An Insightful Recollection for Predicting Protein Subcellular Locations in Multi-Label Systems

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ABSTRACT

A systematic introduction has been presented for the recent advances in predicting protein subcellular localization in the multi-label systems, where the constituent proteins may simultaneously occur or move between two or more location sites and hence have exceptional biological functions worthy of our special notice. All the predictors included in this review each have a user-friendly web-server, by which the majority of experimental scientists can very easily acquire their desired data without the need to go through the complicated mathematics involved.

1. INTRODUCTION

As elucidated in two recent comprehensive review papers [1, 2], to develop a really useful bioinformatics tool, one needs to observe the guidelines of the Chou's 5-steps rule [2-36] to go through the following five steps: 1) select or construct a valid benchmark dataset to train and test the predictor; 2) represent the samples with an effective formulation that can truly reflect their intrinsic correlation with the target to be predicted; 3) introduce or develop a powerful algorithm to conduct the prediction; 4) properly perform cross-validation tests to objectively evaluate the anticipated prediction accuracy; 5) establish a user-friendly web-server for the predictor that is accessible to the public. The bioinformatics or computational tool established by observing the guidelines of Chou's 5-step rules have the following remarkable merits: a) crystal clear in logic development, b) completely transparent in operation, c) easily to repeat the reported results by other investigators, d) with high potential in stimulating other new bioinformatics tools, and e) very convenient to be used by the majority of experimental scientists. As for more about the importance of the 5-steps rule, see an insightful Wikipedia article at https://en.wikipedia.org/wiki/5-step_rules. It is instructive to point out that, although the present minireview was focused on the recent development in subcellular prediction for the multi-label proteins [37, 38], the 5-steps rule can also be used to deal with many different systems, such as those in material science [39] and even those in commercial science (e.g., analyzing the effect of bank credit card versus mobile payment). The only difference between the biological systems and other disciplines' systems is how to formulate the statistical samples or events with an effective mathematical expression that can truly reflect their intrinsic correlation with the target to be predicted. This is just like the case of many machine-learning algorithms. They can be used in nearly all the areas of statistical analysis.

2. PREDICTING SUBCELLULAR LOCALIZATION OF PROTEINS

The smallest unit of life is a cell, which contains numerous protein molecules. Most of the functions critical to the cell's survival are performed by these proteins located in its different organelles, usually called "subcellular locations" (**Figure 1**). Information of subcellular localization for a protein can provide useful clues about its function. To reveal the intricate pathways at the cellular level, knowledge of the subcellular localization of proteins in a cell is prerequisite. Unfortunately, it is both time-consuming and costly to determine the subcellular locations of proteins purely based on experiments. With the avalanche of protein sequences generated in the post-genomic age, it is highly desired to develop computational tools for rapidly and effectively identifying the subcellular locations of uncharacterized proteins based on their sequences information alone. The demand has become even more challenging owing to the fact that many protein molecules may simultaneously exist or move between two or more subcellular location sites [40]. Actually, it is these multiplex proteins that are of significance for in-depth understanding the biological processes in a living cell.

3. FOUR SERIES OF PREDICTORS

In the last decade or so, a number of predictors were developed for predicting the subcellular localization of proteins with both single site and multiple sites based on their sequences information alone. They can be generally classified into four series: 1) 𝕏-mPLoc, 2) iLoc𝕏, 3) pLoc-m𝕏, and 4) pLoc_bal-m𝕏, where the wildcard may denote "Euk" (eukaryotic), "Hum" (human), "Animal", "Plant", "Virus", "Gneg"

