of WHISTLE for comparison.
#The SRAMP method was originally trained on A549, CD8T, HEK293 (sysy) and HEK293 (abacm). To avoid overfitting, only Hela and MOLM13 were considered when evaluating its average performance.

With competing approaches (Table 2). By combining additional genome-derived features, the performance of our approach was substantially higher in all the tested conditions than MethyRNA and SRAMP, which rely only on information extracted from sequences. WHISTLE achieved AUCs of 0.948 and 0.880 under the full transcript and mature mRNA modes, respectively, representing a major improvement compared to MethyRNA (0.790 and 0.732) and SRAMP (0.761 and 0.706).

A predicted map of human m6A epitranscriptome

With the extensive study of RNA epigenetics, especially the accumulation of large number of m6A-seq datasets, the transcriptome-wide distribution of m6A sites has been summarized and made available from bioinformatics databases, such as MeT-DB (55) and RMBase (56). MeT-DB is the first transcriptome m6A database that provides condition-specific distribution of m6A RNA methylation in human and mouse initially, and later in other species as well; while RMBase is a more comprehensive RNA modification database, supporting more species and more RNA modification types. However, as these two databases overwhelmingly rely on m6A-seq data, and implemented a data processing pipeline that could not differentiate between true and randomly-occurring m6A motif located in close proximity within an m6A peak, the information they provide may not be accurate and should be re-assessed.

The reliability of a specific m6A site in epitranscriptome databases has been measured by the number of experiments that support the record. This metric will be used when evaluating the accuracy of the two databases. Interestingly, as shown in Table 3, when comparing the two epitranscriptome databases, the exomePeak-based MeT-DB database is slightly more accurate than primarily the MACS-based RMBase database. However, even with hundreds of high-throughput sequencing datasets accumulated, existing epitranscriptome databases are still far less accurate than what we may achieve with machine learning approaches (see Table 2).

We thus performed a whole transcriptome prediction of m6A RNA-methylation sites in human to generate a map of human m6A epitranscriptome using our proposed WHISTLE approach. Our predicted map is of substantially higher accuracy (average AUC of 0.948 and 0.880) compared with existing epitranscriptome databases MeT-DB (average AUC of 0.798 and 0.744) and RMBase (average AUC of 0.786 and 0.736) when evaluated on independent base-resolution datasets under both full transcript and mature mRNA mode, respectively. Additionally, we calculated a posterior probability of RNA-methylation site under a specific experimental condition. This provided a more empirical evaluation of the methylation status by taking into consideration the prior probability of an m6A motif being an m6A site, which is estimated from the base-resolution databases.

Besides CLIP-based approaches, we also tested the accuracy of the proposed method on a high resolution m6A-seq dataset (57). Although still antibody-based, this m6A-seq dataset was generated from an improved protocol and achieved near base resolution (58). As shown in Table 4, when antibody-based m6A-seq technique is used as the ground truth, WHISTLE still substantially outperformed competing approaches under both the full transcript and mature mRNA models.

Website interface

An online database has been built to host the predicted human m6A epitranscriptome. The individual RNA-methylation sites were then functionally annotated with gene expression data, RNA methylation data and protein–protein interaction data according to the ‘guilt-by-association’ principle (detailed in the Supplementary File S2). As is shown in Figure 2, the website supports queries that may be a methylation site, a gene or a specific biological function under the Gene Ontology framework (59). It also supports the download of the original base-resolution datasets (Table 1) used for site prediction and the entire predicted epitranscriptome map with the functional annotations for large-scale analysis.

CONCLUSIONS

Along with recent advances in RNA epigenetics, especially, the development of new techniques for profiling the RNA methylome (60,61), computationally deciphering the epitranscriptome from various omic data presents a major...
challenge to the bioinformatics community. In the past few years, sequence-derived features have been widely used for the prediction of RNA modification sites in human (24), mouse (24), other mammals (23,25), yeast (30,62) and other species; and a few major bioinformatics databases, including MeT-DB (18), RMBase (17), m6AVar (63), MOD-Omics (64) and RNAMDB (65) have been built. These databases address various aspects of the RNA modifications including transcriptome-wide distribution, mechanism pathway, relevance to mRNA and RNA-binding proteins, functional variants, etc., and have greatly benefited researchers in this field.

