**iLearnPlus**: a comprehensive and automated machine-learning platform for nucleic acid and protein sequence analysis, prediction and visualization

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**ABSTRACT**

Sequence-based analysis and prediction are fundamental bioinformatic tasks that facilitate understanding of the sequence-structure-function paradigm for DNAs, RNAs and proteins. Rapid accumulation of sequences requires equally pervasive development of new predictive models, which depends on the availability of effective tools that support these efforts. We introduce **iLearnPlus**, the first machine-learning platform with graphical- and web-based interfaces for the construction of machine-learning pipelines for analysis and predictions using nucleic acid and protein sequences. **iLearnPlus** provides a comprehensive set of algorithms and automates sequence-based feature extraction and analysis, construction and deployment of models, assessment of predictive performance, statistical analysis, and data visualization; all without programming. **iLearnPlus** includes a wide range of feature sets which encode information from the input sequences and over twenty machine-learning algorithms that cover several deep-learning approaches, outnumbering the current solutions by a wide margin. Our solution caters to experienced bioinformaticians, given the broad range of options, and biologists with no programming background, given the point-and-click interface and easy-to-follow design process. We showcase **iLearnPlus** with two case studies concerning prediction of long noncoding RNAs (lncRNAs) from RNA transcripts and prediction of crotonylation sites in protein chains. **iLearnPlus** is an open-source platform available at https://github.com/Superzchen/iLearnPlus/ with the web-server at http://ilearnplus.erc.monash.edu/.

**INTRODUCTION**

High-throughput sequencing has significantly advanced and experienced widespread use over the past few decades, generating the unprecedented volume of the DNAs, RNAs and protein sequence data. With the fast accumulation of these data, effectively analyzing, mining and visualizing biological sequences have become a non-trivial task (1). Among a variety of computational solutions, machine-learning methods are a popular and efficient solution for the accurate function prediction/analysis for biological se-
quences (2–7). Many sequence-based machine-learning approaches have been proposed, contributing to a better understanding of the functions and structures of DNAs, RNAs and proteins (8–10), particularly in the context of human disease (11–13). Despite the diversity of machine-learning frameworks for the sequence analysis and prediction, in general, they follow the same set of five major steps after the sequence data was collected: feature extraction, feature analysis, classifier construction, performance evaluation, and data/result visualization, as demonstrated in Figure 1.

Bioinformatics-driven data analysis is an essential part of biological studies. The sequence-based analysis and predictions often require complex processing steps, data science expertise, and access to sophisticated software. These requirements have become a significant hurdle, especially for biologists with limited bioinformatics expertise. Several web servers and standalone software packages for the sequence-based analysis and prediction have been recently developed to meet these needs. Representative tools include iFeature (14), iLearn (15), Selene (16), Kipoi (17), Janggu (18), BioSeq-Analysis (19) and BioSeq-Analysis2.0 (20). Selene is a Python-based deep-learning library for rapid development, training, and application of deep-learning models from biological sequences. Janggu is a Python package that similarly focuses on the deep learning models. Kipoi is a collaborative initiative that defines standards and fosters reuse of trained models. However, these three tools cover only a portion of the complete pipeline outlined in Figure 1. We provide a detailed side-by-side comparison in Supplementary Table S1. BioSeq-Analysis is regarded as the first automated platform for machine-learning-based bioinformatics analysis and predictions at the sequence level (19). Its subsequent version, BioSeq-Analysis2.0 covers residue-level analysis, further improving the scope of this platform (20). In 2018, we released the first computational pipeline, iFeature, that generates features for both protein and peptide sequences. Later, we extended iFeature to design and implement iLearn, which is an integrated platform and meta-learner for feature engineering, machine-learning analysis and modelling of DNA, RNA and protein sequence data. Both platforms, iFeature and iLearn, have been applied in many areas of bioinformatics and computational biology including but not limited to the prediction and identification of mutational effects (21), protein-protein interaction hotspots (22), drug-target interactions (23), protein crystallization propensity (24), DNA-binding sites (25) and DNA-binding proteins (26), protein families (27,28), and DNA, RNA and protein modifications (29–32). The breadth and number of these applications show a substantial need for such solutions. However, further work is needed. First, new platforms need to overcome limitations of the current solutions in terms of streamlined and easiness of use, so that they make a sophisticated machine-learning based analysis of biological sequence accessible to both experienced bioinformaticians and biologists with limited programming background. This means that the development of the complex predictive and analytical pipelines should be streamlined by providing one platform that handles and offers support for the entire computational process. Second, the current platforms offer limited facilities for feature extraction, feature analysis and classifier construction. This calls for new approaches that provide a more comprehensive and molecule-specific (DNA versus RNA versus protein) set of feature descriptors, a broader range of tools for feature analysis, and which should ideally cover state-of-the-art machine-learning algorithms including deep learning.

To this end, we release a comprehensive and automated sequence analysis and prediction platform, iLearnPlus, implemented in Python/PyQt5. iLearnPlus works across all major operating systems (i.e. Windows, macOS and Linux). Our platform includes four modules: iLearnPlus-Basic, iLearnPlus-Estimator, iLearnPlus-AutoML and iLearnPlus-LoadModel. These modules support a wide range of functionality, such as feature extraction, feature analysis, construction of machine-learning framework, training of machine-learning models/classifiers, assessment of predictive performance for these models, statistical analysis, and data/result visualization. iLearnPlus is geared to be used by both experienced bioinformaticians and biologists with limited bioinformatics expertise. When compared to the currently available tools (Supplementary Table S2), iLearnPlus offers the following key advantages:

(i) To the best of our knowledge, iLearnPlus is the first GUI-based platform that facilitates machine learning-based analysis and prediction of biological sequences;

(ii) iLearnPlus outperforms the existing platforms in the number of the available machine-learning algorithms and the coverage of features produced from the input sequences: 21 machine-learning algorithms (12 conventional machine-learning methods, two ensemble-learning frameworks and seven deep-learning approaches) and 19 classes of features that cover 147 feature sets;