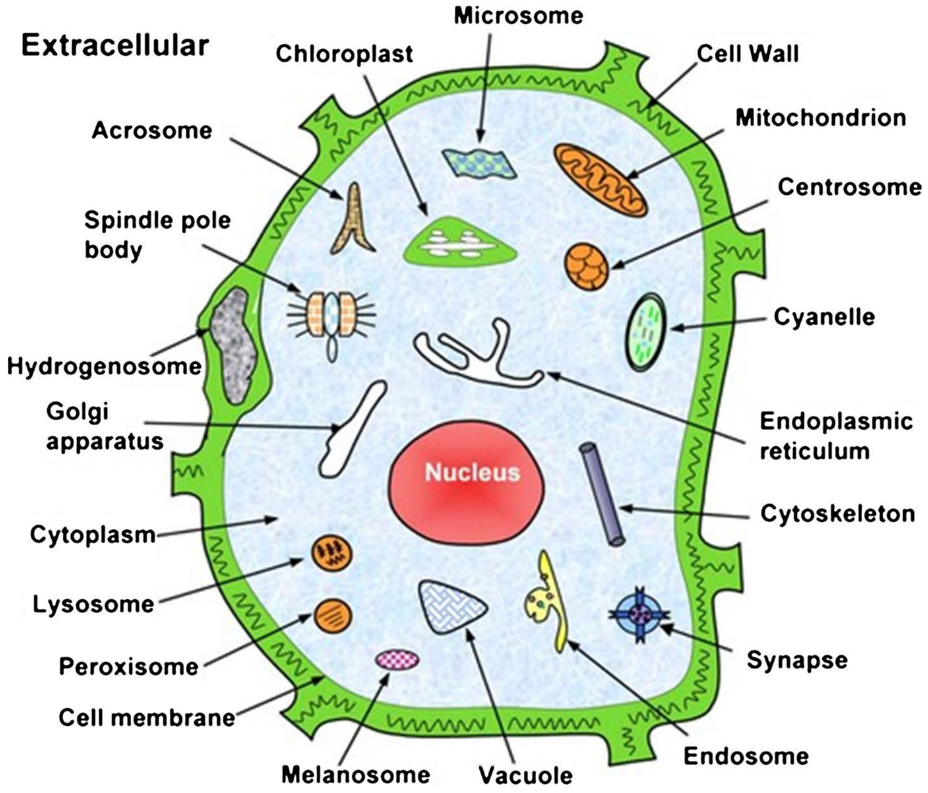


Figure 1. Schematic illustration to show the 22 organelles or subcellular locations in an eukaryotic cell. Adapted from Chou and Shen with permission [189].

(Gram-negative bacterial), “Gpos” (Gram-positive bacterial) proteins, respectively, as formulated by

$$\mathbb{X} \in \begin{cases} \text{Euk} \\ \text{Hum} \\ \text{Animal} \\ \text{Plant} \\ \text{Virus} \\ \text{Gneg} \\ \text{Gpos} \end{cases} \tag{1}$$

The protein samples in the $\mathbb{X}$-mPLoc series [41-46] were formulated by hybridizing the GO (Gene Ontology) information, FunD (Functional Domain) information, and PSSM (Sequential Evolutionary) information into the general PseAAC [3], which was extended from pseudo amino acid composition [47, 48].

The protein samples in the iLoc-$\mathbb{X}$series [49-55] were formulated by incorporating the GO information and PSSM information into the general PseAAC.

The protein samples in the pLoc-m $\mathbb{X}$series [56-62] were formulated by extracting the key or optimal GO information into the general PseAAC.

The protein samples in the pLoc_bal-m $\mathbb{X}$series [2, 26, 29, 63-65] were formulated by further balancing out the protein samples used in pLoc-m $\mathbb{X}$series.

As for the justification of using the GO information for predicting the subcellular localization of proteins, see Section 4 of a review paper [66], where an insightful analysis has been elaborated and there is no need to repeat here.

3.1. Benchmark Dataset

All the predictors in the above four series were developed based on a very stringent benchmark dataset in which none of proteins had ≥25% pairwise sequence identity to any other in a same subset. But such a strict cutoff treatment was not imposed for the protein sequences in the “viral capsid” subset because otherwise it would contain too few proteins to be of statistical significance as explained in [46].

3.2. Sample Formulation

The most straightforward expression for a protein sample is its sequential model as given by

$$\mathbf{P} = \mathrm{R}_1\mathrm{R}_2\mathrm{R}_3\mathrm{R}_4\mathrm{R}_5\mathrm{R}_6\mathrm{R}_7\cdots\mathrm{R}_L \tag{2}$$

