Ovarian Cancer Histotypes: Report of Statistical Findings

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April 11, 2025

Table of contents

Preface **6**

1 Introduction **7**

2 Methods **8**
2.1 Pre-Processing . . . 8
2.1.1 Case Selection . . . 8
2.1.2 Quality Control . . . 8
2.1.3 Normalization . . . 8
2.1.4 Final Processing . . . 10
2.2 Classifiers . . . 11
2.2.1 Resampling of Training Set . . . 11
2.2.2 Hyperparameter Tuning . . . 12
2.2.3 Subsampling . . . 12
2.2.4 Workflows . . . 13
2.3 Two-Step Algorithm . . . 13
2.3.1 Aggregating Predictions . . . 14
2.4 Sequential Algorithm . . . 15
2.4.1 Aggregating Predictions . . . 16
2.5 Gene Optimization . . . 17
2.5.1 Variable Importance . . . 19
2.6 Performance Evaluation . . . 19
2.6.1 Class Metrics . . . 19
2.6.2 AUC . . . 21

3 Distributions **22**
3.1 Histotype Distribution . . . 23
3.2 Cohort Distribution . . . 24
3.3 Quality Control . . . 24
3.3.1 Failed Samples . . . 24
3.3.2 %GD vs. SNR . . . 26
3.4 Pairwise Gene Expression . . . 28

4 Results **32**
4.1 Training Set . . . 32
4.1.1 Accuracy . . . 32
4.1.2 Sensitivity . . . 36
4.1.3 Specificity . . . 39
4.1.4 F1-Score . . . 42
4.1.5 Balanced Accuracy . . . 45
4.1.6 Kappa . . . 48

4.1.7 G-mean . . . 50
4.2 Rank Aggregation . . . 53
4.2.1 Across Classes . . . 54
4.2.2 Across Metrics . . . 57
4.2.3 Top Workflows . . . 57
4.3 Optimal Gene Sets . . . 61
4.3.1 Sequential Algorithm . . . 61
4.3.2 SMOTE-Random Forest . . . 62
4.4 Test Set Performance . . . 62
4.4.1 Confirmation Set . . . 63
4.4.2 Validation Set . . . 67

References **69**

List of Figures

2.1 Venn diagram of common and unique gene targets covered by each CodeSet 10
2.2 Cohorts Selection 11
2.3 Visualization of Subsampling Techniques 13
2.4 Two-Step Algorithm 14
2.5 Aggregating Predictions for Two-Step Algorithm 15
2.6 Sequential Algorithm 16
2.7 Aggregating Predictions for Sequential Algorithm 17

3.1 % Genes Detected vs. Signal to Noise Ratio 26
3.2 % Genes Detected vs. Signal to Noise Ratio (Zoomed) 27
3.3 Random1-Normalized CS1 vs. CS3 Gene Expression 28
3.4 Random1-Normalized CS2 vs. CS3 Gene Expression 29
3.5 HKgenes-Normalized CS1 vs. CS3 Gene Expression 30
3.6 HKgenes-Normalized CS2 vs. CS3 Gene Expression 31

4.1 Training Set Mean Accuracy 32
4.2 Training Set Class-Specific Mean Accuracy 34
4.3 Training Set Mean Sensitivity 36
4.4 Training Set Class-Specific Mean Sensitivity 37
4.5 Training Set Mean Specificity 39
4.6 Training Set Class-Specific Mean Specificity 40
4.7 Training Set Mean F1-Score 42
4.8 Training Set Class-Specific Mean F1-Score 43
4.9 Training Set Mean Balanced Accuracy 45
4.10 Training Set Class-Specific Mean Balanced Accuracy 46
4.11 Training Set Mean Kappa 48
4.12 Training Set Class-Specific Mean Kappa 49
4.13 Training Set Mean G-mean 51
4.14 Training Set Class-Specific Mean G-mean 52
4.15 Top 5 Workflow Per-Class Evaluation Metrics by Metric 58
4.16 Gene Optimization for Sequential Classifier 61
4.17 Gene Optimization for SMOTE-Random Forest Classifier 62
4.18 Confusion Matrices for Confirmation Set Models 64
4.19 ROC Curves for Sequential Full Model in Confirmation Set 65
4.20 ROC Curves for Sequential, Optimal Model in Confirmation Set 66
4.21 ROC Curves for SMOTE-Random Forest, Full Set Model in Confirmation Set 67
4.22 ROC Curves for SMOTE-Random Forest, Optimal Set Model in Confirmation Set . 67
4.23 Confusion Matrix for Validation Set Model 68
4.24 ROC Curves for SMOTE-Random Forest, Optimal Set Model in Validation Set . . . 68

List of Tables

2.1 Gene Distribution 18

3.1 Histotype Distribution in Training Set by Processing Stage 23
3.2 Histotype Distribution in Training, Confirmation, and Validation Sets 24
3.3 Pre-QC Cohort Distribution by CodeSet 24
3.4 Quality Control Summary 25

4.1 Training Set Mean Accuracy 33
4.2 Training Set Class-Specific Mean Accuracy 35
4.3 Training Set Mean Sensitivity 36
4.4 Cross-Validated Training Set Class-Specific Mean Sensitivity 38
4.5 Training Set Mean Specificity 39
4.6 Training Set Class-Specific Mean Specificity 41
4.7 Training Set Mean F1-Score 42
4.8 Training Set Class-Specific Mean F1-Score 44
4.9 Training Set Mean Balanced Accuracy 45
4.10 Training Set Class-Specific Mean Balanced Accuracy 47
4.11 Training Set Mean Kappa 48
4.12 Training Set Class-Specific Mean Kappa 50
4.13 Training Set Mean G-mean 51
4.14 Training Set Class-Specific Mean G-mean 53
4.15 54
4.16 55
4.17 56
4.18 Rank Aggregation Comparison of Metrics Used 57
4.19 Top 5 Workflows from Final Rank Aggregation 57
4.20 Evaluation Metrics on Confirmation Set Models 63
4.21 Evaluation Metrics on Validation Set Model, SMOTE-Random Forest, Optimal Set . 67

Preface

This report of statistical findings describes the classification of ovarian cancer histotypes using data from NanoString CodeSets.

Marina Pavanello conducted the initial exploratory data analysis, Cathy Tang implemented class imbalance techniques, Derek Chiu conducted the normalization and statistical analysis, and Lauren Tindale and Aline Talhouk are the project leads.

1 Introduction

Ovarian cancer has five major histotypes: high-grade serous carcinoma (HGSC), low-grade serous carcinoma (LGSC), endometrioid carcinoma (ENOC), mucinous carcinoma (MUC), and clear cell carcinoma (CCOC). A common problem with classifying these histotypes is that there is a class imbalance issue. HGSC dominates the distribution, commonly accounting for 70% of cases in many patient cohorts, while the other four histotypes are spread over the rest of the cases. Subsampling methods like up-sampling, down-sampling, and SMOTE can be used to mitigate this problem.

The supervised learning is performed under a consensus framework: we consider various classification algorithms and use evaluation metrics like accuracy, F1-score, and Kappa, to inform the decision of which methods to carry forward for prediction in confirmation and validation sets.

2 Methods

2.1 Pre-Processing

2.1.1 Case Selection

Raw data comes from three NanoString CodeSets (CS): CS1, CS2, and CS3. We divide the data into training, confirmation, and validation sets by using samples from these sets of cohorts:

- Training
  - CS1: MAYO, OOU, OOUE, VOA, MTL
  - CS2: MAYO, OOU, OOUE, OVAR3, VOA, ICON7, JAPAN, MTL, POOL-CTRL
  - CS3: OOU, OOUE, VOA, POOL-1, POOL-2, POOL-3
- Confirmation:
  - CS3: TNCO
- Validation:
  - CS3: DOVE4

2.1.2 Quality Control

Samples that failed any of the following NanoString quality control conditions were removed:

- `linFlag`: linearity of positive controls with positive control concentrations is less than 0.95, or linearity measures are unknown
- `imagingFlag`: percent of field of view is less than 75%
- `spcFlag`: smallest positive control is less than the lower limit of detection (negative control average expression less two times the negative control standard deviation), or negative control average expression equals zero
- `normFlag`: signal to noise ratio less than 100, or percent of genes detected is less than 50. Note: these thresholds were determined by examining the [%GD vs. SNR] relationship below.

2.1.3 Normalization

The full training set (n=1243) is comprised of data from CodeSets (CS) 1, 2, and 3. All CodeSets were first normalized to housekeeping genes, then different approaches were taken for subsequent normalizations of each CodeSet.

CS1 was normalized to CS3 using five "Random1" reference samples. These reference samples are randomly selected from CS1 among all samples in the three CodeSets that share common otta IDs, such that we obtain one sample from each of the five histotypes. Then, we use the reference-based method to normalize CS1 to CS3 across their common genes, for the remaining expression samples Talhouk et al. (2016).

Similarly, CS2 was normalized to CS3 using the same "Random1" reference samples, now taken from CS2. Normalization was performed across common genes between CS2 and CS3.

For CS3, we first split the dataset into three sites: Vancouver, USC, and AOC. We use the CS3-Vancouver subset as a "reference standard", and normalized CS3-USC and CS3-AOC to CS3-Vancouver using a "Random1" reference set randomly selected among samples common between Vancouver, USC, and AOC. Finally, the CS3-Vancouver expression samples are included in the training set without further normalization.

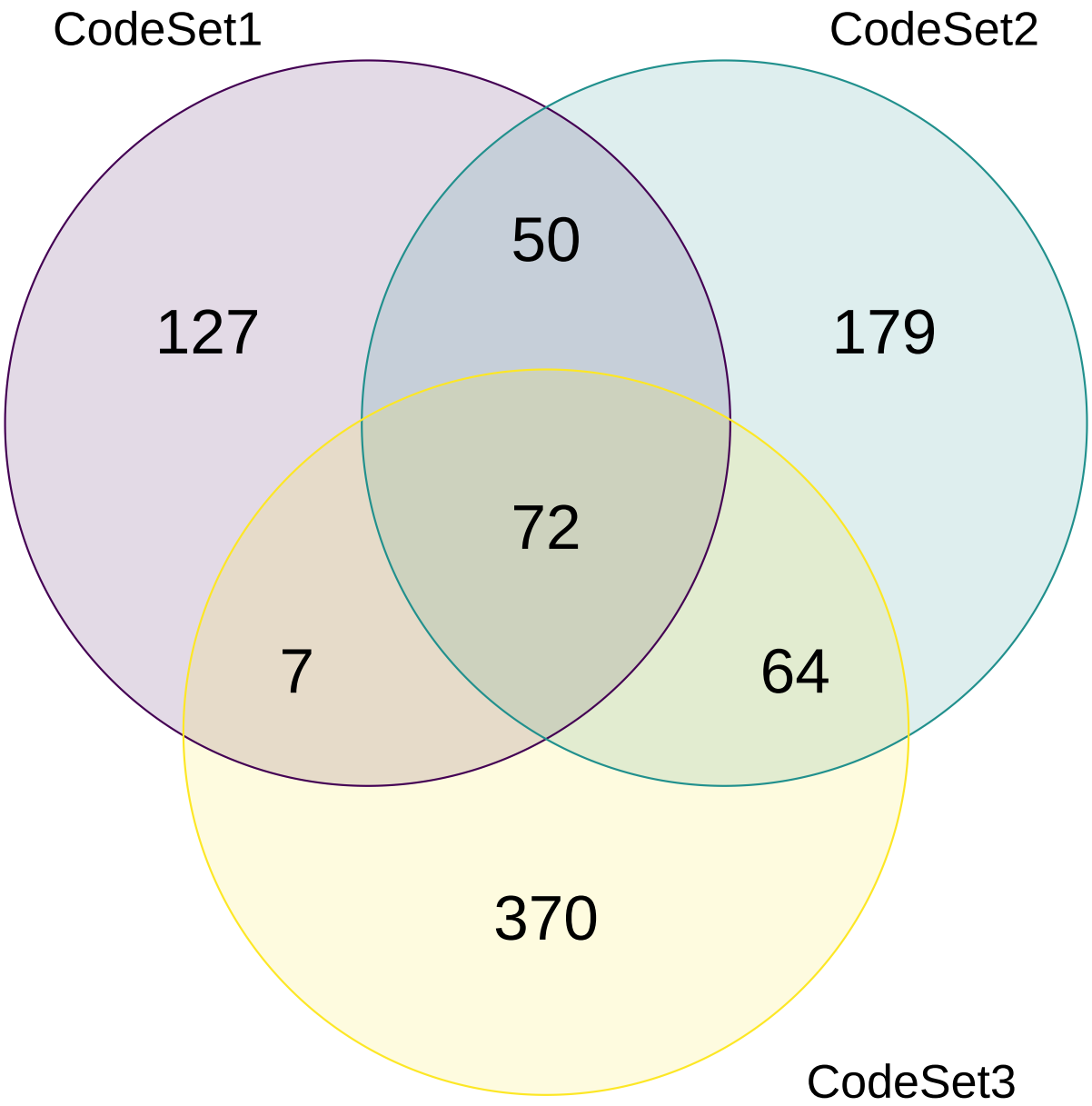


Figure 2.1: Venn diagram of common and unique gene targets covered by each CodeSet

2.1.4 Final Processing

We map ovarian histotypes to all remaining samples and keep the major histotypes for building the predictive model: high-grade serous carcinoma (HGSC), clear cell ovarian carcinoma (CCOC), endometrioid ovarian carcinoma (ENOC), low-grade serous carcinoma (LGSC), mucinous carcinoma (MUC).

Duplicate cases (two samples with the same ottaID) were removed before generating the final training set to use for fitting the classification models. All CS3 cases were preferred over CS1

and CS2, and CS3-Vancouver cases were preferred over CS3-AOC and CS3-USC when selecting duplicates.

The final training set used only genes that were common across all three CodeSets.

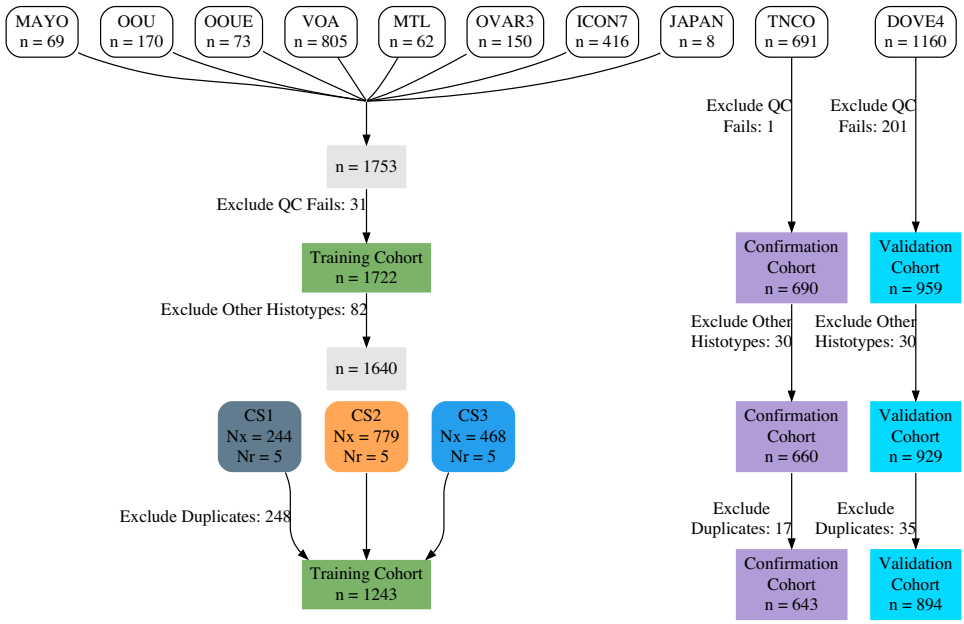


Figure 2.2: Cohorts Selection

2.2 Classifiers

We use 4 classification algorithms in the supervised learning framework for the Training Set. The pipeline was run using SLURM batch jobs submitted to a partition on a CentOS 7 server. All resampling techniques, pre-processing, model specification, hyperparameter tuning, and evaluation metrics were implemented using the `tidymodels` suite of packages. The classifiers we used are:

- Random Forest (`rf`)
- Support Vector Machine (`svm`)
- XGBoost (`xgb`)
- Regularized Multinomial Regression (`mr`)

2.2.1 Resampling of Training Set

We used a nested cross-validation design to assess each classifier while also performing hyperparameter tuning. An outer 5-fold CV stratified by histotype was used together with an inner 5-fold CV with 2 repeats stratified by histotype. This design was chosen such that the test sets of the inner resamples would still have a reasonable number of samples belonging to the smallest minority class.

The outer resampling method cannot be the bootstrap, because the inner training and inner test sets will likely contain the same samples as a result of sampling with replacement in the outer training set. This phenomenon might result in inflated performance as some observations are used both to train and evaluate the hyperparameter tuning in the inner loop.

2.2.2 Hyperparameter Tuning

The following specifications for each classifier were used for tuning hyperparameters:

- `rf` and `xgb`: The number of trees were fixed at 500. Other hyperparameters were tuned across 10 randomly selected points in a latin hypercube design.
- `svm`: Both the cost and sigma hyperparameters were tuned across 10 randomly selected points in a latin hypercube design. We tuned the cost parameter in the range [1, 8]. The range for tuning the sigma parameter was obtained from the 10% and 90% quantiles of the estimation using the `kernlab::sigest()` function.
- `mr`: We generated 10 randomly selected points in a latin hypercube design for the penalty (lambda) parameter. Then, we generated 10 evenly spaced points in [0, 1] for the mixture (alpha) parameter in the regularized multinomial regression model. These two sets of 10 points were crossed to generate a tuning grid of 100 points.

The hyperparameter combination that resulted in the highest average F1-score across the inner training sets was selected for each classifier to use as the model for assessing prediction performance in the outer training loop.

2.2.3 Subsampling

Here are the specifications of the subsampling methods used to handle class imbalance:

- None: No subsampling is performed
- Down-sampling: All levels except the minority class are sampled down to the same frequency as the minority class
- Up-sampling: All levels except the majority class are sampled up to the same frequency as the majority class
- SMOTE: All levels except the majority class have synthetic data generated until they have the same frequency as the majority class
- Hybrid: All levels except the majority class have synthetic data generated up to 50% of the frequency of the majority class, then the majority class is sampled down to the same frequency as the rest.

The figure below helps visualize how the distribution of classes changes when we apply subsampling techniques to handle class imbalance:

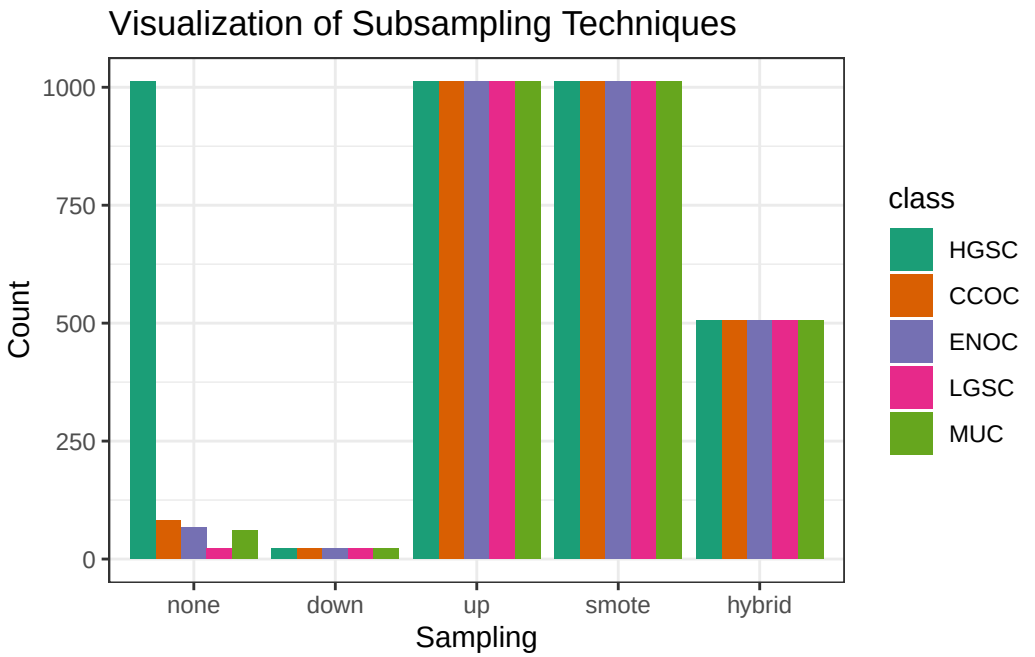


Figure 2.3: Visualization of Subsampling Techniques

2.2.4 Workflows

The 4 **algorithms** and 5 **subsampling** methods are crossed to create 20 different classification **workflows**. For example, the `hybrid_xgb` workflow is a classifier that first pre-processes a training set by applying a hybrid subsampling method, and then proceeds to use the XGBoost algorithm to classify ovarian histotypes.

