Ovarian Cancer Histotypes: Report of Statistical Findings

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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, Kappa, and G-mean to inform the decision of which methods to carry forward for prediction in confirmation and validation sets.

2. Methods

2.1 Normalization

The full training set was comprised of data from CodeSet (CS) 1, 2, and 3. All CodeSets were first normalized to housekeeping genes, then a different approach was taken for each of the CodeSets.

CS1 was normalized to CS3 using "Random1" reference samples. These reference samples are common samples between CS1 and CS3, randomly selected such that we obtain one from each of the five histotypes. Then we use the reference method to normalize CS1 to CS3.

Similarly, CS2 was normalized to CS3 using "Random1" reference samples using five common samples between CS2 and CS3 such that there is one from each histotype.

For CS3, we first split the dataset by site: Vancouver, USC, and AOC. We use the CS3-Vancouver subset as a "reference standard", so we normalized CS3-USC and CS3-AOC to CS3-Vancouver using a "Random1" reference method where we reference samples are common between USC and Vancouver, and between AOC and Vancouver. The CS3-Vancouver is also included without further normalization.

2.2 Case Selection

Duplicate cases (two samples with the same ottaID) were removed from the training set before fitting the classification models. CS3 cases were preferred over CS1 and CS2, and CS3-Vancouver were preferred over CS3-AOC and CS3-USC.

The training, confirmation, and validation sets all used a different set of cohorts.

2.3 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`)

2.3.1 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.

2.3.2 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.

2.3.3 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:

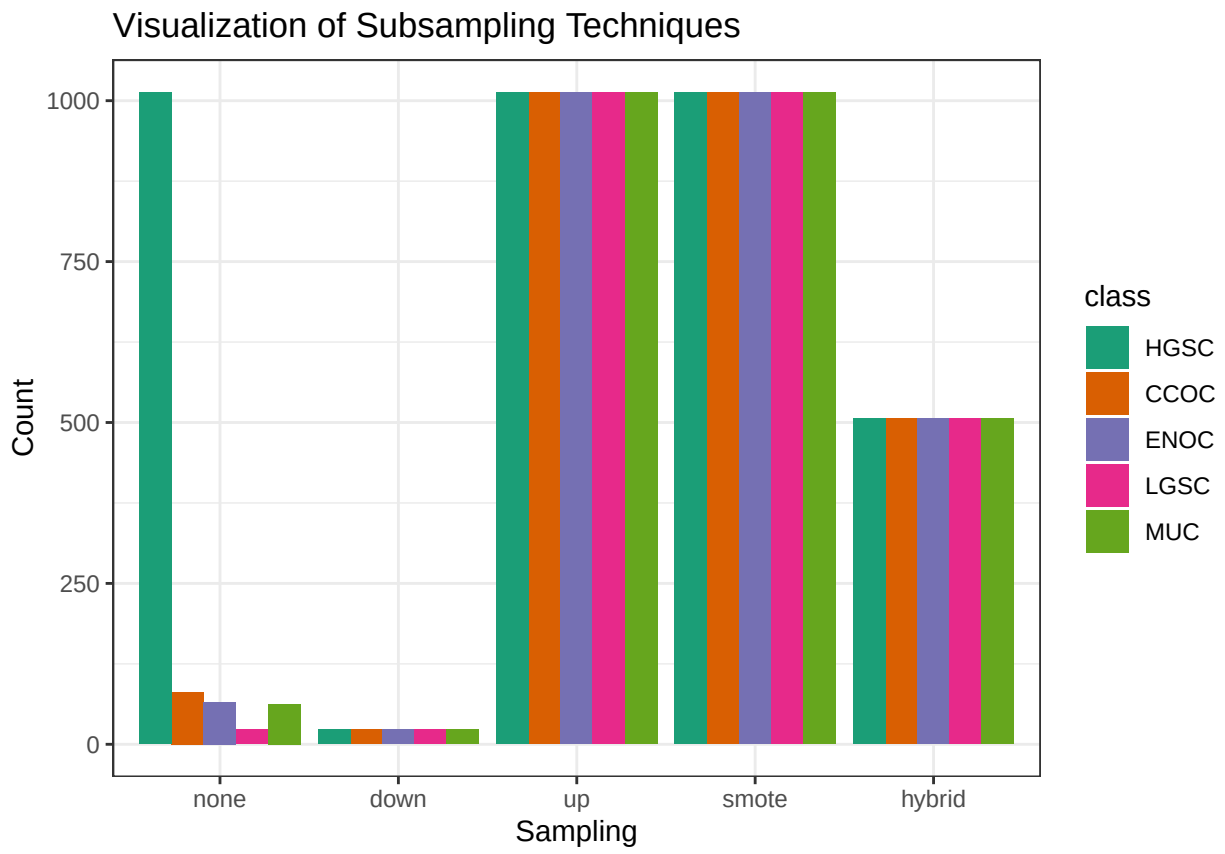


Figure 2.1: Visualization of Subsampling Techniques

2.3.4 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.

2.4 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

$$X_k = \text{Training data with k classes}$$
$$C_k = \text{Class with highest } F_1 \text{ score from training } X_k$$
$$W_k = \text{Workflow associated with } C_k$$

Figure 2.2 shows how the two-step algorithm works:

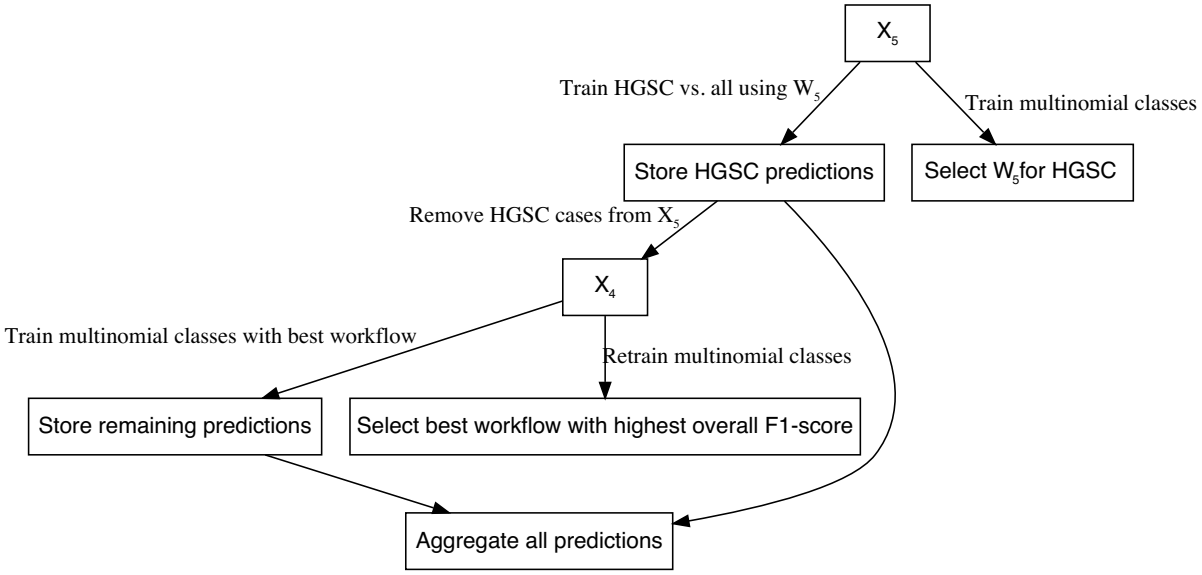


Figure 2.2: Two-Step Algorithm

Although the class imbalance problem is mostly eliminated in Step 2 after removing the HGSC cases, we still use the same subsampling method in Step 2 as was used in Step 1 to keep the algorithm consistent.

2.5 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 2.3 illustrates how the sequential algorithm works for K=5, using ovarian histotypes as an example for the classes.

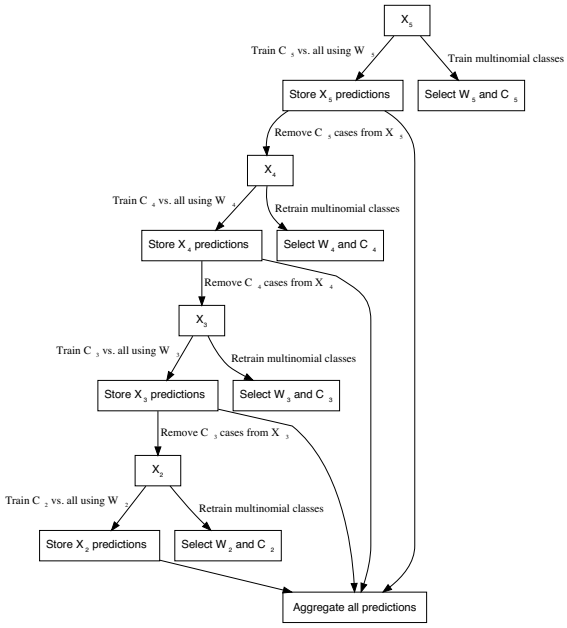


Figure 2.3: Sequential Algorithm

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.

2.5.1 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 2.4 illustrates how the final predictions are assigned:

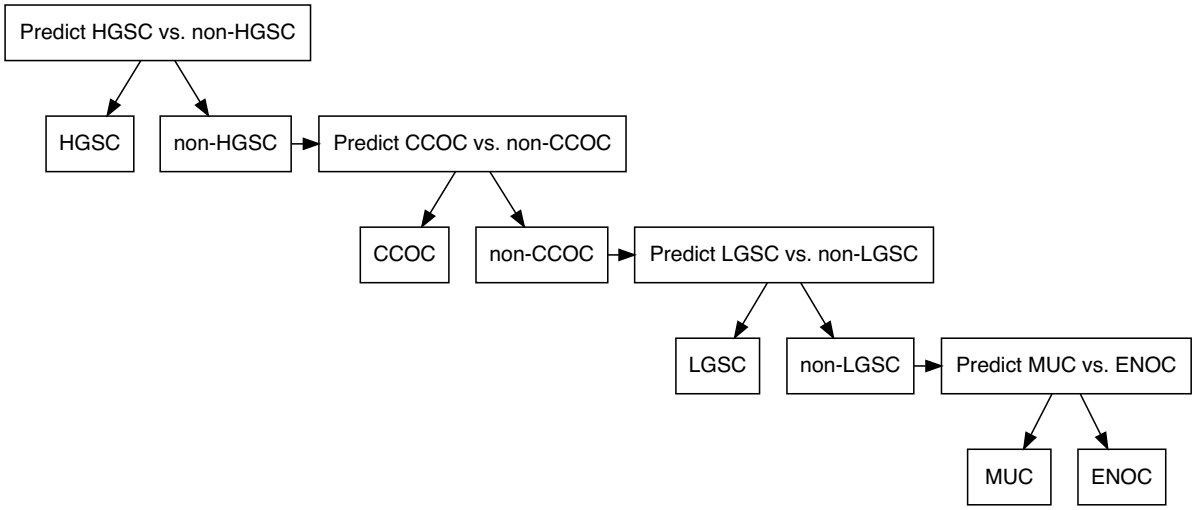


Figure 2.4: Aggregating Predictions for Sequential Algorithm

2.6 Gene Optimization

We want to discover an optimal set of genes for the classifiers while including specific genes from other studies. 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.

Taking the union of PrOTYPE and SPOT genes we obtain a total of 28 unique genes that we want to use for the final classifier, regardless of model performance. We then incrementally add genes from the remaining 44 candidate genes based on an overall variable importance rank to this list 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.

2.6.1 Variable Importance

Variable importance is calculated using either a model-based approach if it is available, or a permutation-based VI score otherwise (e.g. for SVM). 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.

2.7 Evaluation Metrics

We use the accuracy, kappa, F1-score, area under the ROC (AUC), and geometric mean as evaluation metrics to compare training 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, except for AUC because it is potentially misleading to combine predicted probabilities in a one-vs-all fashion.

2.7.1 Accuracy

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

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

2.7.2 Kappa

Kappa is the defined as:

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

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

2.7.3 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.