where L denotes the protein’s length or the number of its constituent amino acid residues, R_1 is the 1st residue, R_2 the 2nd residue, R_3 the 3rd residue, and so forth. Since all the existing machine-learning algorithms (e.g., “Support Vector Machine” or SVM algorithm [4, 5], “Covariance Discriminant” or CD algorithm [67-69], “Nearest Neighbor” or NN algorithm [70, 71], and “Random Forest” or RF algorithm [72, 73]) can only handle vectors as elaborated in [74], we have to convert the sequential expression of Equation (2) into a vector. But a vector defined in a discrete model might completely lose all the sequence order or pattern information. To deal with this problem, the concept of PseAAC (Pseudo Amino Acid Composition) was introduced [47, 48]. Ever since then, the concept of PseAAC has been widely used in nearly all the areas of computational proteomics with the aim to grasp various different sequence patterns that are essential to the targets investigated (see, e.g., [20, 21, 28, 75-170] as well as a long list of references cited in [171]). Because it has been widely and increasingly used, four powerful open access soft-wares, called “PseAAC” [172], “PseAAC-Builder” [88], “propy” [98], and “PseAAC-General” [109], were established: the former three are for generating various modes of special PseAAC [173]; while the fourth one for those of general PseAAC [3], including not only all the special modes of feature vectors for proteins but also the higher level feature vectors such as “Functional Domain” or “FunD” mode, “Gene Ontology” or “GO” mode, and "Sequential Evolution" or "PSSM" [174] mode. Encouraged by the successes of using PseAAC to deal with protein/peptide sequences, its idea and approach were extended to PseKNC (Pseudo K-tuple Nucleotide Composition) to generate various feature vectors for DNA/RNA sequences [175-178] that have proved very successful as well [14, 179-190]. According to the concept of general PseAAC [3], any protein sequence can be formulated as a PseAAC vector given by

$$\mathbf{P} = \begin{bmatrix} \Psi_1 & \Psi_2 & \cdots & \Psi_u & \cdots & \Psi_\Omega \end{bmatrix}^{\mathbf{T}} \tag{3}$$

where **T** is a transpose operator, while the integer Ω is a parameter and its value as well as the components $\Psi_u \left(u = 1, 2, \cdots, \Omega \right)$ will depend on how to extract the desired information from the amino acid sequence of **P**.

3.3. Operation Engine

The operation engine for ×-mPLoc series was constructed by fusing an array of OET-KNN (Optimized Evidence-Theoretic K-Nearest Neighbor) classifiers [191-193].

The operation engine for iLoc-×series was the multi-labeled KNN (K-Nearest Neighbor) classifier [49].

The operation engine for the pLoc-m and pLoc_bal-m ×series was the ML-GKR (multi-label Gaussian kernel regression) classifier [56].

3.3.1. Metrics and Cross-Validation

In order to objectively evaluate the prediction quality of a multi-label predictor, one needs to consider the following two issues. 1) What metrics should be used to quantitatively reflect its accuracy? 2) What test approach should be adopted to score the metrics?

Quite different from the metrics used to measure the prediction quality of a single-label predictor, the metrics for a multi-label predictor are much more complicated. To quantitatively evaluate the power of a multi-label predictor, we need to use two sets of metrics: one for its global accuracy and the other for its local accuracy.

The global accuracy is defined by a set of five metrics as given in [66]

$$\begin{cases} \text{Aiming} \uparrow = \dfrac{1}{N^{\mathrm{q}}} \sum_{k=1}^{N^{\mathrm{q}}} \left(\dfrac{\left\| \mathbb{L}_k \cap \mathbb{L}_k^* \right\|}{\left\| \mathbb{L}_k^* \right\|} \right), \quad [0,1] \\ \text{Coverage} \uparrow = \dfrac{1}{N^{\mathrm{q}}} \sum_{k=1}^{N^{\mathrm{q}}} \left(\dfrac{\left\| \mathbb{L}_k \cap \mathbb{L}_k^* \right\|}{\left\| \mathbb{L}_k \right\|} \right), \quad [0,1] \\ \text{Accuracy} \uparrow = \dfrac{1}{N^{\mathrm{q}}} \sum_{k=1}^{N^{\mathrm{q}}} \left(\dfrac{\left\| \mathbb{L}_k \cap \mathbb{L}_k^* \right\|}{\left\| \mathbb{L}_k \cup \mathbb{L}_k^* \right\|} \right), \quad [0,1] \\ \text{Absolute true} \uparrow = \dfrac{1}{N^{\mathrm{q}}} \sum_{k=1}^{N^{\mathrm{q}}} \Delta\left(\mathbb{L}_k, \mathbb{L}_k^* \right), \quad [0,1] \\ \text{Absolute false} \downarrow = \dfrac{1}{N^{\mathrm{q}}} \sum_{k=1}^{N^{\mathrm{q}}} \left(\dfrac{\left\| \mathbb{L}_k \cup \mathbb{L}_k^* \right\| - \left\| \mathbb{L}_k \cap \mathbb{L}_k^* \right\|}{M} \right), \quad [1,0] \end{cases} \tag{4}$$

where *N*q is the total number of query proteins or tested proteins, *M* is the total number of different labels for the investigated system, || || means the operator acting on the set therein to count the number of its elements, ⋃ means the symbol for the "union" in the set theory, ⋂ denotes the symbol for the "intersection", $\mathbb{L}_k$ denotes the subset that contains all the labels observed by experiments for the *k*-th tested sample, represents the subset that contains all the labels predicted for the *k*-th sample, and $\mathbb{L}_k^*$ represents the subset that contains all the labels predicted for the *k*-th sample, and

$$\Delta\left(\mathbb{L}_k,\mathbb{L}_k^*\right)=\begin{cases}1, & \text{if all the labels in } \mathbb{L}_k^* \text{ are identical to those in } \mathbb{L}_k\\ 0, & \text{otherwise}\end{cases} \tag{5}$$

