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MultiClassMetabo: A Superior Classification Model Constructed Using Metabolic Markers in Multiclass Metabolomics

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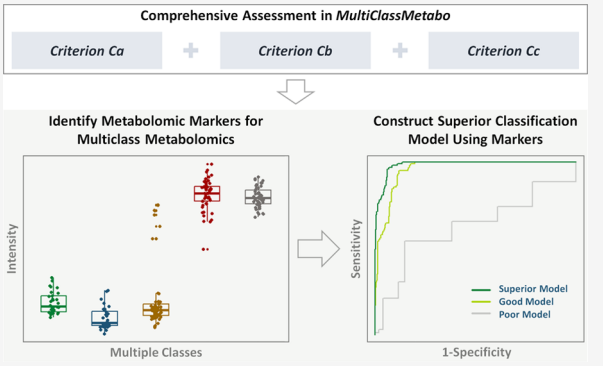
ABSTRACT: Multiclass metabolomics has become a popular technique for revealing the mechanisms underlying certain physiological processes, different tumor types, or different therapeutic responses. In multiclass metabolomics, it is highly important to uncover the underlying biological information on biosamples by identifying the metabolic markers with the most associations and classifying the different sample classes. The classification problem of multiclass metabolomics is more difficult than that of the binary problem. To date, various methods exist for constructing classification models and identifying metabolic markers consisting of well-established techniques and newly emerging machine learning algorithms. However, how to construct a superior classification model using these methods remains unclear for a given multiclass metabolomic data set. Herein, *MultiClassMetabo* has been developed for constructing a superior classification model using metabolic markers identified in multiclass metabolomics. *MultiClassMetabo* can enable online services, including (*a*) identifying metabolic markers by marker identification methods, (*b*) constructing classification models by classification methods, and (*c*) performing a comprehensive assessment from multiple perspectives to construct a superior classification model for multiclass metabolomics. In summary, *MultiClassMetabo* is distinguished for its capability to construct a superior classification model using the most appropriate method through a comprehensive assessment, which makes it an important complement to other available tools in multiclass metabolomics. *MultiClassMetabo* can be accessed at http://idrblab.cn/multiclassmetabo/.



INTRODUCTION

Metabolomics can provide unique insights into biological processes and is a valuable resource for specific diseases through the study of thousands of small molecules. In particular, multiclass metabolomics has become a hotspot, attracting much attention to the study of the trends of metabolic markers among multiple sample groups.[1] It can directly reveal different process stages of complex diseases,[2] different types of drug action effectiveness,[3,4] and differences among similar diseases in the clinic.[5] Moreover, the construction of classification models is an integral part of classifying different samples into multiple classes in multiclass metabolomics.[6] Compared with that of case-control studies, the classification of multiclass metabolomics is much more complicated as the number of sample classes increases.[7]

To date, various methods have been used for constructing classification models in multiclass metabolomics, such as random forests.[8,9] Due to the differences in the principles of these classification methods, conflicting outcomes are observed when different classification methods are applied, even for the same data set.[10,11] Therefore, it is highly necessary to apply an appropriate classification method with superior performance for a specific data set. To select the most suitable method, the performance of all classification methods must be assessed using well-established criteria.[12,13] Apart from classification methods, methods of identifying metabolic markers are also of importance for the performance of the classification model in multiclass metabolomics.[14,15]

When suitable methods for identifying markers and classification are applied, a well-performing classification model can be constructed for a given data set. To select suitable methods, the assessment of these methods is indispensable before applying the methods directly.[16,17] Moreover, a single criterion is insufficient for a comprehensive assessment of these models.[18] Multiple different criteria are important for obtaining reliable results.[7,19] Therefore, a comprehensive assessment from different perspectives using

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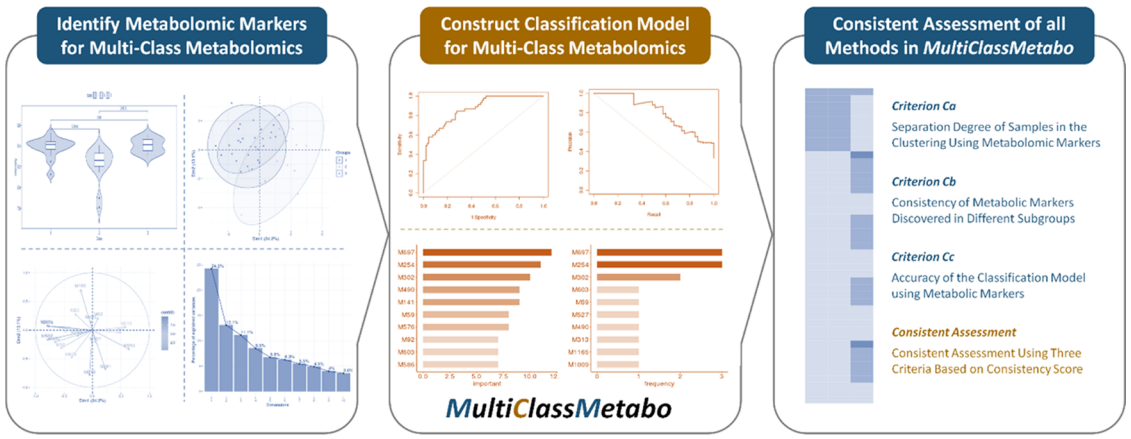