(iii) iLearnPlus provides a variety of ways to visualize the user-defined data and prediction performance including scatter plots, ROC (Receiver Operating Characteristic) curves, PRC (Precision-Recall Curves), histograms, kernel density plots, heatmaps and boxplots;

(iv) iLearnPlus supports two popular statistical tests: the Student’s t-test and bootstrap test (33), to assess the statistical significance of differences and improvements in the context of the model performance;

(v) iLearnPlus provides the iLearnPlus-AutoML module for evaluating the prediction performance of different machine-learning models and selecting the best-performing model via automatic parameter optimization, to support less data science-savvy users in maximizing the predictive capability of machine-learning pipelines;

(vi) iLearnPlus facilitates the deployment of the developed models with the iLearnPlus-LoadModel module. This module applies the already trained machine-learning models on new data;

(vii) iLearnPlus provides more options for model integration by exploring possible combinations of the prediction outcomes of separate models as the input, and retrain another machine-learning model (excluding the
Figure 1. A summary of the five major steps involved in the development of machine learning-based models for biological sequences analysis. These steps include feature extraction, feature analysis, classifier construction, performance evaluation, and data/result visualization.

deep-learning approaches), to assess if the prediction performance can be further improved; and 
(viii) **iLearnPlus** provides auxiliary functionalities for data preprocessing, such as file format transformations and combination of multiple feature encodings into one file.

**iLearnPlus** offers user-friendly interface and integrates four functional modules that streamline the entire computational process related to analysis and sequence-based prediction of the DNA, RNA and protein sequences. This 'one-stop' solution facilitates generation of biological hypotheses by supporting the design, testing and deployment of accurate predictive models. Following, we describe the features and capabilities of our platform for each of the five major steps defined above. We also demonstrate its application with two case studies that concern the development and testing of novel machine-learning models for the predictions of long noncoding RNAs (lncRNAs) and crotonylation sites in protein chains.

**MATERIALS AND METHODS**

**Feature extraction**

The feature extraction functionality in the **iLearnPlus**-Basic module generates numeric vectors from biological sequences. These vectors encode biochemical, biophysical, and compositional properties of the input sequences in the format that is compatible with the subsequent machine-learning tasks. **iLearnPlus** incorporates 19 major classes of features for protein, RNA and DNA sequences (Tables 1 and 2). To compare, **iLearnPlus** outnumbers the current platforms, including iLearn (15), iFeature (14) and BioSeq-Analysis2.0 (20) by 50, 94 and 31 feature sets. Supplementary Table S2 provides a detailed side-by-side comparison of the feature set numbers that these platforms offer for the DNA, RNA and protein sequences. The input sequences of **iLearnPlus** are required to be in the FASTA format. We designed an extended version of the header line (standard FASTA format is accepted; refer to the online instructions for more detail) which is processed by the graphical input data explorer. The biological sequence type (i.e. DNA, RNA or protein) is detected automatically based on the input sequences. The lists of the feature sets are provided in Table 1 (for protein sequences) and Table 2 (for DNA and RNA sequences), and can be customized by users. The selected subset of feature sets is output with a convenient table widget, which includes the molecule name, molecule label, feature (column) names and the corresponding values. **iLearnPlus** supports four formats for saving the calculated features, including LIBSVM format, Comma-Separated Values (CSV), Tab Separated Values (TSV), and Waikato Environment for Knowledge Analysis (WEKA) format. This variety of popular formats facilitates direct use of the features in third-party computational tools, such as scikit-learn (34), WEKA (35) and its web interface.

Besides the data tables, **iLearnPlus** provides advanced facilities to visualize the data. For instance, it generates hybrid plots that overlay kernel density curves and histograms (Figure 2) that can be used to shed light on the statistical distributions of the extracted features. The histogram provides a visual representation of feature values grouped into discrete intervals, while the kernel density approach produces a smooth curve that represents the probability density function for continuous variables (36) (Figure 2A). The visualization can be conducted for a specific dataset as well as a selected subset of features in that dataset.
Table 1. Feature descriptors calculated by iLearnPlus for protein sequences

| Descriptor group                     | Descriptor (abbreviation) | Reference |
|--------------------------------------|---------------------------|-----------|
| Amino acid composition               | Amino acid composition (AAC) | (37)      |
|                                     | Enhanced amino acid composition (EAAC) | (14,15) |
|                                     | Composition of \( k \)-spaced amino acid pairs (CKSAAP) | (38,39) |
|                                     | Kmer (dipeptides and tripeptides) composition (DPC and TPC) | (37,40) |
|                                     | Dipeptide deviation from expected mean (DDE) | (40) |
|                                     | Composition (CTDC) | (41–45) |
|                                     | Transition (CTDT) | (41–45) |
|                                     | Distribution (CTDD) | (41–45) |
|                                     | Conjoint triad (CTriad) | (46) |
|                                     | Conjoint \( k \)-spaced Triad (KSCTriad) | (14,15) |
|                                     | Adaptive skip dipeptide composition (ASDC) | (47) |
|                                     | PseAAC of distance-pairs and reduced alphabet (DistancePair) | (20,48) |
| Grouped amino acid composition      | Grouped amino acid composition (GAAC) | (14,15) |
|                                     | Grouped enhanced amino acid composition (GEAAC) | (14,15) |
|                                     | Composition of \( k \)-spaced amino acid group pairs (CKSAAGP) | (14,15) |
|                                     | Grouped dipeptide composition (GDPC) | (14,15) |
|                                     | Grouped tripeptide composition (GTPC) | (14,15) |
| Autocorrelation                     | Moran (Moran) | (49,50) |
|                                     | Geary (Geary) | (51) |
|                                     | Normalized Moreau-Broto (NMBroto) | (52) |
|                                     | Auto covariance (AC) | (53–55) |
|                                     | Cross covariance (CC) | (53–55) |
|                                     | Auto-cross covariance (ACC) | (53–55) |
| Quasi-sequence-order                | Sequence-order-coupling number (SOCNumber) | (56–58) |
|                                     | Quasi-sequence-order descriptors (QSOrder) | (56–58) |
| Pseudo-amino acid composition       | Pseudo-amino acid composition (PAAC) | (59,60) |
|                                     | Amphiphilic PAAC (APAAC) | (59,60) |
|                                     | Pseudo \( K \)-tuple reduced amino acids composition (PseKRAAC_type 1 to type 16) | (61) |
| Residue composition                 | Binary - 20bit (binary) | (62,63) |
|                                     | Binary - 6bit (binary_6bit) | (20,64) |
|                                     | Binary - 5bit (binary_5bit_type 1 and type 2) | (20,65) |
|                                     | Binary - 3bit (binary_3bit_type 1 to type 7) | (47) |
|                                     | Learn from alignments (AESNN3) | (20,66) |
|                                     | Overlapping property features - 10 bit (OPF_10bit) | (47) |
|                                     | Overlapping property features - 7 bit (OPF_7bit type 1 to type 3) | (47) |
| Physicochemical property            | AAIndex (AAIndex) | (67) |
| BLOSUM matrix                       | BLOSUM62 (BLOSUM62) | (68) |
| Z-Scale index                       | Z-Scale (Zscale) | (69) |
| Similarity-based descriptor         | \( K \)-nearest neighbor (KNN) | (70) |