In Equation (4), the first four metrics with an upper arrow ↑ are called positive metrics, meaning that the larger the rate is the better the prediction quality will be; the 5th metrics with a down arrow is called negative metrics, implying just the opposite meaning. As we can see from Equation (5): 1) the "Aiming" defined by the 1st sub-equation is for checking the rate or percentage of the correctly predicted labels over the practically predicted labels; 2) the "Coverage" defined in the 2nd sub-equation is for checking the rate of the correctly predicted labels over the actual labels in the system concerned; 3) the "Accuracy" in the 3rd sub-equation is for checking the average ratio of correctly predicted labels over the total labels including correctly and incorrectly predicted labels as well as those real labels but are missed in the prediction; 4) the "Absolute true" in the 4th sub-equation is for checking the ratio of the perfectly or completely correct prediction events over the total prediction events; 5) the "Absolute false" in the 5th sub-equation is for checking the ratio of the completely wrong prediction over the total prediction events.

The five metrics in Equation (4) reflect the quality of a multi-label predictor from five different angles at the global level. It is instructive to point out, however, among the five global metrics the most important one and also the most difficult to improve its success rate is the "Absolute true" or "perfectly correct" rate [66]. Why? This is because the score standard for the absolute true rate is very harsh. According to its definition, for a protein sample that is actually simultaneously located at the subcellular locations ("A", "B", "C"). If the predicted result is not exactly the three locations but ("A", "B") or ("A", "B", "C", "D"), no score whatsoever will be given. In other words, when and only when the predicted localization for the protein sample is perfectly identical to its actual localization, can we add one point for the absolute true rate; otherwise, zero.

The set of metrics in Equation (4) are used to evaluate the prediction quality of a multi-label predictor for all the proteins in the entire cell, and hence is called the "set of metrics for the global accuracy" or the "set of global metrics".

To evaluate the local accuracy of a multi-label predictor, we use a set of Chou's four intuitive metrics that were derived by Chou *et al.* [4, 69] based on the symbols introduced by Chou [194-196] for studying the cleavage sites of signal peptides. The set of metrics are given below

$$\begin{cases}\mathrm{Sn}(i)=1-\dfrac{N_-^+(i)}{N^+(i)} & 0\le \mathrm{Sn}(i)\le 1\\[2ex] \mathrm{Sp}(i)=1-\dfrac{N_+^-(i)}{N^-(i)} & 0\le \mathrm{Sp}(i)\le 1\\[2ex] \mathrm{Acc}(i)=1-\dfrac{N_-^+(i)+N_+^-(i)}{N^+(i)+N^-(i)} & 0\le \mathrm{Acc}(i)\le 1\\[2ex] \mathrm{MCC}(i)=\dfrac{1-\left(\dfrac{N_-^+(i)}{N^+(i)}+\dfrac{N_+^-(i)}{N^-(i)}\right)}{\sqrt{\left(1+\dfrac{N_+^-(i)-N_-^+(i)}{N^+(i)}\right)\left(1+\dfrac{N_-^+(i)-N_+^-(i)}{N^-(i)}\right)}} & -1\le \mathrm{MCC}(i)\le 1\\[2ex] (i=1,2,\cdots,M) & \end{cases} \tag{6}$$

where Sn, Sp, Acc, and MCC represent the sensitivity, specificity, accuracy, and Mathew's correlation coefficient, respectively [15], *i* denotes the *i*-th subcellular location (or subset) in the benchmark dataset, and *M* has exactly the same meaning as in Equation (5). $N^+(i)$ is the total number of the samples investigated in the i-th subset, whereas $N_{-}^{+}(i)$ is the number of the samples in $N^{+}(i)$ that are incorrectly predicted to be of other locations; $N^{-}(i)$ is the total number of samples in any location but not the i-th location, whereas $N_{+}^{-}(i)$ is the number of the samples in $N^{-}(i)$ that are incorrectly predicted to be of the i-th location.