2.7.4 F1-Score

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

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

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:

$$F_{1macro} = \frac{1}{k} \sum_{i=1}^{k} F_{1i}$$

where each F_{1i} 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_{1i} is undefined because the per-class precision of class i is undefined. Those F_{1i} terms are removed from the F_{1macro} equation and the resulting value may be inflated. Interpreting the F1-score in such a case would be misleading.

2.7.5 Geometric Mean

The geometric mean (G-mean) is the k^{th} root of the product of class-specific sensitivities for k classes:

$$\text{G-mean} = \sqrt[k]{\prod_{i=1}^{k} \text{Sensitivity}_k}$$

The G-mean generalizes easily for the multiclass scenario.

3. Distributions

3.1 Histotypes in Classifier Data

3.2 Cohort Counts

3.3 Cohorts in Classifier Data

3.4 Quality Control

3.4.1 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 %GD vs. SNR relationship below.

3.4.2 %GD vs. SNR

\begin{figure}[H]

Table 3.1: Pre-QC Training Set Histotype Distribution by CodeSet

Variable	Levels	CS1	CS2	CS3	Total
Histotype	HGSC	120 (45%)	643 (79%)	515 (92%)	1278 (78%)
	CCOC	48 (18%)	61 (7%)	11 (2%)	120 (7%)
	ENOC	60 (22%)	32 (4%)	11 (2%)	103 (6%)
	MUC	19 (7%)	62 (8%)	12 (2%)	93 (6%)
	LGSC	20 (7%)	21 (3%)	9 (2%)	50 (3%)
Total	N (%)	267 (16%)	819 (50%)	558 (34%)	1644 (100%)

Table 3.2: Training Set (with duplicates) Histotype Distribution by CodeSet

Variable	Levels	CS1	CS2	CS3	Total
Histotype	HGSC	116 (48%)	623 (80%)	475 (94%)	1214 (79%)
	CCOC	44 (18%)	54 (7%)	8 (2%)	106 (7%)
	ENOC	55 (23%)	27 (3%)	8 (2%)	90 (6%)
	MUC	15 (6%)	59 (8%)	9 (2%)	83 (5%)
	LGSC	14 (6%)	19 (2%)	6 (1%)	39 (3%)
Total	N (%)	244 (16%)	782 (51%)	506 (33%)	1532 (100%)

Table 3.3: Final Training Set Histotype Distribution by CodeSet

Variable	Levels	CS1	CS2	CS3	Total
Histotype	HGSC	9 (12%)	553 (79%)	451 (96%)	1013 (81%)
	CCOC	25 (32%)	52 (7%)	4 (1%)	81 (7%)
	ENOC	37 (48%)	25 (4%)	4 (1%)	66 (5%)
	MUC	3 (4%)	55 (8%)	5 (1%)	63 (5%)
	LGSC	3 (4%)	16 (2%)	4 (1%)	23 (2%)
Total	N (%)	77 (6%)	701 (56%)	468 (38%)	1246 (100%)

Table 3.4: Histotype Distribution in Confirmation and Validation Sets

Variable	Levels	Confirmation	Validation
Histotype	HGSC	422 (66%)	674 (74%)
	CCOC	75 (12%)	80 (9%)
	ENOC	106 (16%)	108 (12%)
	MUC	27 (4%)	26 (3%)
	LGSC	13 (2%)	18 (2%)
Total	N (%)	643 (42%)	906 (58%)

Table 3.5: Training Set counts by CodeSet and Processing Stage

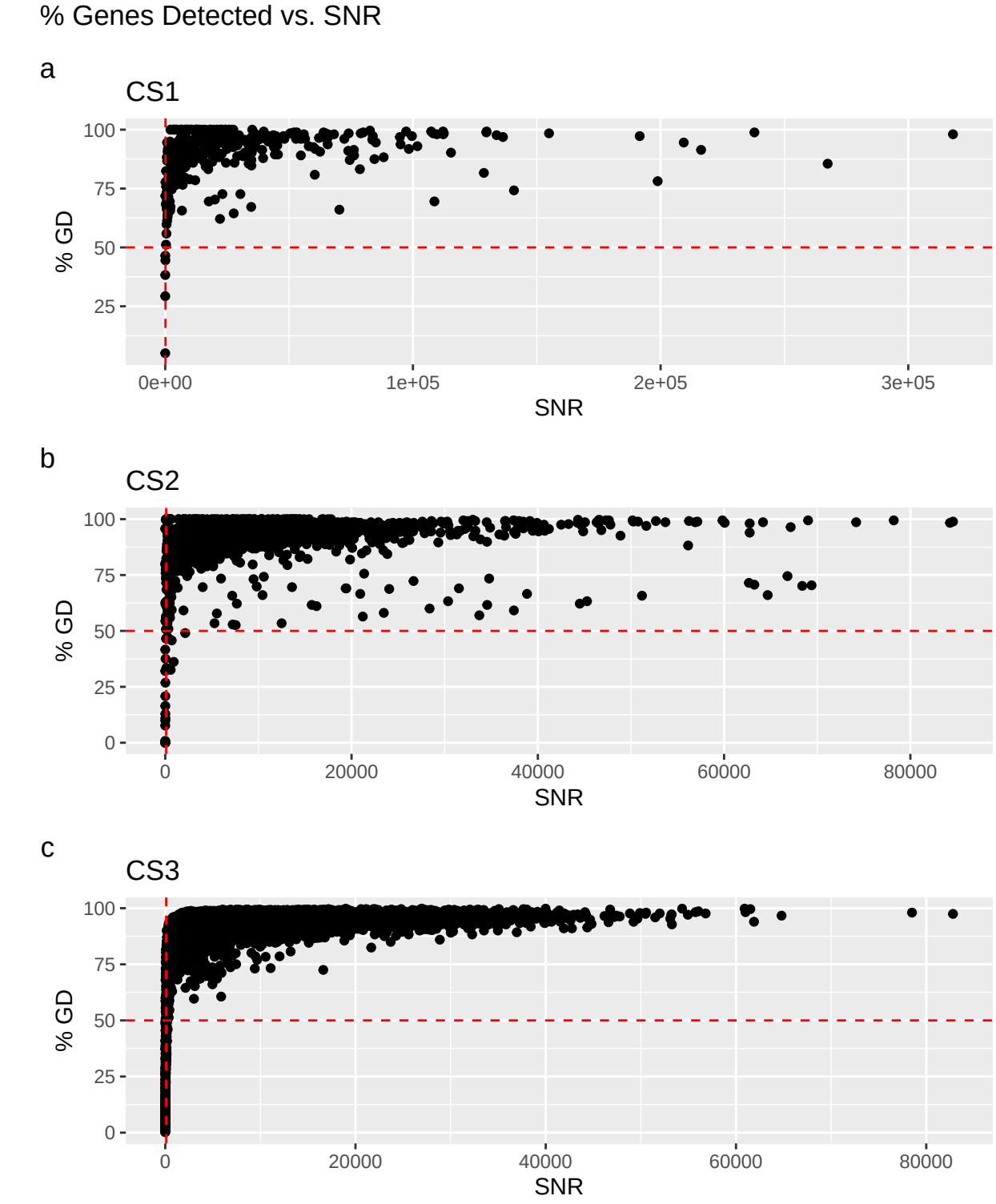
Processing Stage	CS1	CS2	CS3	Total
Raw Data	412	1223	5424	7059
Selected Cohorts	294	903	2477	3674
QC	286	888	2285	3459
Normalized to Reference	263	832	2107	3202
CS3: remove test sets, add AOC/USC	263	832	514	1609
Major Histotypes	244	782	506	1532
Removed Duplicates	77	701	468	1246

Table 3.6: Cohort Distribution in Training, Confirmation, and Validation Sets

CodeSet	Cohort	Training	Confirmation	Validation
CS1	MAYO	2	0	0
CS1	MTL	1	0	0
CS1	OOU	53	0	0
CS1	OOUE	1	0	0
CS1	VOA	20	0	0
CS2	ICON7	365	0	0
CS2	JAPAN	8	0	0
CS2	MAYO	42	0	0
CS2	MTL	59	0	0
CS2	OOU	27	0	0
CS2	OOUE	18	0	0
CS2	OVAR3	136	0	0
CS2	VOA	46	0	0
CS3	OOU	18	0	0
CS3	OOUE	11	0	0
CS3	VOA	439	0	0
CS3	TNCO	0	643	0
CS3	DOVE4	0	0	906

Table 3.7: Number of failed samples by CodeSet and fail condition

CodeSet	CodeSet Total	linFlag	imagingFlag	spcFlag	normFlag	QCFlag	n
CS1	8	Passed	Failed	Passed	Passed	Failed	3
		Passed	Passed	Passed	Failed	Failed	5
CS2	32	Failed	Failed	Failed	Failed	Failed	2
		Failed	Passed	Failed	Failed	Failed	3
		Failed	Passed	Passed	Passed	Failed	3
		Passed	Failed	Passed	Passed	Failed	3
		Passed	Passed	Passed	Failed	Failed	21
CS3	274	Failed	Failed	Failed	Failed	Failed	1
		Failed	Failed	Passed	Failed	Failed	3
		Failed	Passed	Passed	Failed	Failed	11
		Passed	Failed	Passed	Passed	Failed	7
		Passed	Passed	Passed	Failed	Failed	252



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\caption{% Genes Detected vs. Signal to Noise Ratio} \end{figure}

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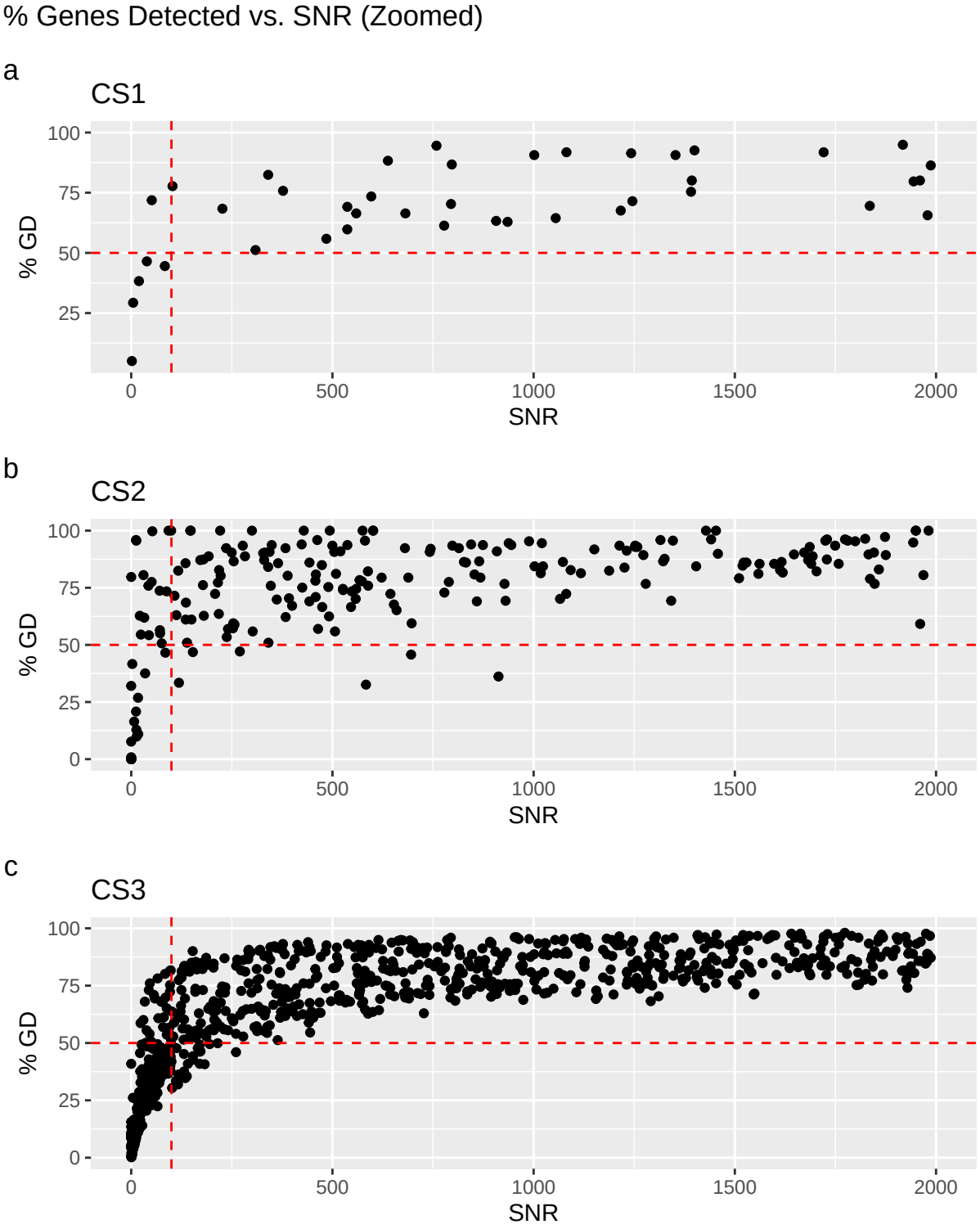