**Feature analysis**

Feature analysis is an optional but highly-recommended step that helps to eliminate irrelevant, noisy, or redundant features from the original feature set, with the overarching goal to optimize the predictive performance of the subsequently used machine-learning algorithm(s) (32). iLearnPlus provides multiple options to facilitate feature analysis, including ten feature clustering, three dimensionality reduction, two feature normalization and five feature selection approaches (Table 3). Compared with iLearn, the currently most comprehensive platform in the context of feature analysis (Supplementary Table S1), iLearnPlus provides four additional clustering algorithms: the Mini Batch \( k \)-means Clustering (85,86), Markov Clustering (MCL) (87), Agglomerative Clustering (88), and Spectral Clustering (89). The feature analysis supports the same comprehensive list of the file formats as the feature extraction tools (i.e. LIB-SVM format, CSV, TSV and WEKA format).

The clustering groups similar objects (molecules) in a given dataset described by a specific set of features. Upon completion of the clustering process, molecules are grouped, and each group is assigned with a cluster ID. The cluster IDs are displayed in the table widget. The feature selection and dimensionality reduction approaches serve to reduce the number of features, while potentially boosting the prediction performance by eliminating irrelevant (to a given predictive task) and redundant (mutually correlated) features. Finally, feature normalization rescales the feature values to a specific range, so different features can be used together in the same dataset. We provide two widely used normalization algorithms: \( Z \)-score normalization and Min-Max normalization. In the \( Z \)-score normalization, features are rescaled to the normal distribution with the mean of 0 and the standard deviation of 1. In the MinMax normalization, features are scaled to the unit range between 0 and 1. In iLearnPlus, the results produced by the feature selection and normalization methods can be conveniently visualized using the hybrid plots, while a scatter plot can be used to display the outputs produced by the clustering and dimensionality reduction tools (Figure 2B).

**Classifier construction and integration**

Many objectives related to the analysis of the DNA/RNA/protein sequences can be formulated as a classification problem. Examples include the prediction of structures and functions of protein and nucleic acid
Table 2. Feature descriptors calculated by *iLearnPlus* for DNA and RNA sequences