In addition to being widely used in proteome and genome analyses (see, e.g., [6, 8, 10, 13, 15, 33, 36, 180, 181, 185, 197-203]), the set of metrics in Equation (6) can be used to evaluate the prediction quality of a multi-label predictor for the proteins in each of subcellular locations concerned (see, e.g., [58, 62]), and hence is called the "set of metrics for local accuracy" or the "set of local metrics".

3.3.2. Cross-Validation and Jackknife Test

Three cross-validation methods are often used in statistical prediction. They are: 1) independent dataset test, 2) subsampling (or K-fold cross-validation) test, and 3) jackknife test [204]. Of these three, however, the jackknife test was deemed the least arbitrary that can always yield a unique result for a given benchmark dataset [37, 38], as clearly elucidated in a comprehensive review paper [3] and demonstrated by Eqs. (28)-(32) therein. Therefore, the jackknife test has been increasingly recognized and widely adopted by investigators to test the power of various prediction methods (see, e.g., [80, 82, 101, 110, 205-208]).

Therefore, all the predictors in Section 2 were examined by the jackknife tests.

3.4. Web Servers

The last but not least important guideline in the 5-step rules is about the web-server. As pointed out in [209] and demonstrated in a series of recent publications (see, e.g., [5-15, 17-19, 174, 180-185, 197-201, 203, 210-244]), user-friendly and publicly accessible web-servers represent the future direction for developing practically more useful predictors. Actually, many practically useful web-servers have significantly increased bioinformatics impacts on medicinal chemistry [74], driving medicinal chemistry into an unprecedented revolution [171].

All the multi-label predictors listed in Section 2 have their web-servers well established as summarized below.

3.4.1. mPLoc Series

This series contains six publicly accessible web-servers: (1) "Euk-mPLoc" at http://www.csbio.sjtu.edu.cn/bioinf/euk-multi-2/ [43] for predicting the subcellular localization of eukaryotic proteins. (2) "Hum-mPLoc" at http://www.csbio.sjtu.edu.cn/bioinf/hum-multi-2/ [41] for predicting the subcellular localization of human proteins. (3)"Plant-mPLoc" at http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/ [44] for predicting the subcellular localization of plant proteins. (4)"Virus-mPLoc" at http://www.csbio.sjtu.edu.cn/bioinf/virus-multi/ [46] for predicting the subcellular localization of virus proteins. (5)"Gneg-mPLoc" at http://www.csbio.sjtu.edu.cn/bioinf/Gneg-multi/ [45] for predicting the subcellular localization of Gram-negative bacterial proteins. (6) "Gpos-mPLoc" at http://www.csbio.sjtu.edu.cn/bioinf/Gpos-multi/ [42] for predicting subcellular localization of Gram-positive bacterial proteins.

The aforementioned six web-servers have also been integrated into a package called "Cell-PLoc" at PLoc/ [37] and its updated version "Cell-PLoc 2.0" at http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/ [38].

3.4.2. iLoc-Series

It contains the following seven web-servers. 1) "iLoc-Euk" at http://www.jci-bioinfo.cn/iLoc-Euk [49] for predicting the subcellular localization of eukaryotic proteins. 2) "iLoc-Hum" at http://www.jcibioinfo.cn/iLoc-Hum [52] for predicting the subcellular localization of human proteins. 3) "iLoc-Animal" at Animal [55] for predicting the subcellular localization of animal proteins. 4) "iLoc-Plant" at http://www.jci-bioinfo.cn/iLoc-Plant [50] for predicting the subcellular localization of plant proteins. 5) "iLoc-Virus" at http://www.jci-bioinfo.cn/iLoc-Virus [51] for predicting the subcellular localization of virus proteins. 6) "iLoc-Gneg" at http://www.jcibioinfo.cn/iLoc-Gneg [53] for predicting the subcellular localization of Gram-negative proteins. 7) "iLoc-Gpos" at http://www.jci-bioinfo.cn/iLoc-Gpos [54].