2.3 Two-Step Algorithm

The HGSC histotype comprises of approximately 80% of cases among ovarian carcinoma patients, while the remaining 20% of cases are relatively, evenly distributed among ENOC, CCOC, LGSC, and MUC histotypes. We can implement a two-step algorithm as such:

- Step 1: use binary classification for HGSC vs. non-HGSC
- Step 2: use multinomial classification for the remaining non-HGSC classes

Let

$$\begin{aligned} X_k &= \text{Training data with k classes} \\ C_k &= \text{Class with highest } F_1 \text{ score from training } X_k \\ W_k &= \text{Workflow associated with } C_k \end{aligned} \tag{2.1}$$

Figure 2.4 shows how the two-step algorithm works:

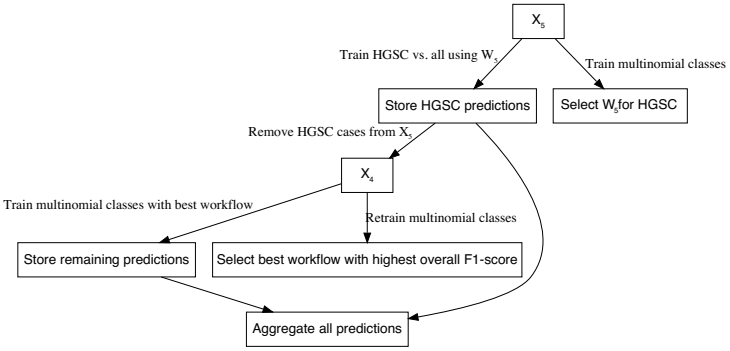


Figure 2.4: Two-Step Algorithm

2.3.1 Aggregating Predictions

The aggregation for two-step predictions is quite straightforward:

1. Predict HGSC vs. non-HGSC
2. Among all non-HGSC cases, predict CCOC vs. LGSC vs. MUC vs. ENOC

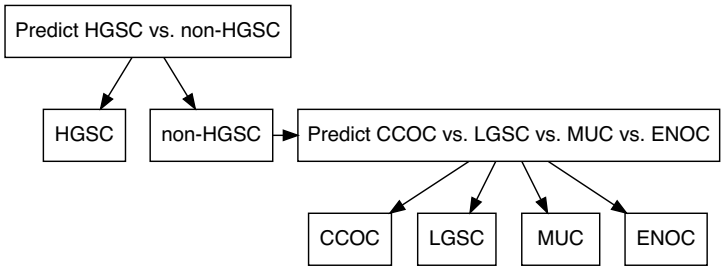


Figure 2.5: Aggregating Predictions for Two-Step Algorithm

2.4 Sequential Algorithm

Instead of training on k classes simultaneously using multinomial classifiers, we can use a sequential algorithm that performs k-1 one-vs-all binary classifications iteratively to obtain a final prediction of all cases. At each step in the sequence, we classify one class vs. all other classes, where the classes that make up the "other" class are those not equal to the current "one" class and excluding all "one" classes from previous steps. For example, if the "one" class in step 1 was HGSC, the "other" classes would include CCOC, ENOC, LGSC, and MUC. If the "one" class in step 2 was CCOC, the "other" classes include ENOC, LGSC, and MUC.

The order of classes and workflows to use at each step in the sequential algorithm must be determined using a retraining procedure. After removing the data associated with a particular class, we retrain using the remaining data using multinomial classifiers as described before. The class and workflow to use for the next step in the sequence is selected based on the best per-class evaluation metric value (e.g. F1-score).

Figure 2.6 illustrates how the sequential algorithm works for K=5, using ovarian histotypes as an example for the classes.

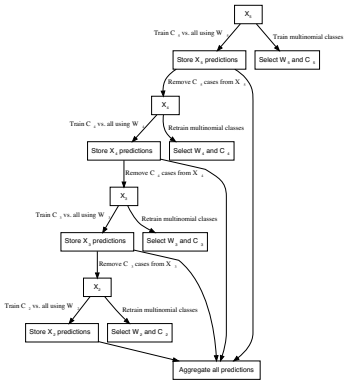


Figure 2.6: Sequential Algorithm

The subsampling method used in the first step of the sequential algorithm is used in all subsequent steps in order to maintain data pre-processing consistency. As a result, we are only comparing classification algorithms within one subsampling method across the entire sequential algorithm.

2.4.1 Aggregating Predictions

We have to aggregate the one-vs-all predictions from each of the sequential algorithm workflows in order to obtain a final class prediction on a holdout test set. Each sequential workflow has to be assessed on every sample to ensure that cases classified into the "all" class from a previous step of the sequence are eventually assigned a predicted class. For example, say that based on certain class-specific metrics we determined that the order of classes in the sequential algorithm was to predict HGSC vs. non-HGSC, CCOC vs. non-CCOC, LGSC vs. non-LGSC, and then MUC vs. ENOC. Figure 2.7 illustrates how the final predictions are assigned:

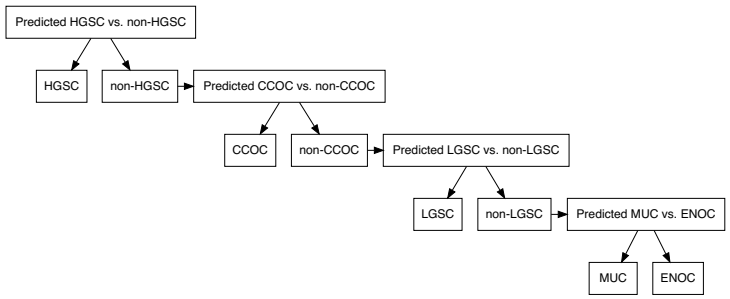


Figure 2.7: Aggregating Predictions for Sequential Algorithm

2.5 Gene Optimization

We want to discover an optimal set of genes for the classifiers while including specific genes from other studies such as PrOTYPE and SPOT. A total of 72 genes are used in the classifier training set.

There are 16 genes in the classifier set that overlap with the PrOTYPE classifier: COL11A1, CD74, CD2, TIMP3, LUM, CYTIP, COL3A1, THBS2, TCF7L1, HMGA2, FN1, POSTN, COL1A2, COL5A2, PDZK1IP1, FBN1.

There are also 13 genes in the classifier set that overlap with the SPOT signature: HIF1A, CXCL10, DUSP4, SOX17, MITF, CDKN3, BRCA2, CEACAM5, ANXA4, SERPINE1, TCF7L1, CRABP2, DNAJC9.

We obtain a total of 28 genes from the union of PrOTYPE and SPOT genes that we want to include in the final classifier, regardless of model performance. We then incrementally add genes one at a time from the remaining 44 candidate genes based on an overall variable importance rank to the set of 28 base genes and recalculate performance metrics. The number of genes at which the performance peaks or starts to plateau may indicate an optimal gene set model for us to compare with the full set model.

Here is the breakdown of genes used and whether they belong to the PrOTYPE and/or SPOT sets:

Table 2.1: Gene Distribution

[H]

Genes	PrOTYPE	SPOT
TCF7L1	v	v
COL11A1	v	
CD74	v	
CD2	v	
TIMP3	v	
LUM	v	
CYTIP	v	
COL3A1	v	
THBS2	v	
HMGA2	v	
FN1	v	
POSTN	v	
COL1A2	v	
COL5A2	v	
PDZK1IP1	v	
FBN1	v	
HIF1A		v
CXCL10		v
DUSP4		v
SOX17		v
MITF		v
CDKN3		v
BRCA2		v
CEACAM5		v
ANXA4		v
SERPINE1		v
CRABP2		v
DNAJC9		v
C10orf116		
GAD1		
TPX2		
KGFLP2		
EGFL6		
KLK7		
PBX1		
LIN28B		
TFF3		
MUC5B		
FUT3		
STC1		
BCL2		
PAX8		
GCNT3		
GPR64		
ADCYAP1R1		
IGKC		
BRCA1		
IGJ		
TFF1		

2.5.1 Variable Importance

Variable importance is calculated using either a model-based approach if it is available, or a permutation-based VI score otherwise. The variable importance scores are averaged across the outer training folds, and then ranked from highest to lowest.

For the sequential and two-step classifiers, we calculate an overall VI rank by taking the cumulative union of genes at each variable importance rank across all sequences, until all genes have been included.

The variable importance measures are:

- Random Forest: impurity measure (Gini index)
- XGBoost: gain (fractional contribution of each feature to the model based on the total gain of the corresponding features's splits)
- SVM: permutation based p-values
- Multinomial regression: absolute value of estimated coefficients at cross-validated lambda value

2.6 Performance Evaluation

2.6.1 Class Metrics

We use the accuracy, sensitivity, specificity, F1-score, kappa, balanced accuracy, and geometric mean, as class metrics to measure both training and test performance between different workflows. Multiclass extensions of these metrics can be calculated except for F1-score, where we use macro-averaging to obtain an overall metric. Class-specific metrics are calculated by recoding classes into one-vs-all categories for each class.

2.6.1.1 Accuracy

The accuracy is defined as the proportion of correct predictions out of all cases:

$$\text{accuracy} = \frac{TP}{TP + FP + FN + TN} \tag{2.2}$$

2.6.1.2 Sensitivity

Sensitivity is the proportional of correctly predicted positive cases, out of all cases that were truly positive

$$\text{sensitivity} = \frac{TP}{TP + FN} \tag{2.3}$$

2.6.1.3 Specificity

Specificity is the proportional of correctly predicted negative cases, out of all cases that were truly negative.

$$\text{specificity} = \frac{TN}{TN + FP} \tag{2.4}$$

2.6.1.4 F1-Score

The F-measure can be thought of as a harmonic mean between precision and recall:

$$F_{meas} = \frac{(1 + \beta^2) \times precision \times recall}{(\beta^2 \times precision) + recall} \tag{2.5}$$

The β value can be adjusted to place more weight upon precision or recall. The most common value is β is 1, which is also commonly known as the F1-score. A multiclass extension doesn't exist for the F1-score, so we use macro-averaging to calculate this metric when there are more than two classes. For example, with k classes, the macro-averaged F1-score is equal to:

$$F_{1_{macro}} = \frac{1}{k} \sum_{i=1}^{k} F_{1_i} \tag{2.6}$$

where each F_{1_i} is the F1-score computed frrom recoding classes into $k = i$ vs. $k \neq i$.

In situations where there is not at least one predicted case for each of the classes (e.g. for a poor classifier), F_{1_i} is undefined because the per-class precision of class i is undefined. Those F_{1_i} terms are removed from the $F_{1_{macro}}$ equation and the resulting value may be inflated. Interpreting the F1-score in such a case would be misleading.

2.6.1.5 Balanced Accuracy

Balanced accuracy is the arithmetic mean of sensitivity and specificity.

$$\text{Balanced Accuracy} = \frac{\text{Sensitivity} + \text{Specificity}}{2} \tag{2.7}$$

2.6.1.6 Kappa

Kappa is the defined as:

$$\text{kappa} = \frac{p_0 - p_e}{1 - p_e} \tag{2.8}$$

where p_0 is the observed agreement among raters and p_e is the hypothetical probability of agreement due to random chance.

2.6.2 AUC

The area under the receiver operating curve (AUC) is calculated by adding up the area under the curve formed by plotting sensitivity vs. 1 - specificity. The Hand-till method is used as a multiclass extension for the AUC.

We did not use AUC to measure class-specific training set performance because combining predicted probabilities in a one-vs-all fashion might be potentially misleading. The sum of probabilities that add up to the "other" class is not equivalent to the predicted probability of the "other" class when using a multiclass classifier.

Instead, we only reported ROC curves and their associated AUCs for the test set performance of the sequential and two-step algorithms.

3 Distributions

3.1 Histotype Distribution

Table 3.1: Histotype Distribution in Training Set by Processing Stage

[H]

Variable	Levels	CS1	CS2	CS3	Total
Selected Cohorts					
Histotype	HGSC	126 (43%)	655 (73%)	1779 (72%)	2560 (70%)
	CCOC	48 (16%)	61 (7%)	181 (7%)	290 (8%)
	ENOC	60 (20%)	34 (4%)	268 (11%)	362 (10%)
	MUC	20 (7%)	62 (7%)	77 (3%)	159 (4%)
	LGSC	21 (7%)	21 (2%)	42 (2%)	84 (2%)
	Other	19 (6%)	70 (8%)	130 (5%)	219 (6%)
Total	N (%)	294 (8%)	903 (25%)	2477 (67%)	3674 (100%)
QC					
Histotype	HGSC	120 (42%)	641 (73%)	1636 (72%)	2397 (70%)
	CCOC	48 (17%)	61 (7%)	173 (8%)	282 (8%)
	ENOC	60 (21%)	32 (4%)	229 (10%)	321 (9%)
	MUC	19 (7%)	60 (7%)	69 (3%)	148 (4%)
	LGSC	20 (7%)	21 (2%)	40 (2%)	81 (2%)
	Other	19 (7%)	67 (8%)	126 (6%)	212 (6%)
Total	N (%)	286 (8%)	882 (26%)	2273 (66%)	3441 (100%)
Main Histotypes					
Histotype	HGSC	120 (45%)	641 (79%)	1636 (76%)	2397 (74%)
	CCOC	48 (18%)	61 (7%)	173 (8%)	282 (9%)
	ENOC	60 (22%)	32 (4%)	229 (11%)	321 (10%)
	MUC	19 (7%)	60 (7%)	69 (3%)	148 (5%)
	LGSC	20 (7%)	21 (3%)	40 (2%)	81 (3%)
Total	N (%)	267 (8%)	815 (25%)	2147 (66%)	3229 (100%)
Removed Duplicates					
Histotype	HGSC	117 (47%)	623 (79%)	1540 (77%)	2280 (75%)
	CCOC	45 (18%)	55 (7%)	159 (8%)	259 (9%)
	ENOC	56 (22%)	28 (4%)	216 (11%)	300 (10%)
	MUC	16 (6%)	58 (7%)	59 (3%)	133 (4%)
	LGSC	15 (6%)	20 (3%)	36 (2%)	71 (2%)
Total	N (%)	249 (8%)	784 (26%)	2010 (66%)	3043 (100%)
Normalized and Recombined					
Histotype	HGSC	116 (48%)	622 (80%)	451 (96%)	1189 (80%)
	CCOC	44 (18%)	54 (7%)	4 (1%)	102 (7%)
	ENOC	55 (23%)	27 (3%)	4 (1%)	86 (6%)
	MUC	15 (6%)	57 (7%)	5 (1%)	77 (5%)
	LGSC	14 (6%)	19 (2%)	4 (1%)	37 (2%)
Total	N (%)	244 (16%)	779 (52%)	468 (31%)	1491 (100%)
Removed Replicates					
Histotype	HGSC	9 (12%)	552 (79%)	451 (96%)	1012 (81%)
	CCOC	25 (32%)	52 (7%)	4 (1%)	81 (7%)
	ENOC	37 (48%)	25 (4%)	4 (1%)	66 (5%)
	MUC	3 (4%)	53 (8%)	5 (1%)	61 (5%)
	LGSC	3 (4%)	16 (2%)	4 (1%)	23 (2%)
Total	N (%)	77 (6%)	698 (56%)	468 (38%)	1243 (100%)

Table 3.2: Histotype Distribution in Training, Confirmation, and Validation Sets

[H]

Variable	Levels	Training	Confirmation	Validation
Histotype	HGSC	1012 (81%)	422 (66%)	666 (74%)
	CCOC	81 (7%)	75 (12%)	79 (9%)
	ENOC	66 (5%)	106 (16%)	105 (12%)
	MUC	61 (5%)	27 (4%)	26 (3%)
	LGSC	23 (2%)	13 (2%)	18 (2%)
Total	N (%)	1243 (45%)	643 (23%)	894 (32%)

3.2 Cohort Distribution

Table 3.3: Pre-QC Cohort Distribution by CodeSet

CodeSet	**CS1**, N = 294	**CS2**, N = 903	**CS3**, N = 2,477
Cohort			
OOU	108 (37%)	43 (4.8%)	19 (0.8%)
OOUE	32 (11%)	30 (3.3%)	11 (0.4%)
VOA	145 (49%)	122 (14%)	538 (22%)
OVAR3	0 (0%)	150 (17%)	0 (0%)
ICON7	0 (0%)	416 (46%)	0 (0%)
MAYO	6 (2.0%)	63 (7.0%)	0 (0%)
DOVE4	0 (0%)	0 (0%)	1,160 (47%)
TNCO	0 (0%)	0 (0%)	691 (28%)
MTL	3 (1.0%)	59 (6.5%)	0 (0%)
JAPAN	0 (0%)	8 (0.9%)	0 (0%)
POOL-CTRL	0 (0%)	12 (1.3%)	0 (0%)
POOL-1	0 (0%)	0 (0%)	31 (1.3%)
POOL-2	0 (0%)	0 (0%)	14 (0.6%)
POOL-3	0 (0%)	0 (0%)	13 (0.5%)

3.3 Quality Control

3.3.1 Failed Samples

We use an aggregated `QCFlag` that considers a sample to have failed QC if any of the following conditions are true:

- `linFlag`: linearity of positive controls with positive control concentrations is less than 0.95, or linearity measures are unknown
- `imagingFlag`: percent of field of view is less than 75%
- `spcFlag`: smallest positive control is less than the lower limit of detection (negative control average expression less two times the negative control standard deviation), or negative control average expression equals zero

- `normFlag`: signal to noise ratio less than 100, or percent of genes detected is less than 50. Note: these thresholds were determined by examining the Section 3.3.2 relationship below.