| Descriptor group                                      | Descriptor (abbreviation)                                                                 | Sequence type | Reference |
|------------------------------------------------------|------------------------------------------------------------------------------------------|---------------|-----------|
| Nucleic acid composition                             | Nucleic acid composition (NAC)                                                           | DNA/RNA       | (15)      |
|                                                      | Enhanced nucleic acid composition (ENAC)                                                 | DNA/RNA       | (15)      |
|                                                      | *k*-spaced nucleic acid pairs (CKSNAP)                                                    | DNA/RNA       | (15)      |
|                                                      | Basic kmer (Kmer)                                                                         | DNA/RNA       | (71)      |
|                                                      | Reverse compliment kmer (RCKmer)                                                         | DNA/RNA       | (72,73)   |
|                                                      | Accumulated nucleotide frequency (ANF)                                                   | DNA/RNA       | (74)      |
|                                                      | Nucleotide chemical property (NCP)                                                       | DNA/RNA       | (74)      |
|                                                      | The occurrence of kmers, allowing at most m mismatches (Mismatch)                         | DNA/RNA       | (20)      |
|                                                      | The occurrence of kmers, allowing non-contiguous matches (Subsequence)                   | DNA/RNA       | (20)      |
|                                                      | Adaptive skip dinucleotide composition (ASDC)                                             | DNA/RNA       | (47)      |
|                                                      | Local position-specific dinucleotide frequency (LPDF)                                     | DNA/RNA       | (75)      |
|                                                      | The Z curve parameters for frequencies of phase-specific mononucleotides (Z\text{curve,9bit}) | DNA/RNA       | (76)      |
|                                                      | The Z curve parameters for frequencies of phase-independent dinucleotides (Z\text{curve,12bit}) | DNA/RNA       | (76)      |
|                                                      | The Z curve parameters for frequencies of phase-specific dinucleotides (Z\text{curve,36bit}) | DNA/RNA       | (76)      |
|                                                      | The Z curve parameters for frequencies of phase-independent trinucleotides (Z\text{curve,48bit}) | DNA/RNA       | (76)      |
|                                                      | The Z curve parameters for frequencies of phase-specific trinucleotides (Z\text{curve,144bit}) | DNA/RNA       | (76)      |
| Residue composition                                  | Binary (binary)                                                                           | DNA/RNA       | (62,63)   |
|                                                      | Dinucleotide binary encoding (DBE)                                                       | DNA/RNA       | (75)      |
|                                                      | Position-specific of two nucleotides (PS2)                                                | DNA/RNA       | (20,77)   |
|                                                      | Position-specific of three nucleotides (PS3)                                              | DNA/RNA       | (20,77)   |
|                                                      | Position-specific of four nucleotides (PS4)                                               | DNA/RNA       | (20,77)   |
| Position-specific tendencies of trinucleotides       | Position-specific trinucleotide propensity based on single-strand (PSTNPSSs)             | DNA/RNA       | (78,79)   |
|                                                      | Position-specific trinucleotide propensity based on double-strand (PSTNPDSs)              | DNA/RNA       | (78,79)   |
| Electron-ion interaction pseudopotentials            | Electron-ion interaction pseudopotentials value (EIIP)                                   | DNA/RNA       | (80,81)   |
|                                                      | Electron-ion interaction pseudopotentials of trinucleotide (PseEIIP)                      | DNA/RNA       | (80,81)   |
| Autocorrelation and cross-covariance                 | Dinucleotide-based auto covariance (DAC)                                                 | DNA/RNA       | (53–55)   |
|                                                      | Dinucleotide-based cross covariance (DCC)                                                 | DNA/RNA       | (53–55)   |
|                                                      | Dinucleotide-based auto-cross covariance (DACC)                                            | DNA/RNA       | (53–55)   |
|                                                      | Trinucleotide-based auto covariance (TAC)                                                 | DNA/RNA       | (53)      |
|                                                      | Trinucleotide-based cross covariance (TCC)                                                | DNA/RNA       | (53)      |
|                                                      | Trinucleotide-based auto-cross covariance (TACC)                                          | DNA/RNA       | (53)      |
|                                                      | Moran (Moran)                                                                             | DNA/RNA       | (49,50)   |
|                                                      | Geary (Geary)                                                                             | DNA/RNA       | (51)      |
|                                                      | Normalized Moreau-Broto (NMBroto)                                                       | DNA/RNA       | (52)      |
| Physicochemical property                             | Dinucleotide physicochemical properties (DPCP type 1 and type 2)                         | DNA/RNA       | (82)      |
|                                                      | Trinucleotide physicochemical properties (TPCP type 1 and type 2)                        | DNA/RNA       | (82)      |
| Mutual information                                   | Multivariate mutual information (MMI)                                                    | DNA/RNA       | (83)      |
| Similarity-based descriptor                          | K-nearest neighbor (KNN)                                                                  | DNA/RNA       | (83)      |
| Pseudo nucleic acid composition                      | Pseudo dinucleotide composition (PseDNC)                                                 | DNA/RNA       | (53,84)   |
|                                                      | Pseudo *k*-tupler composition (PseKNC)                                                    | DNA/RNA       | (53,84)   |
|                                                      | Parallel correlation pseudo dinucleotide composition (PCPseDNC)                          | DNA/RNA       | (53,84)   |
|                                                      | Parallel correlation pseudo trinucleotide composition (PCPseTNC)                         | DNA/RNA       | (53,84)   |
|                                                      | Series correlation pseudo dinucleotide composition (SCPseDNC)                           | DNA/RNA       | (53,84)   |
|                                                      | Series correlation pseudo trinucleotide composition (SCPseTNC)                         | DNA/RNA       | (53,84)   |
sequences (19,100,101). iLearnPlus supports both binary classification (two outcomes) and multi-class classification (multiple outcomes). It offers 12 conventional machine-learning algorithms, two ensemble-learning frameworks, and seven deep-learning approaches (Table 4). This broad selection of algorithms is more comprehensive than what the current platforms offer, i.e. 21 versus 5 in iLearn and BioSeq-Analysis2.0 (Supplementary Table S2). We use the implementation from four popular third-party machine-learning platforms, including scikit-learn (34), XGBoost (102), LightGBM (103) and PyTorch (104). Deep-learning approaches are implemented using the PyTorch library, while LightGBM and XGBoost algorithms are imple-

Table 3. The feature analysis approaches provided in iLearnPlus

| Method            | Algorithm (abbreviation) | Reference |
|-------------------|---------------------------|-----------|
| Clustering        | $k$-means (kmeans)        | (85,86)   |
|                   | Mini-Batch $K$-means      | (85,86)   |
|                   | (MiniBatchKMeans)         |           |
|                   | Gaussian mixture (GM)     | (85,86)   |
|                   | Agglomerative (Agglomerative) | (88)     |
|                   | Spectral (Spectral)       | (89)      |
|                   | Markov clustering (MCL)   | (87)      |
|                   | Hierarchical clustering (hcluster) | (85,90)|
|                   | Affinity propagation clustering (APC) | (91) |
|                   | Mean shift (meanshift)    | (92)      |
|                   | DBSCAN (dbscan)           | (93)      |
| Feature selection | Chi-square test (CHI2)    | (38)      |
|                   | Information gain (IG)     | (38,39)   |
|                   | F-score value (FScore)     | (94)      |
|                   | Mutual information (MIC)  | (95)      |
|                   | Pearson’s correlation coefficient (Pearson) | (96) |
| Dimensionality reduction | Principal component analysis (PCA) | (97) |
|                   | Latent dirichlet allocation (LDA) | (98) |
|                   | $t$-distributed stochastic neighbor embedding ($t$-SNE) | (99) |
| Feature normalization | Z-Score (ZScore)         | (15)      |
|                   | MinMax (MinMax)           | (15)      |