3.4.3. pLoc-m Series

There are seven web-servers in this series as listed below. 1) "pLoc-mEuk" at http://www.jci-bioinfo.cn/pLoc-mEuk/ [60] for predicting the subcellular localization of eukaryotic proteins. 2) "pLoc-mHum" at http://www.jci-bioinfo.cn/pLoc-mHum/ [62] for predicting the subcellular localization of human proteins. 3) "pLoc-mAnimal" at http://www.jci-bioinfo.cn/pLoc-mAnimal/ [58] for predicting the subcellular localization of animal proteins. 4) "pLoc-mPlant" at http://www.jci-bioinfo.cn/pLoc-mPlant/ [56] for predicting the subcellular localization of plant proteins. 5) "pLoc-mVirus" at http:// www.jci-bioinfo.cn/pLoc-mVirus/ [57] for predicting the subcellular localization of virus proteins. 6) "pLoc-mGneg" at http://www.jcibioinfo.cn/pLoc-mGneg/ [61] for predicting the subcellular localization of Gram-negative proteins. 7) "pLoc-mGpos" at http://www.jcibioinfo.cn/pLoc-mGpos/ [59] for predicting the subcellular localization of Gram-positive proteins.

3.4.4. pLoc_bal-m Series

There are seven web-servers in this series as listed below. 1) "pLoc_bal-mEuk" at [2]. 2) "pLoc_bal-mHum" [2]. 3) "pLoc_bal-mAnimal" [65]. 4) "pLoc_bal-mPlant" [26]. 5) "pLoc_bal-mVirus" [29]. 6) "pLoc_bal-mGneg" [63]. 7) "pLoc_bal-mGpos" [29].

Listed in **Table 1** are the global accuracy rates (cf. Equation (4)) predicted with the aforementioned seven web-servers, while the corresponding the local accuracy rates (cf. Equation (6)) are given in **Table 2**. As shown from the rates in the two tables, all the seven web-servers have yielded very high prediction quality in both the global and local cases. Therefore, using these web-servers, the majority of experimental scientists can easily obtain their desired results without the need to go through the detailed mathematics involved.

Below, let us take the multi-label predictor of pLoc_bal-mEuk [2] as a showcase. 1) Click the link at mEuk/, you'll see the top page for predicting the eukaryotic protein subcellular localization prompted on your computer screen (**Figure 2**). 2) You can either type or copy/paste the sequences of query eukaryotic proteins into the input box at the center of **Figure 2**. The input sequence should be in the FASTA format. You can click the Example button right above the input box to see the sequences in FASTA

Table 1. List of the five global metrics rates reported from each of the seven predictors in the pLoc_bal-m series.

No	Predictor[a]	Aiming[b]	Coverage[b]	Accuracy[b]	Absolutetrue[b]	Absolutefalse[b]
1	pLoc_bal-mEuk	88.31%	85.06%	84.34%	78.78%	0.07%
2	pLoc_bal-mHum	90.57%	82.75%	84.39%	79.14%	1.20%
3	pLoc_bal-mAnimal	87.96%	85.33%	84.64%	73.11%	1.65%
4	pLoc_bal-mPlant	91.74%	87.39%	88.02%	84.87%	0.78%
5	pLoc_bal-mVirus	88.97%	92.86%	89.77%	82.13%	2.66%
6	pLoc_bal-mGneg	96.61%	95.81%	96.05%	94.68%	0.36%
7	pLoc_bal- mGpos	97.69%	97.13 %	97.40 %	97.11%	0.14%

[a] See Equation (1) of Section 2 for further explanation. [b] See Equation (5) for the definition of the global metrics.

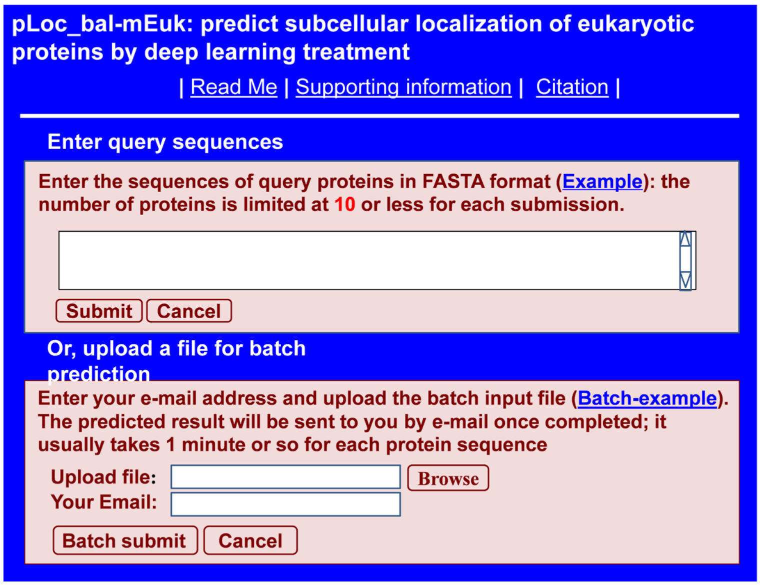
Table 2. Performance of pLoc_bal-mEuk for each of the 22 subcellular locations.