Figure 1. Key features of *MultiClassMetabo*. *MultiClassMetabo* can identify metabolomic markers (left panel), construct a classification model (middle panel) for multiclass metabolomics, and discover the appropriate method by consistent assessment from a set of methods for identifying metabolomic markers and constructing classification models (right panel).

multiple criteria is highly necessary for selecting suitable methods for discovering metabolic markers and constructing classification models.

Currently, several popular online tools have been developed for metabolomic analysis. These web servers include *XCMS Online*,[20] *MetaboAnalyst*,[21] *MetaDB*,[22] *NOREVA*,[1] *MetDAT*,[23] *Metabolomics Workbench*,[24] *Workflow4MeTabolomics*,[25] etc. As shown in Supporting Table S1, most of these web servers focus on analyzing multiclass metabolomic data, and only *XCMS Online* and *MetaboAnalyst* provide a classification function. However, none of them employ multiple criteria for assessing the performance of classification methods in multiclass metabolomics. In addition, none of these web services have been developed to consider the effects of metabolic marker methods on the classification results, especially in multiclass metabolomics, which seriously limits the wide application of these well-performing methods. Therefore, it is necessary to develop an online tool that not only provides many methods for identifying metabolic markers and constructing classification models in multiclass metabolomics but also can select the method with the best performance through a comprehensive assessment. However, such a tool is not yet available for identifying the most appropriate methods and building a superior classification model in multiclass metabolomics.

Herein, *MultiClassMetabo* was developed to construct a superior classification model in multiclass metabolomics (as shown in Figure 1). *MultiClassMetabo* enables online services, including (*a*) discovering metabolic markers for multiclass metabolomic data using 5 methods, (*b*) constructing multiclass classification models using 9 methods, and (*c*) enabling consistent assessment for all methods of discovering metabolic markers and building classification models from multiple perspectives. In summary, *MultiClassMetabo* is unique in that it can select the most appropriate method to obtain a superior classification model using a comprehensive assessment and enhance its popularity for a given multiclass metabolomic data set. *MultiClassMetabo* is freely accessible at http://idrblab.cn/multiclassmetabo/.

MATERIALS AND METHODS

Comprehensive Collection of Methods for Metabolic Markers and Classification. Both methods of discovering metabolic markers and methods of building classification models have a key effect on the classification model performance in multiclass metabolomics. In *MultiClassMetabo*, five methods of identifying metabolic markers were collected, including the Kruskal−Wallis test (KWT),[26] one-way analysis of variance (ANOVA),[27] partial least-squares-discriminant analysis (PLS-DA),[28] variable selection using random forests (VSURF),[29] and support vector machine-recursive feature elimination (SVM-RFE).[30] In addition, nine methods of building classification models frequently adopted in multiclass metabolomics were collected, including adaptive boosting (AdaBoost),[31] bagging,[32] decision trees (DT),[33] k-nearest neighbor (KNN),[34] linear discriminant analysis (LDA),[35] native Bayes (NB),[36] partial least-squares (PLS),[37] random forest (RF),[38] and support vector machine (SVM).[39] The descriptions and applications of these methods are illustrated in detail in Supporting Table S2. As described in Supporting Table S2, an abbreviation is applied to represent each corresponding method in this study. Moreover, 45 strategies were obtained by combining metabolic marker identification and classification methods, which were indicated by the corresponding abbreviations. For instance, the strategy combining support vector machine-recursive feature elimination as a marker identification method and decision trees as a classification method is depicted as SVM-RFE + DT. The 45 combined strategies for classification in multiclass metabolomics were used for a comprehensive assessment in *MultiClassMetabo*. To our knowledge, these 45 combined strategies represent the largest set of strategies provided by an available web server thus far to identify metabolomic markers and construct classification models in multiclass metabolomics.