Table 4. The integrated machine-learning and deep-learning algorithms in iLearnPlus

| Algorithm category | Algorithm                        | Reference |
|--------------------|----------------------------------|-----------|
| Conventional machine-learning algorithms | Random forest (RF) | (105) |
|                     | Decision tree (DecisionTree)     | (106)     |
|                     | Support vector machine (SVM)     | (107)     |
|                     | $K$-nearest neighbors (KNN)       | (108)     |
|                     | Logistic regression (LR)         | (109)     |
|                     | Gradient boosting decision tree (GBDT) | (110) |
|                     | Light gradient boosting machine (LightBGM) | (111) |
|                     | Extreme gradient boosting (XGBoost) | (102) |
|                     | Stochastic gradient descent (SGD) | (34)      |
|                     | Naive Bayes (NaiveBayes)         | (112)     |
|                     | Linear discriminant analysis (LDA) | (113) |
|                     | Quadratic discriminant analysis (QDA) | (113) |
| Ensemble-learning frameworks | Bagging (Bagging) | (114) |
| Deep-learning algorithms | Adaptive boosting (AdaBoost) | (115) |
|                     | Convolutional neural network (CNN) | (30)     |
|                     | Attention based convolutional neural network (ABCNN) | (116) |
|                     | Recurrent neural network (RNN)    | (117)     |
|                     | Bidirectional recurrent neural network (BRNN) | (118,119) |
|                     | Residual network (ResNet)        | (120)     |
|                     | Auto-encoder (AE)                | (121)     |
|                     | Multilayer perceptron (MLP)      | (122)     |

sequences (19,100,101). iLearnPlus supports both binary classification (two outcomes) and multi-class classification (multiple outcomes). It offers 12 conventional machine-learning algorithms, two ensemble-learning frameworks, and seven deep-learning approaches (Table 4). This broad selection of algorithms is more comprehensive than what the current platforms offer, i.e. 21 versus 5 in iLearn and BioSeq-Analysis2.0 (Supplementary Table S2). We use the implementation from four popular third-party machine-learning platforms, including scikit-learn (34), XGBoost (102), LightGBM (103) and PyTorch (104). Deep-learning approaches are implemented using the PyTorch library, while LightGBM and XGBoost algorithms are imple-
users can either directly specify the values of the penalty and gamma parameters for the RBF (Radial Basis Function) kernel of the SVM classifier, or select the ‘Auto optimization’ option to optimize these two parameters automatically. The default parameter search space, which for the gamma values ranges from $2^{-10}$ to $2^2$, can be modified to a user-defined range. iLearnPlus also provides two classifier-dependent ensemble-learning frameworks: Bagging and AdaBoost. These frameworks are typically used to boost predictive performance. Importantly, iLearnPlus supports parallelization (via the use of multiple processors) to improve the computational efficiency for parallelizable algorithms, such as RF, Bagging, XGBoost and LightGBM.

Another key advantage of iLearnPlus is the availability of multiple modern deep-learning classifiers. The deep-learning techniques rely on multi-layer (deep) neural networks (NNs) to train complex predictive models from high-dimensional data that can be produced from the biological sequences (123,124). To facilitate applications of deep-learning techniques in the analysis of DNA, RNA and protein sequences, iLearnPlus incorporates deep-learning architectures including convolutional NN, attention-based convolutional NN, recurrent NN, bidirectional recurrent NN, residual NN, auto-encoder NN and the traditional multilayer perceptron NN (Table 4). These frameworks rely on a wide range of recent advancements including the convolution operation, attention mechanism, stacked residual blocks, long short-term memory (LSTM) units, and gated recurrent units (GRUs). Their inclusions are motivated by recent successful applications to predict protein contact maps (125,126), protein function (127), DNA-protein binding (128) and compound-protein affinity (129), to name but a few examples. Details concerning the architectures and parameters of these deep-learning networks can be found in the iLearnPlus online manual. We highlight the fact that iLearnPlus automatically detects and uses GPU devices to optimize performance and reduce the computational burden. When training deep-learning models, our platform utilizes the following default parameters: cross-entropy as the loss function, learning rate set as $10^{-3}$, maximum number of epochs set as 1000, termination of training with no performance improvement within 100 epochs, and parameter optimization utilizing the widely used Adam algorithm (130). Alternatively, these parameters can also be configured manually by users.

iLearnPlus also provides an option to perform meta-learning (131), where results produced by multiple predictive models (so called base models) are used in tandem to train new machine-learning models; the deep-learning approaches are excluded from the meta-learning. The underlying objective is to improve predictive performance compared to the performance of the base models. iLearnPlus assesses the performance for the constructed meta-models and identifies the best one (i.e. model producing the best predictive performance).

Performance evaluation

iLearnPlus implements the $K$-fold cross-validation and independent test assessments to evaluate the performance of the constructed classifiers. The $K$-fold cross-validation test divides the dataset at random into $K$ equally-sized subsets of sequences (i.e. folds). One of these folds is used as the validation dataset, and the remaining $K-1$ folds are used as the training dataset to train the machine-learning model and optimize its parameters. After repeating this process $K$ times, each fold is used once as the validation dataset (132). The independent test aims to evaluate and compare the predictive performance of multiple classifiers using a non-overlapping test dataset. This allows users to control the level of similarity between the training and test sequences. In iLearnPlus, the samples labeled as ‘training’ are used to implement the $K$-fold cross-validation test, while the samples labeled as ‘testing’ are used as the test dataset.

For a binary classification task, we provide eight commonly employed measures that quantify the predictive performance including sensitivity (Sn; Recall), specificity (Sp), accuracy (Acc), Matthews correlation coefficient (MCC), precision ($F_1$ score ($F_1$), the area under ROC curve (AUROC) and the area under the PRC curve (AUPRC) (15,20,133,134), which are defined as:

$$Sn = \frac{TP}{TP + FN}.$$  

(1)

$$Sp = \frac{TN}{TN + FP}.$$  

(2)

$$Acc = \frac{TP + TN}{TP + TN + FP + FN}.$$  

(3)

$$MCC = \frac{TP \times TN - FP \times FN}{\sqrt{(TP+FP)(TP+FN)(TN+FP)(TN+FN)}}.$$  

(4)

$$Precision = \frac{TP}{TP + FP}.$$  

(5)

$$F1 = 2 \times \frac{Precision \times Recall}{Precision + Recall}.$$  

(6)

where $TP$, $FP$, $TN$ and $FN$ represent the numbers of true positives, false positives, true negatives and false negatives, respectively. The AUROC and AUPRC values, which range between 0 and 1, are calculated based on the receiver-operating-characteristic (ROC) curve and the precision–recall curve, respectively. The higher the AUROC and AUPRC values, the better predictive performance of the underlying model.