Here, we constructed a functionally annotated high-accuracy predicted map of the human m^6^A epitranscriptome and named it WHISTLE. The most stringent validation strategy was implemented, in which the performance of WHISTLE was assessed on six independent datasets (Tables 2 and 3) and on dataset generated from a different technique (Table 4). By integrating 35 genome-derived features with the conventional sequence-derived features, WHISTLE achieved a substantial improvement in accuracy, under both the full transcript model and the mature mRNA

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**Table 3. Performance evaluation for bioinformatics databases**

| Mode               | Method | A549 | CD8T | Hela | HEK293 (sysy) | HEK293 (abacm) | MOLM13 | Average AUC |
|--------------------|--------|------|------|------|---------------|---------------|--------|-------------|
| Full Transcript    | RMBase | 0.825 | 0.788 | 0.832 | 0.837         | 0.701         | 0.733  | 0.786       |
|                   | MetDB  | 0.835 | 0.802 | 0.843 | 0.848         | 0.719         | 0.744  | 0.798       |
| Mature mRNA       | RMBase | 0.768 | 0.716 | 0.790 | 0.752         | 0.707         | 0.682  | 0.736       |
|                   | MetDB  | 0.775 | 0.730 | 0.795 | 0.762         | 0.716         | 0.683  | 0.744       |

**Note:** To ensure the results are comparable to Table 2, only the unique sites not previously reported in the training data of predictors were considered. Please see Supplementary Table S4 for the results when all sites from the independent testing samples were considered.

**Table 4. Performance assessment using high resolution m^6^A-seq data**

| Mode                  | AUC under full transcript model | AUC under mature mRNA model |
|-----------------------|---------------------------------|-----------------------------|
| WHISTLE               | 0.980                           | 0.904                       |
| MethylRNA             | 0.904                           | 0.826                       |
| SRAMP                 | 0.825                           | 0.783                       |
| RMBase                | 0.774                           | 0.758                       |
| MeTDB                 | 0.775                           | 0.767                       |

**Note:** The high confidence consensus m^6^A sites detected in more than two of the total six high resolution m^6^A-seq experiments (57) were considered. Similar as before, MethylRNA and WHISTLE used the same m^6^A datasets for training. Only the unique sites not previously reported in the training data were considered here. Please see Supplementary Table S5 for the results when all sites from the independent testing samples were considered.
model, compared with existing machine learning-based m6A predictors and the latest epitranscriptome databases. It is worth noting that, the prediction performance achieved on the full transcript model (AUC: 0.948) may be significantly over-estimated due to the library preparation (polyA selection) of the miCLIP and m6A-CLIP samples used, because they cannot effectively capture the intrinsic m6A sites. The performance achieved on the mature mRNA model (AUC: 0.880) is probably a more realistic estimate.

A web server WHISTLE was built to enable the direct query of predicted RNA-methylation sites, their putative functions and their potential association to other methylation sites or genes, which provides the requisite data for the further epitranscriptome studies in human.

Our work has provided a computational scheme to study the m6A epitranscriptome based on multi-omics datasets using machine learning and network-based method. In the future, it can be easily expanded to the study of other RNA modifications, such as m1A (66) and Pseudouridine (67), as well as in other species, such as mouse and yeast.

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

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REFERENCES

1. Roundtree, I.A., Evans, M.E., Pan, T. and He, C. (2017) Dynamic RNA modifications in gene expression regulation. Cell, 169, 1187–1200.

2. Boccaletto, P., Machnicka, M.A., Purta, E., Piatkowski, P., Baginski, B., Wiricki, T.K., de Crecy-Lagard, V., Ross, R., Limbach, P.A., Kotter, A. et al. (2018) MODOMICS, a database of RNA modification pathways 2017 update. Nucleic Acids Res., 46, D303–D307.

3. Meyer, K.D. and Jaffrey, S.R. (2017) Rethinking m6A readers, writers, and erasers. Annu. Rev. Cell Dev. Biol., 33, 319–342.

4. Niu, Y., Zhao, X., Wu, Y.S., Li, M.M., Wang, X.J. and Yang, Y.G. (2013) N6-methyl-adenosine (m6A) in RNA: an old modification with a novel epigenetic function. Genomics Proteomics Bioinformatics, 11, 8–17.

