TENTACLES workflow for: Supervised Ensemble Learning Identifies Minimal Consensus Gene Signatures for Crohn's Disease Classification

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1 Introduction

This vignette demonstrates the application of **TENTACLES** to bulk RNA-seq datasets from patients with Crohn's disease (CD) and healthy controls (HC), reproducing the analysis described in the manuscript *"Supervised Ensemble Learning Identifies Minimal Consensus Gene Signatures for Crohn's Disease Classification."*

1.1 Structure of the document

- **Setup**
  Preparation of libraries and datasets required to run the TENTACLES workflow.
- **Consensus gene-signature discovery on Dataset 1**
  - Preprocessing of Dataset 1 with `preProcess()` (normalization, filtering, optional batch correction).
  - Training of multiple supervised classifiers with `runClassifiers()` and embedded feature selection.
  - Derivation of a consensus gene-signature via `getConsensus()`.
  - DEG analysis via DESeq2.
  - Evaluation and comparison of DEG- vs consensus-based signatures using univariate and multivariate analyses (PCA, per-gene AUROC, MLP) with `testConsensus()`.
- **Cross-cohort validation on Dataset 2 (`testConsensus`)**
  - Preprocessing of Dataset 2.
  - Application of `testConsensus()` to assess consensus gene-signature reproducibility on a second independent dataset.
  - Benchmarking of consensus gene-signature performance against DEG-derived gene sets.
  - DEG Analysis on Dataset 2 and identification of common DEGs across Dataset 1 and 2.
- **Unsupervised external validation on Dataset 3**
  - Preprocessing of Dataset 3.
  - Use of `valConsensus()` to assess the discriminatory power of the signature via unsupervised clustering across six different methods.

2 Setup

This section imports the required libraries and the datasets used throughout the analysis.

```r
# Increase max size for parallel processing
options(future.globals.maxSize = 2 * 1024^3)
# Set seed for reproducibility
set.seed(1789)
# Load the package
library(cowplot)
library(scales)
library(tibble)
library(ggrepel)
library(DESeq2)
library(tidyverse)
library(TENTACLES)
```

The datasets used in this vignette correspond to publicly available intestinal bulk RNA-Seq samples from Crohn's disease (CD) patients and healthy controls. Raw sequencing data were originally retrieved from the NCBI Sequence Read Archive (SRA) under the following BioProjects:

- PRJNA248469 – **Dataset 1** (training cohort)
- PRJNA565216 and PRJNA985602 – **Dataset 2** (testing cohort)
- PRJNA702434 – **Dataset 3** (validation cohort)

All samples were selected based on the inclusion criteria described in the manuscript: intestinal biopsies from individuals clinically diagnosed with CD or from HC.

In the original study, the raw FASTQ files were processed through a uniform bioinformatics pipeline to ensure cross-study consistency. Briefly, quality control was performed with *fastp* (v1.0.1), reads were aligned to the GRCh38.p14 human reference genome using *STAR* (v2.7.10), strandedness was verified with *RSeQC* (v5.0.1), and gene-level quantification was obtained with *Rsubread* (v2.22.1).

For the present vignette, these processed count matrices and their sample metadata are re-used as a single ready-to-load object (`ibd_dataset.RData`, containing `ibd.count` (samples on rows, genes on columns) and `ibd.clin` (sample-level annotations)), deposited on Zenodo as cited in the paper.

```r
# Load all datasets
load("ibd_dataset.RData")
```

2.1 Dataset cleaning

For all downstream analyses we focus on CD versus HC (labeled `Not IBD` in the metadata), excluding *ulcerative colitis* (UC). The helper function below subsets a given study accession and returns harmonized objects (`desc`, `count`) aligned by sample ID, keeping only CD and Not IBD:

```r
# Helper Function
filter_study_dataset <- function(clin, count, study, class_exclude = "UC") {
  desc <- clin[clin$Study %in% study & !clin$class %in% class_exclude, ]
  count_sub <- count[rownames(count) %in% rownames(desc), ]
  rownames(desc) <- desc$ID
  list(desc = desc, count = count_sub)
}
```

3 Consensus gene-signature discovery on Dataset 1

In this section, we focus on **Dataset 1 (PRJNA248469)**, which serves as the **training dataset** for the construction of a robust gene signature distinguishing CD from HC.