For a multi-class classification task, we implement the popular $Acc$ measure, which is defined as (134,135):

$$Acc = \frac{TP(i) + TN(i)}{TP(i) + TN(i) + FP(i) + FN(i)}.$$  

(7)

where $TP(i)$, $FP(i)$, $TN(i)$ and $FN(i)$ represent the numbers of the samples (molecules) predicted correctly to be in the $i$th class, the total number of the samples in the $i$th class that are predicted as one of the other classes, the total number of the samples predicted correctly not to be in the $i$th class, and the total number of the samples not in the $i$th class that are predicted as the $i$th class, respectively.
Table 5. Graphical display options and statistical analysis methods in iLearnPlus

| Category           | Types        | Purpose                          |
|--------------------|--------------|----------------------------------|
| Graphic display    | Histogram    | Display data distribution        |
|                    | Kernel density plot | Display data distribution         |
|                    | Heatmap      | Display P-value/correlation matrix between different models |
|                    | Scatter plot | Display clustering and dimensionality reduction result |
|                    | Boxplot      | Depict the group values in the K-fold cross-validation for each of the eight metrics |
|                    | ROC curve    | Depict the overall performance of a model for balanced data |
|                    | PRC curve    | Depict the overall performance of a model for un-balanced data |
| Statistical analysis| Student’s t-test | Compare the means of two evaluate metric |
|                    | Bootstrap test | Evaluate the significance of performance difference between all pairs of ROC or PRC curves |

Data visualization and statistical analysis

iLearnPlus provides a wide range of tools to support analysis and visualization of the prediction results. It offers a variety of statistical plots including histograms, kernel density curves, heatmaps, boxplots, ROC and PRC curves, to assist users to interpret the prediction outcomes effectively (Table 5). As discussed above, histograms and kernel density plots are particularly suitable to visualize data distributions while the scatter plots should be used to analyze feature clustering and dimensionality reduction results. We supplement the predictive performance quantified with AUROC and AUPRC with the corresponding ROC and PRC curves. Users should employ boxplots to illustrate the distribution of the evaluation metrics values from the K-fold cross-validation experiments, allowing for comparison of predictive quality across different models (e.g. the performance based on different feature descriptors and/or machine-learning algorithms). We use the matplotlib library (136) to generate plots in iLearnPlus. The corresponding graphics can be saved using a variety of image formats (e.g. PNG, JPG, PDF, TIFF etc.).

iLearnPlus supports two statistical tests that can be used to compare the predictive performance across different models or tests. The student’s t-test compares the means of two sets of performance measures, typically obtained via the K-fold cross-validation test. The bootstrap test (33) is typically used to assess the significance of differences between data quantified with the ROC and PRC curves. For example, to compare the AUROC values, we apply the following formula:

\[
D = \frac{AUROC1 - AUROC2}{Sd(AUROC1 - AUROC2)}
\] (8)

where \(AUROC1\) and \(AUROC2\) denote the two original AUROC values, while \(AUROC1'\) and \(AUROC2'\) are the bootstrap resampled values of \(AUROC1\) and \(AUROC2\), respectively and \(Sd\) represents the standard deviation. By default, we perform 500 bootstrap replicates. In each replicate, we resample the original measurements with replacement to produce new ROC curves. After resampling, we compute \(AUROC1'\), \(AUROC2'\) and their difference (i.e. \(AUROC1' - AUROC2'\)) and use these values to calculate \(P\)-values. We also visualize these results with a heatmap.

RESULTS AND DISCUSSION

The functions and modules in iLearnPlus

iLearnPlus covers the five major steps needed to build effective models for analysis and prediction of nucleic acid and proteins sequences: feature extraction, feature analysis, classifier construction, performance evaluation and data/result visualization (Figure 1). We implement these steps by developing four modules in iLearnPlus: iLearnPlus-Basic, iLearnPlus-Estimato, iLearnPlus-AutoML and iLearnPlus-LoadModel (Figure 3). The iLearnPlus-Basic module facilitates analysis and prediction using a selected feature-based representation of the input protein/RNA/DNA sequences (sequence descriptors) and a selected machine-learning classifier. This module is particularly instrumental when interrogating the impact of using different sequence feature descriptors and machine-learning algorithms on the predictive performance. The iLearnPlus-Estimator module provides a flexible way to perform feature extraction by allowing users to select multiple feature descriptors. The iLearnPlus-AutoML module focuses on automated benchmarking and maximization of the predictive performance across different machine-learning classifiers that are applied on the same set or combined sets of feature descriptors. In addition, by combining the iLearnPlus-Estimator and iLearnPlus-AutoML modules, users can conveniently and efficiently evaluate and compare the predictive quality across different selected sequence descriptors and different machine-learning algorithms. Moreover, models generated by iLearnPlus can be exported and saved as model files with the ‘.pkl’ extension in both the stand-alone software and using the web server. Using the iLearnPlus-LoadModel module, users can upload, deploy and test their models on new (test) data. Moreover, the saved models that rely on conventional machine-learning algorithms can be directly applied in the scikit-learn environment, whereas the exported deep-learning models can be applied using the PyTorch library. Section 8 of the user manual provides detailed instructions.