5. Desnories, R., Friderici, K. and Rottman, F. (1974) Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. Proc. Natl. Acad. Sci. U.S.A., 71, 3971–3975.

6. Fustin, J.M., Doi, M., Yamaguchi, Y., Hida, H., Nishimura, S., Yoshida, M., Isagawa, T., Morioka, M.S., Kakeya, H., Manabe, I. et al. (2013) RNA-methylation-dependent RNA processing controls the speed of the circadian clock. Cell, 155, 793–806.

7. Meyer, K.D. and Jaffrey, S.R. (2014) The dynamic epitranscriptome: N6-methyladenosine and gene expression control. Nat. Rev. Mol. Cell Biol., 15, 313–326.

8. Zhou, J., Wan, J., Gao, X., Zhang, X., Jaffrey, S.R. and Qian, S.B. (2015) Dynamic m6A mRNA methylation directs translational control of heat shock response. Nature, 526, 591–594.

9. Alarcon, C.R., Lee, H., Goodarzi, H., Halberg, N. and Tavazoie, S.F. (2015) N6-methyladenosine marks primary microRNAs for processing. Nature, 519, 482–485.

10. Xiang, Y., Laurent, B., Hsu, C.H., Nachtergaele, S., Lu, Z., Sheng, W., Xu, C., Chen, H., Ouyang, J., Wang, S. et al. (2017) RNA m6A methylation regulates the ultra-violet-induced DNA damage response. Nature, 543, 573–576.

11. Liu, N., Dai, Q., Zheng, G., He, C., Parisien, M. and Pan, T. (2015) N(6)-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions. Nature, 518, 560–564.

12. Wang, X., Lu, Z., Gomez, A., Hon, G.C., Yue, Y., Han, D., Fu, Y., Parisien, M., Dai, Q., Jia, G. et al. (2014) N6-methyladenosine-dependent regulation of messenger RNA stability. Nature, 505, 117–120.

13. Dominissini, D., Mosshich-Moshkovitz, S., Schwartz, S., Salmon-Divon, M., Ungar, L., Osenberg, S., Cesarkas, K., Jacob-Hirsch, J., Amariglio, N., Kupiec, M. et al. (2012) Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature, 485, 201–206.

14. Meyer, K.D., Saletore, Y., Zumo, P., Elemento, O., Mason, C.E. and Jaffrey, S.R. (2012) Comprehensive analysis of mRNA methylation reveals enrichment in 3′ UTRs and near stop codons. Cell, 149, 1635–1646.

15. Meng, J., Lu, Z., Liu, H., Zhang, L., Zhang, S., Chen, Y., Yao, M.K. and Huang, Y. (2014) A protocol for RNA methylation differential analysis with MeRIP-Seq data and exomePeak R/Bioconductor package. Methods, 69, 274–281.

16. Dominissini, D., Mosshich-Moshkovitz, S., Salmon-Divon, M., Amariglio, N. and Rechavi, G. (2013) Transcriptome-wide mapping of N(6)-methyladenosine by m6A-seq based on immunocapturing and massively parallel sequencing. Nat. Protoc., 8, 176–189.

17. Xuan, J.-J., Sun, W.-J., Lin, P.-H., Zhou, K.-R., Liu, S., Zheng, L.-L., Qu, L.-H. and Yang, J.-H. (2018) RMBase v2.0: deciphering the map of RNA modifications from epitranscriptome sequencing data. Nucleic Acids Res., 46, D327–D334.

18. Liu, H., Wang, H., Wei, Z., Zhang, S., Hua, G., Zhang, S.-W., Zhang, L., Gao, S.-J., Meng, J., Chen, X. et al. (2018) MeT-DB V2.0: elucidating context-specific functions of N6-methyl-adenosine methyltransferases. Nucleic Acids Res., 46, D281–D287.

19. Linder, B., Grozich, A.V., Olarerin-George, A.O., Meydan, C., Mason, C.E. and Jaffrey, S.R. (2015) Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. Nature Methods, 12, 767–772.

20. Ke, S., Alemu, E.A., Mertens, C., Gantman, E.C., Fak, J.J., Mele, A., Haripal, B., Zucker-Scharff, I., Moore, M.J., Park, C.Y. et al. (2018) A majority of m6A residues are in the last exons, allowing the potential for 3′ UTR regulation. Genes Dev., 29, 2037–2053.