i	Location[a]	Sn(*i*)[b]	Sp(*i*)[b]	Acc(*i*)[b]	MCC(*i*)[b]
1	Acrosome	1.0000	0.9997	0.9997	0.9353
2	Cell membrane	0.9986	0.9907	0.9914	0.9505
3	Cell wall	0.9796	0.9990	0.9988	0.9158
4	Centrosome	1.0000	0.9961	0.9961	0.8712
5	Chloroplast	0.9948	0.9988	0.9986	0.9851
6	Cyanelle	1.0000	1.0000	1.0000	1.0000
7	Cytoplasm	0.8477	0.9559	0.9254	0.8137
8	Cytoskeleton	1.0000	0.9959	0.9960	0.9024
9	Endoplasmic reticulum	0.9978	0.9970	0.9970	0.9741
10	Endosome	1.0000	0.9992	0.9992	0.9336
11	Extracell	0.9962	0.9955	0.9956	0.9815
12	Golgi apparatus	0.9961	0.9963	0.9963	0.9452
13	Hydrogenosome	1.0000	1.0000	1.0000	1.0000
14	Lysosome	1.0000	0.9999	0.9999	0.9913
15	Melanosome	1.0000	1.0000	1.0000	1.0000
16	Microsome	1.0000	0.9995	0.9995	0.8742
17	Mitochondrion	1.0000	0.9940	0.9945	0.9636
18	Nucleus	0.8858	0.9550	0.9343	0.8429
19	Peroxisome	1.0000	0.9988	0.9988	0.9609
20	Spindle pole body	1.0000	0.9991	0.9991	0.9518
21	Synapse	1.0000	0.9994	0.9994	0.9504
22	Vacuole	1.0000	0.9984	0.9985	0.9657

[a]See Table 1 and the relevant context for further explanation. [b]See Equation (7) for the metrics definition.

format. 3) Click on the Submit button to see the predicted result; e.g., if you use the four protein sequences in the Example window as the input, after 10 seconds or so, you will see a new screen shown up (Figure 3). Listed on its upper part are the names of the subcellular locations numbered from "1" to "22" that are covered by the predictor for the eukaryotic proteins. Shown in its lower part is a table of two columns. Listed in the left-column are the IDs of query proteins; listed in the right column are the predicted subcellular locations denoted by the integer numbers within the range of 1 to 22. As we can see from the figure, the output for the query protein Q63564 of example-1 is "1," meaning it belonging to "acrosome" only; the output for the query protein P23276 of example-2 is "2, 8" meaning it belonging to "cell membrane" and "cytoskeleton"; the output for the query protein Q9VVV9 of example-3 is "2, 7, 18", meaning it belonging to "cell membrane", "cytoplasm", and "nucleus"; the output for the query protein Q673G8 of example-4 is "2, 7, 10, 18", meaning it belonging to "cell membrane", "cytoplasm", "endosome", and "nucleus". All these results are perfectly consistent with experimental observations.

As shown on the lower panel of Figure 2, you may also choose the batch prediction by entering your



pLoc_bal-mEuk: predict subcellular localization of eukaryotic proteins by deep learning treatment

Enter query sequences

Enter the sequences of query proteins in FASTA format (Example): the number of proteins is limited at 10 or less for each submission.