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\caption{% Genes Detected vs. Signal to Noise Ratio (Zoomed)} \end{figure}

3.5 Pairwise Gene Expression

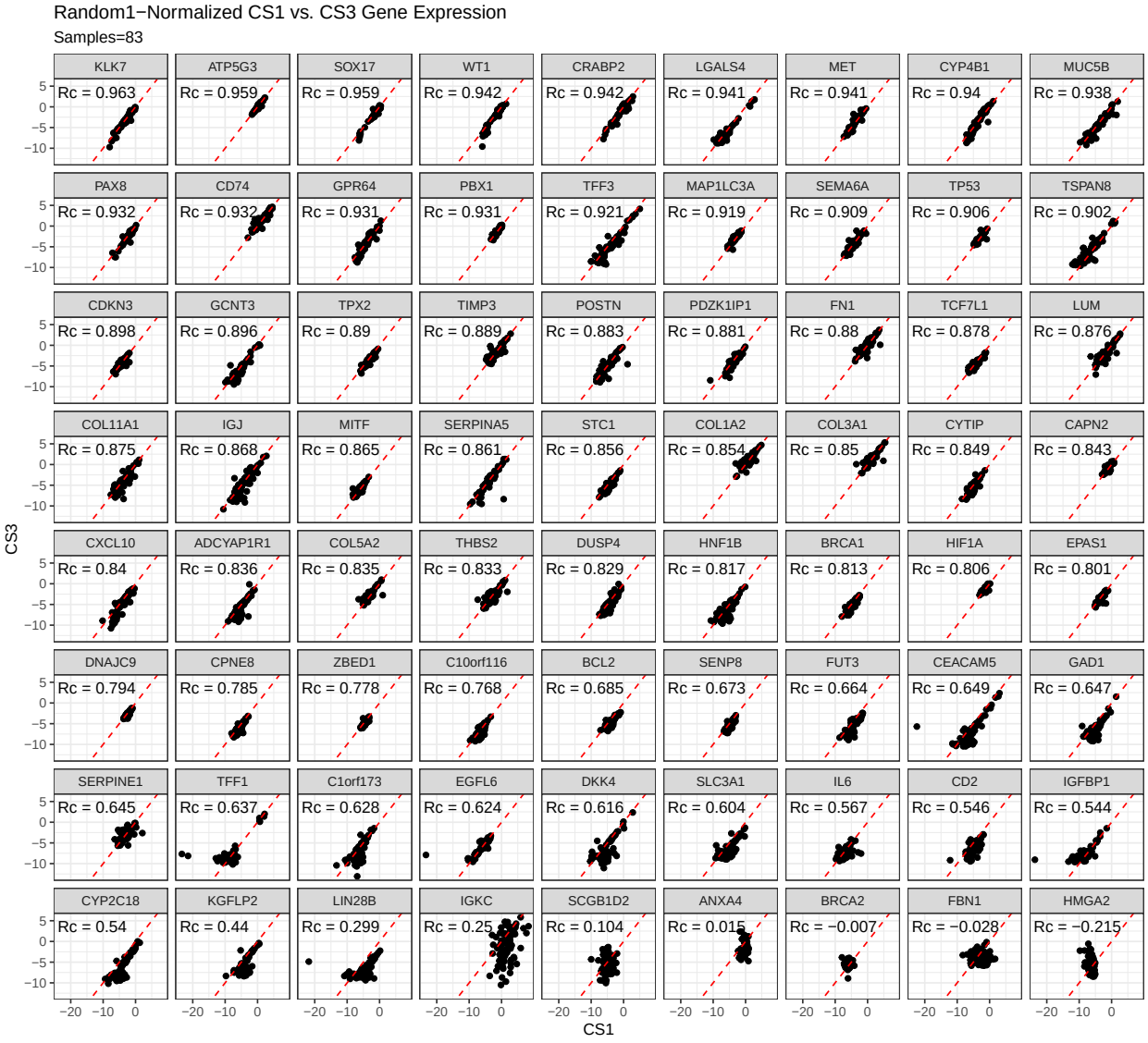

Figure 3.1: Random1-Normalized CS1 vs. CS3 Gene Expression

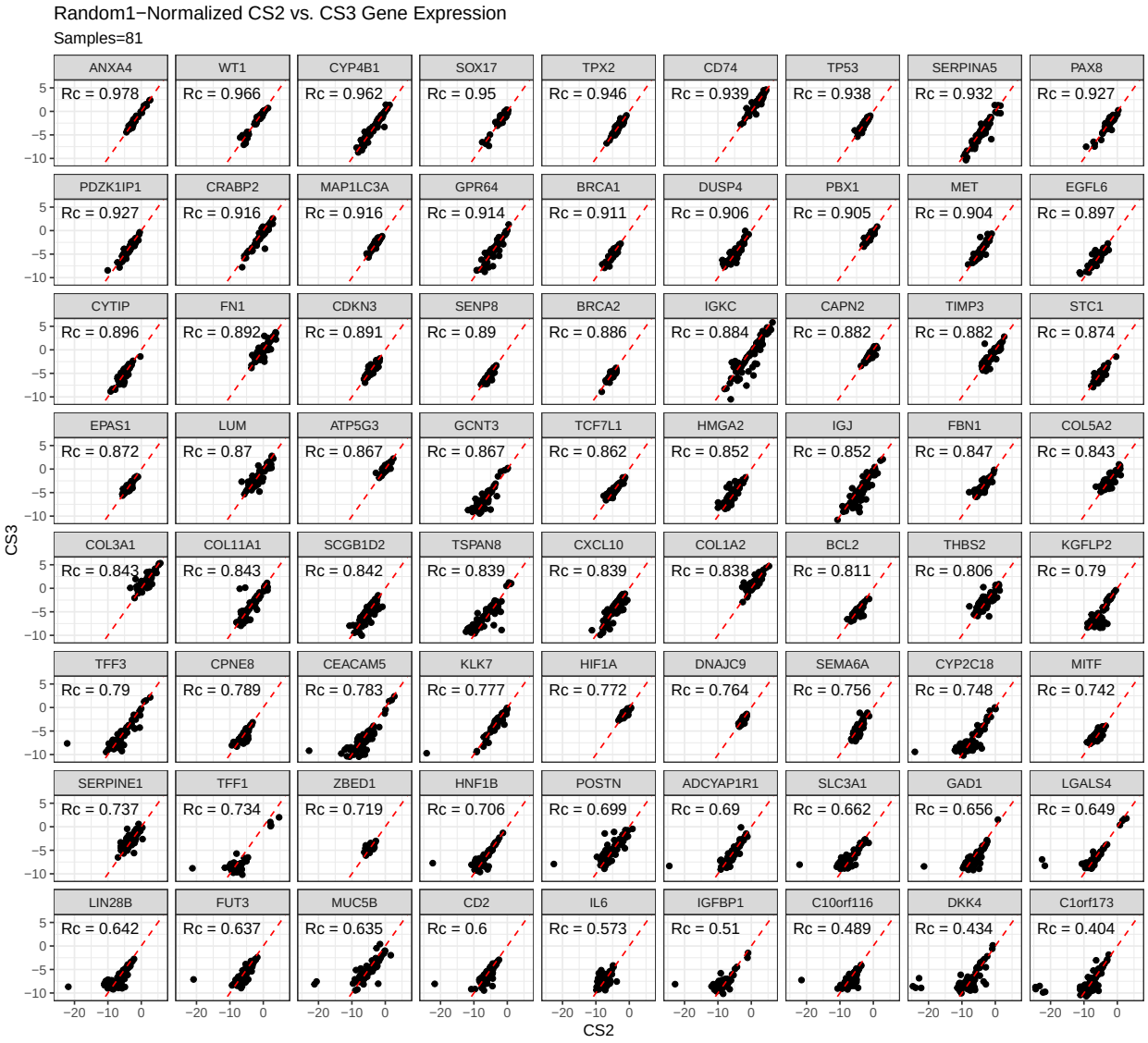

Figure 3.2: Random1-Normalized CS2 vs. CS3 Gene Expression

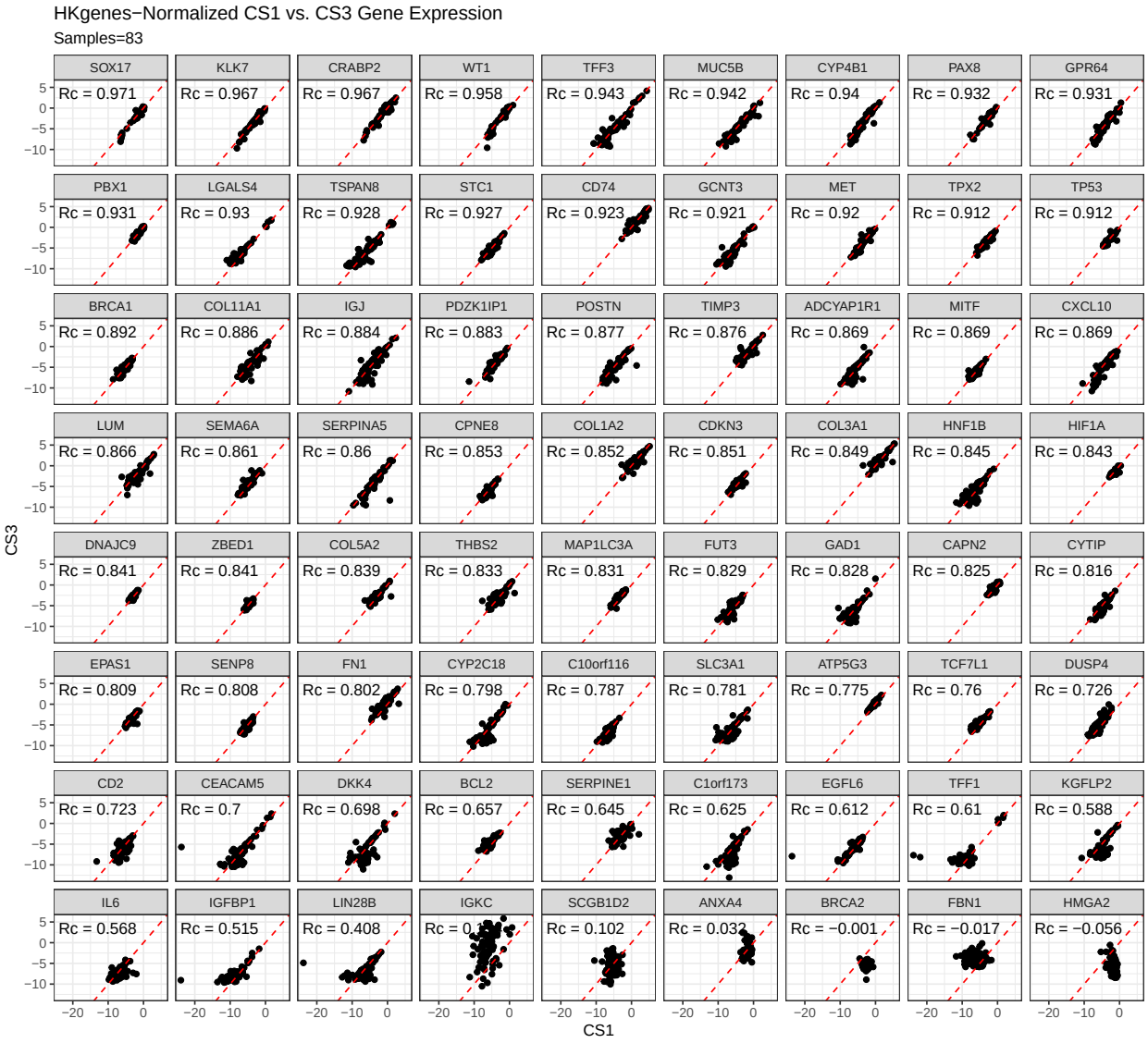

Figure 3.3: HKgenes-Normalized CS1 vs. CS3 Gene Expression

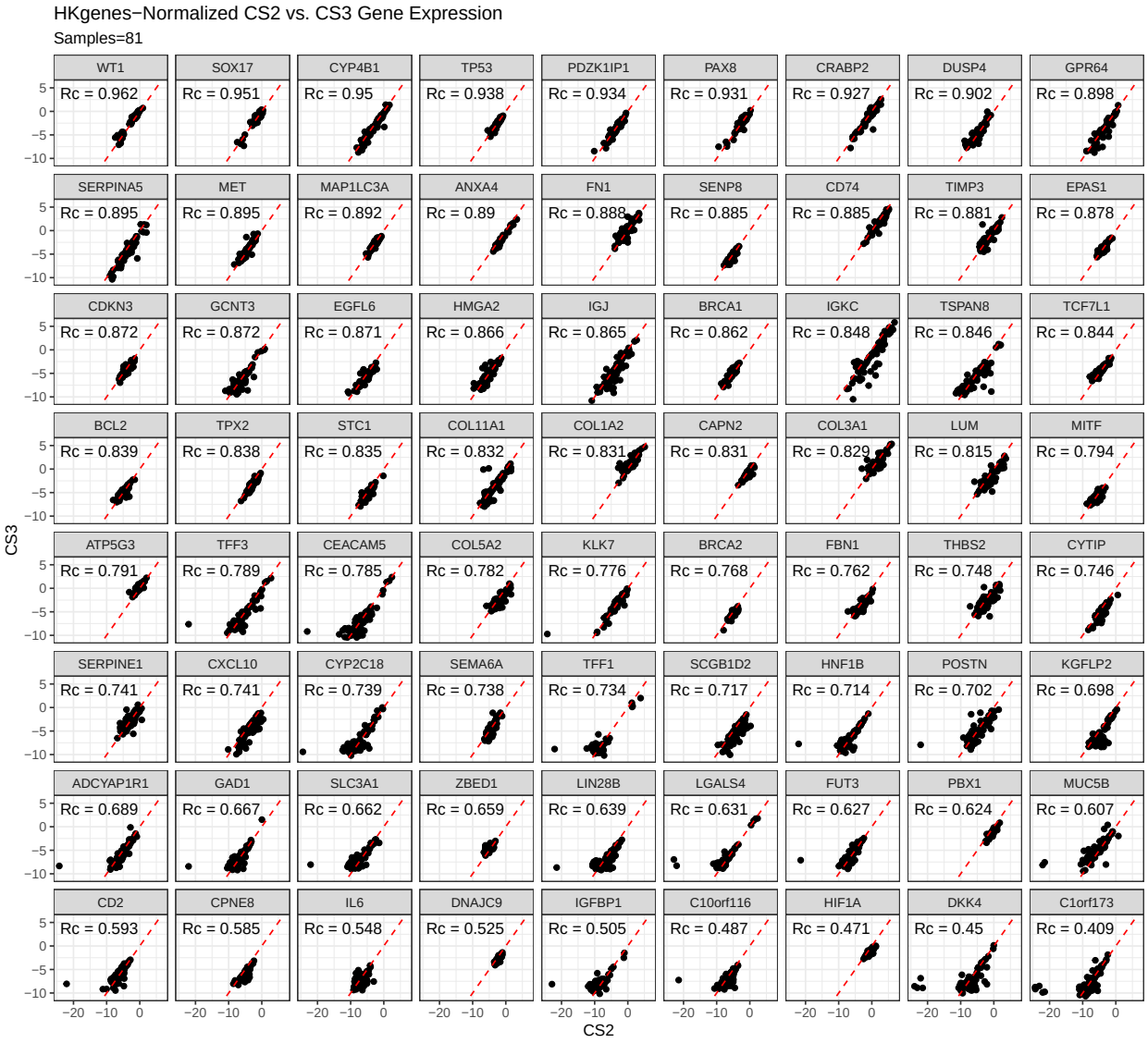

Figure 3.4: HKgenes-Normalized CS2 vs. CS3 Gene Expression

4. Results

We summarize cross-validated training performance of class metrics in the training set. The accuracy, F1-score, kappa, and G-mean 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

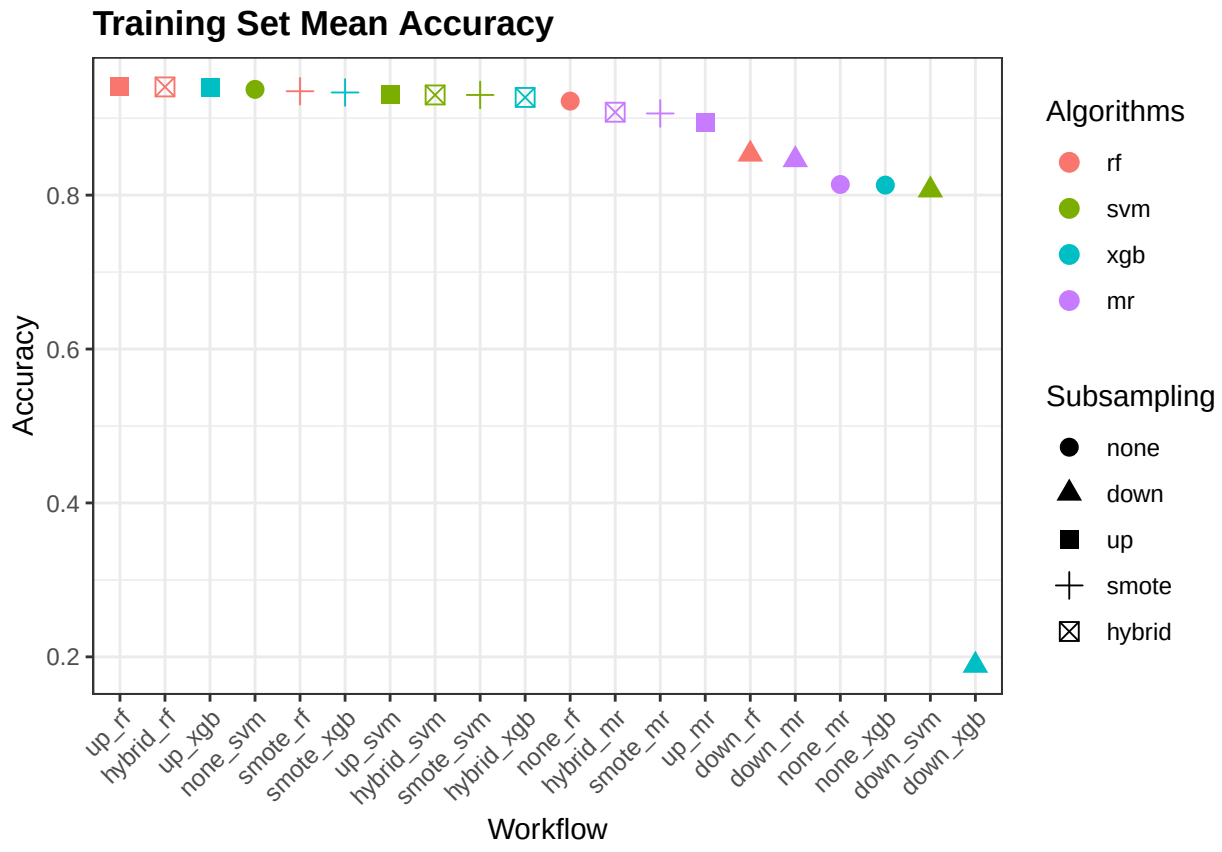

Figure 4.1: Training Set Mean Accuracy

Table 4.1: Training Set Mean Accuracy

Subsampling	Algorithms			
	rf	svm	xgb	mr
none	0.922	0.937	0.813	0.814
down	0.853	0.807	0.189	0.846