Multiple Criteria for Comprehensive Performance Assessment. In this study, the comprehensive assessment of methods for identifying metabolomic markers and constructing classification models was achieved by using three evaluation criteria for multiclass metabolomic studies. Additionally, one metric was selected to quantify the performance of the methods under each criterion. Based on the well-defined cutoff of each metric, the performance of different methods can be categorized into superior, good, or poor.

Criterion *Ca*: Separation Degree of Samples in Clustering Analysis Using Metabolomic Markers. Clustering analysis was adopted to indicate the separation degree of samples in multiple classes using metabolic markers for multiclass metabolomic data.[40,41] For a clustering outcome,

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Table 1. Six Multiclass Metabolic Benchmarks Were Used for the Comprehensive Assessment of Methods for Discovering Metabolic Markers and Constructing Classification Models in This Study[a]

data set ID	no. samples	no. classes	platform	data set description
ST000584	208	5	LC-MS HILIC pos	751 metabolites from the tissue of the zebrafish in five groups (2-, 24-, and 48-h postfeeding of fasting wild type, as well as *tfeb* and *lmna* knockout mutant).[53]
MTBLS146	179	3	LC-MS RP pos	1162 metabolites from the maternal plasma of 179 healthy pregnant women in each of three trimesters.[54]
MTBLS315	61	3	LC-MS RP pos	774 metabolites from the plasma of 61 children in three groups (malaria, nonmalarial febrile illness, and bacterial bloodstream infection).[55]
MTBLS413	60	3	LC-MS HILIC pos	462 metabolites from Angolan patients infected with Trypanosomiasis at different disease stages (stage 1, advanced stage 2, and control).[56]
ST000294	45	5	LC-MS RP pos	538 metabolites from the diapausing pupae of the flesh fly at five sampling points across the metabolic cycles.[57]
ST000047	45	3	LC-MS HILIC pos	831 metabolites from the cerebrospinal fluid of Alzheimer's disease patients, mild cognitive impairment, and cognitively normal individuals.[58]

[a]RP: reversed phase, pos: positive, and HILIC: hydrophilic interaction liquid chromatography.

a method of identifying metabolic markers was regarded as superior when an obvious separation was observed for different sample classes. A well-established metric (*purity*) in the clustering analysis was used as a representative measure to assess the quality of clustering.[42] If the *purity* value is equal to 0, the quality of the clustering is poor; if the *purity* value is equal to 1, the quality of the clustering is excellent.[43] When the *purity* values are within the ranges of >0.8, ≤ 0.8, > 0.5, and ≤0.5, the corresponding methods are categorized into those with superior, good, and poor performance, respectively.

Criterion *Cb*: Consistency of Metabolic Markers Identified in Different Subgroups. The consistency of metabolic markers identified in different subgroups was regarded as an indispensable criterion to assess different methods.[44,45] In this study, the raw data were first divided into three subgroups by random sampling. Three lists of metabolic markers were discovered from three subgroups based on the same method of identifying metabolic markers. Then, the relative weighted consistency (*CWrel*) was calculated and used as a powerful metric to quantitatively assess the stability of metabolic markers from the three subgroups.[44,46] If the *CWrel* value is equal to 1, the consistency of metabolomic markers from different subgroups is high.[47] When the *CWrel* values are within the ranges of >0.3, ≤ 0.3, > 0.15, and ≤0.15, the corresponding methods are categorized into those with superior, good, and poor performance, respectively.

Criterion *Cc*: Accuracy of the Classification Model Using Metabolic Markers. For a multiclass metabolomic data set, the first step is identifying the metabolic markers by the most suitable method. Based on the identified metabolic markers, a multiclass classification model can be constructed using a specific classification method.[48,49] Both the receiver operating characteristic (*ROC*) curve and the area under the curve (*AUC*) value were applied to quantitatively assess the performance of the classification model.[50] If the *AUC* value is close to 1, the performance of the classification method using the identified metabolic markers is excellent for the studied multiclass metabolomic data.[51] When the *AUC* values are within the ranges of >0.9, ≤ 0.9, > 0.7, and ≤0.7, the corresponding methods are categorized into those with superior, good, and poor performances, respectively.