Table 3.4: Quality Control Summary

Quality Control Flag	**CS1**, N = 294	**CS2**, N = 903	**CS3**, N = 2,477
___Linearity___			
Failed	0 (0%)	4 (0.4%)	0 (0%)
Passed	294 (100%)	899 (100%)	2,477 (100%)
___Imaging___			
Failed	3 (1.0%)	0 (0%)	4 (0.2%)
Passed	291 (99%)	903 (100%)	2,473 (100%)
___Smallest Positive Control___			
Failed	0 (0%)	2 (0.2%)	0 (0%)
Passed	294 (100%)	901 (100%)	2,477 (100%)
___Normality___			
Failed	5 (1.7%)	19 (2.1%)	200 (8.1%)
Passed	289 (98%)	884 (98%)	2,277 (92%)
___Overall QC___			
Failed	8 (2.7%)	21 (2.3%)	204 (8.2%)
Passed	286 (97%)	882 (98%)	2,273 (92%)

3.3.2 %GD vs. SNR

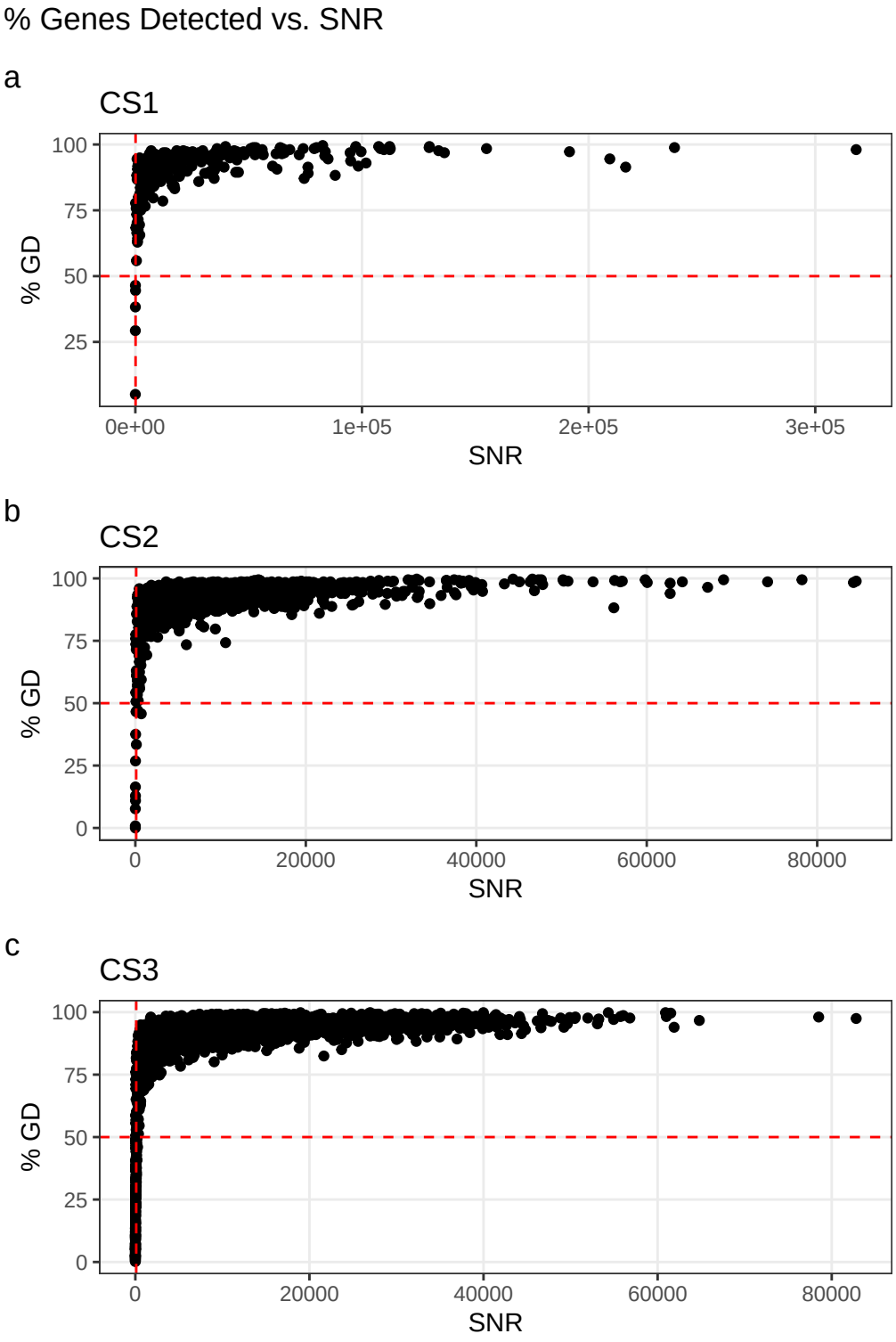


Figure 3.1: % Genes Detected vs. Signal to Noise Ratio

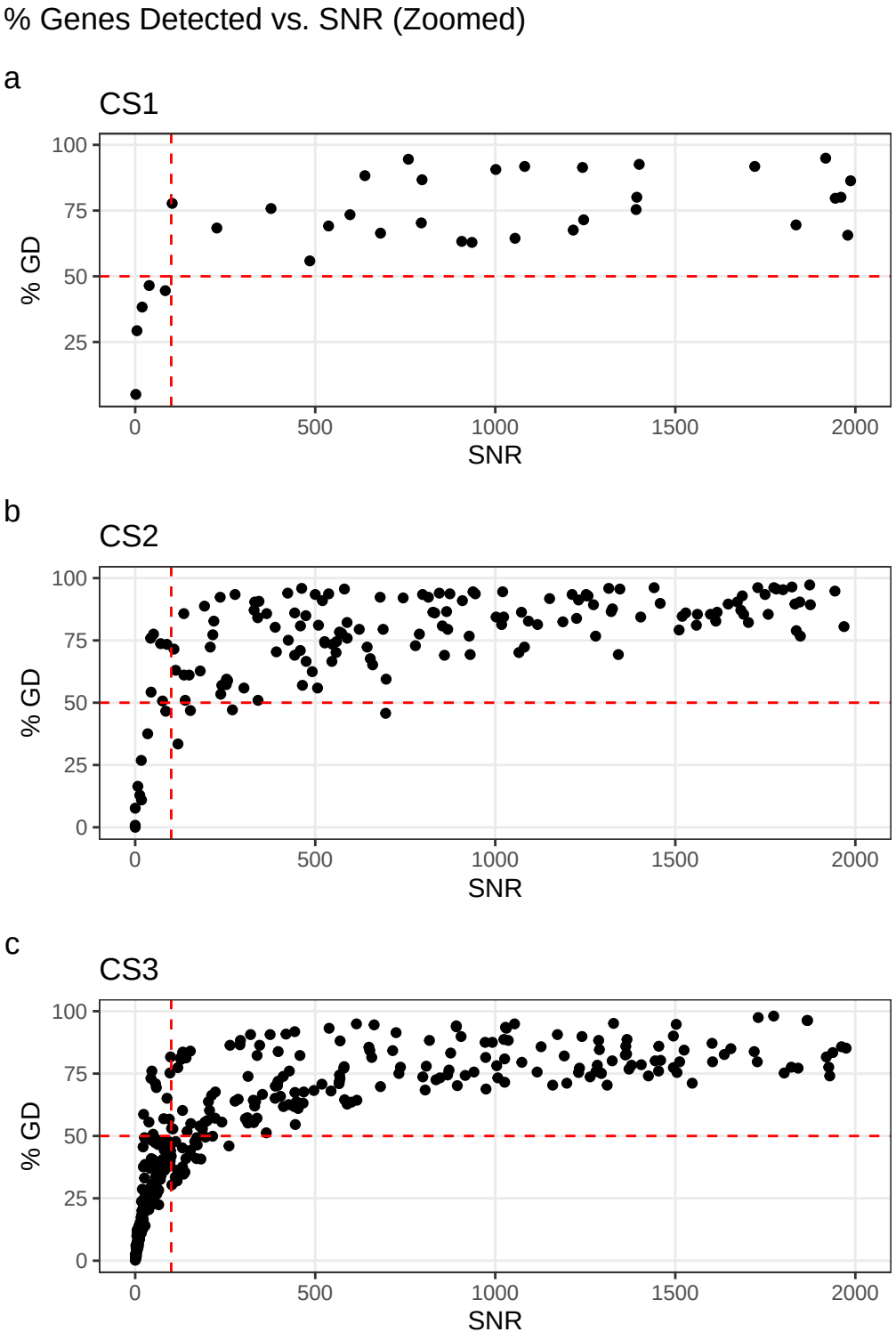


Figure 3.2: % Genes Detected vs. Signal to Noise Ratio (Zoomed)