up	0.941	0.931	0.94	0.894
smote	0.935	0.93	0.933	0.906
hybrid	0.941	0.93	0.927	0.908

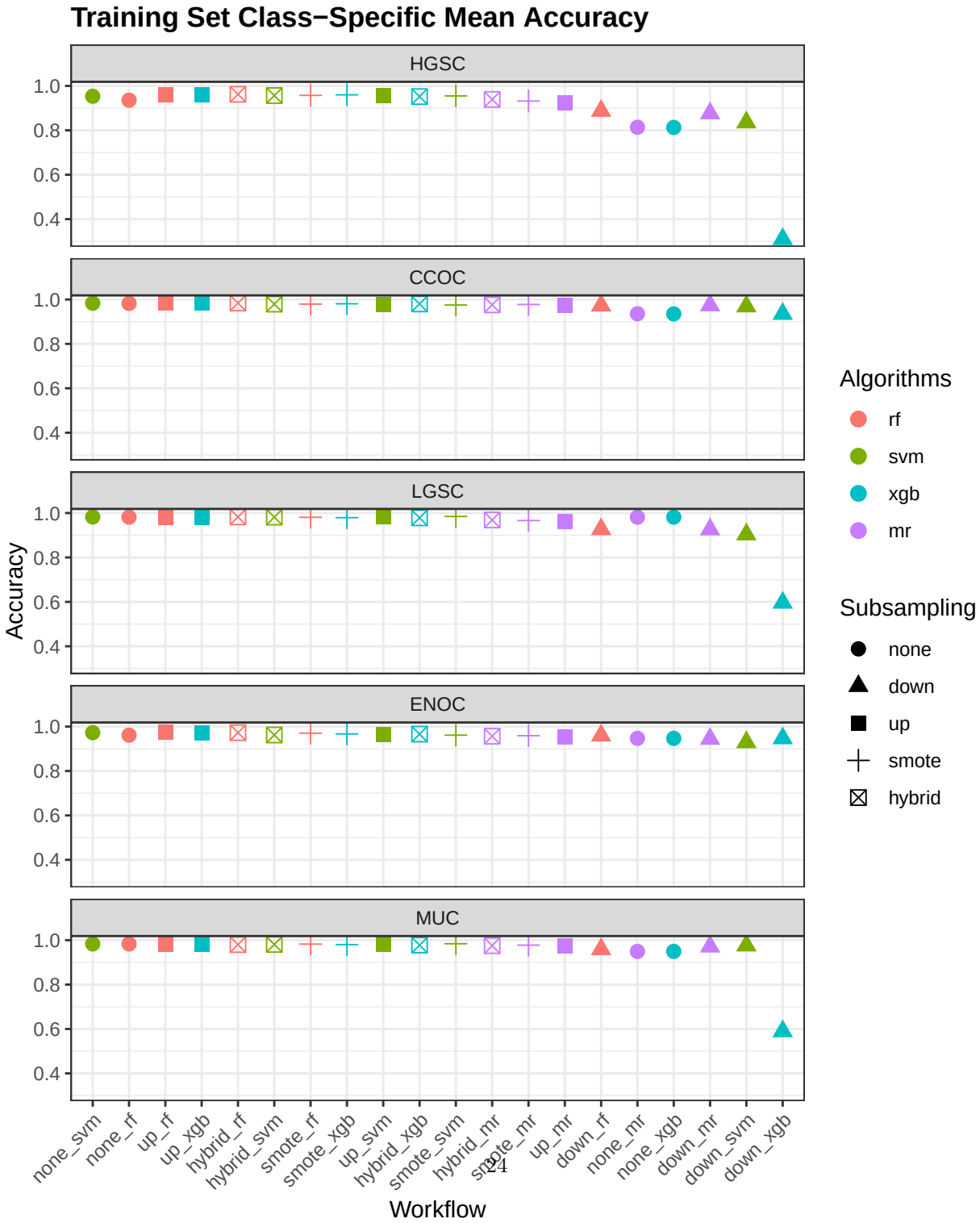

Table 4.2: Training Set Class-Specific Mean Accuracy

		Algorithms			
Subsampling	Histotype	rf	svm	xgb	mr
none	HGSC	0.936	0.953	0.813	0.814
	CCOC	0.982	0.983	0.935	0.936
	LGSC	0.982	0.982	0.982	0.982
	ENOC	0.961	0.973	0.947	0.947
	MUC	0.983	0.983	0.949	0.949
down	HGSC	0.888	0.835	0.31	0.876
	CCOC	0.973	0.969	0.935	0.974
	LGSC	0.927	0.904	0.596	0.926
	ENOC	0.96	0.929	0.947	0.945
	MUC	0.959	0.976	0.59	0.971
up	HGSC	0.96	0.955	0.961	0.924
	CCOC	0.984	0.978	0.984	0.975
	LGSC	0.981	0.982	0.982	0.961
	ENOC	0.975	0.964	0.972	0.954
	MUC	0.983	0.982	0.981	0.974
smote	HGSC	0.957	0.955	0.96	0.932
	CCOC	0.979	0.975	0.981	0.978
	LGSC	0.981	0.985	0.979	0.966
	ENOC	0.97	0.961	0.967	0.959
	MUC	0.982	0.984	0.98	0.978
hybrid	HGSC	0.962	0.957	0.952	0.939
	CCOC	0.984	0.979	0.98	0.976
	LGSC	0.982	0.982	0.978	0.969
	ENOC	0.973	0.962	0.966	0.957
	MUC	0.98	0.981	0.978	0.975