Multiclass Metabolomic Benchmarks Collected for the Case Study. As shown in Table 1, six multiclass metabolomic benchmark data sets from the publicly available *MetaboLights*[52] or *Metabolomics Workbench*[24] were applied to test the utility of *MultiClassMetabo*. Among these benchmarks, there were dozens to hundreds of samples divided into five or three sample classes. There were hundreds to thousands of metabolites in various species (including zebrafish, human, and flesh fly) in these six multiclass metabolomic data sets. Particularly, data set ST000584 contains 751 metabolites from the tissue of zebrafish, where 208 samples are divided into five groups (2-, 24-, 48-h postfeeding of fasting wild type, as well as *tfeb* and *lmna* knockout mutant).[53] Data set MTBLS146 consists of 1,162 metabolites from the maternal plasma of 180 healthy pregnant women in each trimester.[54] Data set MTBLS315 is composed of 774 metabolites from the plasma of 61 children in three groups (malaria, nonmalarial febrile illness, and bacterial bloodstream infection).[55] Data set MTBLS413 consists of 462 metabolites in 60 samples from Angolan patients infected with trypanosomiasis at different disease stages (stage 1, advanced stage 2, and control).[56] Data set ST000294 contains 538 metabolites in 45 samples from the diapausing pupae of the flesh fly at five sampling points across the metabolic cycles.[57] Data set ST000047 is composed of 831 metabolites from 45 samples in the cerebrospinal fluid of Alzheimer's disease patients, mild cognitive impairment patients, and cognitively normal individuals.[58] Each raw data set was filtered using 80% rules, and the missing values were imputed through the KNN imputation method. Then, the imputed data were processed by the log transformation method and the Pareto scaling method.

Server Implementation Details and Format of Input Files Required. *MultiClassMetabo* was deployed on a web service based on the Cent OS Linux v6.5 operating system. *MultiClassMetabo* can be accessed by all users without a login requirement. Users can visit *MultiClassMetabo* using popular web browsers, including Google Chrome, Microsoft Edge, Safari, and Internet Explorer. The sample-by-feature matrix in *csv* format is the input file of this server. In this file, samples are set in rows and metabolites are set in columns. The sample ID is in the first column and must be unique for all samples. The class of samples is given in the second column. The metabolite ID is in the first row and should be unique across all metabolites. The exemplar file is provided on the *MultiClassMetabo* Web site, and users can directly download this exemplar file.

RESULTS AND DISCUSSION

Exploration of Multiclass Metabolomic Data Enabled by *MultiClassMetabo*. To test the utility of *MultiClassMetabo*, six multiclass metabolomic benchmark data sets were

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Table 2. Performance under Three Criteria for the Representative Methods of Discovering Metabolic Markers and Building Classification Models Based on Different Benchmark Data Sets[a]

Dataset	Methods	Criterion *Ca*	Criterion *Cb*	Criterion *Cc*
ST000584	PLS-DA + Bagging	**0.80**	**0.79**	**0.99**
	VSURF + SVM	0.61	0.13	**0.96**
	KWT + LDA	0.43	0.13	0.63
MTBLS146	VSURF + KNN	**0.89**	**0.40**	**0.99**
	SVM-RFE + DT	0.74	0.26	0.89
	ANOVA + NB	0.47	0.54	0.69
MTBLS315	VSURF + AdaBoost	0.70	**0.31**	**0.92**
	KWT + LDA	0.66	0.29	0.81
	SVM-RFE + PLS	0.64	0.29	0.69
MTBLS413	VSURF + SVM	**0.80**	**0.39**	**0.98**
	KWT + DT	0.47	**0.39**	0.85
	SVM-RFE + DT	0.73	0.13	0.80
ST000294	PLS-DA + RF	**0.80**	**0.48**	**0.94**
	ANOVA + PLS	0.47	**0.34**	0.87
	SVM-RFE + KNN	0.31	0.06	0.56
ST000047	KWT + SVM	**0.80**	**0.40**	**0.92**
	ANOVA + RF	0.51	**0.33**	0.74
	PLS-DA + LDA	0.47	0.28	0.75

[a]The font of each assessment result is colored dark green, light green, and gray for superior, good, and poor performance, respectively.

applied in this study. The performance of the methods for identifying metabolic markers and constructing classification models is demonstrated in Table 2. Three representative methods were selected to illustrate the differential performance of various methods for each of the benchmark data. As shown in Table 2, the font of each assessment result for the superior, good, and poor methods is colored dark green, light green, and gray, respectively. The performance of different methods varied significantly even for the same data set. For data set ST000584, the performance of the PLS-DA + Bagging method was consistently superior based on all 3 criteria, the performance of the VSURF + SVM method was good or superior using some criteria but poor using other criteria, and the performance of the KWT + LDA method was consistently poor based on all 3 criteria. For data set MTBLS146, the performance of the VSURF + KNN method was consistently superior based on all 3 criteria, the performance of the SVM-RFE + DT method was good or superior using some criteria but poor using other criteria, and the performance of the ANOVA + NB method was consistently poor based on all 3 criteria. For data set MTBLS315, the performance of the VSURF + AdaBoost method was consistently superior based on all 3 criteria, the performance of the KWT + LDA method was good or superior using some criteria but poor using other criteria, and the performance of the SVM-RFE + PLS method was consistently poor based on 3 criteria.