3.4 Pairwise Gene Expression

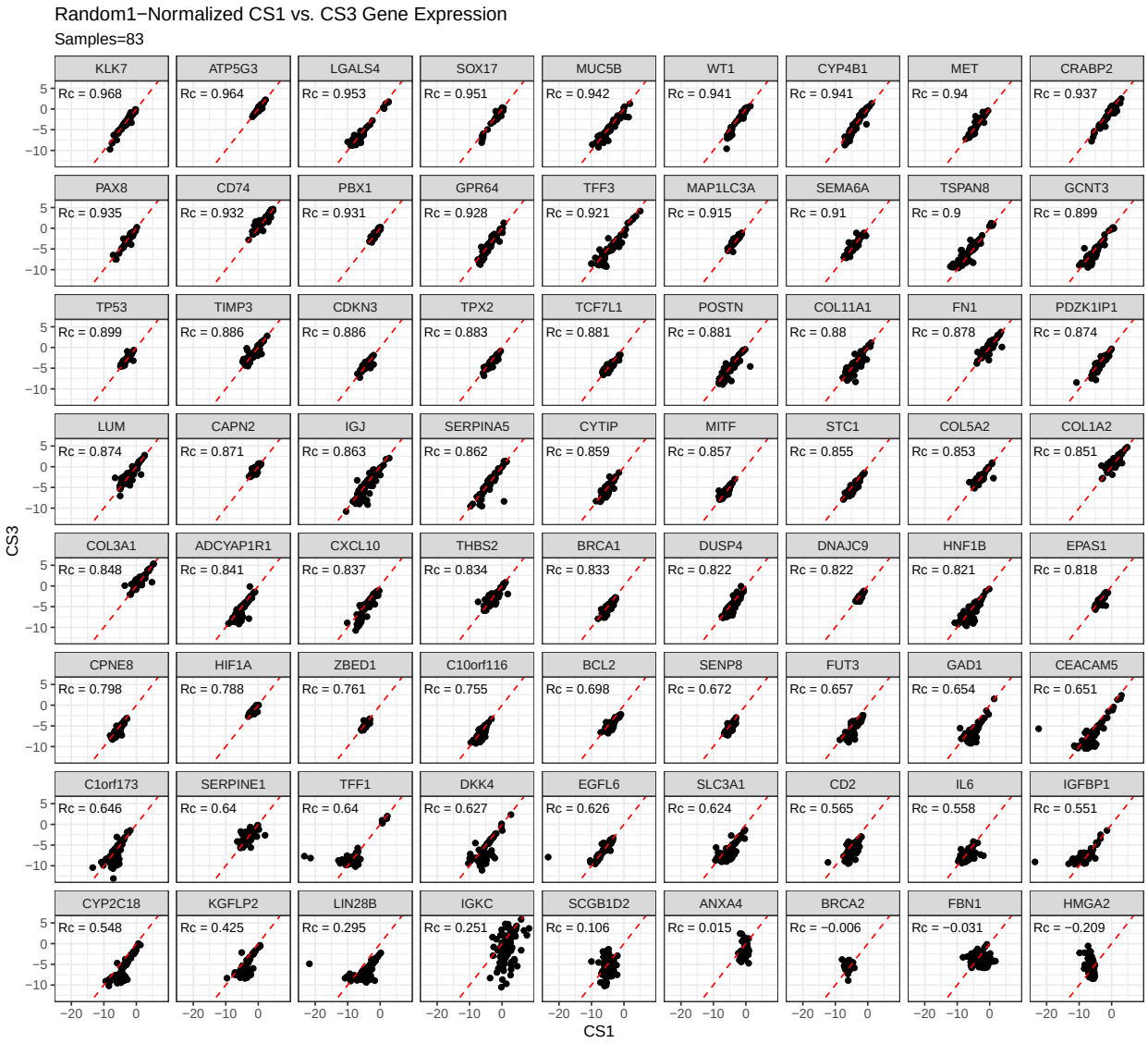


Figure 3.3: Random1-Normalized CS1 vs. CS3 Gene Expression

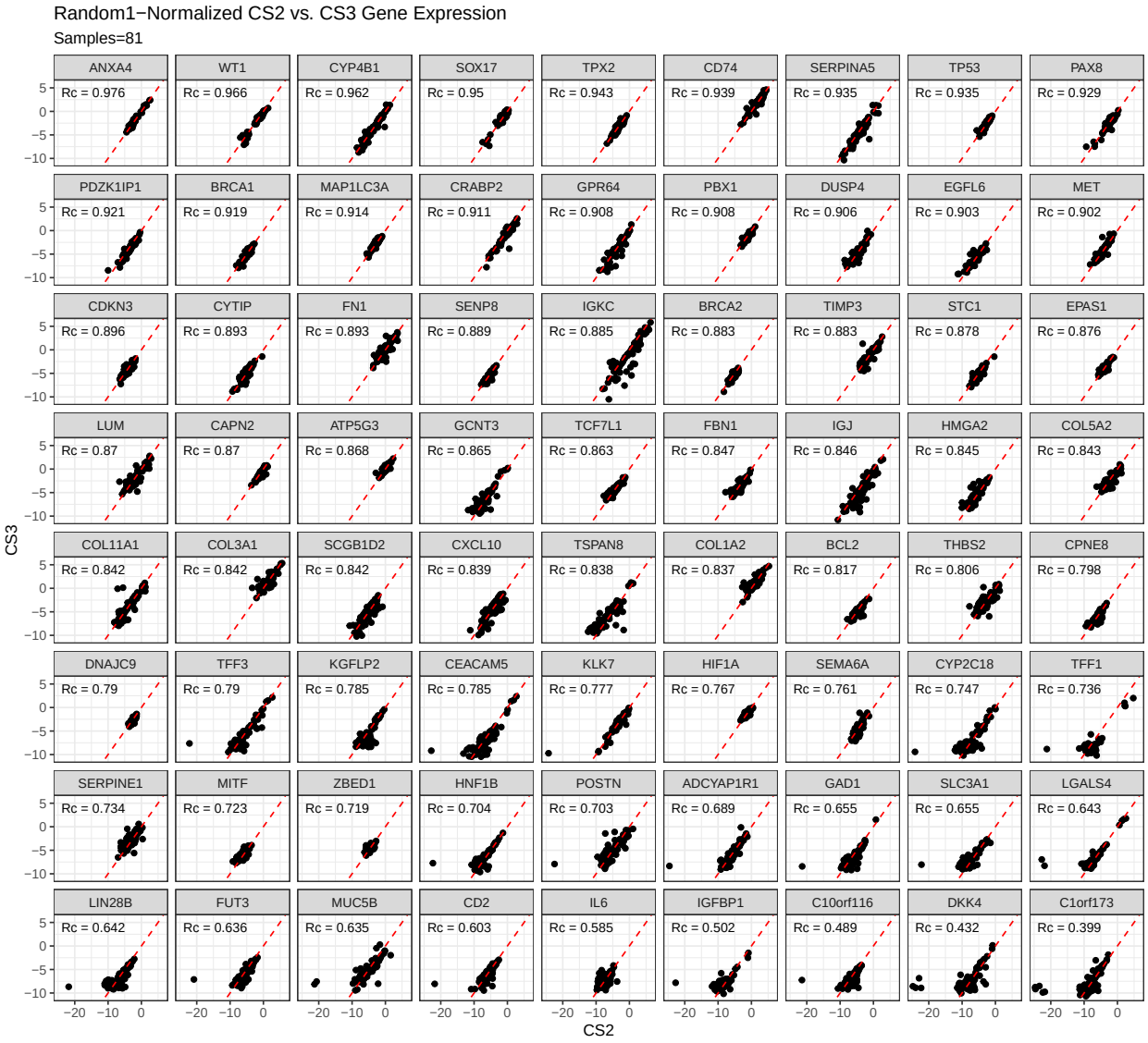


Figure 3.4: Random1-Normalized CS2 vs. CS3 Gene Expression

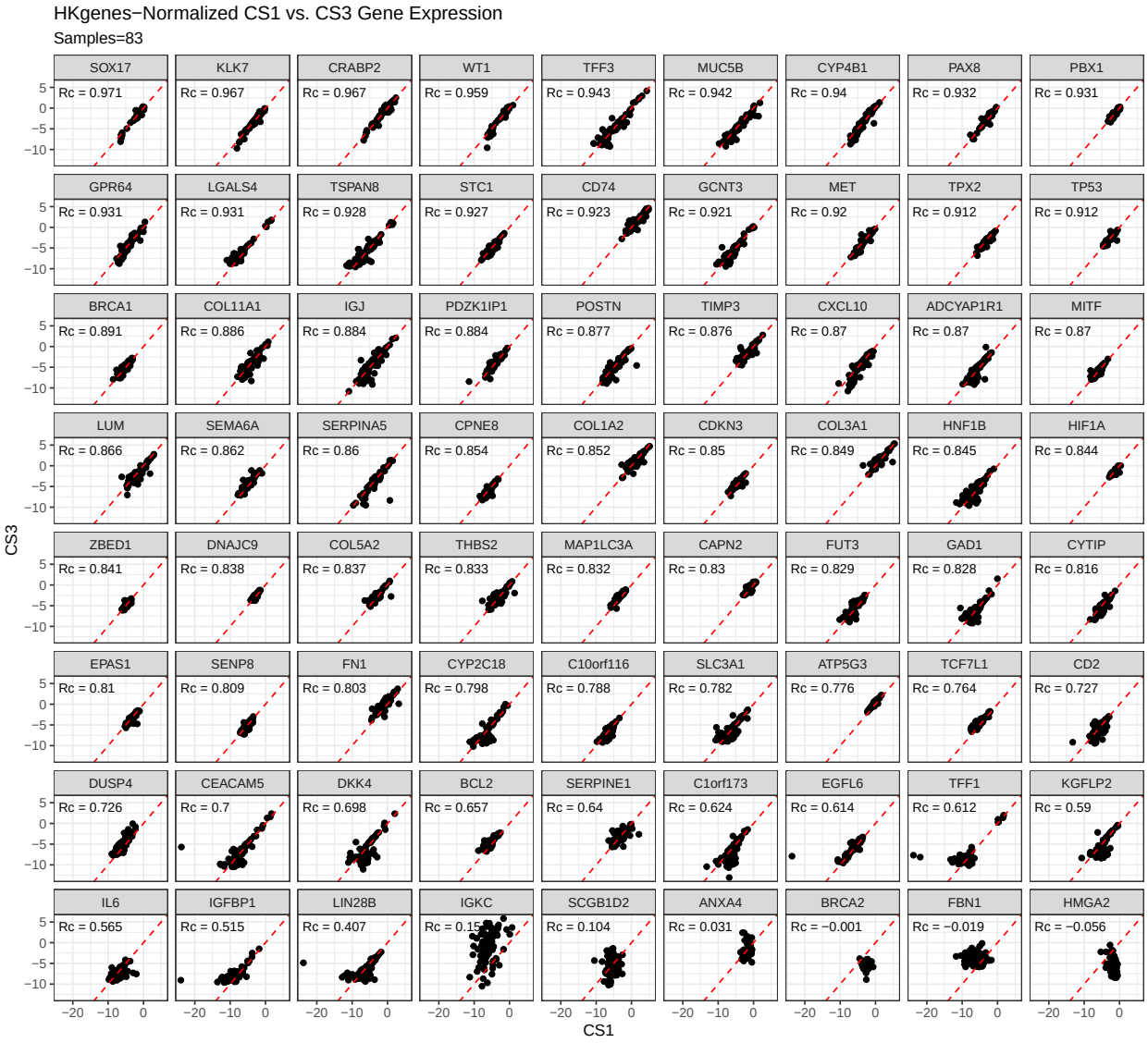


Figure 3.5: HKgenes-Normalized CS1 vs. CS3 Gene Expression

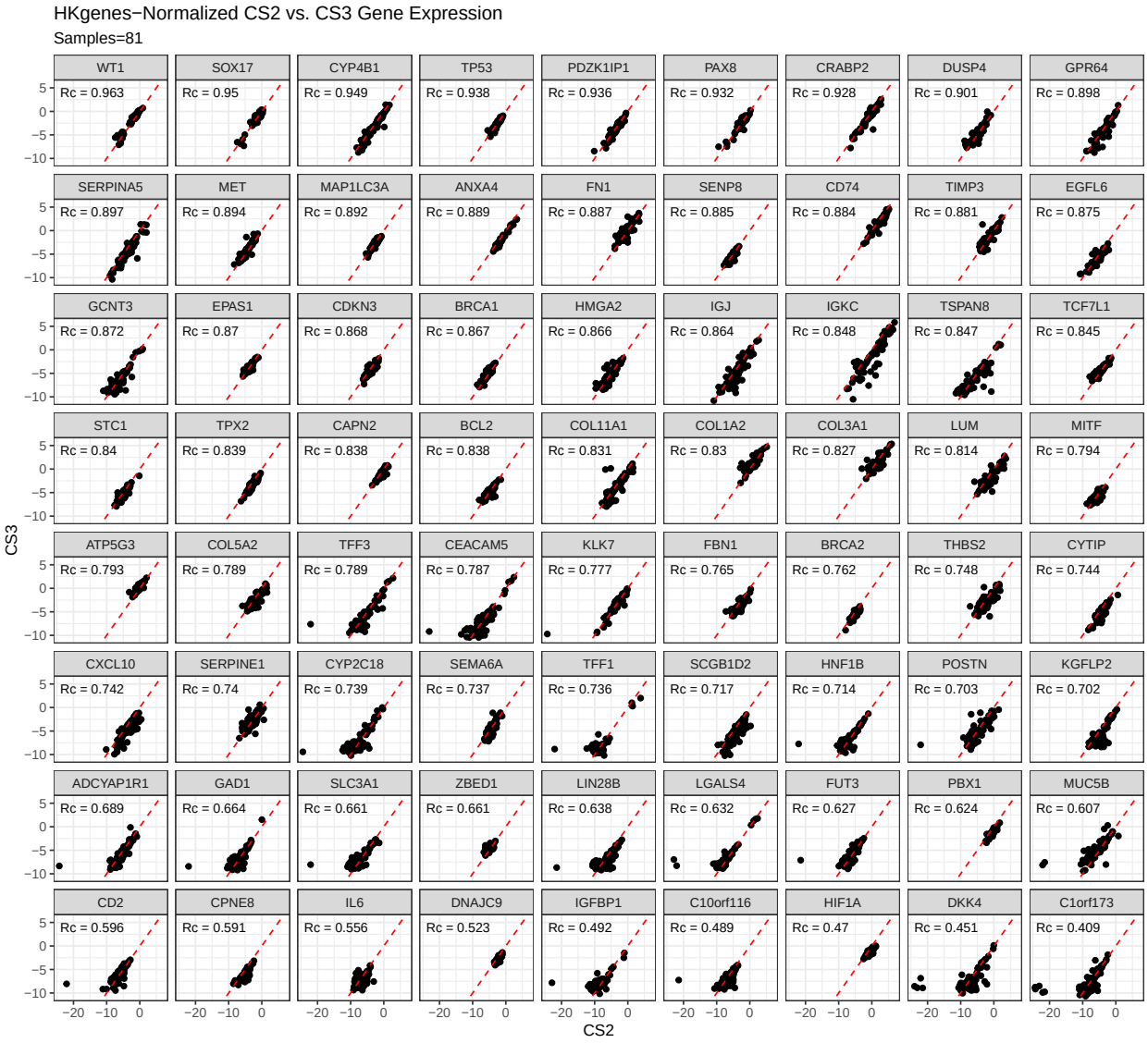


Figure 3.6: HKgenes-Normalized CS2 vs. CS3 Gene Expression

4 Results

We summarize cross-validated training performance of class metrics in the training set. The accuracy, F1-score, and kappa, are the metrics of interest. Workflows are ordered by their mean estimates across the outer folds of the nested CV for each metric.

4.1 Training Set

4.1.1 Accuracy

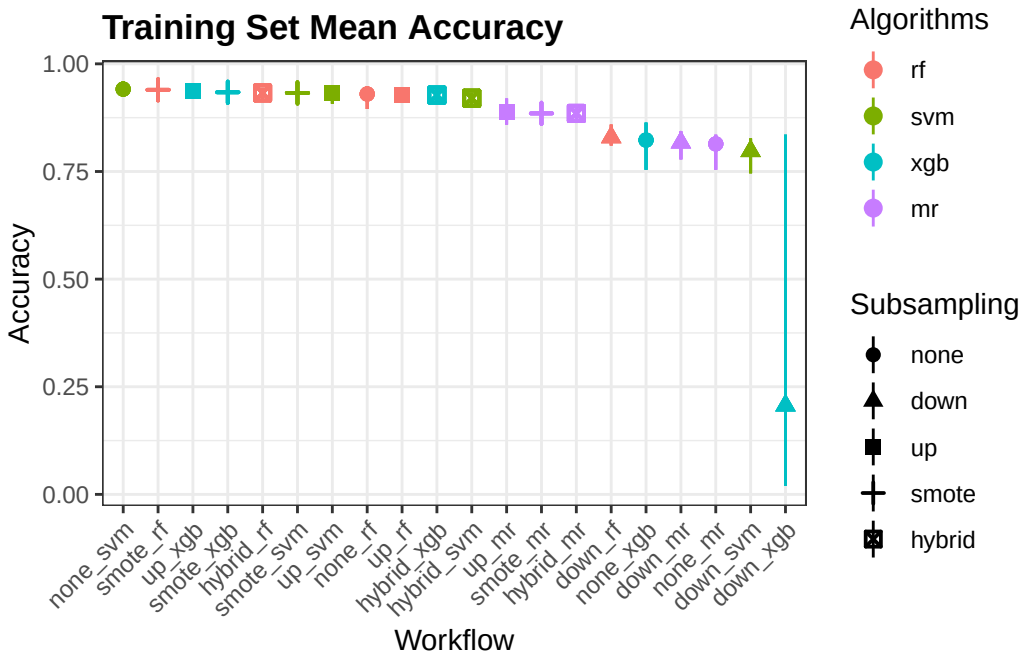


Figure 4.1: Training Set Mean Accuracy

Table 4.1: Training Set Mean Accuracy

[H]

	Algorithms			
Subsampling	rf	svm	xgb	mr
none	0.93	0.941	0.823	0.814
down	0.829	0.797	0.206	0.817
up	0.928	0.932	0.937	0.889
smote	0.94	0.932	0.934	0.885
hybrid	0.932	0.92	0.928	0.885

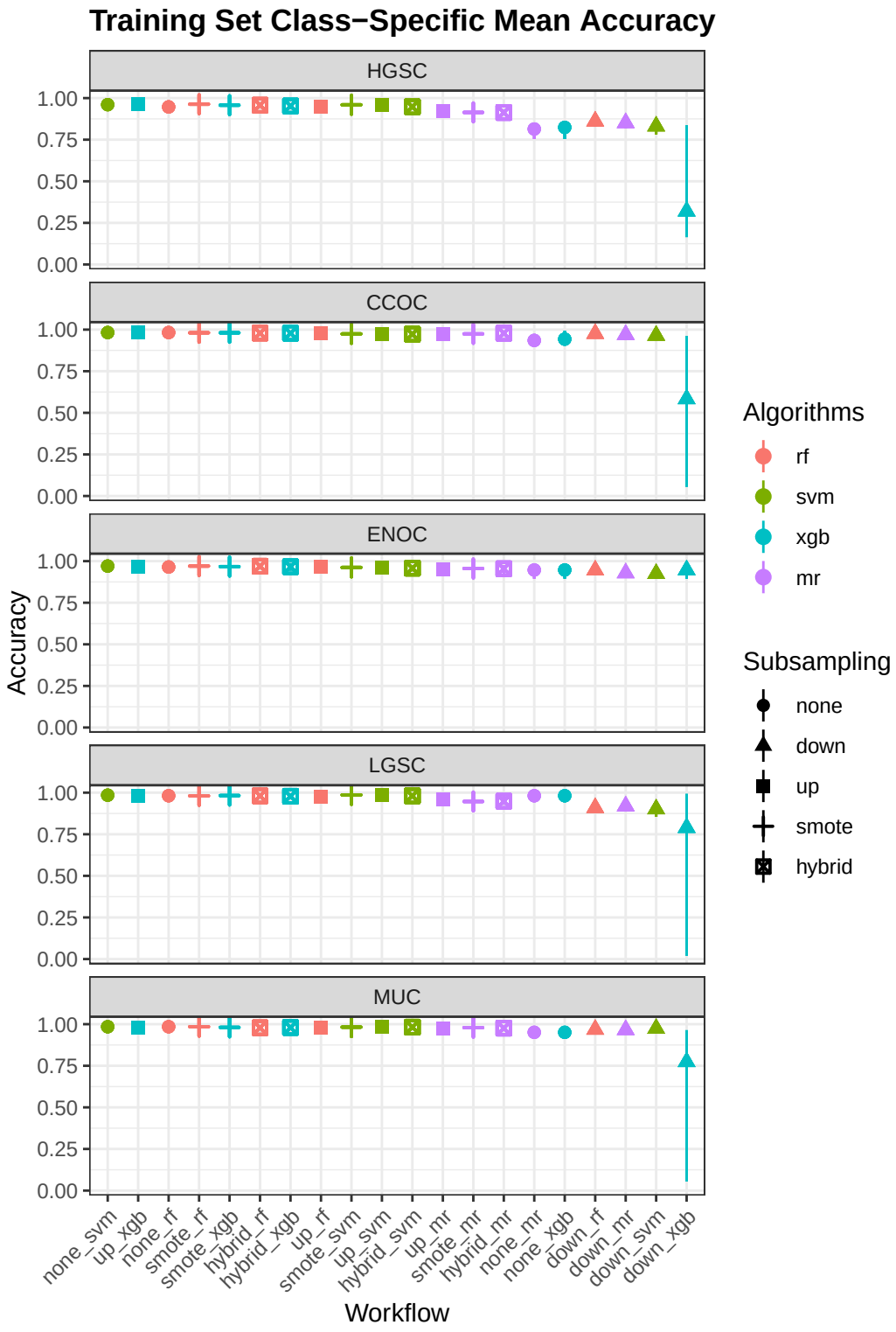


Figure 4.2: Training Set Class-Specific Mean Accuracy

Table 4.2: Training Set Class-Specific Mean Accuracy

[H]

		Algorithms			
Subsampling	Histotype	rf	svm	xgb	mr
none	HGSC	0.947	0.961	0.824	0.814
	CCOC	0.982	0.982	0.943	0.935
	ENOC	0.965	0.97	0.947	0.947
	LGSC	0.981	0.985	0.982	0.982
	MUC	0.985	0.985	0.951	0.951
down	HGSC	0.861	0.831	0.32	0.851
	CCOC	0.976	0.965	0.583	0.969
	ENOC	0.945	0.924	0.947	0.928
	LGSC	0.909	0.901	0.79	0.92
	MUC	0.968	0.973	0.773	0.966
up	HGSC	0.949	0.959	0.962	0.92
	CCOC	0.979	0.973	0.982	0.974
	ENOC	0.969	0.963	0.967	0.95
	LGSC	0.977	0.986	0.982	0.961
	MUC	0.982	0.982	0.982	0.972
smote	HGSC	0.963	0.96	0.957	0.914
	CCOC	0.981	0.974	0.981	0.974
	ENOC	0.97	0.962	0.967	0.956
	LGSC	0.981	0.986	0.982	0.947
	MUC	0.985	0.982	0.981	0.979
hybrid	HGSC	0.957	0.948	0.953	0.913
	CCOC	0.979	0.973	0.978	0.978
	ENOC	0.969	0.957	0.966	0.954
	LGSC	0.981	0.981	0.978	0.949
	MUC	0.978	0.982	0.98	0.976

4.1.2 Sensitivity

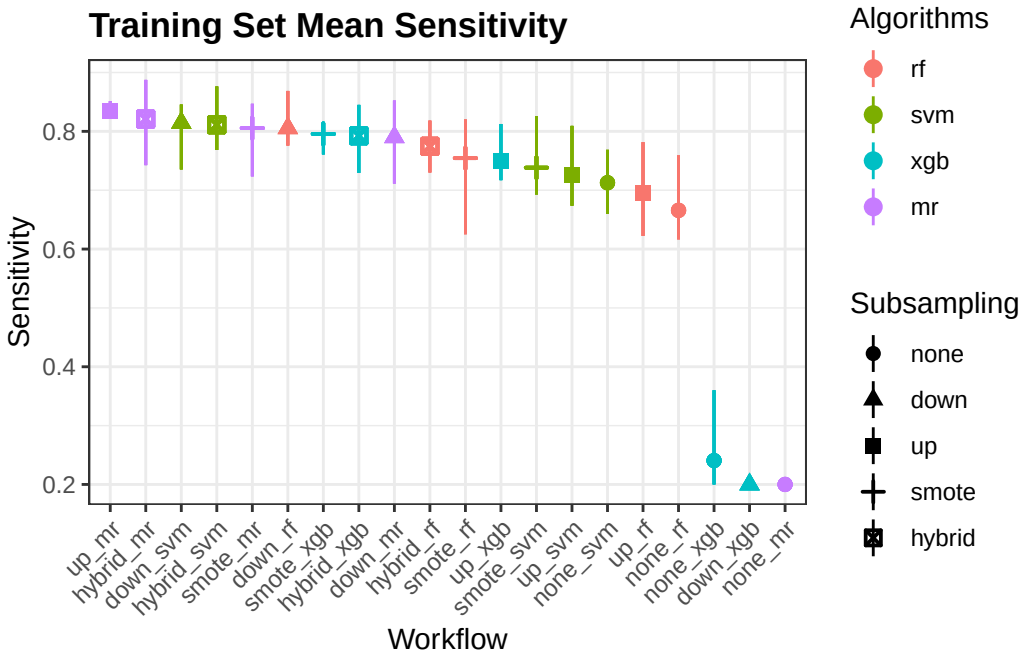


Figure 4.3: Training Set Mean Sensitivity

Table 4.3: Training Set Mean Sensitivity

[H]

	Algorithms			
Subsampling	rf	svm	xgb	mr
none	0.666	0.713	0.241	0.2
down	0.805	0.814	0.2	0.79
up	0.695	0.725	0.75	0.834
smote	0.755	0.738	0.796	0.806
hybrid	0.775	0.811	0.792	0.821

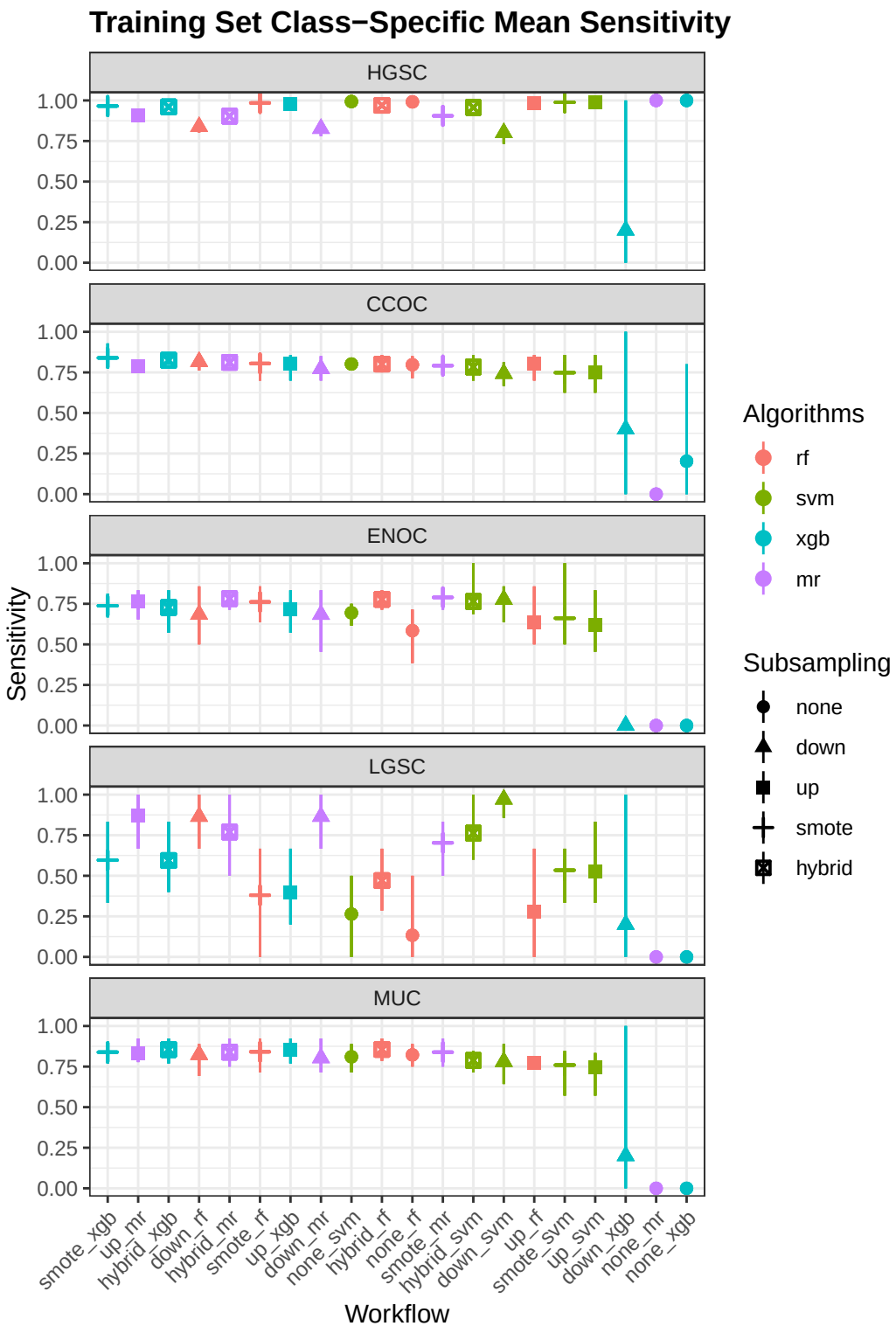


Figure 4.4: Training Set Class-Specific Mean Sensitivity

Table 4.4: Cross-Validated Training Set Class-Specific Mean Sensitivity

[H]

		Algorithms			
Subsampling	Histotype	rf	svm	xgb	mr
none	HGSC	0.991	0.993	1	1
none	CCOC	0.797	0.802	0.203	0
none	ENOC	0.585	0.695	0	0
none	LGSC	0.133	0.264	0	0
none	MUC	0.822	0.81	0	0
down	HGSC	0.838	0.8	0.2	0.826
down	CCOC	0.816	0.743	0.4	0.772
down	ENOC	0.684	0.777	0	0.683
down	LGSC	0.865	0.971	0.2	0.865
down	MUC	0.824	0.781	0.2	0.804
up	HGSC	0.981	0.989	0.98	0.91
up	CCOC	0.805	0.748	0.805	0.789
up	ENOC	0.636	0.617	0.715	0.766
up	LGSC	0.28	0.528	0.397	0.871
up	MUC	0.77	0.744	0.854	0.833
smote	HGSC	0.984	0.988	0.965	0.905
smote	CCOC	0.805	0.748	0.84	0.792
smote	ENOC	0.762	0.661	0.738	0.79
smote	LGSC	0.38	0.534	0.596	0.703
smote	MUC	0.841	0.76	0.839	0.839
hybrid	HGSC	0.97	0.957	0.96	0.903
hybrid	CCOC	0.801	0.783	0.825	0.812
hybrid	ENOC	0.777	0.764	0.727	0.782
hybrid	LGSC	0.47	0.763	0.594	0.77
hybrid	MUC	0.855	0.788	0.854	0.839