Table 4.3: Training Set Mean F1-Score

Subsampling	Algorithms			
	rf	svm	xgb	mr
none	0.787	0.801	0.897	0.822
down	0.657	0.645	0.231	0.664
up	0.755	0.726	0.776	0.709
smote	0.748	0.75	0.747	0.724
hybrid	0.77	0.751	0.728	0.72

4.1.2 F1-Score

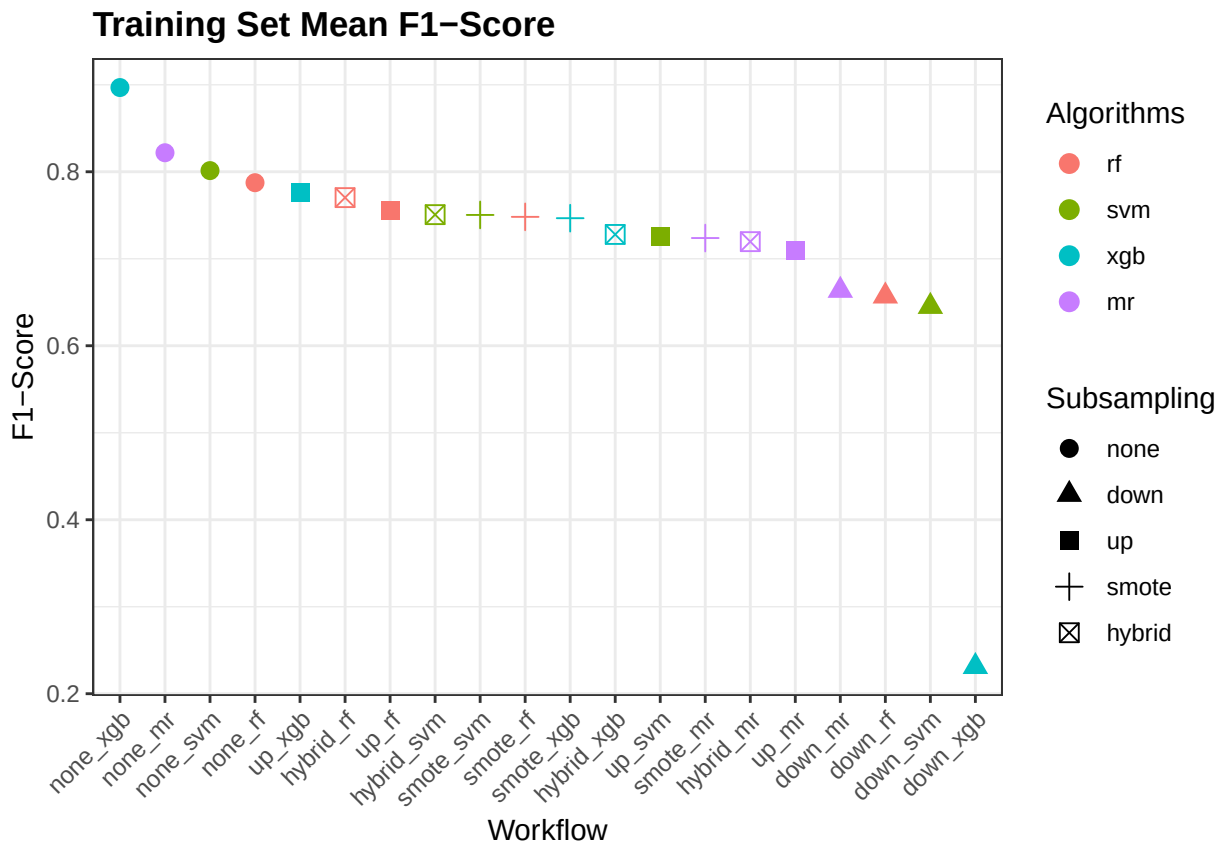

Figure 4.3: Training Set Mean F1-Score

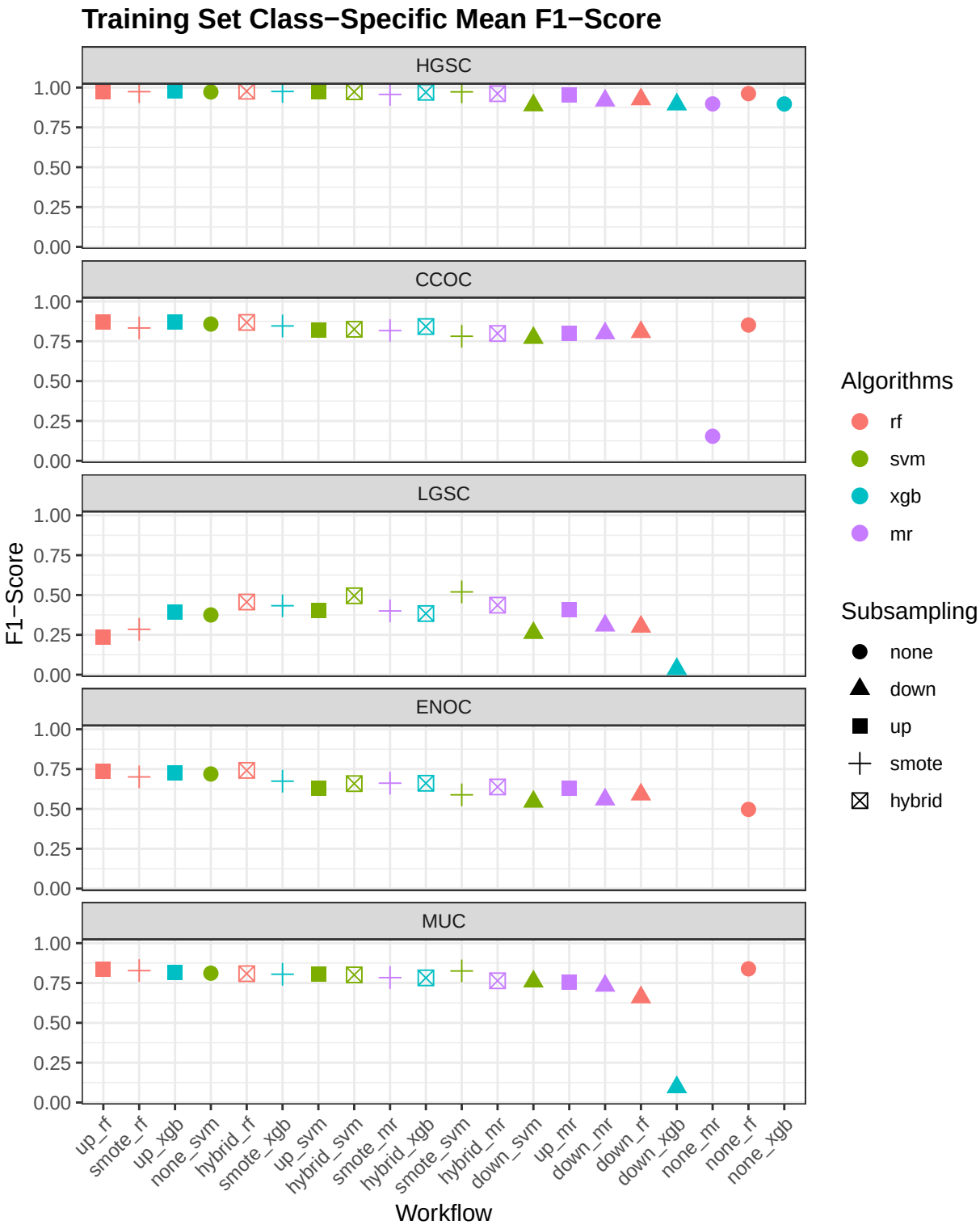

Figure 4.4: Training Set Class-Specific Mean F1-Score

Table 4.4: Cross-Validated Training Set Class-Specific Mean F1-Score

		Algorithms			
Subsampling	Histotype	rf	svm	xgb	mr
none	HGSC	0.962	0.972	0.897	0.897
	CCOC	0.852	0.858	NA	0.154
	LGSC	NA	0.375	NA	NA
	ENOC	0.497	0.719	NA	NA
	MUC	0.839	0.811	NA	NA
down	HGSC	0.926	0.888	0.894	0.918
	CCOC	0.808	0.771	NA	0.8
	LGSC	0.301	0.262	0.035	0.308
	ENOC	0.59	0.545	NA	0.559
	MUC	0.66	0.759	0.096	0.732
up	HGSC	0.976	0.973	0.976	0.951
	CCOC	0.869	0.822	0.87	0.8
	LGSC	0.236	0.402	0.394	0.409
	ENOC	0.734	0.629	0.728	0.63
	MUC	0.835	0.804	0.814	0.756
smote	HGSC	0.974	0.973	0.975	0.957
	CCOC	0.833	0.782	0.846	0.817
	LGSC	0.285	0.519	0.433	0.4
	ENOC	0.701	0.588	0.674	0.662
	MUC	0.827	0.825	0.805	0.784
hybrid	HGSC	0.977	0.973	0.97	0.962
	CCOC	0.868	0.825	0.843	0.799
	LGSC	0.456	0.495	0.384	0.436
	ENOC	0.742	0.659	0.661	0.638
	MUC	0.808	0.801	0.782	0.764

Table 4.5: Training Set Mean Kappa

Subsampling	Algorithms			
	rf	svm	xgb	mr
none	0.727	0.79	0	0.008
down	0.639	0.569	0	0.63
up	0.81	0.783	0.814	0.717
smote	0.796	0.772	0.799	0.743
hybrid	0.819	0.788	0.783	0.744