For data set MTBLS413, the performance of the VSURF + SVM method was consistently superior based on all 3 criteria, the performance of the KWT + DT method was good or superior using some criteria but poor using other criteria, and the performance of the SVM-RFE + DT method was consistently poor based on all 3 criteria. For data set ST000294, the performance of the PLS-DA + RF method was consistently superior based on all 3 criteria, the performance of the ANOVA + PLS method was good or superior using some criteria but poor using other criteria, and the performance of the SVM-RFE + KNN method was consistently poor based on all 3 criteria. For data set ST000047, the performance of the KWT + SVM method was consistently superior based on all 3 criteria, the performance of the ANOVA + RF method was good or superior using some criteria but poor using other criteria, and the performance of the PLS-DA + LDA method was consistently poor based on all 3 criteria. There were conflicting outcomes when different methods of discovering metabolic markers and building classification models were applied even for the same data set. Therefore, in multiclass metabolomics, the superior classification model using appropriate metabolic markers needed a systematic evaluation using multiple criteria proposed in *MultiClassMetabo*.

Assessment of Methods for Identifying Metabolic Markers. For the six benchmarks, the differences in the metabolic markers among multiple classes are displayed using boxplots, as shown in Figure 2. In the boxplots, if the *p*-value from the *t*-test in two classes was less than 0.05, it was flagged with one star above the corresponding boxplot. If the *p*-value was less than 0.01, two stars were flagged above the corresponding boxplot. If the *p*-value was less than 0.001, three stars were flagged above the corresponding boxplot. The differences in the metabolic markers among multiple classes

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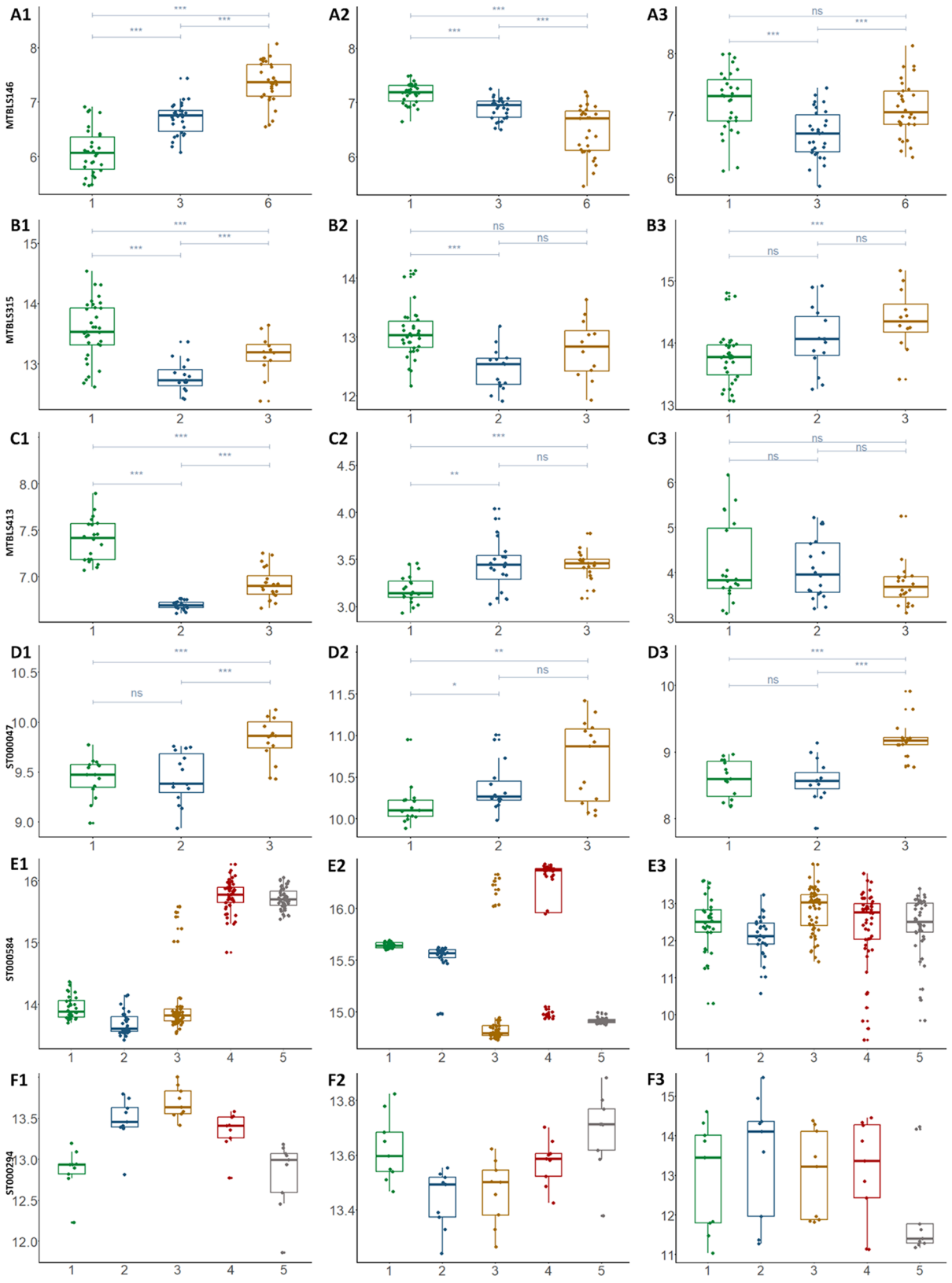