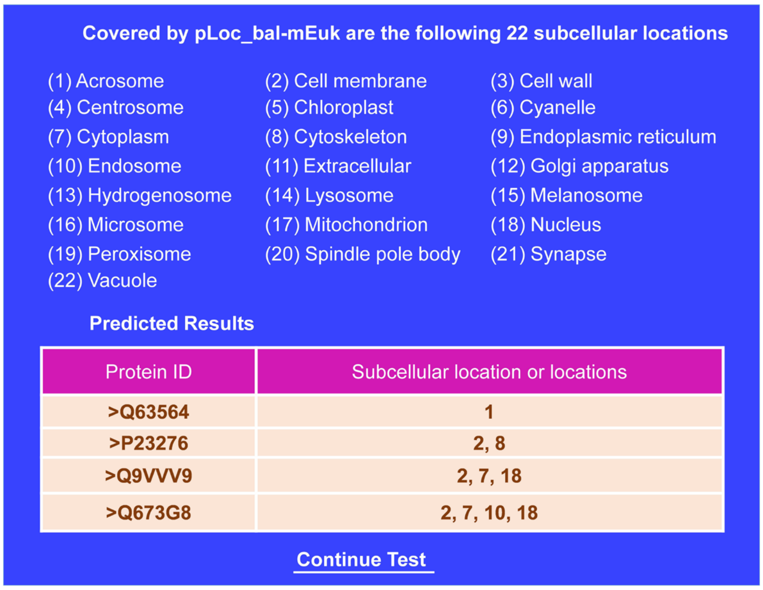
Or, upload a file for batch prediction

Enter your e-mail address and upload the batch input file (Batch-example). The predicted result will be sent to you by e-mail once completed; it usually takes 1 minute or so for each protein sequence

Upload file:

Your Email:

Figure 2. A semi screenshot for the top page of pLoc_bal-mEuk.



Covered by pLoc_bal-mEuk are the following 22 subcellular locations

(1) Acrosome (2) Cell membrane (3) Cell wall
(4) Centrosome (5) Chloroplast (6) Cyanelle
(7) Cytoplasm (8) Cytoskeleton (9) Endoplasmic reticulum
(10) Endosome (11) Extracellular (12) Golgi apparatus
(13) Hydrogenosome (14) Lysosome (15) Melanosome
(16) Microsome (17) Mitochondrion (18) Nucleus
(19) Peroxisome (20) Spindle pole body (21) Synapse
(22) Vacuole

Predicted Results

Protein ID	Subcellular location or locations
>Q63564	1
>P23276	2, 8
>Q9VVV9	2, 7, 18
>Q673G8	2, 7, 10, 18

Figure 3. A semi screenshot for the webpage obtained by following Step 3 of Section 3.5.4.

e-mail addresses and your batch input file (in FASTA format of course) via the Browse button. To see the sample of batch input file, click on the button Batch-example. After clicking the button Batch-submit, you will see "Your batch job is under computation; once the results are available, you will be notified by e-mail."

4. CONCLUSIONS AND PERSPECTIVE

The development of protein subcellular location prediction can be separated into two stages. In the early stage, all the prediction methods were developed with the assumption that each of the constituent proteins in a cell was located in one and only one location (organelle). Although those methods did play important roles in stimulating the development of such a fundamental area in cell molecular biology and proteomics, the aforementioned original hypothesis has been proved not completely correct. With more experimental data available, it has been found that many protein molecules may simultaneously exist or move between two or more subcellular location sites. It is these multiplex proteins that are of significance for in-depth understanding the biological processes in a living cell.

Since a multiplex protein needs the multiple labels to mark its locations, the multi-label theory and techniques [66] have been introduced into this frontier area of molecular biology. Meanwhile, to examine the power of a multi-label predictor, two sets of metrics have been introduced: one is the set of global metrics for evaluating its accuracy for an entire cell or in the global level, and the other is the set of local metrics for evaluating its accuracy for a specific subcellular location or in the local level. Of these metrics, the most important is the one for measuring the success rate of "absolute true" at the global level, which is also the harshest one for improvement.

The predictors introduced in this review paper have been all established by following the 5-steps rule [3], and hence they each have a user-friendly web server for the majority of experimental scientists to easily get their desired data. Also, their cornerstones are based on PseAAC [3, 47, 48, 173, 245], and hence their prediction quality is usually higher than the other methods.

It has not escaped our notice that since multi-label proteins usually have some unique or exceptional functions [37, 38, 74, 246], the advance in predicting this kind of proteins is far beyond the meaning of merely understanding the biological process concerned. It will play increasingly important roles for designing multi-target drugs [247-251], which represents a very hot trend currently in drug development [252].

It is instructive to point out that, in comparison with their counterparts, the benchmark datasets in Section 3.1 have the following two merits: 1) more stringent in excluding homology bias, and 2) cover more location sites. It is expected, however, with more experimental data available in future, they will also need updated in both the stringent criteria and coverage scope, so as to further empower the multi-label predictors in Section 3.5.4.

Finally, it is illuminative to point out that using graphic approaches to study biological and medical systems can provide an intuitive vision and useful insights for helping analyze complicated relations therein as shown in the systems of enzyme fast reaction [253-255], graphical rules in molecular biology [256-259], and low-frequency internal motion in biomacromolecules (such as protein and DNA) [260]. Particularly, what happened is that this kind of insightful implication has also been demonstrated in [261] and many follow-up publications [262-285].

CONFLICTS OF INTEREST

The author declares no conflicts of interest regarding the publication of this paper.

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