4.1.3 Kappa

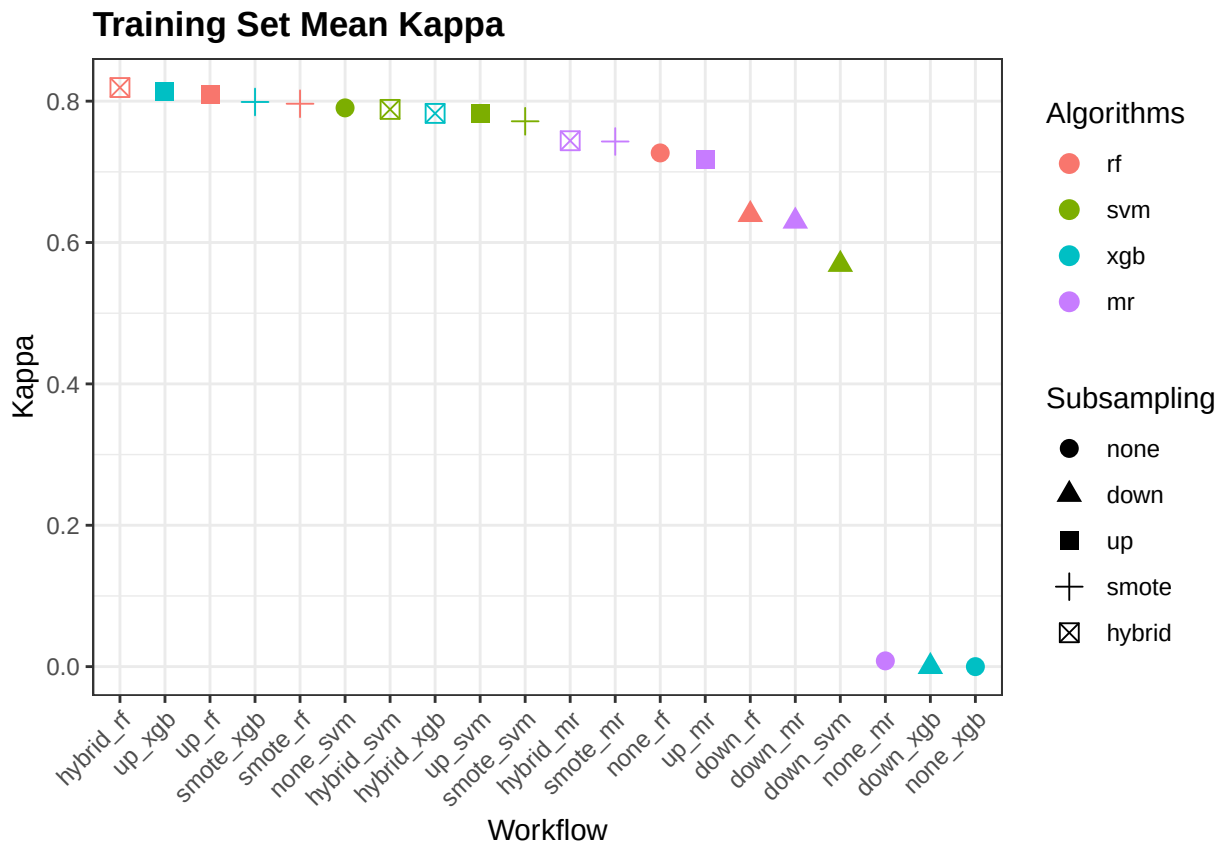

Figure 4.5: Training Set Mean Kappa

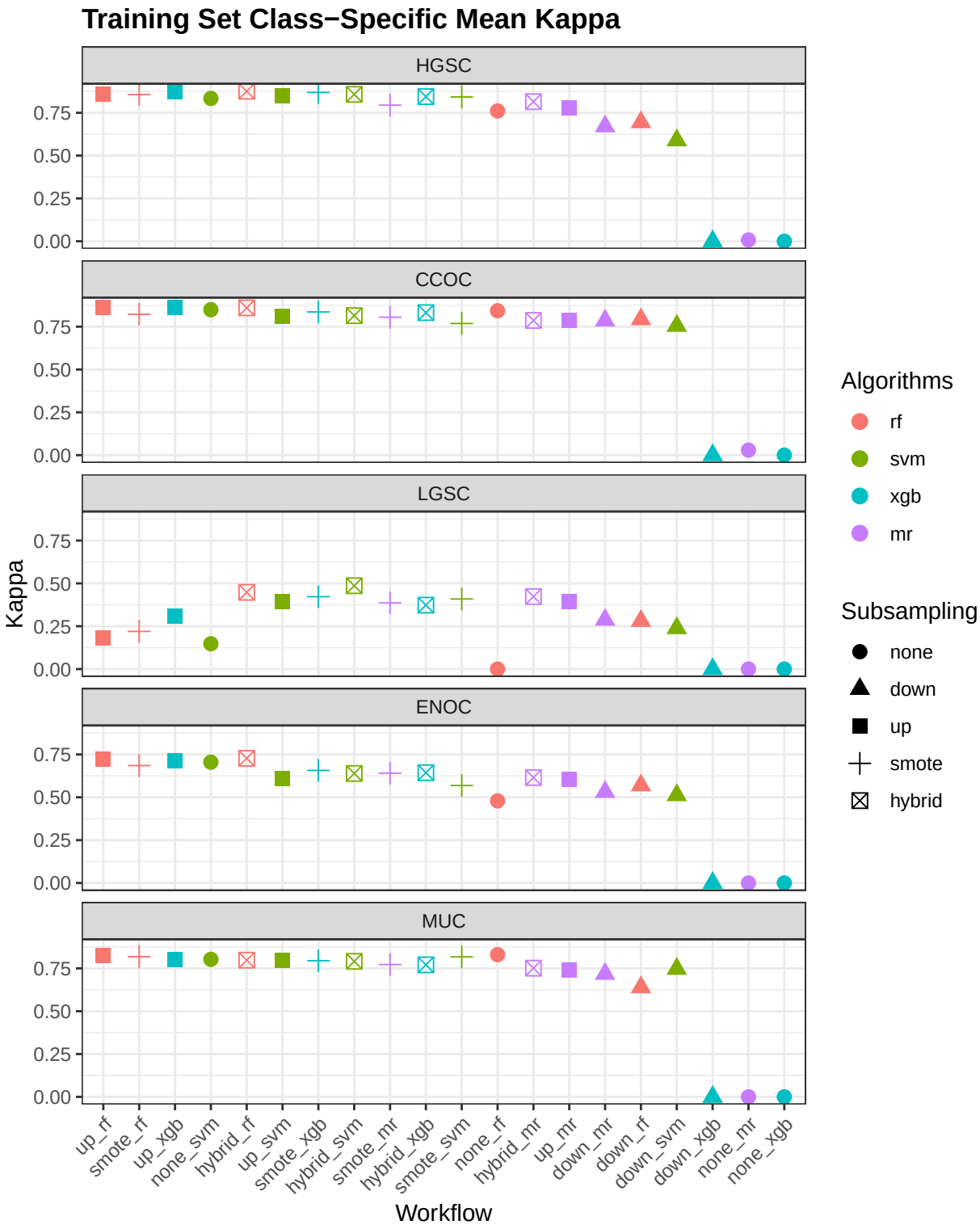

Figure 4.6: Training Set Class-Specific Mean Kappa

Table 4.6: Training Set Class-Specific Mean Kappa

		Algorithms			
Subsampling	Histotype	rf	svm	xgb	mr
none	HGSC	0.761	0.834	0	0.008
	CCOC	0.843	0.849	0	0.03
	LGSC	0	0.148	0	0
	ENOC	0.479	0.705	0	0
	MUC	0.83	0.803	0	0
down	HGSC	0.695	0.589	0	0.671
	CCOC	0.793	0.755	0	0.786
	LGSC	0.281	0.238	0	0.288
	ENOC	0.57	0.512	0	0.531
	MUC	0.64	0.747	0	0.718
up	HGSC	0.86	0.848	0.872	0.776
	CCOC	0.86	0.81	0.861	0.787
	LGSC	0.181	0.394	0.307	0.394
	ENOC	0.721	0.61	0.713	0.607
	MUC	0.826	0.795	0.804	0.743
smote	HGSC	0.856	0.842	0.869	0.795
	CCOC	0.822	0.769	0.836	0.805
	LGSC	0.22	0.409	0.422	0.386
	ENOC	0.685	0.569	0.657	0.64
	MUC	0.818	0.817	0.795	0.772
hybrid	HGSC	0.876	0.857	0.844	0.815
	CCOC	0.86	0.814	0.832	0.786
	LGSC	0.448	0.486	0.373	0.423
	ENOC	0.728	0.639	0.644	0.615
	MUC	0.798	0.791	0.771	0.751

Table 4.7: Training Set Mean G-mean

Subsampling	Algorithms			
	rf	svm	xgb	mr
none	0.705	0.732	1	0.858
down	0.786	0.837	1	0.821
up	0.717	0.722	0.735	0.817
smote	0.739	0.694	0.738	0.847
hybrid	0.729	0.739	0.769	0.803