4.1.3 Specificity

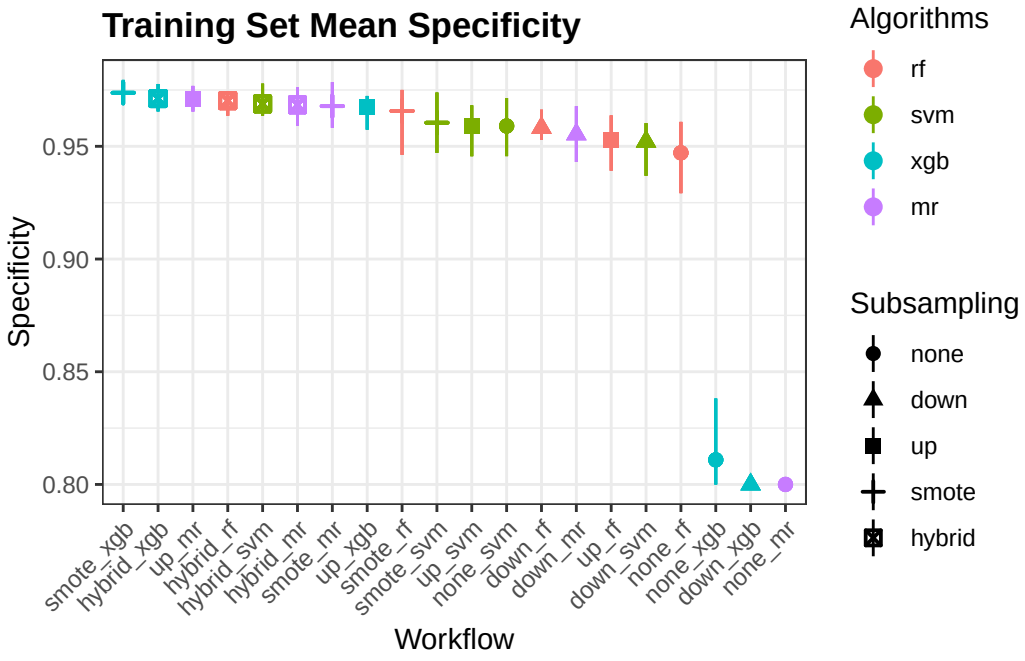


Figure 4.5: Training Set Mean Specificity

Table 4.5: Training Set Mean Specificity

[H]

	Algorithms			
Subsampling	rf	svm	xgb	mr
none	0.947	0.959	0.811	0.8
down	0.958	0.952	0.8	0.955
up	0.953	0.959	0.968	0.971
smote	0.966	0.96	0.974	0.968
hybrid	0.97	0.969	0.971	0.968

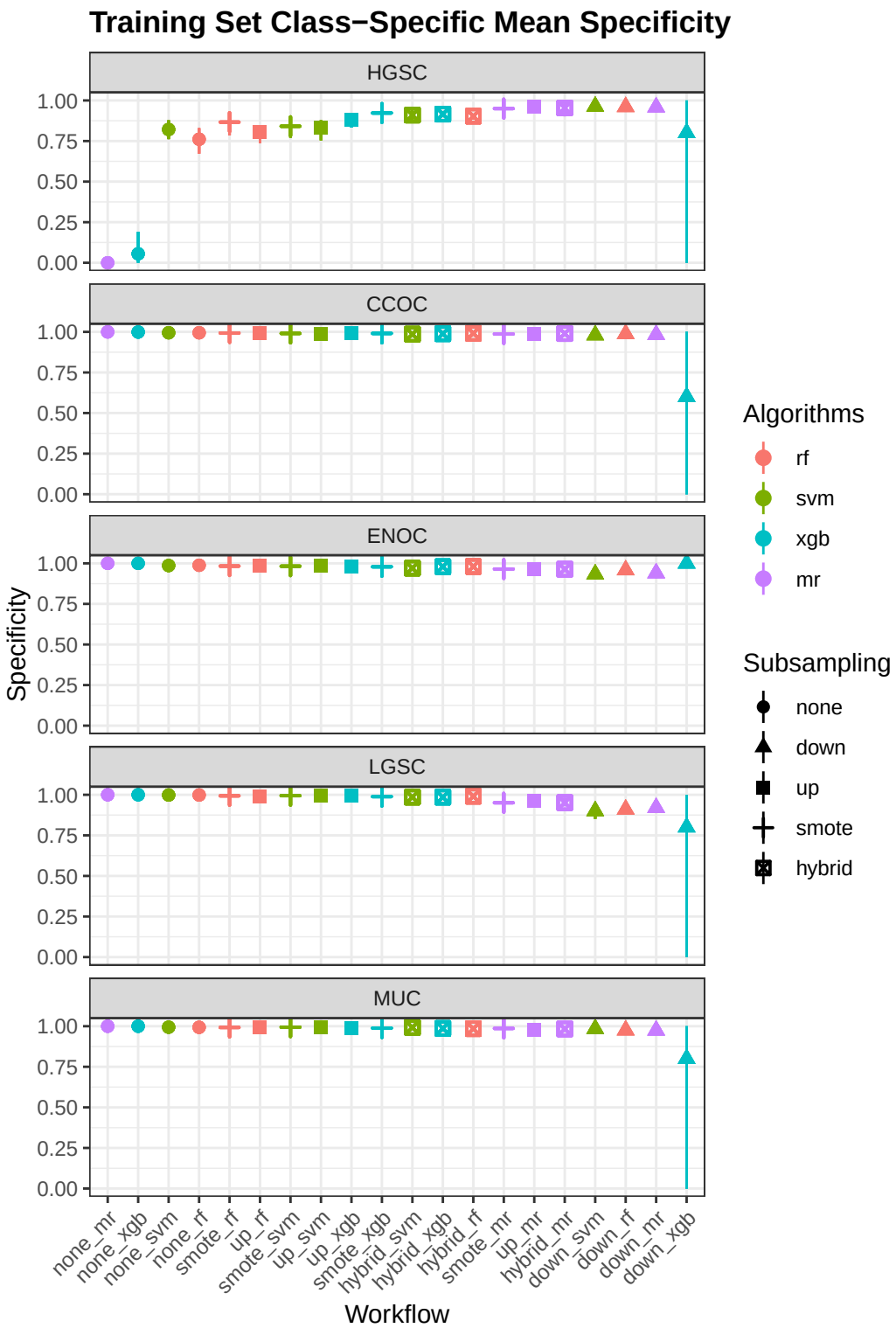


Figure 4.6: Training Set Class-Specific Mean Specificity

Table 4.6: Training Set Class-Specific Mean Specificity

[H]

		Algorithms			
Subsampling	Histotype	rf	svm	xgb	mr
none	HGSC	0.761	0.821	0.055	0
none	CCOC	0.995	0.995	0.999	1
none	ENOC	0.988	0.986	1	1
none	LGSC	0.998	0.998	1	1
none	MUC	0.993	0.994	1	1
down	HGSC	0.961	0.963	0.8	0.959
down	CCOC	0.987	0.98	0.6	0.983
down	ENOC	0.958	0.933	1	0.94
down	LGSC	0.91	0.9	0.8	0.92
down	MUC	0.975	0.984	0.8	0.975
up	HGSC	0.803	0.833	0.881	0.964
up	CCOC	0.991	0.99	0.995	0.987
up	ENOC	0.987	0.984	0.981	0.962
up	LGSC	0.991	0.993	0.993	0.963
up	MUC	0.992	0.995	0.988	0.979
smote	HGSC	0.866	0.841	0.923	0.95
smote	CCOC	0.993	0.991	0.991	0.987
smote	ENOC	0.983	0.982	0.979	0.965
smote	LGSC	0.993	0.994	0.989	0.951
smote	MUC	0.992	0.994	0.988	0.986
hybrid	HGSC	0.903	0.91	0.916	0.954
hybrid	CCOC	0.991	0.986	0.989	0.99
hybrid	ENOC	0.981	0.97	0.979	0.964
hybrid	LGSC	0.991	0.985	0.985	0.952
hybrid	MUC	0.985	0.992	0.987	0.983

4.1.4 F1-Score

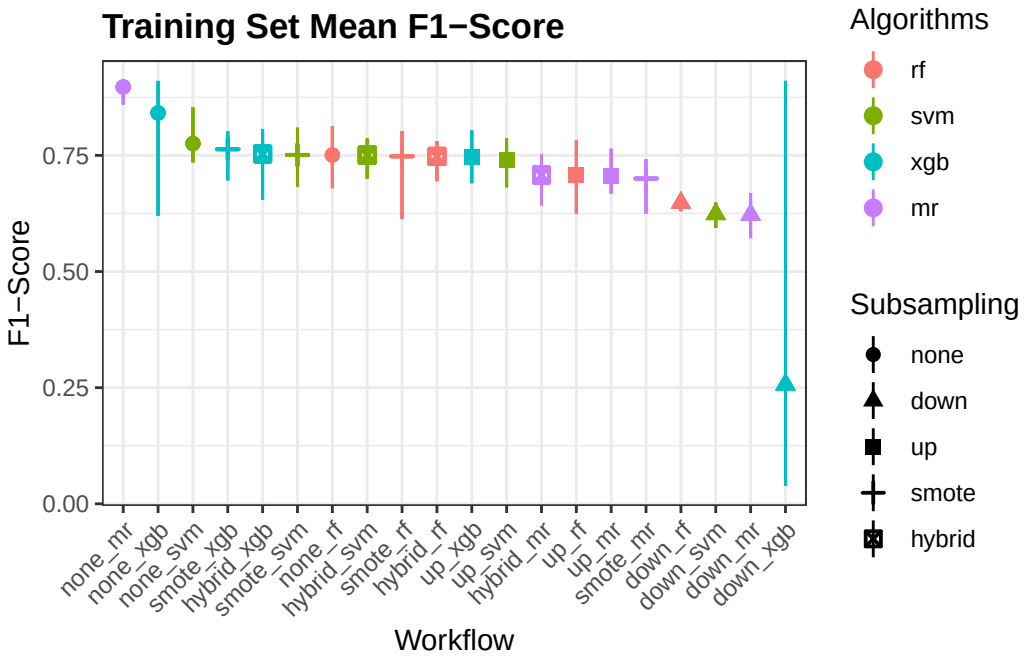


Figure 4.7: Training Set Mean F1-Score

Table 4.7: Training Set Mean F1-Score

[H]

	Algorithms			
Subsampling	rf	svm	xgb	mr
none	0.751	0.775	0.841	0.897
down	0.648	0.623	0.257	0.622
up	0.707	0.741	0.747	0.706
smote	0.748	0.751	0.763	0.7
hybrid	0.748	0.751	0.753	0.708

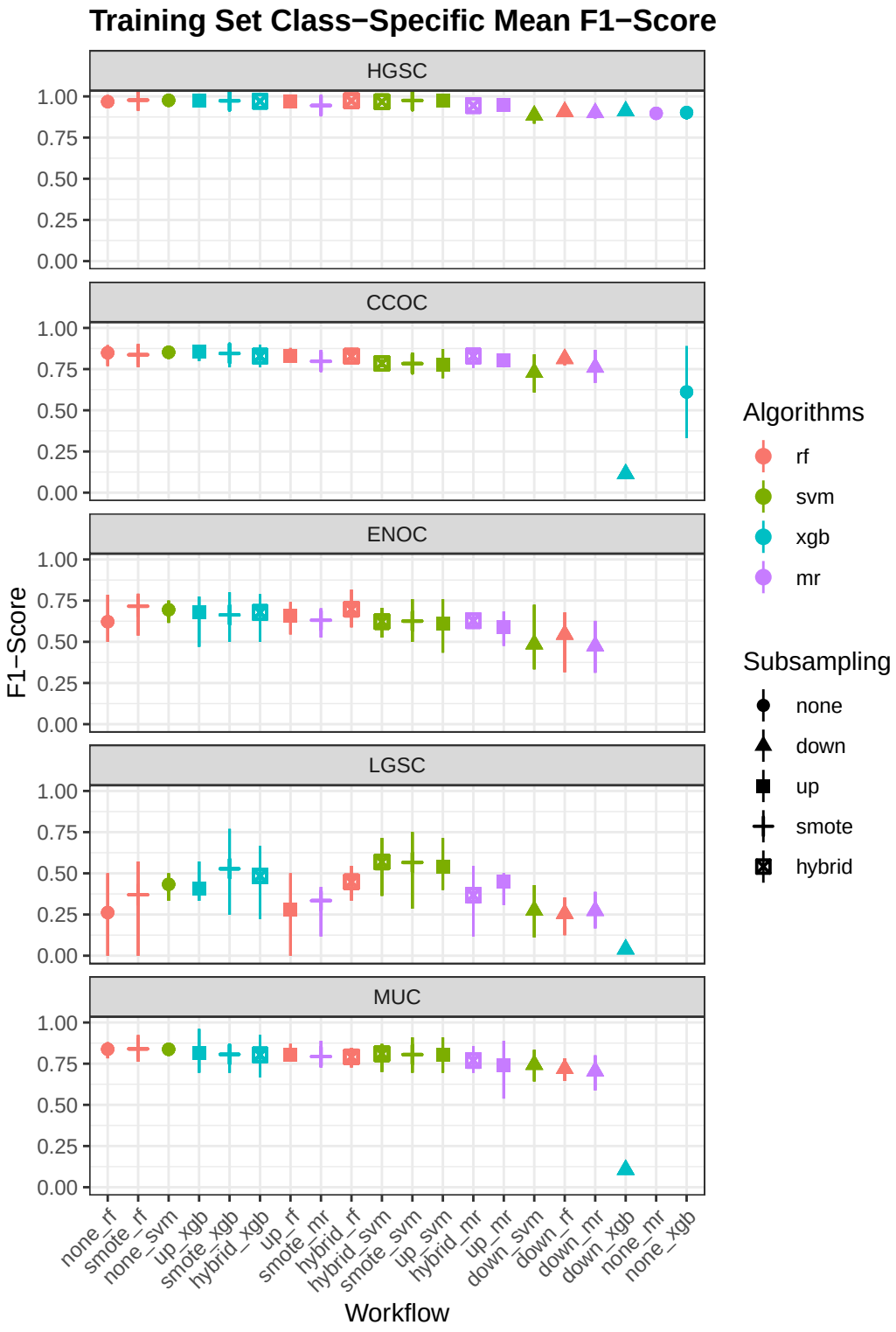


Figure 4.8: Training Set Class-Specific Mean F1-Score

Table 4.8: Training Set Class-Specific Mean F1-Score

[H]

		Algorithms			
Subsampling	Histotype	rf	svm	xgb	mr
none	HGSC	0.968	0.976	0.902	0.897
none	CCOC	0.849	0.852	0.611	NaN
none	ENOC	0.622	0.695	NaN	NaN
none	LGSC	0.262	0.433	NaN	NaN
none	MUC	0.838	0.837	NaN	NaN
down	HGSC	0.907	0.884	0.91	0.9
down	CCOC	0.813	0.728	0.114	0.76
down	ENOC	0.545	0.486	NaN	0.474
down	LGSC	0.255	0.275	0.04	0.273
down	MUC	0.719	0.744	0.106	0.704
up	HGSC	0.969	0.975	0.977	0.949
up	CCOC	0.829	0.776	0.855	0.804
up	ENOC	0.657	0.612	0.678	0.587
up	LGSC	0.278	0.54	0.408	0.448
up	MUC	0.803	0.802	0.817	0.742
smote	HGSC	0.978	0.975	0.973	0.945
smote	CCOC	0.837	0.783	0.845	0.797
smote	ENOC	0.716	0.626	0.663	0.631
smote	LGSC	0.37	0.566	0.528	0.334
smote	MUC	0.84	0.805	0.806	0.793
hybrid	HGSC	0.974	0.967	0.97	0.944
hybrid	CCOC	0.828	0.785	0.829	0.829
hybrid	ENOC	0.698	0.622	0.678	0.628
hybrid	LGSC	0.448	0.569	0.484	0.367
hybrid	MUC	0.791	0.81	0.803	0.769

4.1.5 Balanced Accuracy

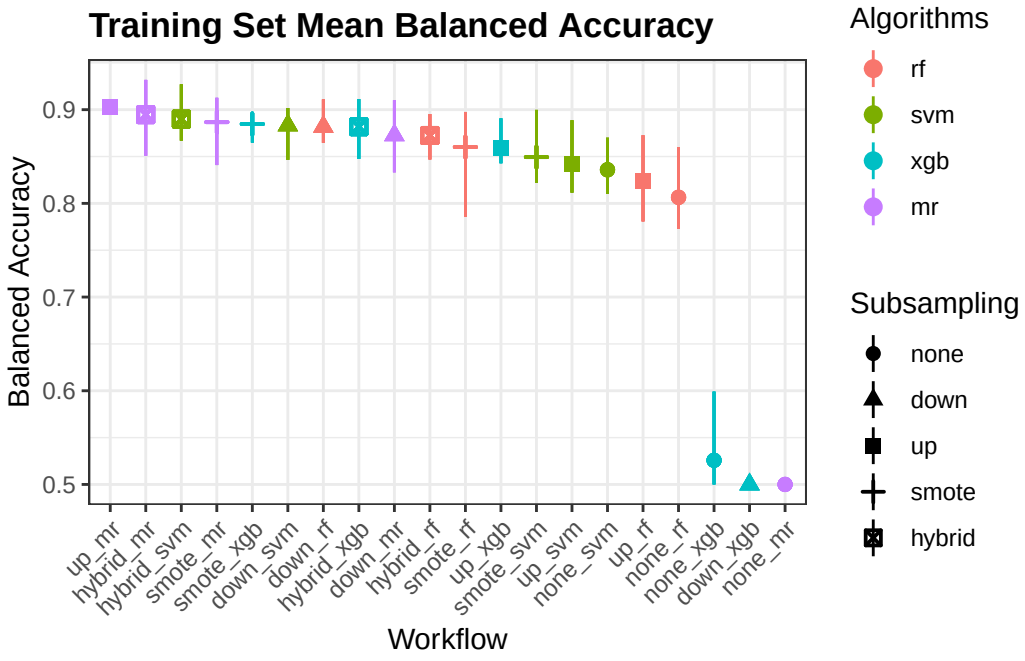


Figure 4.9: Training Set Mean Balanced Accuracy

Table 4.9: Training Set Mean Balanced Accuracy

[H]

	Algorithms			
Subsampling	rf	svm	xgb	mr
none	0.806	0.836	0.526	0.5
down	0.882	0.883	0.5	0.873
up	0.824	0.842	0.859	0.903
smote	0.86	0.849	0.885	0.887
hybrid	0.872	0.89	0.882	0.895