Building and customizing machine-learning pipelines using iLearnPlus

iLearnPlus makes it easy and straightforward to design and optimize machine-learning pipelines to achieve a competitive (if not the best) predictive performance. The design process typically boils down to two key objectives: extraction and selection of features, and selection and parametrization of machine-learning models, both of which are supported by iLearnPlus. Our platform tackles these objectives via a simple example procedure summarized in Figure 4. Users should first apply the iLearnPlus-Estimator module to generate multiple sequence descriptors (feature sets) from the input sequences and test them by constructing and evaluating a machine-learning model in a batch mode.
Figure 3. The iLearnPlus architecture with four major built-in modules, including iLearnPlus-Basic, iLearnPlus-Estimator, iLearnPlus-AutoML, and iLearnPlus-LoadModel.

This allows to establish a point of reference for subsequent optimization/parameterization of the model. The corresponding results and models can be saved for future reference. Subsequently, the iLearnPlus-Basic module should be used to analyze and rank the feature descriptors. Based on the ranking, users should select and evaluate a subset of well-performing features (e.g. a subset of top $N$ features). Next, the evaluation should be performed with the help of the iLearnPlus-AutoML module that optimizes different machine-learning classifiers to the selected feature set. This module also performs statistical comparative analysis of the results and provides the option to save the best model.

The iLearnPlus web server and source code
The full version of iLearnPlus that covers the four modules (iLearnPlus-Basic, iLearnPlus-Estimator, iLearnPlus-AutoML, and iLearnPlus-LoadModel) and a graphical user-interface is available on the GitHub repository at https://github.com/Superzchen/iLearnPlus/. The GUI for the four modules is shown in Figure 5.
Figure 4. An example of building and customizing machine-learning pipelines using \textit{iLearnPlus}. First, the \textit{iLearnPlus-Estimator} module is used to evaluate the performance of multiple feature descriptors based on the input sequences. Next, the feature descriptors with satisfactory performance are selected and the \textit{iLearnPlus-Basic} module is then used to select the top $N$ important features and save the top $N$ features into a file. Finally, users can upload the feature selection results to the \textit{iLearnPlus-AutoML} module to evaluate the performance of the machine-learning algorithms of interests in an automated manner.

\textit{iLearnPlus} is also freely available as a web server at \url{http://ilearnplus.erc.monash.edu/}. In this case, the calculations are performed on the server side, freeing the users from engaging their own computational resources. This server relies on the Nectar (The National eResearch Collaboration Tools and Resources, which is an online infrastructure that supports researchers to connect with colleagues in Australia and around the world) cloud computing infrastructure, which is managed by the eResearch Centre at Monash University. The \textit{iLearnPlus} web server was implemented using the open-source web platform LAMP (Linux-Apache-MySQL-PHP) and is equipped with 16 cores, 64GB memory and 2TB hard disk. The server supports five popular web browsers including the Internet Explorer (\textgeq v.7.0), Microsoft Edge, Mozilla Firefox, Google Chrome and Safari. Given the high computational cost, the web server runs only the \textit{iLearnPlus-Basic} module that supports basic analysis and machine-learning modeling of DNA, RNA and protein sequence data. Figure 6 shows a screenshot of the main page of the \textit{iLearnPlus} web server, where the inputs and parameters of the analysis are entered.

\textbf{Case studies}

We showcase real-world applications of \textit{iLearnPlus} with two bioinformatic scenarios: identification of the long non-coding RNAs (lncRNAs) and prediction of the protein crotonylation sites. We emphasize that the underlying objective is to illustrate how to use our platform for two such diverse applications, rather than securing the top predictive performance compared to the state-of-the-art.

The lncRNAs are the transcripts that are over 200 bp long which do not code for proteins (137). Approximately 70\% of the non-coding sequences are transcribed into lncRNAs. They regulate a variety of biological processes and are linked to several human diseases (138,139). We applied the \textit{iLearnPlus-Basic} module to extract the feature sets and train a classifier that accurately differentiated between lncRNAs and mRNA sequences. We used the datasets from a recent study by Han \textit{et al.} (137). The training and validation datasets contain 4200 lncRNA and 4200 mRNA sequences, while the test dataset includes 1,800 lncRNA and 1,800 mRNA chains from \textit{Mus musculus}. Several studies demonstrate that the distribution of adjoining bases is different for lncRNAs and mRNAs (140,141). Thus, we selected the
‘Kmer’ (size = 3) feature descriptor to extract the features. We applied the random forest algorithm (number of trees = 1000) to train the classifier using these features and optimized the classifier based on the 5-fold cross-validation test. This simple design secured AUC = 0.897, Acc = 81.89% and MCC = 0.642 on the test dataset. The entire process took about 10 mins to complete with the iLearnPlus web server using one CPU. Figures 6 and 7 show the parameter configurations and prediction results that we obtained using the online version of the iLearnPlus-Basic model. We note that the kernel density curves and histograms for distribution visualization of the extracted features that are included in Figure 7 are the new features of iLearnPlus that are not available in the other current tools. This case study demonstrates that the entire process that produces a simple and well-performing model can be conveniently completed in a matter of minutes. We used the entire datasets (a total of 12 000 sequences) with the iLearnPlus web server. However, considering the computational burden on the server side, we set the maximum number of sequences that can be submitted to the server to 2000. In cases where users need to process larger amount of sequence data, we encourage to use the GUI version of iLearnPlus which does not limit the number of input sequences.