4.1.4 G-mean

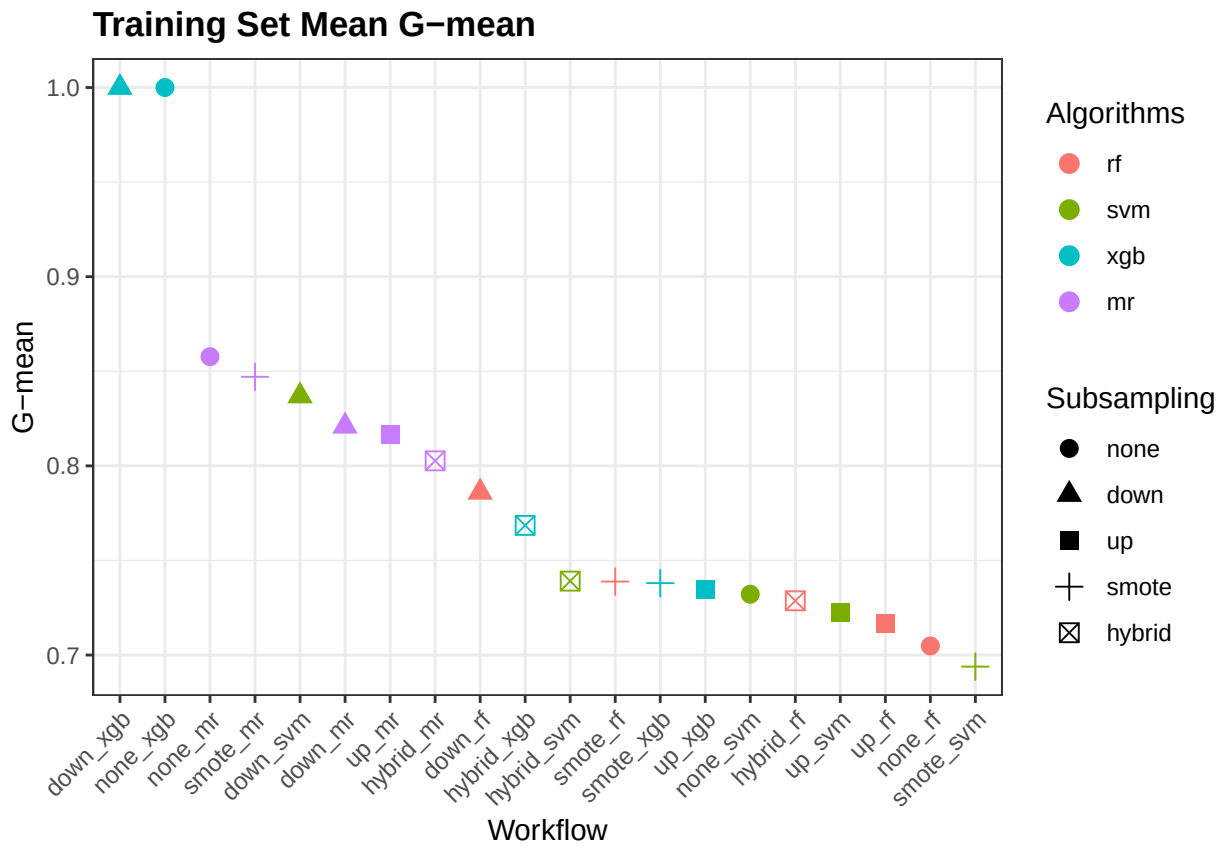

Figure 4.7: Training Set Mean G-mean

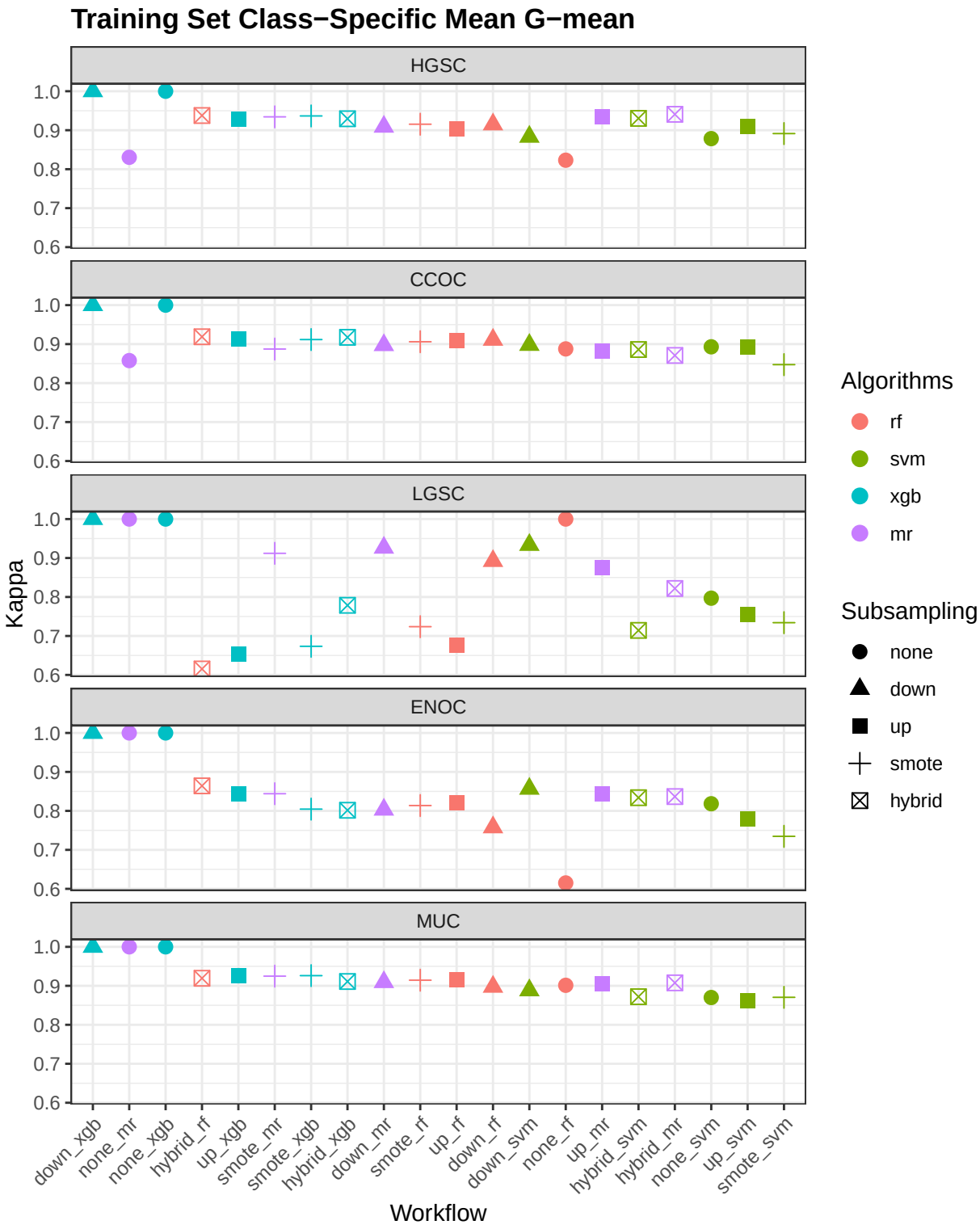

Figure 4.8: Training Set Class-Specific Mean G-mean

Table 4.8: Training Set Class-Specific Mean G-mean

		Algorithms			
Subsampling	Histotype	rf	svm	xgb	mr
none	HGSC	0.823	0.878	1	0.83
	CCOC	0.888	0.893	1	0.858
	LGSC	1	0.797	1	1
	ENOC	0.615	0.818	1	1
	MUC	0.901	0.87	1	1
down	HGSC	0.915	0.883	1	0.909
	CCOC	0.911	0.898	1	0.897
	LGSC	0.892	0.934	1	0.926
	ENOC	0.758	0.857	1	0.803
	MUC	0.897	0.888	1	0.909
up	HGSC	0.904	0.91	0.928	0.935
	CCOC	0.908	0.893	0.913	0.881
	LGSC	0.677	0.754	0.653	0.876
	ENOC	0.82	0.779	0.843	0.844
	MUC	0.915	0.862	0.927	0.906
smote	HGSC	0.915	0.891	0.937	0.934
	CCOC	0.906	0.847	0.912	0.887
	LGSC	0.724	0.734	0.673	0.912
	ENOC	0.814	0.735	0.805	0.844
	MUC	0.914	0.87	0.926	0.925
hybrid	HGSC	0.938	0.93	0.929	0.941
	CCOC	0.919	0.886	0.917	0.871
	LGSC	0.615	0.714	0.779	0.822
	ENOC	0.864	0.834	0.802	0.837
	MUC	0.919	0.872	0.911	0.908

4.2 Optimal Gene Sets

4.2.1 Sequential Algorithm

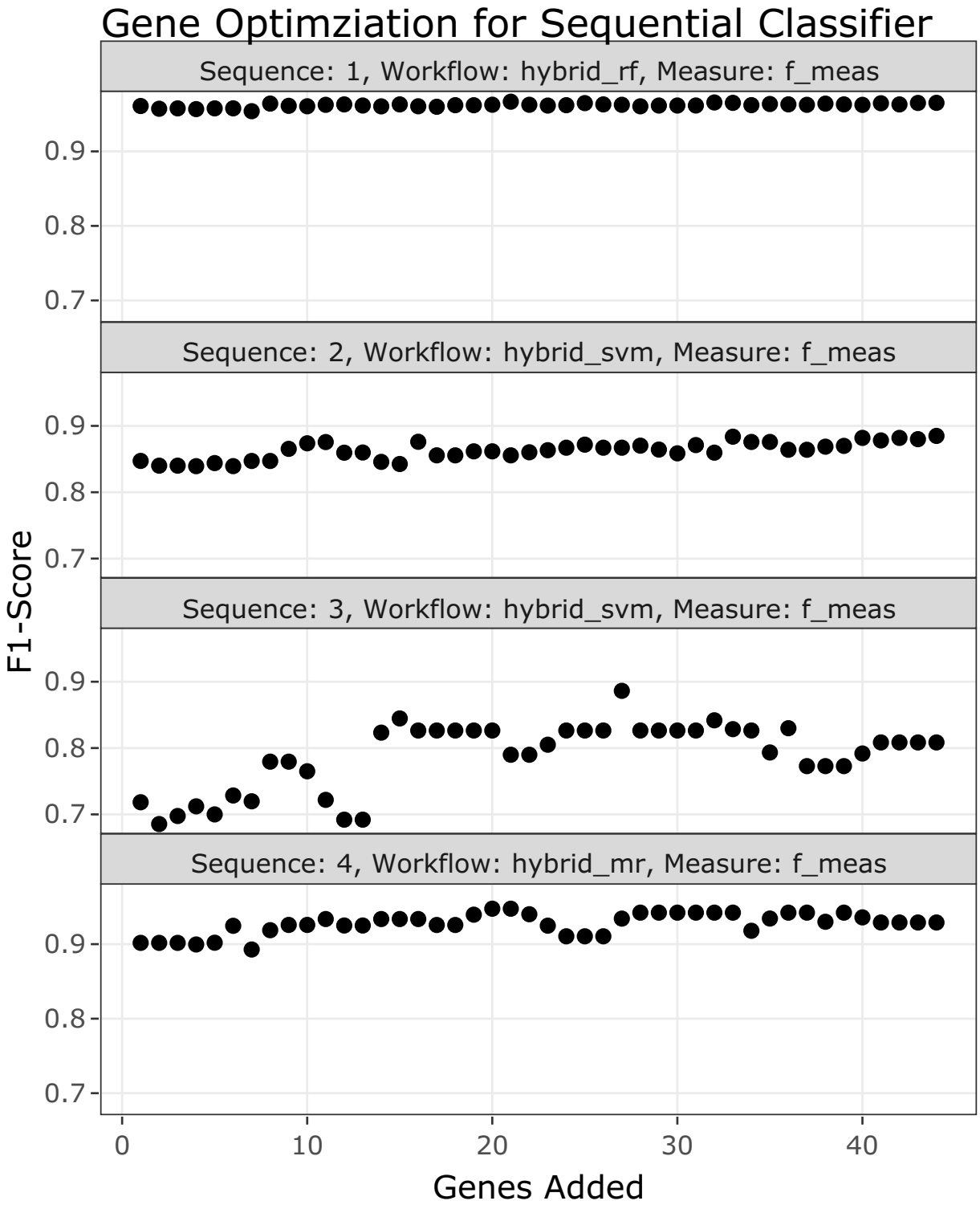

Figure 4.9: Gene Optimization for Sequential Classifier

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 0.9 when we reach 27 genes added, hence the optimal number of genes used will be n=28+27=55 The added genes are: CYP2C18, HNF1B, ATP5G3, TP53, SLC3A1, CPNE8, C1orf173, WT1, MUC5B, MAP1LC3A, EGFL6, ZBED1, GPR64, STC1, MET, IGJ, SERPINA5, KLK7, DKK4, BCL2, SENP8, GCNT3, IGKC, IGFBP1, CAPN2, GAD1 and SCGB1D2.

4.2.2 Two-Step Algorithm

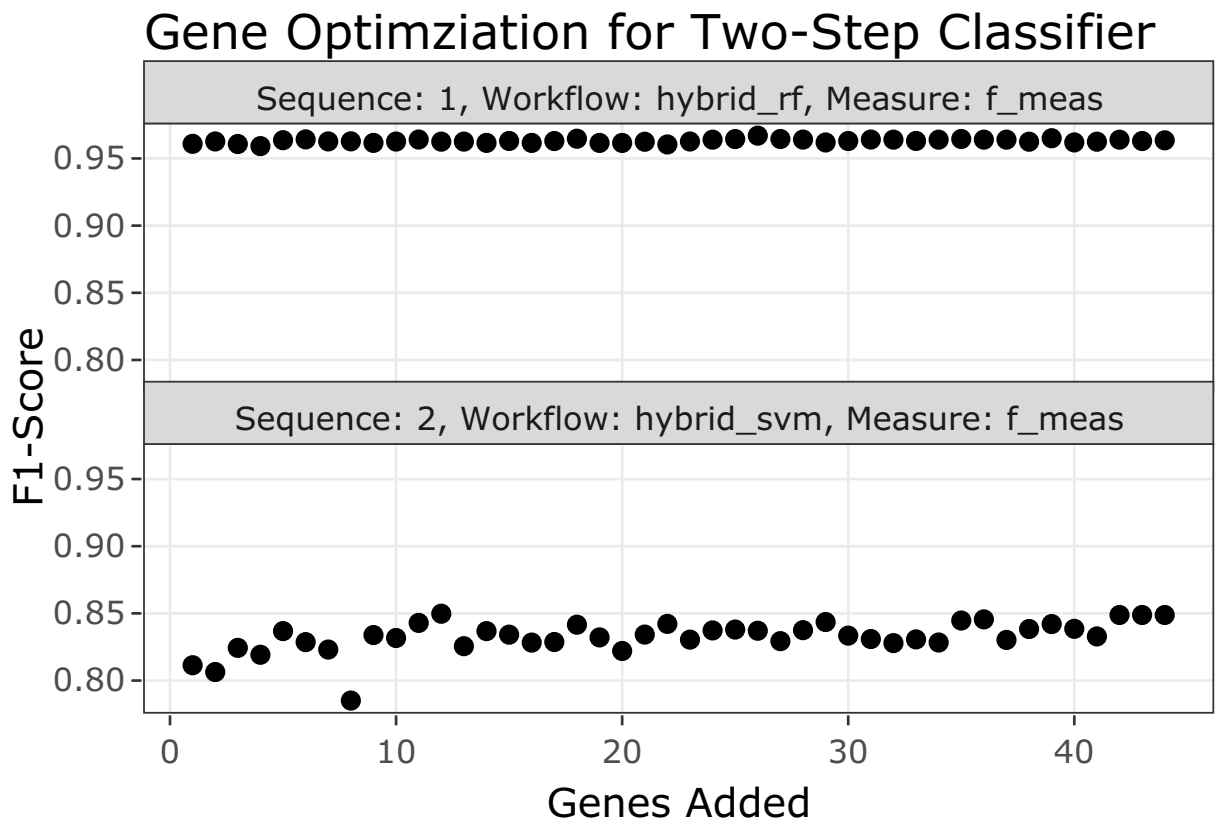

Figure 4.10: Gene Optimization for Two-Step Classifier

Since the second step of the classifier fits a multinomial model, we use the macro F1-score as the measure to analyze gene entry. In the two-step classifier, we see that in Step 2, the F1-score stabilizes at around 0.85 when we reach 12 added. The optimal number of genes used will be n=28+12=40. The added genes are: CYP2C18, MUC5B, HNF1B, SLC3A1, WT1, TSPAN8, EGFL6, TFF1, TFF3, MET, CAPN2 and KLK7.