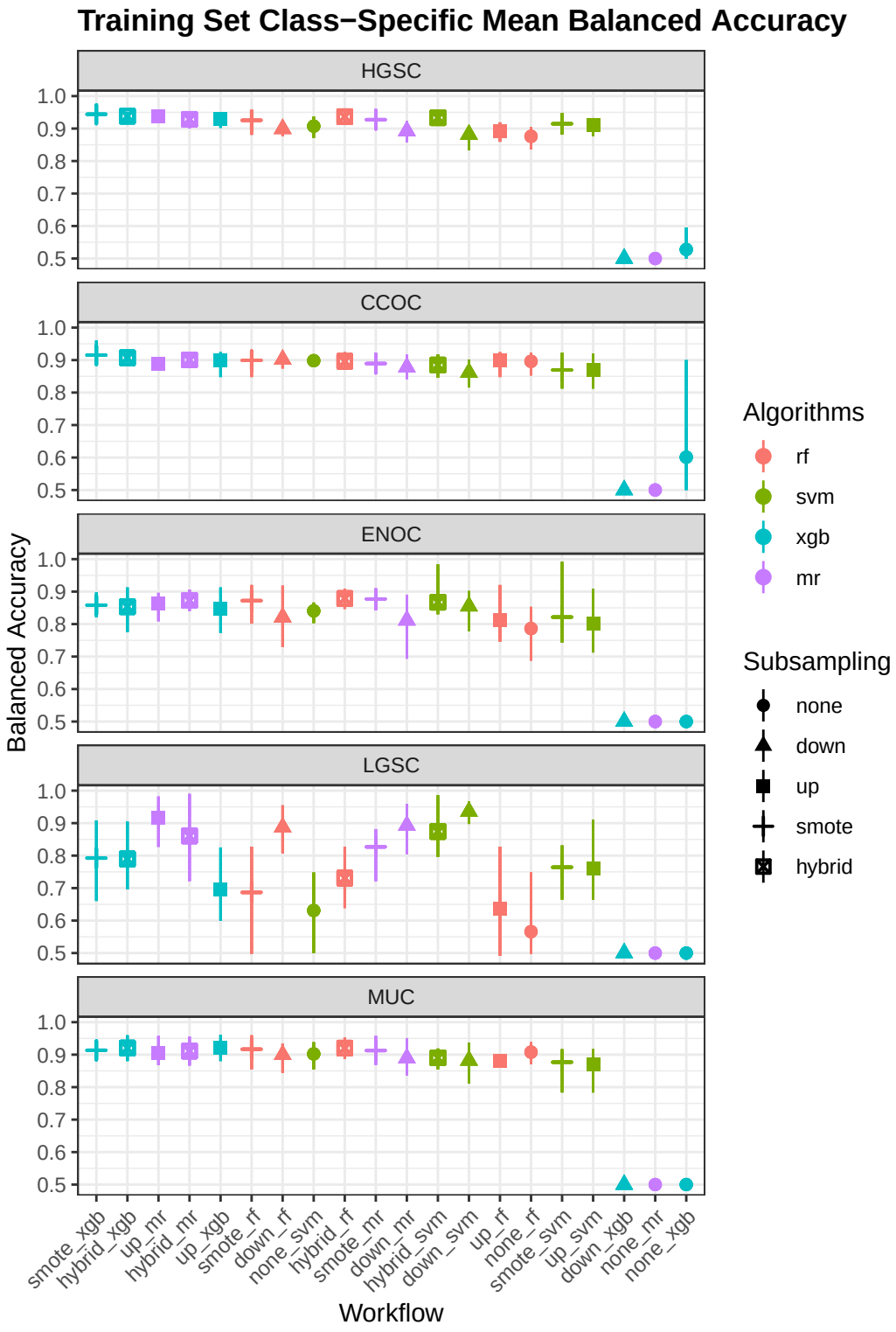


Figure 4.10: Training Set Class-Specific Mean Balanced Accuracy

Table 4.10: Training Set Class-Specific Mean Balanced Accuracy

[H]

		Algorithms			
Subsampling	Histotype	rf	svm	xgb	mr
none	HGSC	0.876	0.907	0.528	0.5
none	CCOC	0.896	0.898	0.601	0.5
none	ENOC	0.786	0.841	0.5	0.5
none	LGSC	0.566	0.631	0.5	0.5
none	MUC	0.908	0.902	0.5	0.5
down	HGSC	0.899	0.882	0.5	0.892
down	CCOC	0.902	0.862	0.5	0.878
down	ENOC	0.821	0.855	0.5	0.812
down	LGSC	0.887	0.936	0.5	0.893
down	MUC	0.9	0.882	0.5	0.889
up	HGSC	0.892	0.911	0.931	0.937
up	CCOC	0.898	0.869	0.9	0.888
up	ENOC	0.812	0.801	0.848	0.864
up	LGSC	0.636	0.761	0.695	0.917
up	MUC	0.881	0.87	0.921	0.906
smote	HGSC	0.925	0.915	0.944	0.927
smote	CCOC	0.899	0.869	0.915	0.889
smote	ENOC	0.872	0.822	0.858	0.877
smote	LGSC	0.687	0.764	0.792	0.827
smote	MUC	0.917	0.877	0.914	0.913
hybrid	HGSC	0.937	0.933	0.938	0.928
hybrid	CCOC	0.896	0.885	0.907	0.901
hybrid	ENOC	0.879	0.867	0.853	0.873
hybrid	LGSC	0.731	0.874	0.79	0.861
hybrid	MUC	0.92	0.89	0.92	0.911

4.1.6 Kappa

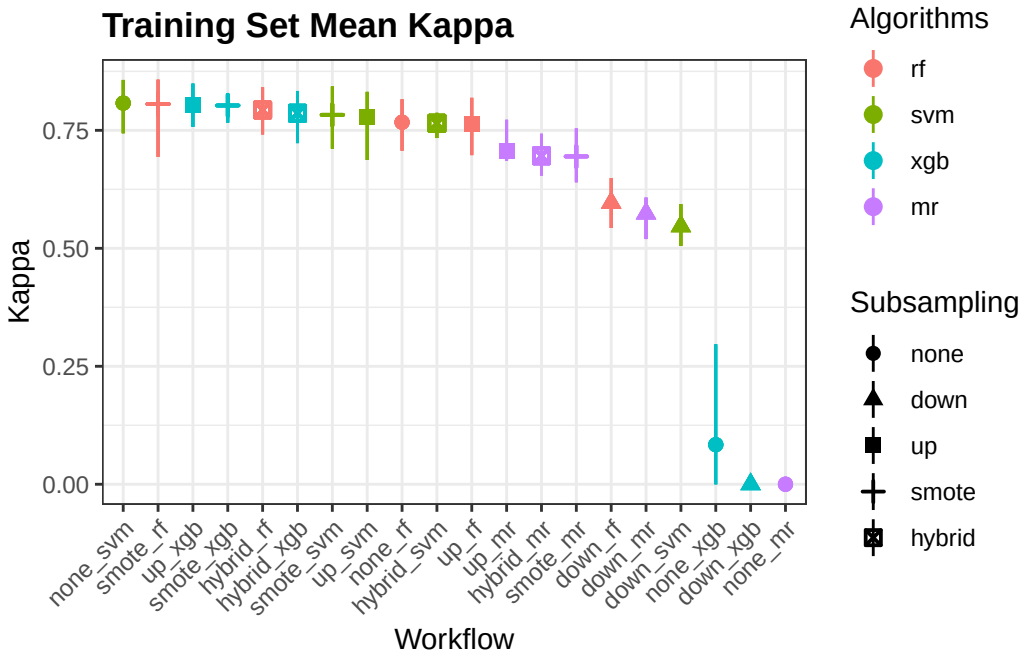


Figure 4.11: Training Set Mean Kappa

Table 4.11: Training Set Mean Kappa

[H]

	Algorithms			
Subsampling	rf	svm	xgb	mr
none	0.767	0.808	0.084	0
down	0.597	0.547	0	0.574
up	0.764	0.779	0.804	0.706
smote	0.806	0.783	0.803	0.695
hybrid	0.793	0.765	0.786	0.696

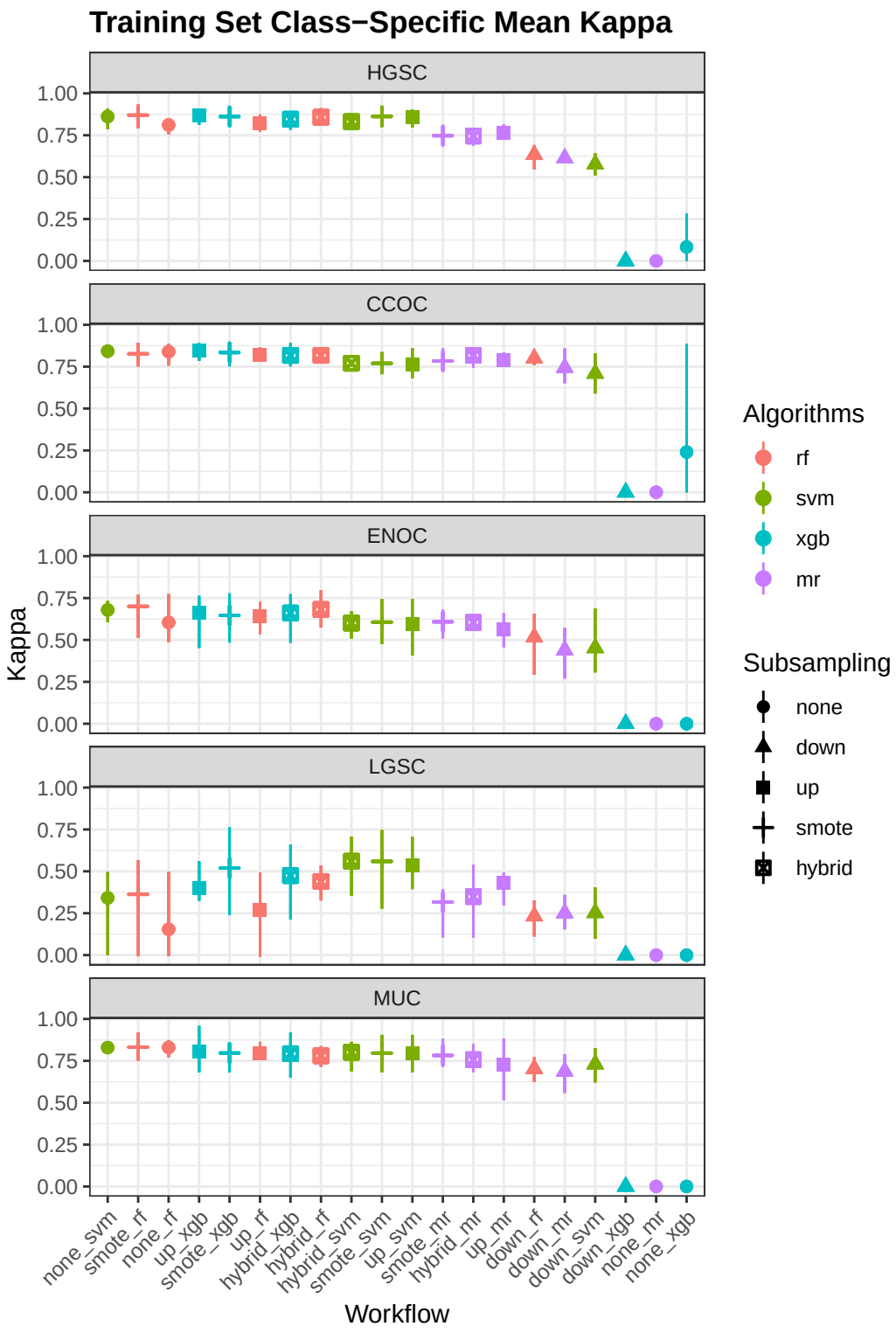


Figure 4.12: Training Set Class-Specific Mean Kappa

Table 4.12: Training Set Class-Specific Mean Kappa

[H]

		Algorithms			
Subsampling	Histotype	rf	svm	xgb	mr
none	HGSC	0.811	0.862	0.083	0
none	CCOC	0.84	0.843	0.24	0
none	ENOC	0.604	0.679	0	0
none	LGSC	0.154	0.342	0	0
none	MUC	0.83	0.829	0	0
down	HGSC	0.633	0.577	0	0.613
down	CCOC	0.8	0.709	0	0.744
down	ENOC	0.517	0.452	0	0.439
down	LGSC	0.232	0.252	0	0.251
down	MUC	0.702	0.73	0	0.687
up	HGSC	0.819	0.859	0.871	0.766
up	CCOC	0.818	0.762	0.845	0.79
up	ENOC	0.641	0.593	0.66	0.562
up	LGSC	0.269	0.533	0.399	0.434
up	MUC	0.793	0.793	0.807	0.728
smote	HGSC	0.87	0.862	0.862	0.747
smote	CCOC	0.827	0.77	0.835	0.783
smote	ENOC	0.7	0.606	0.646	0.608
smote	LGSC	0.363	0.56	0.519	0.316
smote	MUC	0.832	0.796	0.796	0.782
hybrid	HGSC	0.858	0.831	0.846	0.745
hybrid	CCOC	0.817	0.77	0.817	0.818
hybrid	ENOC	0.682	0.601	0.661	0.604
hybrid	LGSC	0.439	0.56	0.475	0.349
hybrid	MUC	0.78	0.801	0.793	0.757

4.1.7 G-mean

DEPRECATED

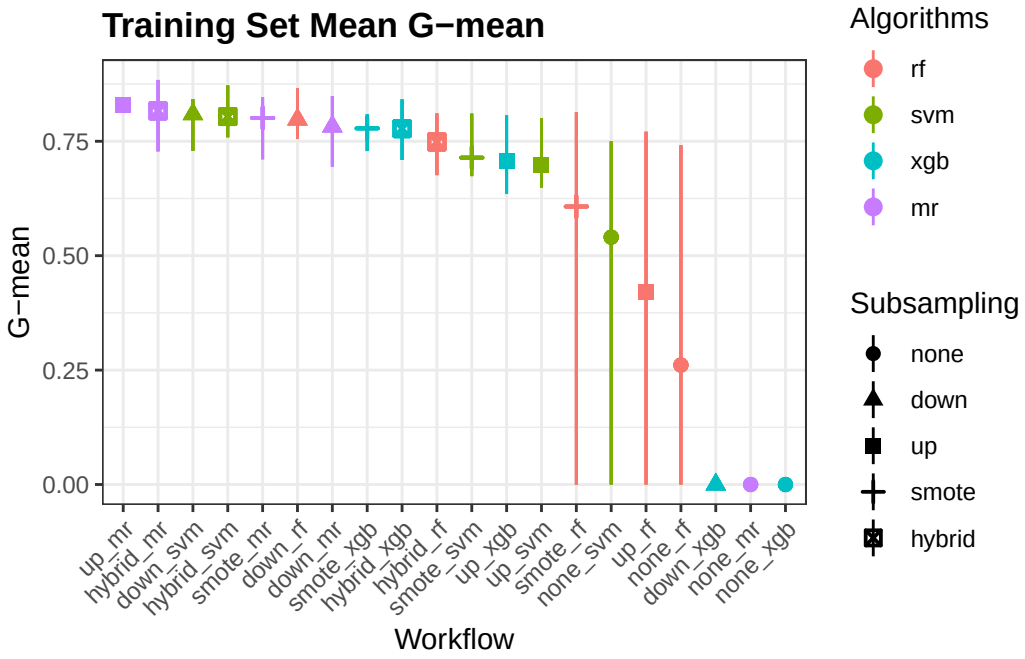


Figure 4.13: Training Set Mean G-mean

Table 4.13: Training Set Mean G-mean

[H]

	Algorithms			
Subsampling	rf	svm	xgb	mr
none	0.261	0.54	0	0
down	0.798	0.809	0	0.782
up	0.421	0.699	0.706	0.829
smote	0.607	0.714	0.778	0.801
hybrid	0.749	0.804	0.778	0.816

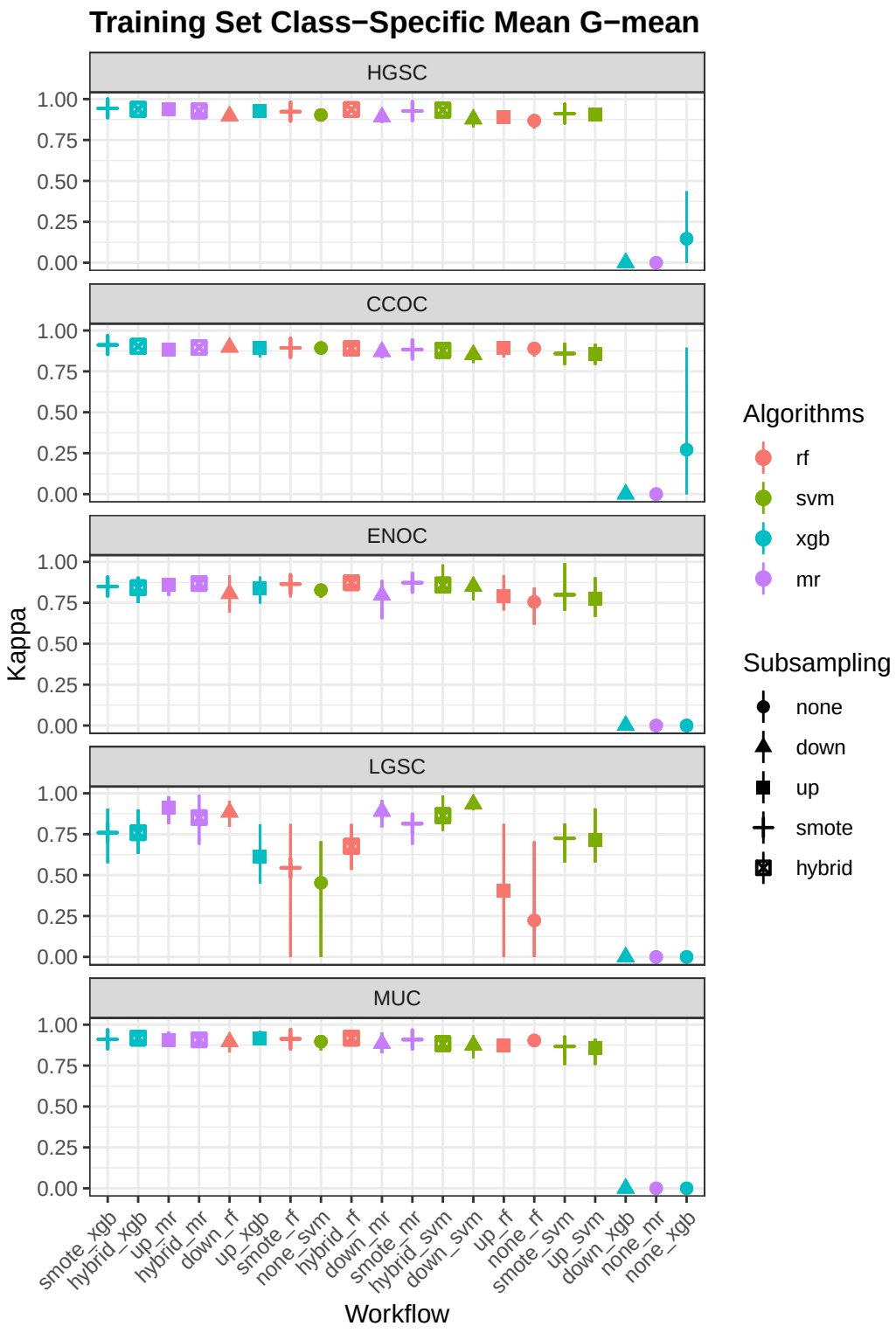


Figure 4.14: Training Set Class-Specific Mean G-mean

Table 4.14: Training Set Class-Specific Mean G-mean

[H]

		Algorithms			
Subsampling	Histotype	rf	svm	xgb	mr
none	HGSC	0.868	0.903	0.146	0
none	CCOC	0.89	0.893	0.271	0
none	ENOC	0.755	0.827	0	0
none	LGSC	0.223	0.453	0	0
none	MUC	0.903	0.897	0	0
down	HGSC	0.897	0.878	0	0.89
down	CCOC	0.897	0.853	0	0.871
down	ENOC	0.806	0.85	0	0.796
down	LGSC	0.884	0.934	0	0.889
down	MUC	0.895	0.875	0	0.884
up	HGSC	0.887	0.907	0.929	0.936
up	CCOC	0.893	0.859	0.894	0.882
up	ENOC	0.789	0.775	0.836	0.858
up	LGSC	0.403	0.715	0.614	0.913
up	MUC	0.874	0.859	0.918	0.903
smote	HGSC	0.923	0.911	0.944	0.927
smote	CCOC	0.894	0.859	0.912	0.884
smote	ENOC	0.864	0.799	0.849	0.872
smote	LGSC	0.545	0.724	0.759	0.814
smote	MUC	0.913	0.867	0.91	0.909
hybrid	HGSC	0.936	0.933	0.938	0.928
hybrid	CCOC	0.891	0.878	0.903	0.896
hybrid	ENOC	0.872	0.859	0.842	0.868
hybrid	LGSC	0.676	0.863	0.759	0.851
hybrid	MUC	0.917	0.884	0.918	0.907

4.2 Rank Aggregation

Multi-step methods:

- **sequential**: sequential algorithm sequence of subsampling methods and algorithms used are:
  - HGSC vs. non-HGSC using SMOTE subsampling and random forest
  - CCOC vs. non-CCOC using hybrid subsampling and XGBoost
  - ENOC vs. non-ENOC using upsampling and support vector machine
  - LGSC vs. MUC using hybrid subsampling and regularized multinomial regression
- **two_step**: two-step algorithm sequence of subsampling methods and algorithms used are:
  - HGSC vs. non-HGSC using SMOTE subsampling and random forest
  - CCOC vs. ENOC vs. MUC vs. LGSC using hybrid subsampling and support vector machine

We conduct rank aggregation using a two-stage nested appraoch:

1. First we rank aggregate the per-class metrics for F1-score, balanced accuracy and kappa.
2. Then we take the aggregated lists from the three metrics and perform a final rank aggregation.
3. The top workflows from the final rank aggregation are used for gene optimization in the confirmation set

4.2.1 Across Classes

Table 4.15

Show 50 entries Search:

F1-Score Rank Aggregation Summary

Workflow	Rank	HGSC	CCOC	ENOC	LGSC	MUC
All	All	All	All	All	All	All
sequential	1	0.969	0.855	0.891	0.919	0.967
two_step	2	0.969	0.865	0.738	0.782	0.864
smote_rf	3	0.978	0.837	0.716	0.37	0.84
none_svm	4	0.976	0.852	0.695	0.433	0.837
up_xgb	5	0.977	0.855	0.678	0.408	0.817
smote_xgb	6	0.973	0.845	0.663	0.528	0.806
hybrid_xgb	7	0.97	0.829	0.678	0.484	0.803
smote_svm	8	0.975	0.783	0.626	0.566	0.805
hybrid_rf	9	0.974	0.828	0.698	0.448	0.791
up_rf	10	0.969	0.829	0.657	0.278	0.803
up_svm	11	0.975	0.776	0.612	0.54	0.802
hybrid_mr	12	0.944	0.829	0.628	0.367	0.769
hybrid_svm	13	0.967	0.785	0.622	0.569	0.81
none_rf	14	0.968	0.849	0.622	0.262	0.838
smote_mr	15	0.945	0.797	0.631	0.334	0.793
up_mr	16	0.949	0.804	0.587	0.448	0.742
down_rf	17	0.907	0.813	0.545	0.255	0.719
down_mr	18	0.9	0.76	0.474	0.273	0.704
down_svm	19	0.884	0.728	0.486	0.275	0.744

Showing 1 to 19 of 19 entries Previous 1 Next

Table 4.16

Show 50 entries Search:

Balanced Accuracy Rank Aggregation Summary

Workflow	Rank	HGSC	CCOC	ENOC	LGSC	MUC
All	All	All	All	All	All	All
sequential	1	0.919	0.889	0.905	0.955	0.955
hybrid_xgb	2	0.938	0.907	0.853	0.79	0.92
smote_xgb	3	0.944	0.915	0.858	0.792	0.914
hybrid_rf	4	0.937	0.896	0.879	0.731	0.92
smote_rf	5	0.925	0.899	0.872	0.687	0.917
up_xgb	6	0.931	0.9	0.848	0.695	0.921
up_mr	7	0.937	0.888	0.864	0.917	0.906
hybrid_mr	8	0.928	0.901	0.873	0.861	0.911
smote_mr	9	0.927	0.889	0.877	0.827	0.913
two_step	10	0.919	0.893	0.819	0.924	0.908
hybrid_svm	11	0.933	0.885	0.867	0.874	0.89
none_svm	12	0.907	0.898	0.841	0.631	0.902
smote_svm	13	0.915	0.869	0.822	0.764	0.877
down_rf	14	0.899	0.902	0.821	0.887	0.9
down_mr	15	0.892	0.878	0.812	0.893	0.889
down_svm	16	0.882	0.862	0.855	0.936	0.882
up_rf	17	0.892	0.898	0.812	0.636	0.881
up_svm	18	0.911	0.869	0.801	0.761	0.87
none_rf	19	0.876	0.896	0.786	0.566	0.908
down_xgb	20	0.5	0.5	0.5	0.5	0.5
none_mr	21	0.5	0.5	0.5	0.5	0.5
none_xgb	22	0.528	0.601	0.5	0.5	0.5

Showing 1 to 22 of 22 entries Previous 1 Next

Table 4.17

Show 50 entries Search:

Kappa Rank Aggregation Summary

Workflow	Rank	HGSC	CCOC	ENOC	LGSC	MUC
All	All	All	All	All	All	All
sequential	1	0.833	0.774	0.819	0.886	0.886
smote_rf	2	0.87	0.827	0.7	0.363	0.832
up_xgb	3	0.871	0.845	0.66	0.399	0.807
none_svm	4	0.862	0.843	0.679	0.342	0.829
two_step	5	0.833	0.796	0.632	0.758	0.818
smote_xgb	6	0.862	0.835	0.646	0.519	0.796
hybrid_rf	7	0.858	0.817	0.682	0.439	0.78
hybrid_xgb	8	0.846	0.817	0.661	0.475	0.793
smote_svm	9	0.862	0.77	0.606	0.56	0.796
up_svm	10	0.859	0.762	0.593	0.533	0.793
hybrid_svm	11	0.831	0.77	0.601	0.56	0.801
up_rf	12	0.819	0.818	0.641	0.269	0.793
none_rf	13	0.811	0.84	0.604	0.154	0.83
hybrid_mr	14	0.745	0.818	0.604	0.349	0.757
smote_mr	15	0.747	0.783	0.608	0.316	0.782
up_mr	16	0.766	0.79	0.562	0.434	0.728
down_rf	17	0.633	0.8	0.517	0.232	0.702
down_mr	18	0.613	0.744	0.439	0.251	0.687
down_svm	19	0.577	0.709	0.452	0.252	0.73
down_xgb	20	0	0	0	0	0
none_mr	21	0	0	0	0	0
none_xgb	22	0.083	0.24	0	0	0

Showing 1 to 22 of 22 entries Previous 1 Next

4.2.2 Across Metrics

Table 4.18: Rank Aggregation Comparison of Metrics Used

[H]

Rank	F1	Balanced Accuracy	Kappa
1	sequential	sequential	sequential
2	two_step	hybrid_xgb	smote_rf
3	smote_rf	smote_xgb	up_xgb
4	none_svm	hybrid_rf	none_svm
5	up_xgb	smote_rf	two_step
6	smote_xgb	up_xgb	smote_xgb
7	hybrid_xgb	up_mr	hybrid_rf
8	smote_svm	hybrid_mr	hybrid_xgb
9	hybrid_rf	smote_mr	smote_svm
10	up_rf	two_step	up_svm
11	up_svm	hybrid_svm	hybrid_svm
12	hybrid_mr	none_svm	up_rf
13	hybrid_svm	smote_svm	none_rf
14	none_rf	down_rf	hybrid_mr
15	smote_mr	down_mr	smote_mr
16	up_mr	down_svm	up_mr
17	down_rf	up_rf	down_rf
18	down_mr	up_svm	down_mr
19	down_svm	none_rf	down_svm
20	NA	down_xgb	down_xgb
21	NA	none_mr	none_mr
22	NA	none_xgb	none_xgb

Table 4.19: Top 5 Workflows from Final Rank Aggregation

[H]

Rank	Workflow
1	sequential
2	smote_rf
3	two_step
4	none_svm
5	up_xgb

4.2.3 Top Workflows

We look at the per-class evaluation metrics of the top 5 workflows.

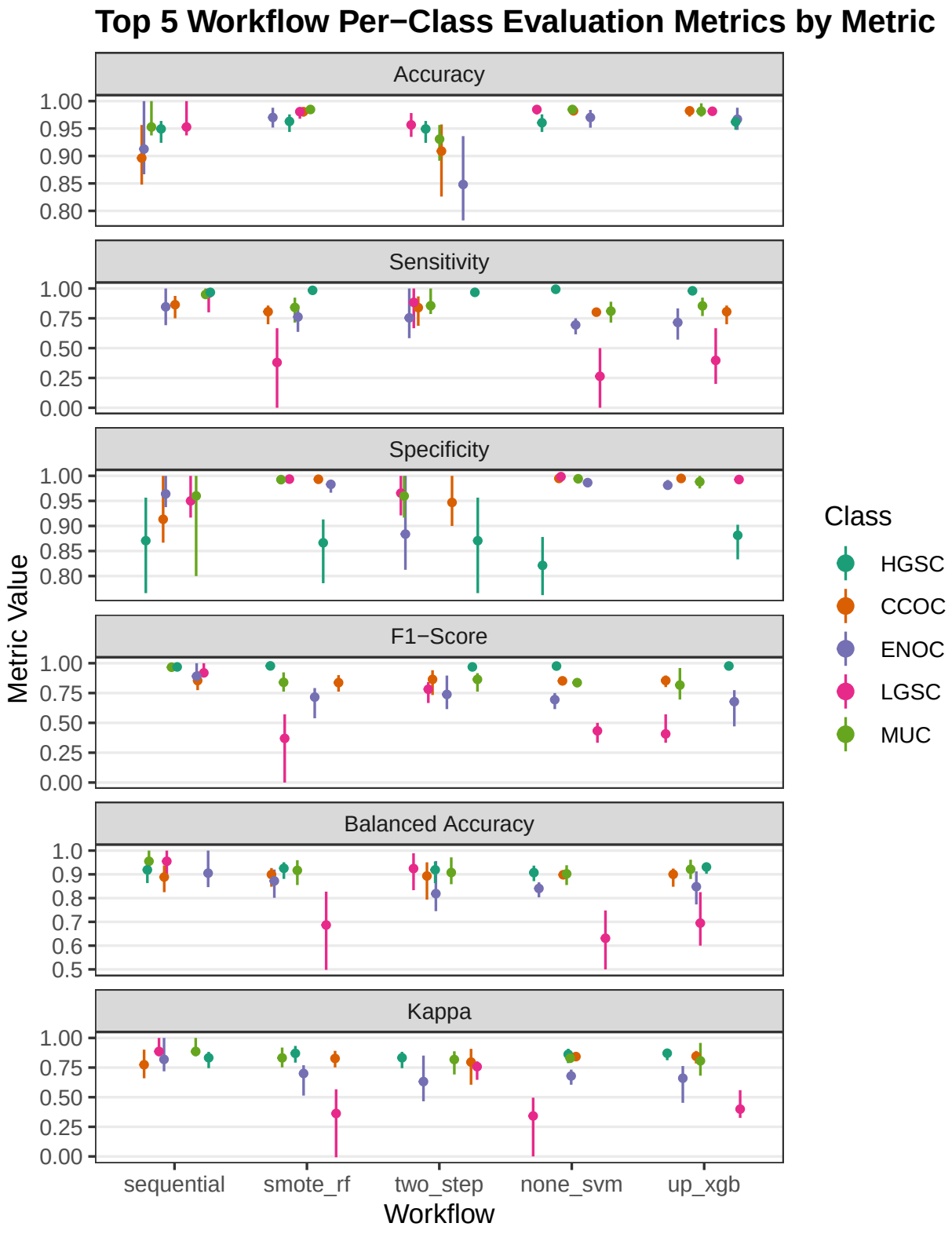
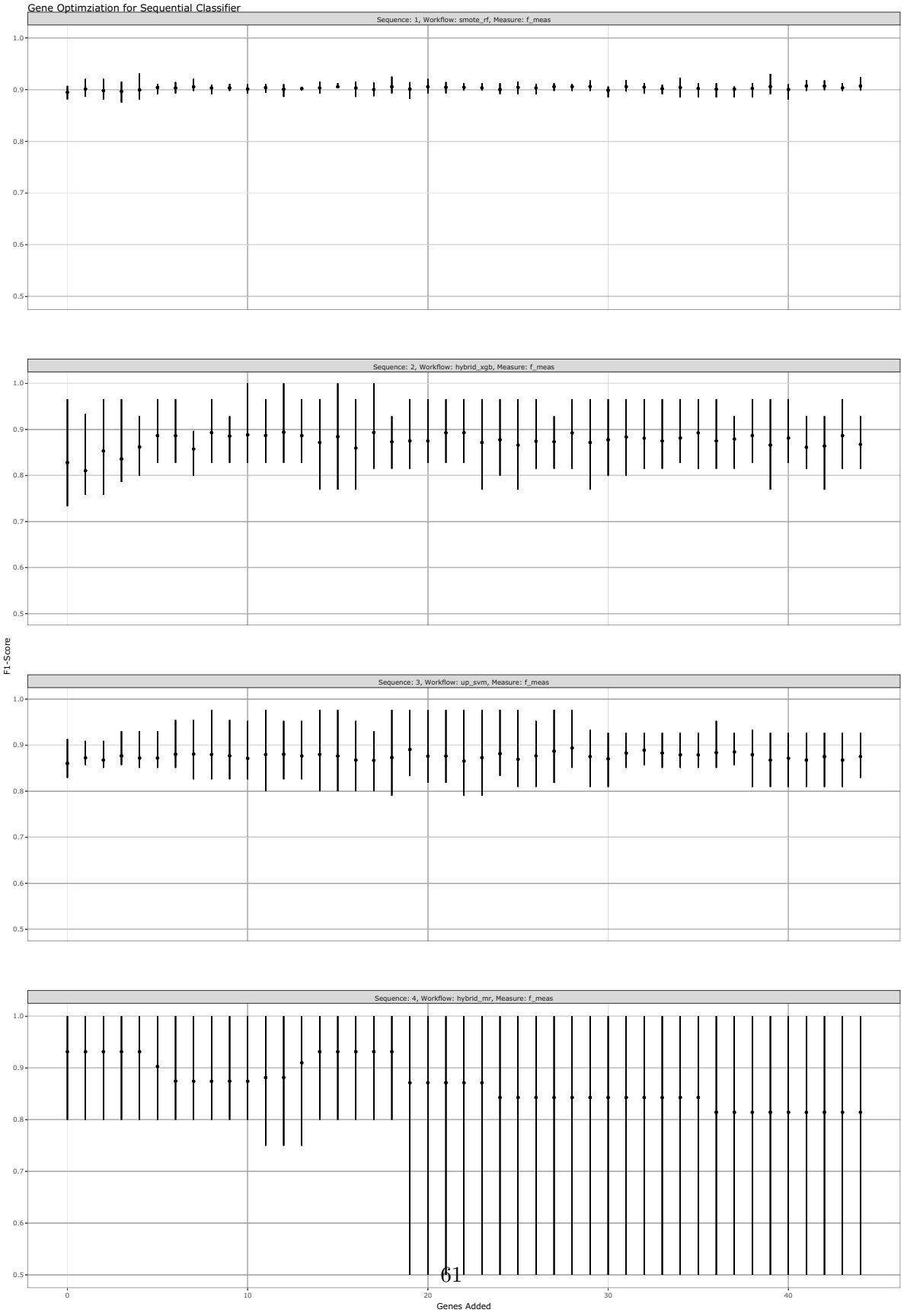


Figure 4.15: Top 5 Workflow Per-Class Evaluation Metrics by Metric

Misclassified cases from a previous step of the sequence of classifiers are not included in subsequent steps of the training set CV folds. Thus, we cannot piece together the test set predictions from the sequential and two-step algorithms to obtain overall metrics.

4.3 Optimal Gene Sets

4.3.1 Sequential Algorithm



In the sequential algorithm, sequences 1, 2, and 4 have relatively flat average F1-scores across the number of genes added. However, we can observe in sequence 3, the F1-score stabilizes at around 0.88 when we reach 7 genes added, hence the optimal number of genes used will be n=28+7=35 The added genes are: CYP2C18, TFF3, TP53, HNF1B, WT1, MAP1LC3A and SLC3A1.

4.3.2 SMOTE-Random Forest

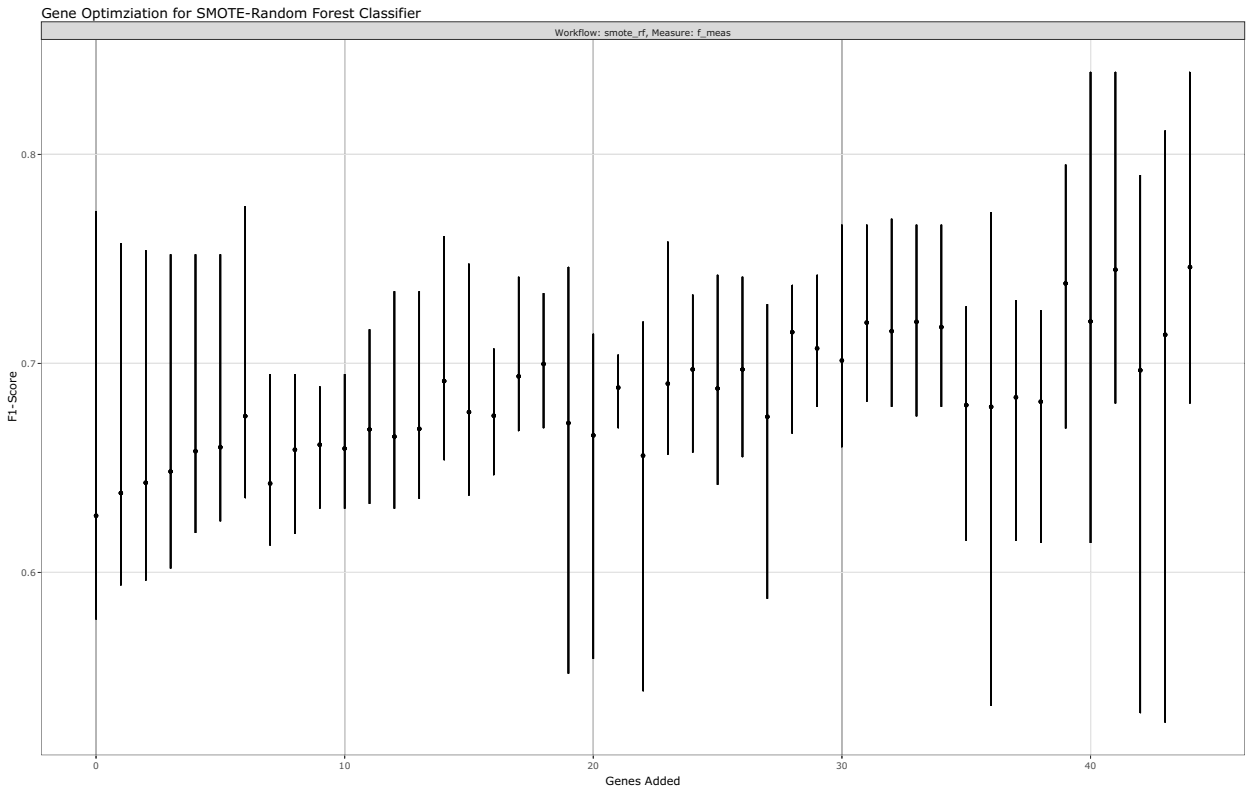


Figure 4.17: Gene Optimization for SMOTE-Random Forest Classifier

In the SMOTE-Random Forest classifier, the F1-score stabilizes at around 0.7 when we reach 18 genes added, hence the optimal number of genes used will be n=28+18=46 The added genes are: TFF1, HNF1B, TFF3, LGALS4, SLC3A1, WT1, KLK7, TPX2, CYP2C18, GAD1, IGFBP1, CAPN2, FUT3, DKK4, C1orf173, GCNT3, C10orf116 and MUC5B.

4.4 Test Set Performance

Now we'd like to see how our best methods perform in the confirmation and validation sets. The class-specific F1-scores will be used.

The top 2 methods are the sequential and SMOTE-Random Forest classifiers. We can test 2 additional methods by using either the full set of genes or the optimal set of genes for both of these classifiers.