Figure 2. Boxplots of the differences in the metabolic markers identified by the representative methods for six benchmarks. The metabolic marker was identified by the method with a superior performance based on the data sets (A1) MTBLS146, (B1) MTBLS315, (C1) MTBLS413, (D1) ST000047, (E1) ST000584, and (F1) ST000294. The metabolic marker was identified by the method with good performance based on data sets (A2) MTBLS146, (B2) MTBLS315, (C2) MTBLS413, (D2) ST000047, (E2) ST000584, and (F2) ST000294. The metabolic marker was identified by the method with poor performance based on the data sets (A3) MTBLS146, (B3) MTBLS315, (C3) MTBLS413, (D3) ST000047, (E3) ST000584, and (F3) ST000294. If the *p*-value from the *t*-test between two classes is less than 0.05, 0.01, and 0.001, the plot is flagged with one star (*), two stars (**), and three stars (***) above the boxplots, respectively.

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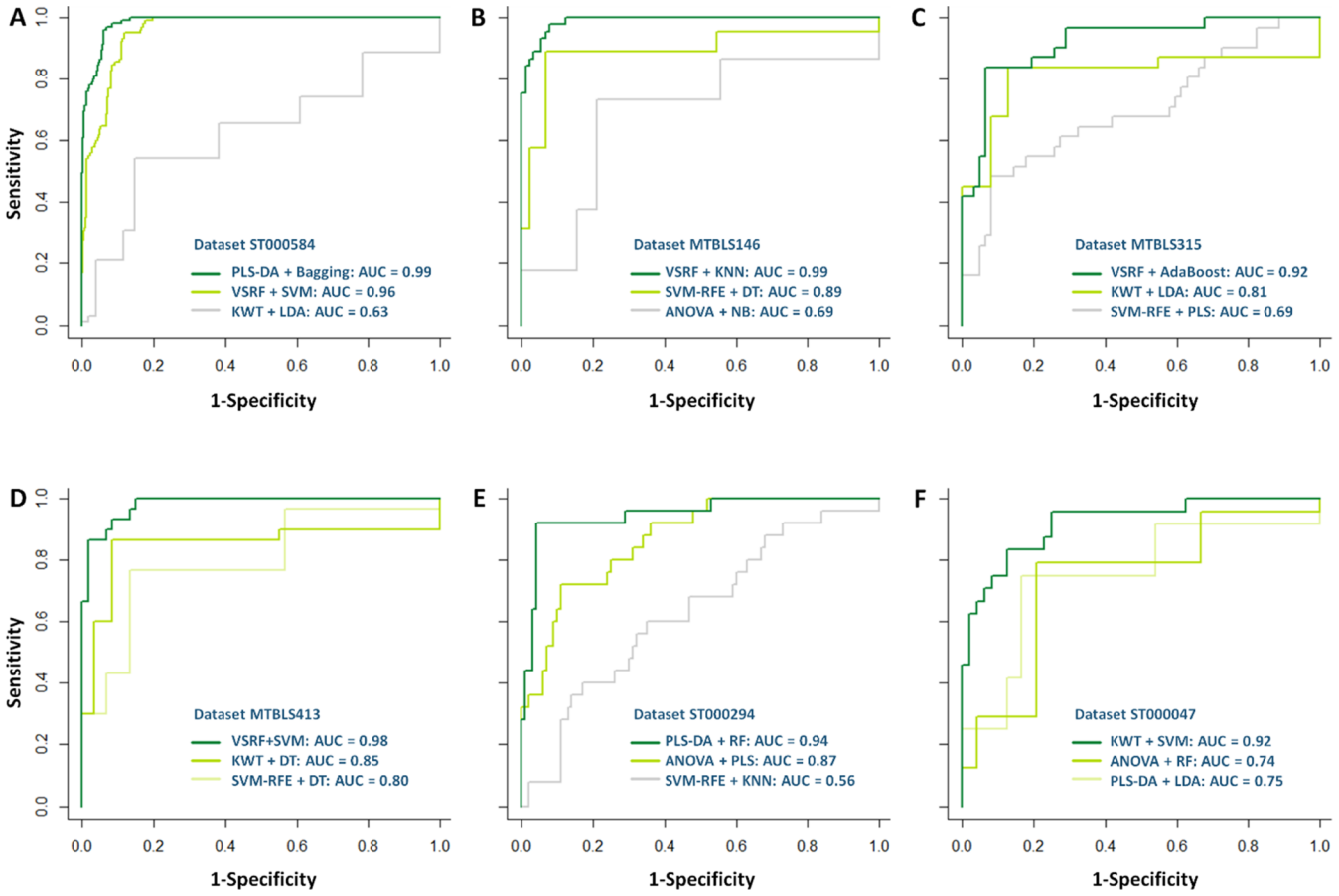