The analysis proceeds through the following steps:

1. **Preprocessing**
   The dataset is processed with `preProcess()`, which performs library-size normalization, low-expression filtering, and metadata preparation for downstream classification.
2. **Classifier training and feature selection**
   A panel of supervised machine-learning models is trained using the `runClassifiers()` function. Each model is coupled with a dedicated **feature selection strategy**, executed within resampling folds to avoid information leakage. During training, **variable importance scores** are computed for all classifiers.
3. **Consensus signature extraction**
   Features that are recurrently selected across multiple high-performing models are aggregated into a unified **consensus gene-signature** using the `getConsensus()` function.
4. **Differential gene expression analysis (DEG)**
   DEGs are computed using **DESeq2** on the same dataset, serving as a classical baseline for comparison.
5. **Benchmarking**
   The discriminative performance of the consensus gene-signature is evaluated using `testConsensus()` and compared to the DEG-based gene set through univariate and multivariate analyses.

3.1 Process the data (`preProcess`)

The `preProcess()` function from **TENTACLES** performs **library-size normalization**, **low-expression filtering**, and —if specified— **batch-effect correction**.
It requires as input:

- `df.count`: a data frame of raw gene counts, with **samples as rows** and **genes as columns**;
- `df.clin`: a data frame containing **sample-level metadata**, with samples as rows and variables (e.g., class labels, batch ID) as columns;
- `class`: the name of the column in `df.clin` indicating the **outcome variable** (e.g., `"class"`), which must be a binary factor (e.g., `"CD"` vs `"Not IBD"`).

Optional arguments allow for batch correction (via `batch` and `covar.mod`), plotting (PCA and PVCA before and after batch correction), and threshold customization for low gene expression filtering (through `mincpm` and `minfraction`).

The `is.normalized` parameter specifies whether the input data are already normalized: it should be set to `TRUE` for **microarray** data and `FALSE` (default) for **RNA-Seq** data.

In this case, since the input consists of raw RNA-Seq counts, we leave `is.normalized = FALSE`:

```r
# Helper Function to select Dataset 1
tmp <- filter_study_dataset(ibd.clin, ibd.count, "PRJNA248469")
# Define Desc and Count
desc_d1 <- tmp$desc
```

```r
count_d1 <- tmp$count
# Preprocess data with preProcess()
pp_d1 <- preProcess(df.count = count_d1, df.clin = desc_d1, class = "class",
                    case.label = "CD", is.normalized = FALSE,
                    mincpm = 1, minfraction = 0.1, plot = FALSE)
rm(tmp)
```

The output of the `preProcess()` function is a `preProcess.obj` object, a structured S4 object containing all intermediate and final elements from the preprocessing pipeline.

```r
str(pp_d1, list.len = 4)
#> Formal class 'preProcess.obj' [package "TENTACLES"] with 4 slots
#>   ..@ raw      :'data.frame':    260 obs. of  10062 variables:
#>   .. ..$ TNFRSF4       : int [1:260] 120 53 146 144 56 74 34 178 101 30 ...
#>   .. ..$ TNFRSF18      : int [1:260] 36 53 111 106 30 93 49 107 57 38 ...
#>   .. ..$ PRDM16        : int [1:260] 45 34 7 46 9 27 9 13 18 29 ...
#>   .. ..$ PRKCZ         : int [1:260] 432 551 232 390 176 263 142 178 154 642 ...
#>   .. .. [list output truncated]
#>   ..@ processed:List of 2
#>   .. ..$ normalized   :'data.frame': 260 obs. of  8426 variables:
#>   .. .. ..$ TNFRSF4      : num [1:260] 4.2 3.24 4.91 4.03 3.9 ...
#>   .. .. ..$ TNFRSF18     : num [1:260] 2.63 3.24 4.53 3.62 3.09 ...
#>   .. .. ..$ PRDM16       : num [1:260] 2.91 2.68 1.26 2.56 1.7 ...
#>   .. .. ..$ PRKCZ        : num [1:260] 5.99 6.47 5.56 5.41 5.49 ...
#>   .. .. .. [list output truncated]
#>   .. ..$ adjusted.data:'data.frame': 260 obs. of  8426 variables:
#>   .. .. ..$ TNFRSF4      : num [1:260] 4.2 3.24 4.91 4.03 3.9 ...
#>   .. .. ..$ TNFRSF18     : num [1:260] 2.63 3.24 4.53 3.62 3.09 ...
#>   .. .. ..$ PRDM16       : num [1:260] 2.91 2.68 1.26 2.56 1.7 ...
#>   .. .. ..$ PRKCZ        : num [1:260] 5.99 6.47 5.56 5.41 5.49 ...
#>   .. .. .. [list output truncated]
#>   ..@ metadata :'data.frame':    260 obs. of  12 variables:
#>   .. ..$ Age           : num [1:260] 12.5 8 9 5.08 10.42 ...
#>   .. ..$ Assay.Type    : chr [1:260] "RNA-Seq" "RNA-Seq" "RNA-Seq" "RNA-Seq" ...
#>   .. ..$ Study         : chr [1:260] "PRJNA248469" "PRJNA248469" "PRJNA248469" "PRJNA248469" ...
#>   .. ..$ ID            : chr [1:260] "SAMN03322967" "SAMN03322968" "SAMN03322969" "SAMN03322970" ..
#>   .. .. [list output truncated]
#>   ..@ data.info:List of 2
#>   .. ..$ type      : chr "rnaseq"
#>   .. ..$ normalized: logi FALSE
```