21. Chen, W., Feng, P., Ding, H., Lin, H. and Chou, K.-C. (2015) iRNA-Methyl: identifying N 6-methyladenosine sites using pseudo nucleotide composition. Anal. Biochem., 490, 26–33.

22. Liu, Z., Xiao, X., Yu, D.-J., Jia, J., Qu, W.-R. and Chou, K.-C. (2016) pRNAm-PC: predicting N 6-methyladenosine sites in RNA sequences via physical-chemical properties. Anal. Biochem., 497, 60–67.

23. Zhou, Y., Zeng, P., Li, Y.H., Zhang, Z. and Cui, Q. (2016) SRAMP: prediction of mammalian N6-methyladenosine (m6A) sites based on sequence-derived features. Nucleic Acids Res., 44, e91.

24. Chen, W., Tang, H. and Lin, H. (2017) MethyRNA: a web server for identification of N(6)-methyladenosine sites. J. Biomol. Struct. Dyn., 35, 683–687.

25. Xiang, S., Liu, K., Yan, Z., Zhang, Y. and Sun, Z. (2016) RNAMethPre: a web server for the prediction and query of mRNA m6A sites. PLoS One, 11, e0162707.
26. Xing,P., Su,R., Guo,F. and Wei,L. (2017) Identifying N(6)-methyladenosine sites using multi-interval nucleotide pair position specificity and support vector machine. *Sci. Rep.*, 7, 46757.

27. Li,G.Q., Liu,Z., Shen,H.B. and Yu,D.J. (2016) TargetM6A: identifying N6-methyladenosine sites from RNA sequences via position-specific nucleotide propensities and a support vector machine. *IEEE Trans. Nanobiosci.*, 15, 674–682.

28. Xiang,S., Yan,Z., Liu,K., Zhang,Y. and Sun,Z. (2016) AthMethPre: a web server for the prediction and query of mRNA m6A sites in Arabidopsis thaliana. *Mol. Biol. Sys.*, 12, 3333–3337.

29. Feng,P., Ding,H., Yang,H., Chen,W., Lin,H. and Chou,K.-C. (2016) iRNA-PseColl: identifying the occurrence sites of different RNA modifications by incorporating collective effects of nucleotides into PseKNC. *Mol. Ther. Nucleic Acids*, 7, 155–163.

30. Wei,L., Chen,H. and Su,R. (2018) M6APred-EL: a sequence-based predictor for identifying N6-methyladenosine sites using ensemble learning. *Mol. Ther. Nucleic Acids*, 12, 635–644.

31. Akbar,S. and Hayat,M. (2018) Methyl-STNC: identification of N6-methyladenosine sites by extending the idea of SAAC into Chou's PseAAC to formulate RNA sequences. *J. Theor. Biol.*, 455, 205–211.

32. Chen,W., Ding,H., Zhou,X., Lin,H. and Chou,K.-C. (2018) iRNA(m6A)-PseDNC: identifying N6-methyladenosine sites using pseudo dinucleotide composition. *Anal. Biochem.*, 561-562, 59–65.

33. Kuksa,P., Leung,Y., Vandiver,L.E., Anderson,Z., Gregory,B.D. and Wang,L.-S. (2017) In Silico Identification of RNA Modifications from High-Throughput Sequencing Data Using HAMR. In: Lusser,A (ed). *RNA Methylation: Methods and Protocols*. Springer, NY, Vol 1562, pp. 211–229.

34. Chen,W., Xing,P. and Zou,Q. (2017) Detecting N6-methyladenosine sites from RNA transcriptions using ensemble support vector machines. *Sci. Rep.*, 7, 40242.

35. Feng,P., Ding,H., Chen,W. and Lin,H. (2016) Identifying RNA 5-methylcytosine sites via pseudo nucleotide compositions. *Mol. Biol. Sys.*, 12, 3307–3311.

36. Chen,W., Feng,P., Tang,H., Ding,H. and Lin,H. (2016) Identifying 2’-O-methylationation by integrating nucleotide chemical properties and nucleotide compositions. *Genomics*, 107, 255–258.

37. Chen,W., Feng,P., Ding,H. and Lin,H. (2016) Identifying N6-methyladenosine sites in the Arabidopsis thaliana transcriptome. *Mol. Genet. Genomics*, 291, 2225–2229.