Figure 3. ROC curves and AUC values were applied to visualize the performance of the classification model for each benchmark data set. The ROC curve is colored dark green, light green, and gray when using the methods with superior, good, and poor performance, respectively.

were more obvious when these markers were discovered by a method with a superior performance for identifying metabolic markers. For data set MTBLS146, the boxplots of differences in the metabolic marker identified by three representative methods are shown in Figure 2A. As shown in Figure 2A1, the metabolic marker ranking first using the VSURF method with superior performance significantly differed among the two sample classes. As shown in Figure 2A2, the metabolic marker ranking first using the SVM-RFE method with good performance was also significantly different among the two sample classes. As shown in Figure 2A3, the metabolic marker ranking first using the ANOVA method with poor performance significantly differed between the second class and the other classes, and there was no significant difference between the first and third classes. For data set MTBLS315, the boxplots of differences in the metabolic marker identified by three representative methods are shown in Figure 2B. As shown in Figure 2B1, the metabolic marker ranking first using the VSURF method with superior performance significantly differed between any two sample classes. As shown in Figure 2B2, the metabolic marker ranking first using the KWT method with good performance was significantly different between only the first and second classes. As shown in Figure 2B3, the metabolic marker ranking first using the SVM-RFE method with poor performance was significantly different only between the first and third classes.

For data set MTBLS413, the boxplots of differences in the metabolic marker identified by three representative methods are shown in Figure 2C. As shown in Figure 2C1, the metabolic marker ranking first using the VSURF method with superior performance significantly differed between the two sample classes. As shown in Figure 2C2, the metabolic marker ranking first using the KWT method with good performance significantly differed, except between the second and third classes. As shown in Figure 2C3, none of the metabolic markers ranking first using the SVM-RFE method with poor performance were significantly different between any two classes. For data set ST000047, the boxplots of differences in the metabolic marker identified by three representative methods are shown in Figure 2D. As shown in Figure 2D1, the metabolic marker ranking first using the KWT method with superior performance significantly differed between the third and first/second classes. As shown in Figure 2D2, the metabolic marker ranking first using the ANOVA method with good performance significantly differed between the first and second/third classes. As shown in Figure 2D3, the metabolic marker ranking first using the PLS-DA method with poor performance significantly differed between the third and first/second classes.

For data set ST000584, the boxplots of differences in the metabolic marker identified by three representative methods are shown in Figure 2E. As shown in Figure 2E1, the difference in the metabolic marker ranking first using the PLS-DA method with superior performance was significant among different classes. As shown in Figure 2E2, the difference in the metabolic marker ranking first using the VSURF method with good performance was slightly significant among different classes. As shown in Figure 2E3, there was almost no difference

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in metabolic marker ranking first using the KWT method, with poor performance among different classes. For data set ST000294, the boxplots of differences in the metabolic marker identified by three representative methods are shown in Figure 2F. As shown in Figure 2F1, the difference in metabolic marker ranking first using the PLS-DA method with superior performance was significant among different classes. As shown in Figure 2F2, the difference in metabolic marker ranking first using the ANOVA method with good performance was slightly significant among different classes. As shown in Figure 2F3, there was almost no difference in metabolic marker ranking first using the SVM-RFE method with poor performance among different classes. Therefore, the difference in the metabolic marker identified using a method with superior performance was more significant among different classes than that of a method with poor performance. Thus, selecting the appropriate method is important for identifying the key metabolic markers.