4.4.1 Confirmation Set

Table 4.20: Evaluation Metrics on Confirmation Set Models

[H]

Method	Metric	Overall	Histotypes				
			HGSC	CCOC	ENOC	LGSC	MU
Sequential, Full Set	Accuracy	0.834	0.869	0.960	0.894	0.977	0.9
	Sensitivity	0.655	0.948	0.853	0.462	0.308	0.7
	Specificity	0.928	0.719	0.974	0.980	0.990	0.9
	F1-Score	0.664	0.905	0.831	0.590	0.348	0.6
	Balanced Accuracy	0.792	0.834	0.913	0.721	0.649	0.8
	Kappa	0.665	0.697	0.808	0.535	0.336	0.6
Sequential, Optimal Set	Accuracy	0.821	0.865	0.949	0.879	0.983	0.9
	Sensitivity	0.633	0.953	0.840	0.396	0.385	0.5
	Specificity	0.923	0.697	0.963	0.974	0.995	0.9
	F1-Score	0.659	0.902	0.792	0.519	0.476	0.6
	Balanced Accuracy	0.778	0.825	0.902	0.685	0.690	0.7
	Kappa	0.635	0.684	0.763	0.457	0.468	0.5
SMOTE-Random Forest, Full Set	Accuracy	0.843	0.871	0.969	0.896	0.980	0.9
	Sensitivity	0.659	0.962	0.867	0.453	0.308	0.7
	Specificity	0.928	0.697	0.982	0.983	0.994	0.9
	F1-Score	0.682	0.907	0.867	0.589	0.381	0.6
	Balanced Accuracy	0.793	0.829	0.925	0.718	0.651	0.8
	Kappa	0.677	0.697	0.849	0.535	0.371	0.6
SMOTE-Random Forest, Optimal Set	Accuracy	0.851	0.876	0.966	0.904	0.981	0.9
	Sensitivity	0.695	0.957	0.853	0.500	0.385	0.7
	Specificity	0.932	0.719	0.981	0.983	0.994	0.9
	F1-Score	0.715	0.910	0.853	0.631	0.455	0.7
	Balanced Accuracy	0.813	0.838	0.917	0.742	0.689	0.8
	Kappa	0.697	0.710	0.834	0.580	0.445	0.7

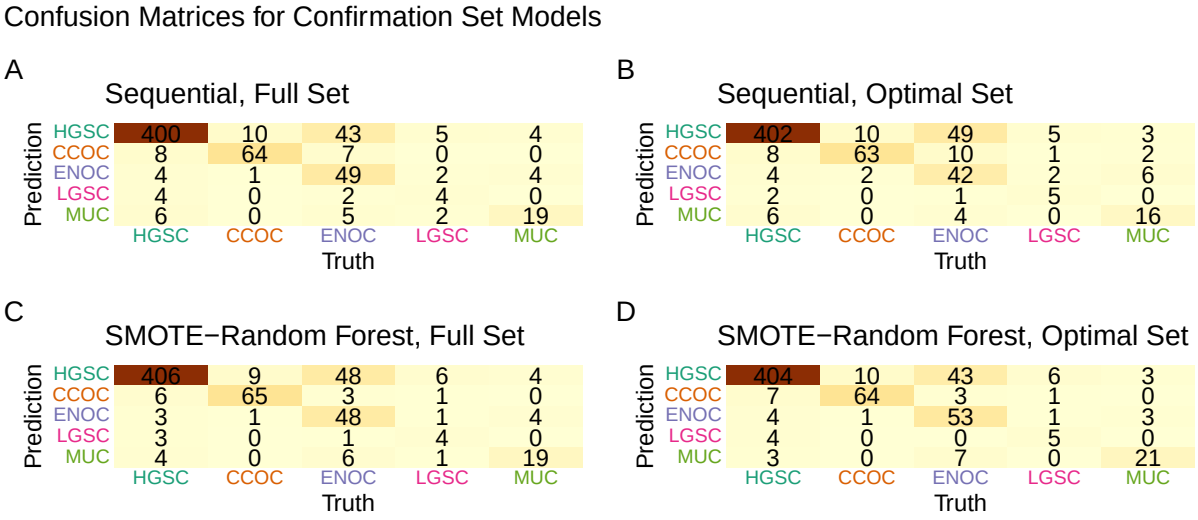


Figure 4.18: Confusion Matrices for Confirmation Set Models

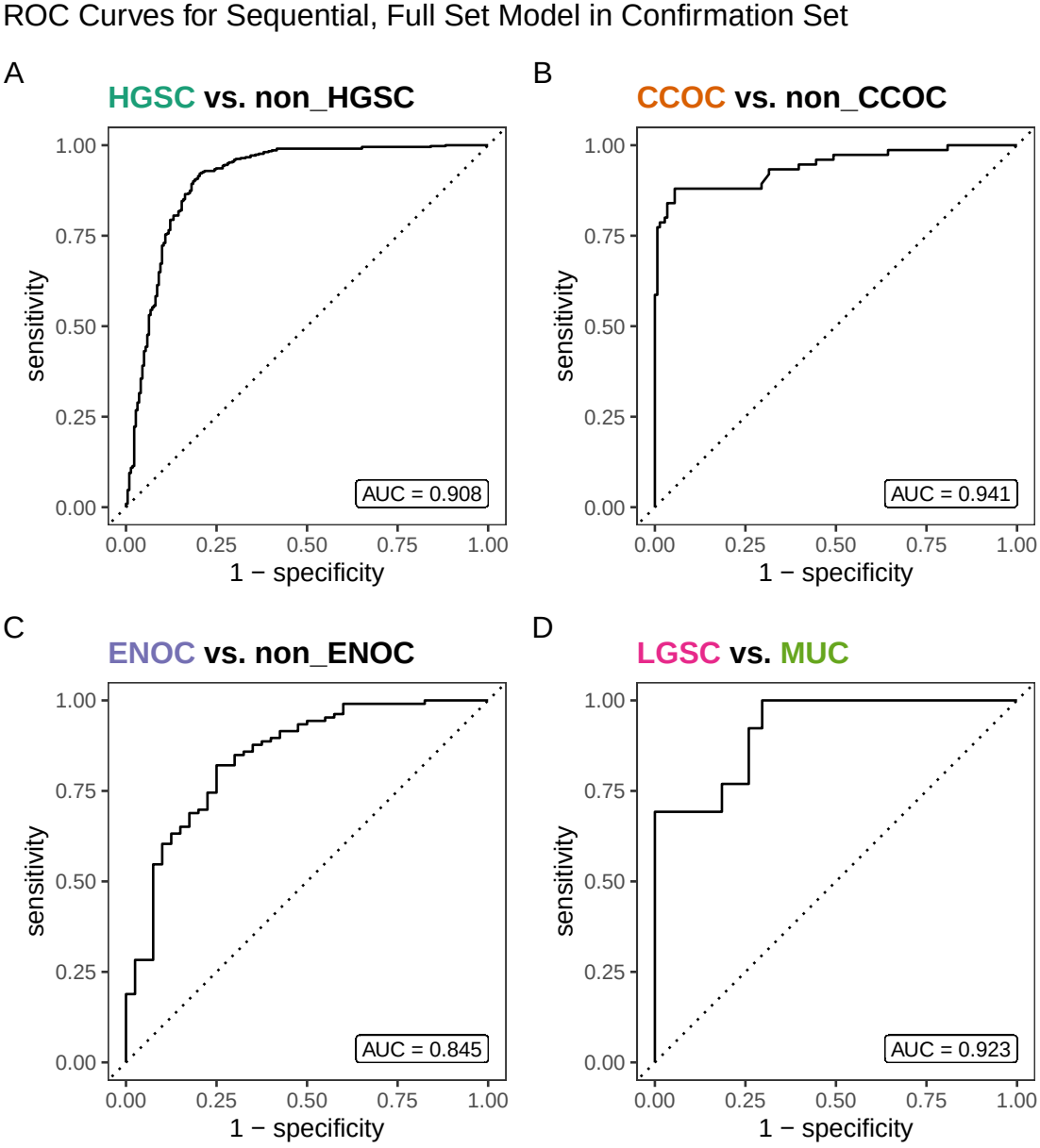


Figure 4.19: ROC Curves for Sequential Full Model in Confirmation Set

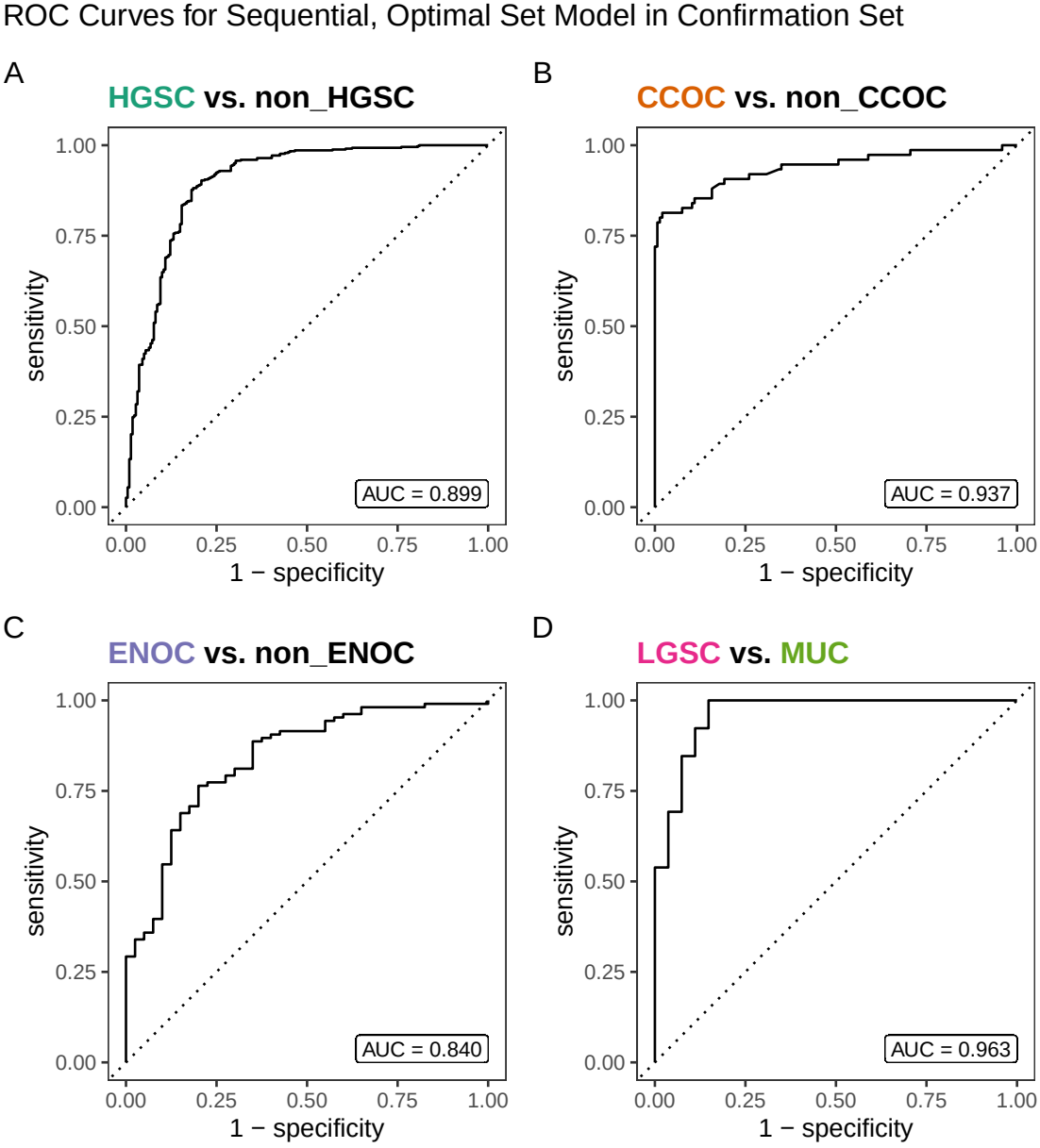


Figure 4.20: ROC Curves for Sequential, Optimal Model in Confirmation Set

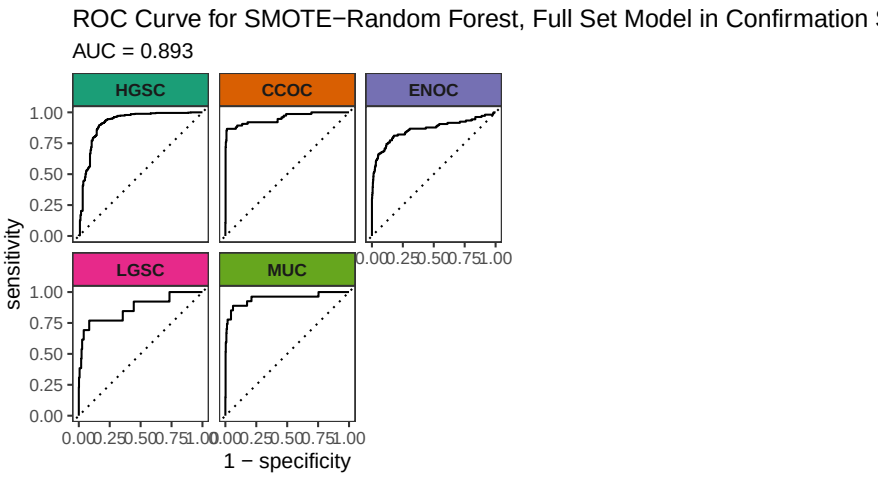


Figure 4.21: ROC Curves for SMOTE-Random Forest, Full Set Model in Confirmation Set

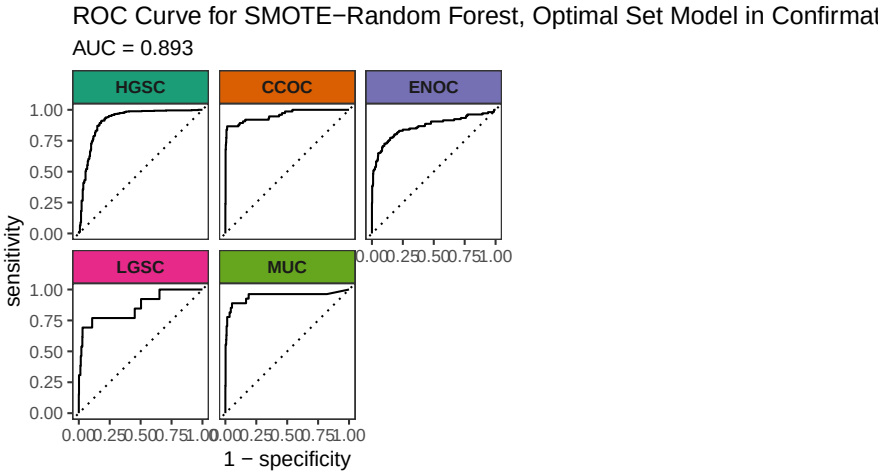


Figure 4.22: ROC Curves for SMOTE-Random Forest, Optimal Set Model in Confirmation Set

4.4.2 Validation Set

Table 4.21: Evaluation Metrics on Validation Set Model, SMOTE-Random Forest, Optimal Set

[H]

		Histotypes				
Metric	Overall	HGSC	CCOC	ENOC	LGSC	MUC
Accuracy	0.890	0.907	0.971	0.952	0.972	0.979
Sensitivity	0.774	0.926	0.937	0.714	0.444	0.846
Specificity	0.955	0.851	0.974	0.984	0.983	0.983
F1-Score	0.731	0.937	0.851	0.777	0.390	0.698
Balanced Accuracy	0.864	0.889	0.955	0.849	0.714	0.914
Kappa	0.748	0.761	0.835	0.750	0.376	0.688

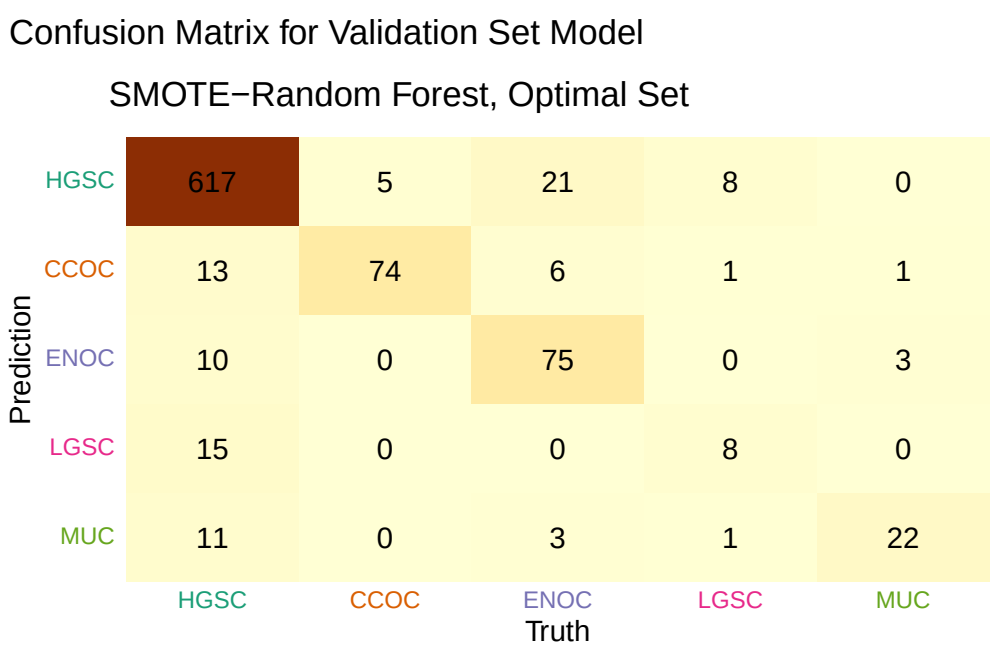


Figure 4.23: Confusion Matrix for Validation Set Model

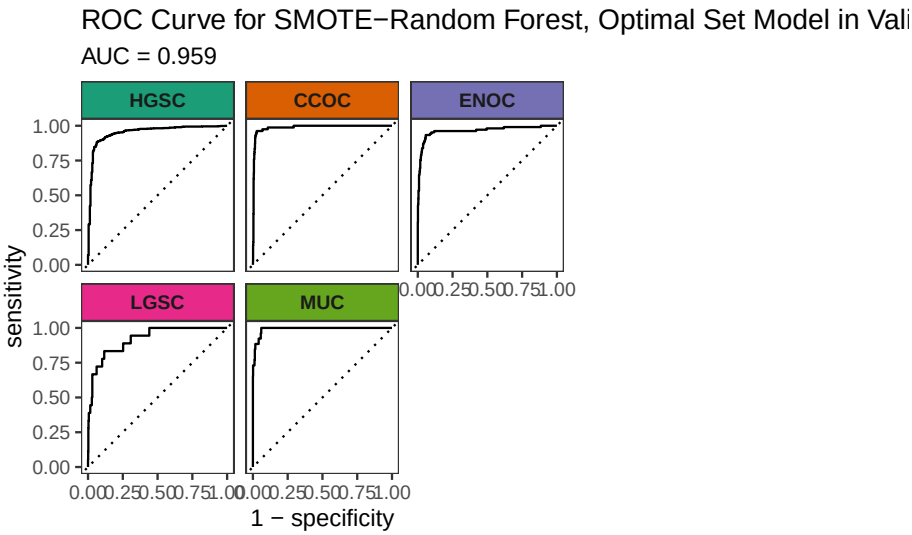


Figure 4.24: ROC Curves for SMOTE-Random Forest, Optimal Set Model in Validation Set

References

Talhouk, Aline, Stefan Kommoss, Robertson Mackenzie, Martin Cheung, Samuel Leung, Derek S. Chiu, Steve E. Kalloger, et al. 2016. "Single-Patient Molecular Testing with NanoString nCounter Data Using a Reference-Based Strategy for Batch Effect Correction." Edited by Benjamin Haibe-Kains. *PLOS ONE* 11 (4): e0153844. https://doi.org/10.1371/journal.pone.0153844.