4.3 Rank Aggregation

Show 50 entries Search:

F1-Score Summary by Workflow and Class

Workflow	HGSC	CCOC	LGSC	ENOC	MUC	Rank
All	All	All	All	All	All	All
sequential	0.962	0.885	0.808	0.929	0.914	1
two_step	0.962	0.888	0.828	0.804	0.875	2
hybrid_rf	0.977	0.868	0.456	0.742	0.808	3
up_rf	0.976	0.869	0.236	0.734	0.835	4
up_xgb	0.976	0.87	0.394	0.728	0.814	5
none_svm	0.972	0.858	0.375	0.719	0.811	6
smote_xgb	0.975	0.846	0.433	0.674	0.805	7
smote_rf	0.974	0.833	0.285	0.701	0.827	8
up_svm	0.973	0.822	0.402	0.629	0.804	9
hybrid_svm	0.973	0.825	0.495	0.659	0.801	10
hybrid_xgb	0.97	0.843	0.384	0.661	0.782	11
smote_mr	0.957	0.817	0.4	0.662	0.784	12
smote_svm	0.973	0.782	0.519	0.588	0.825	13
hybrid_mr	0.962	0.799	0.436	0.638	0.764	14
up_mr	0.951	0.8	0.409	0.63	0.756	15
down_rf	0.926	0.808	0.301	0.59	0.66	16
down_mr	0.918	0.8	0.308	0.559	0.732	17
down_svm	0.888	0.771	0.262	0.545	0.759	18

Showing 1 to 18 of 18 entries Previous 1 Next

The 18 workflows are ordered in the table by their aggregated ranks using the Genetic Algorithm. We see that the best performing methods involve the sequential and two-step algorithms.

4.3.1 Top Workflows

We look at the per-class evaluation metrics of the top 4 workflows.

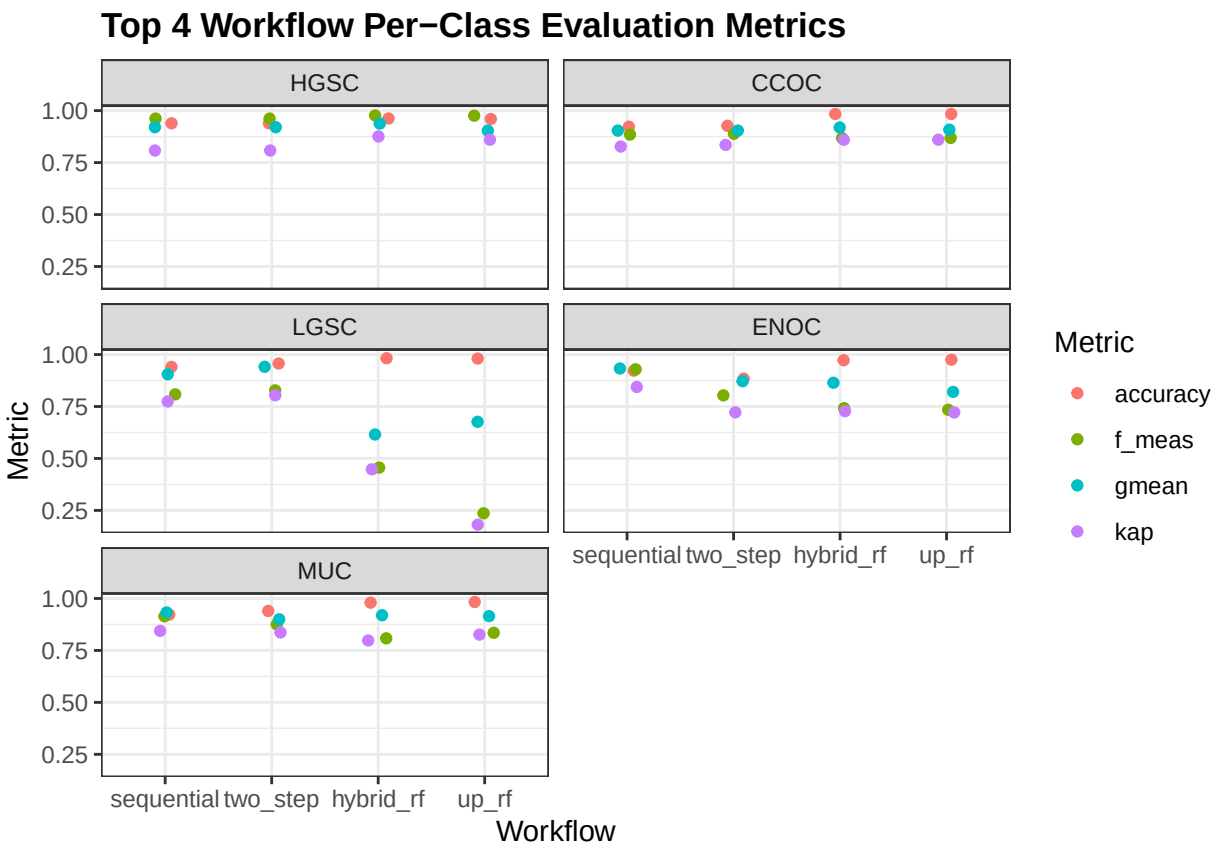

Figure 4.11: Top 4 Workflow Per-Class Evaluation Metrics

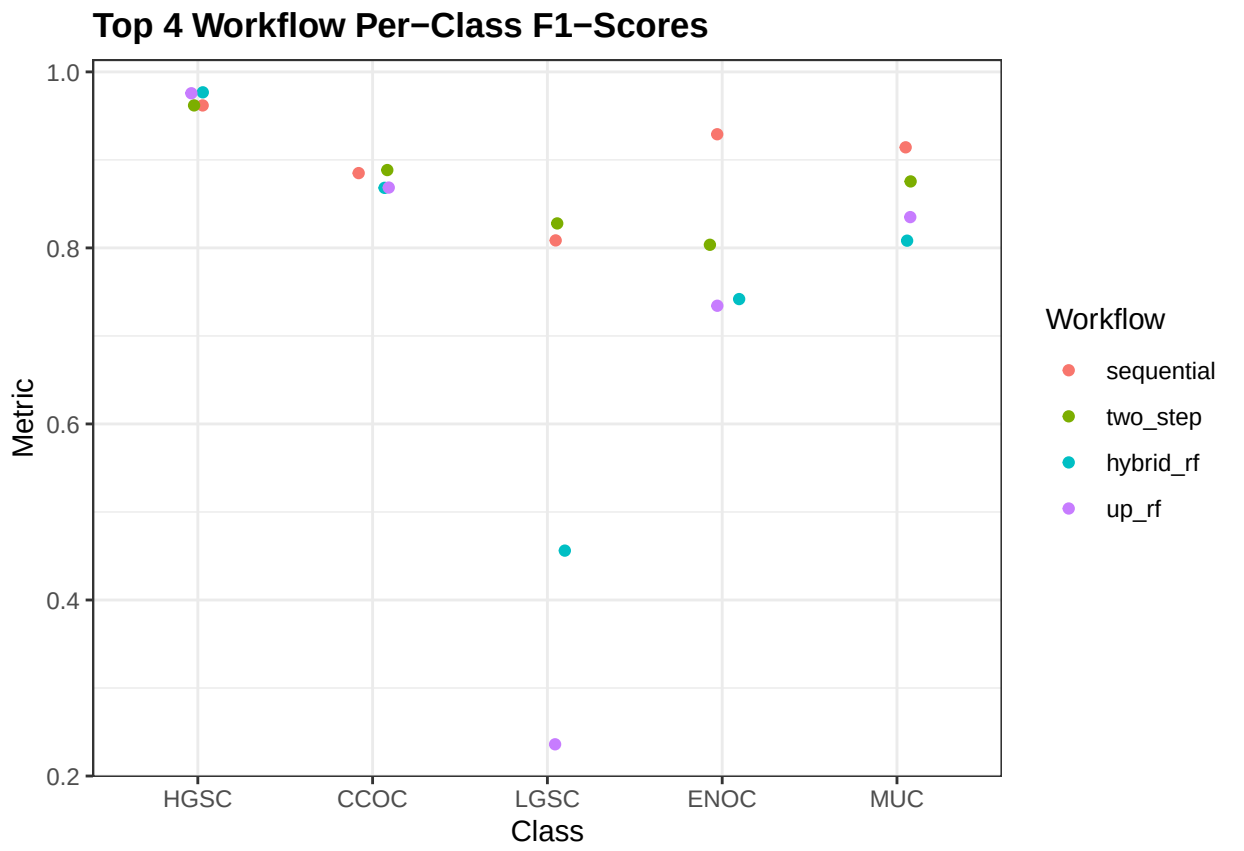

Figure 4.12: Top 4 Workflow Per-Class F1-Scores

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.

4.4 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:

- **sequential**: sequential algorithm with hybrid subsampling at every step. The sequence of algorithms used are:
  - HGSC vs. non-HGSC using random forest
  - CCOC vs. non-CCOC using support vector machine
  - LGSC vs. non-LGSC using support vector machine
  - ENOC vs. MUC using regularized multinomial regression
- **two_step**: two-step algorithm with hybrid subsampling at both steps. The sequence of algorithms used are:
  - HGSC vs. non-HGSC using random forest

Table 4.9: Overall Evaluation Metrics on Confirmation Set Models

Method	accuracy	f1	kappa	gmean
sequential_full	0.834	0.654	0.669	0.574
sequential_optimal	0.827	0.642	0.660	0.574
two_step_full	0.840	0.688	0.682	0.650
two_step_optimal	0.834	0.679	0.668	0.639

Table 4.10: Per-Class Eevaluation Metrics on Confirmation Set Model

		Histotypes				
Method	Metric	HGSC	CCOC	LGSC	ENOC	MUC
two_step_full	accuracy	0.869	0.970	0.969	0.896	0.975
	f1	0.904	0.872	0.333	0.626	0.704
	kappa	0.701	0.856	0.318	0.568	0.691
	gmean	0.833	0.924	0.614	0.715	0.833
two_step_optimal	accuracy	0.866	0.969	0.964	0.890	0.978
	f1	0.902	0.865	0.303	0.594	0.731
	kappa	0.692	0.847	0.286	0.534	0.719
	gmean	0.827	0.916	0.613	0.689	0.835
sequential_full	accuracy	0.869	0.961	0.969	0.893	0.975
	f1	0.904	0.839	0.231	0.619	0.680
	kappa	0.701	0.817	0.215	0.558	0.667
	gmean	0.833	0.919	0.477	0.714	0.790
sequential_optimal	accuracy	0.869	0.950	0.967	0.896	0.972
	f1	0.903	0.800	0.222	0.617	0.667
	kappa	0.702	0.772	0.206	0.560	0.652
	gmean	0.836	0.907	0.476	0.704	0.811

– CCOC vs. ENOC vs. MUC vs. LGSC support vector machine

We can test 2 additional methods by using either the full set of genes or the optimal set of genes for both of these methods.

4.4.1 Confirmation Set

4.4.2 Validation Set

Table 4.11: Overall Evaluation Metrics on Validation Set Model

Method	accuracy	f1	kappa	gmean
two_step_optimal	0.847	0.65	0.653	0.701

Table 4.12: Per-Class Eevaluation Metrics on Validation Set Model

Method	Metric	Histotypes				
		HGSC	CCOC	LGSC	ENOC	MUC
two_step_optimal	accuracy	0.879	0.972	0.964	0.922	0.957
	f1	0.917	0.854	0.353	0.608	0.519
	kappa	0.690	0.839	0.336	0.566	0.499
	gmean	0.853	0.945	0.697	0.706	0.881