38. Zhao,Z., Peng,H., Lan,C., Zheng,Y., Fang,L. and Li,J. (2018) Imbalance learning for the prediction of N(6)-Methylation sites in mRNAs. *BMC Genomics*, 19, 574.

39. Yang,H., Lv,H., Ding,H., Chen,W. and Lin,H. (2018) iRNA-2OM: a sequence-based predictor for identifying 2’-O-Methylation sites by integrating nucleotide chemical properties and nucleotide compositions. *Genomics*, 107, 255–258.

40. Liu,H., Wang,H., Wei,Z., Zhang,S., Hua,G., Zhang,S.W., Zhang,L., Gao,S.J., Meng,J., Chen,X. et al. (2018) MetDB V2.0: elucidating context-specific functions of N6-methyl-adenosine methyltransferase. *Nucleic Acids Res.*, 46, D281–D287.

41. Xuan,J.J., Sun,W.J., Lin,P.H., Zhou,K.R., Liu,S., Zheng,L., Qu,L.H. and Yang,J.H. (2018) RMBase v2.0: deciphering the map of RNA modifications from epitranscriptome sequencing data. *Nucleic Acids Res.*, 46, D327–D334.

42. Schwartz,S., Mumbach,M.R., Jovanovic,M., Wang,T., Maciag,K., Bushkin,G.G., Mertins,P., Ter-Ovanesyan,D., Habib,N., Cacciacerre,D. et al. (2014) Perturbation of m6A writers reveals two distinct classes of mRNA methylation at internal and 5’ sites. *Cell Rep.*, 8, 284–296.

43. Schwartz,S., Agarwala,Sudeep D., Mumbach,Maxwell R., Jovanovic,M., Mertins,P., Shishkin,T., Tabach,Y., Mikkelsten,Tarjei S., Satija,R., Ruvkun,G. et al. (2013) High-resolution mapping reveals a conserved, widespread, dynamic mRNA methylation program in yeast meiosis. *Cell*, 155, 1409–1421.

44. Consortium, G.O. (2016) Expansion of the gene ontology knowledgebase and resources. *Nucleic Acids Res.*, 45, D331–D338.

45. Li,X., Xiong,X. and Yi,C. (2017) Epitranscriptome sequencing technologies: decoding RNA modifications. *Nat. Methods*, 14, 23–31.

46. 2017 Method of the year 2016: epitranscriptome analysis. *Nat Methods*, 14, 1.

47. Chen,W., Tran,H., Liang,Z., Lin,H. and Zhang,L. (2015) Identification and analysis of the N(6)-methyladenosine in the Saccharomyces cerevisiae transcriptome. *Sci. Rep.*, 5, 13859.

48. Zheng,Y., Nie,P., Peng,D., He,Z., Liu,M., Xie,Y., Miao,Y., Zuo,Z. and Ren,J. (2017) m6AVar: a database of functional variants involved in m6A modification. *Nucleic Acids Res.*, 46, D139–D145.

49. Boccaletto,P., Machnicka,M.A., Purta,E., Piątkowski,P., Bągiński, B., Wirczek,T.K., de Crécy-Lagard,V., Ross,R., Limbach,P.A., Kotter,A. et al. (2017) MODOMICS: a database of RNA modification pathways. 2017 update. *Nucleic Acids Res.*, 46, D303–D307.

50. Cantara,WA., Crain,P.F., Rozenksi,J., McCloskey,J.A., Harris,K.A., Zhang,X., Vendeix,F.A., Fabris,D. and Agris,P.F. (2011) The RNA modification database, RNADB: 2011 update. *Nucleic Acids Res.*, 39, D195–D201.

51. Dominissini,D., Nachtigaele,S., Moshitch-Moshkovitz,S., Peer,E., Kol,N., Ben-Haim,M.S., Dai,Q., Segn,D., Salmon-Divon,A., M., Clark,W.C. et al. (2016) The dynamic N(1)-methyladenosine methylome in eukaryotic messenger RNA. *Nature*, 530, 441–446.

52. Cabili,M.N., Trapnell,C., Goff,L., Koziol,M., Tazon-Vega,B., Regue,A. and Rinn,J.L. (2011) Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Genes Dev.*, 25, 1915–1927.