Assessment of Methods for Constructing the Classification Models. The nature of the classification methods played a key role in the performance of the classification model. Moreover, the methods of identifying metabolomic markers also had an important influence on the performance of these models.[59] Therefore, both methods of discovering metabolomic markers and methods of building classification models should be accounted for when selecting superior classification models.[60] Herein, the ROC curve and AUC value can be applied to visualize the performance of different classification models for each benchmark data set. As shown in Figure 3, the ROC curve is colored dark green, light green, and gray when using the methods with superior, good, and poor performance, respectively. For data set ST000584 (shown in Figure 3A), the AUC values were 0.99, 0.96, and 0.63 using the methods with superior (PLS-DA + Bagging), good (VSURF + SVM), and poor (KWT + LDA) performance, respectively. For data set MTBLS146 (shown in Figure 3B), the AUC values were 0.99, 0.89, and 0.69 using the methods with superior (VSURF + KNN), good (SVM-RFE + DT), and poor (ANOVA + NB) performance, respectively. For data set MTBLS315 (shown in Figure 3C), the AUC values were 0.92, 0.81, and 0.69 using methods with superior (VSURF + AdaBoost), good (KWT + LDA), and poor (SVM-RFE + PLS) performance, respectively. For data set MTBLS413 (shown in Figure 3D), the AUC values were 0.98, 0.85, and 0.80 using methods with superior (VSURF + SVM), good (KWT + DT), and poor (SVM-RFE + DT) performance, respectively. For data set ST000294 (shown in Figure 3E), the AUC values were 0.94, 0.87, and 0.56 using methods with superior (PLS-DA + RF), good (ANOVA + PLS), and poor (SVM-RFE + KNN) performance, respectively. For data set ST000047 (shown in Figure 3F), the AUC values were 0.92, 0.74, and 0.75 using methods with superior (KWT + SVM), good (ANOVA + RF), and poor (PLS-DA + LDA) performance, respectively.

Based on the comprehensive assessment in this study, different results are obtained when different methods are applied, even for the same data set. Based on the assessment using the separation degree of samples in clustering analysis, consistency of metabolomic markers in different subgroups, and accuracy of the classification model, PLS-DA, VSURF, and KWT were identified as the superior methods of metabolomic markers for different data sets, and Bagging, KNN, AdaBoost, SVM, and RF were identified as the superior methods of building multiclass classification models for different data sets. The most suitable methods of discovering key metabolic markers and constructing the multiclass model are significantly different for vast data sets. To construct a superior classification model for the given data set, the assessment of methods for discovering metabolic markers and constructing classification models is needed in multiclass metabolomics. Moreover, multiple criteria are especially necessary for the comprehensive assessment of selecting the most appropriate methods for building classification models using the key metabolic markers. Therefore, *MultiClassMetabo* is essential as an online server to assess many methods for metabolic markers and classification to discover a superior classification model for specific data in multiclass metabolomics. In the future, *MultiClassMetabo* might become a popular tool to construct a classification model in multiclass metabolomics.

In this study, there are still some limitations for *MultiClassMetabo*. First, more novel methods based on deep learning of identifying metabolic markers and constructing classification models need to be collected to build the most appropriate multiclass metabolomic model. Second, more assessment criteria are necessary for evaluating different methods in multiple steps of multiclass metabolomics. In the future, multiple criteria can be combined to select the most suitable method combinations from multiple perspectives. Third, similar to multiclass metabolomics, the comprehensive assessment of these methods for identifying metabolic markers and constructing classification models can be expanded for other multiclass omics data (such as transcriptomics, proteomics, microbiomics, and exposomics). It is expected that constructing a superior classification model using metabolic markers can attract increasing attention in multiclass omics.

CONCLUSIONS

In this study, *MultiClassMetabo* was constructed to enable an online service for constructing a superior classification model using metabolic markers in multiclass metabolomics. *MultiClassMetabo* provides (*a*) methods of identifying metabolic markers, (*b*) methods of constructing classification models, and (*c*) the selection of a superior classification model by using a comprehensive assessment in multiclass metabolomics. Overall, *MultiClassMetabo* provides a unique feature for a superior classification model using the most appropriate method in multiclass metabolomics. The *MultiClassMetabo* Web site can be freely accessed at http://idrblab.cn/multiclassmetabo/.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.3c03212.

Coverage of web servers popular in currently available online pipelines of multiclass metabolomics (Table S1) and descriptions of methods popular in multiclass metabolomic studies for identifying metabolic markers and classification (Table S2) (PDF)

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Notes

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