Lysine crotonylation (Kcr) is a post-translational modification (PTM) that was originally found in the histone proteins (142). Recently, this PTM was discovered in non-histone proteins and was found to be involved in the regulation of cell cycle progression and DNA replication cell cycle (143,144). Here, we used the iLearnPlus-Estimator module to comparatively assess the performance of different feature sets using a dataset retrieved from (145,146). We used the data preprocessing strategy from Chen et al. (32) which was developed for a similar prediction problem. Inspired by a recent study by Chen et al. (147), we collected 6687 Kcr sites (positives) and 67 240 non-Kcr sites (negatives) using the sequence segments of 29 residues. We used the iLearnPlus-Estimator module in the standalone GUI version (Figure 8A) to load the data, produced seven feature sets (AAC, EAAC, EGAAC, DDE, binary, ZScale and BLOSUM) and selected a machine-learning algorithm. Similar to the other case study, we used the random forest algorithm (with the default setting of 1000 trees) to construct the classifier via 10-fold cross-validation. We show the corresponding setup in Figure 8A. Figure 8B summarizes the predictive performance quantified with AUROC and AUPRC for models that used each of the seven selected feature sets as inputs. This analysis reveals that the model built utilizing the EGAAC feature descriptors achieved the best performance. This also shows how easy it is to use iLearnPlus to rationally select a well-performing feature encoding for the input sequences. Next, we used the iLearnPlus-AutoML module to comparatively evaluate the predictive performance across seven machine-learning algorithms: SGD, LR, XGBoost, LightGBM, RF, MLP and CNN. We used the bootstrap tests to assess statistical significance of the differences between the ROC curves produced by these algorithms. Figure 9 summarizes the corresponding performance evaluation. More specifically, it shows the evaluation metrics in terms of Sn, Sp, Pre, Acc, MCC and F1 (panel A) whose calculations were based on the default threshold values, correlation matrix that quantifies mutual correlations between classifiers (panel B), ROC (panel C) and PRC (panel D) curves, and boxplots that are used to compare results between classifiers.
Figure 6. A screenshot showing the web server version of iLearnPlus for analyzing DNA (A), RNA (B) and protein (C) sequences. For each sub-server, user can generate their desired analysis pipelines via the major panels marked with (i), (ii), (iii) and (iv). The example input sequences were extracted from the lncRNA dataset prepared by Han et al. (137). The training and validation datasets contain 4,200 IncRNA and 4200 mRNA sequences, while the test dataset includes 1800 IncRNA and 1800 mRNA chains from *Mus musculus*. The category ‘0’ refers to mRNA sequences, while the category ‘1’ denotes the IncRNA sequences.
Figure 7. A screenshot demonstrating the result page of *iLearnPlus* for lncRNA prediction using the *iLearnPlus* web server. The result page includes the summary of basic information (A), including the input sequence type, number of sequences used for training and test, respectively, and the selected feature descriptor type, the generated features and feature analysis result (B), the selected machine-learning algorithm and the evaluation metrics listed for each fold and independent test (C), the ROC and PRC for demonstrating the prediction performance (D) and the hyperlink for downloading all the generated files including the generated feature encoding files, feature analysis result and plots, evaluation metrics matrix file, prediction scores, ROC and PRC curves, and the constructed models (E). The category ‘0’ refers to mRNA sequences, while the category ‘1’ denotes the lncRNA sequences.
Figure 8. The prediction results for protein crotonylation sites using the selected feature descriptors and the local GUI version of iLearnPlus, including a screenshot of the parameter setup using iLearnPlus-Estimator module (A), and the ROC and PRC curves of the seven RF models using different feature descriptors (B).

We found that the deep-learning model, CNN, achieved the best predictive performance among all the seven machine-learning algorithms, with Acc = 85.4% and AUC = 0.823. Overall, this case study demonstrates how to effectively and efficiently address the two key objectives that lead to designing accurate models: extraction and selection of useful features and selection of the best-performing machine-learning models. We considered seven different feature sets, selected the best set and comparatively evaluated seven machine-learning models using a broad and informative set of metrics. This ultimately led to an informed selection of an accurate solution. We emphasize that four of the seven selected algorithms (i.e. CNN, SGD, XGBoost and LightGBM), ability to run statistical tests, and key methods for visualization of results (i.e. boxplots and heatmaps of the correlations between the models) are among the new features offered by iLearnPlus that are not available in the current platforms.

These case studies demonstrate that iLearnPlus is a comprehensive platform for the design, evaluation and analysis of the predictive models for both nucleic acid and protein sequences. It can be used to produce an accurate model ef-
Figure 9. An illustration of the prediction results generated by different machine-learning algorithms using the iLearnPlus-AutoML module for identification of protein crotonylation sites, including the evaluation metrics showing the predictive performance in terms of eight evaluation metrics (A), the correlation matrix of seven selected classifiers (B), ROC curves (C), PRC curves (D) and the boxplots (E) of eight metrics for comparative performance assessment of all the seven selected machine-learning algorithms.

Figure 9. An illustration of the prediction results generated by different machine-learning algorithms using the iLearnPlus-AutoML module for identification of protein crotonylation sites, including the evaluation metrics showing the predictive performance in terms of eight evaluation metrics (A), the correlation matrix of seven selected classifiers (B), ROC curves (C), PRC curves (D) and the boxplots (E) of eight metrics for comparative performance assessment of all the seven selected machine-learning algorithms.

CONCLUSION

Massive accumulation of sequence data calls for the equally aggressive efforts to develop computational models that can analyze and make an inference from these data. In this article, we addressed this need by delivering a comprehensive automated pipeline, iLearnPlus, which provides ‘one-stop’ services for machine learning-based predictions from the DNA, RNA and protein sequence data. iLearnPlus includes four built-in modules, calculates a variety of feature set and provides 21 machine-learning algorithms including seven popular and modern deep-learning methods. Our platform offers a diverse collection of strategies to conceptualize, design, test, comparatively assess and deploy predictive models. iLearnPlus caters to a broad range of users, including biologists with limited bioinformatics expertise who can benefit from the easy-to-use web server. We provide two
case studies using iLearnPlus: predictions of lncRNAs and protein crotonylation sites. The first highlights the fact that our platform supports rapid development of accurate models, while the second demonstrates a sophisticated process that performs feature and classifier selection to maximize the predictive performance of the constructed model. We conclude that iLearnPlus is an effective tool for the design, testing and deployment of machine-learning pipelines for analysis and prediction of the rapidly increasing volume of sequence data for biologists and bioinformaticians.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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