Its main slots include:

- `raw`: the original count matrix provided as input;
- `processed`: a list of data frames containing the results of each preprocessing step, including:
  - `normalized`: the library-size normalized counts data frame;
  - `filtered.data`: genes passing expression thresholds;
  - `adjusted.data`: the final matrix used for classification (after optional batch correction);
- `metadata`: a data frame with sample-level annotations, **filtered and aligned** to the final count matrix;

- `data.info`: a list with metadata about the dataset, including data type (`\"RNA-Seq\"` or `\"microarray\"`), normalization status, number of samples and features, and whether batch correction was applied.

This object is compatible with all downstream TENTACLES functions (e.g., `runClassifiers()`, `testConsensus()`, `valConsensus()`), ensuring reproducibility and consistent sample tracking. The resulting `preProcess.obj` provides the input structure for the multi-model training step described next.

3.2 Training multiple classification algorithms (`runClassifiers`)

The function `runClassifiers()` trains, tunes, and evaluates several supervised models through a unified interface. Each classifier can be coupled with a specific feature-selection strategy, and model hyperparameters are optimized through cross-validation. Variable importance (VIP) is computed for each model, with a permutation-based fallback when direct computation is unavailable.

It takes as inputs:

- `preProcess.obj`: output of `preProcess()` containing the adjusted counts and aligned metadata. Alternatively, `df.count` and `df.clin` can be passed directly if preprocessing was performed outside TENTACLES.
- `models`: character vector of classifiers to fit. Supported options include: `"xgboost"`, `"bag_tree"`, `"lightGBM"`, `"pls"`, `"logistic"`, `"C5_rules"`, `"mars"`, `"bag_mars"`, `"mlp"`, `"bag_mlp"`, `"decision_tree"`, `"rand_forest"`, `"svm_linear"`, `"svm_poly"`, `"svm_rbf"`.
- `selector.recipes`: feature-selection strategy for each model. Supported: `"base"`, `"boruta"`, `"roc"`, `"infgain"`, `"mrmr"`, `"corr"`. If a single recipe is provided, it is applied to all models; otherwise, pairing follows position order.
- `tuning.method`: hyperparameter search. Options: `"tune_grid"` (default), `"tune_race_anova"`, `"tune_race_win_loss"`, `"tune_bayes"`, `"tune_sim_anneal"`.
- `n`: an integer specifying the number of iterations for the tuning method.
- `v`: an integer specifying the number of folds for the cross-validation during the hyperparameters tuning.
- **Thresholds and selectors**: `selector.threshold` (threshold parameter used for `"roc"`, `"infgain"`, `"mrmr"`, `"corr"`), `boruta.maxRuns` (for `"boruta"`).
- **Balancing and filtering**: `downsample = TRUE` to downsample the majority class; `filter = TRUE` to remove genes not annotated in GO/KEGG databases before modeling.
- **Other controls**: `metric` (default `"accuracy"`), `nsim` (# simulations for permutation VIP), `seed`, `plot`, and `parallel` (to speed-up computation).

In the manuscript, ten models were evaluated with model-specific selectors and 5-fold CV grid search; the optimal configuration per model was chosen by the highest F1-score across folds:

```r
# runClassifiers()
rc <- runClassifiers(
  pp_d1,
  models = c("C5_rules", "pls", "rand_forest", "bag_mlp", "decision_tree", "mlp",
             "svm_linear", "xgboost", "bag_tree", "lightGBM", "mars"),
  selector.recipes = c("boruta", "boruta", "corr", "roc", "boruta", "roc",
                       "boruta", "base", "mrmr", "roc", "boruta"),
  tuning.method = "tune_grid",
  filter = TRUE, downsample = TRUE,
  selector.threshold = 0.95, boruta.maxRuns = 100,
  parallel = TRUE,
  metric = "f_meas",
  n = 5, v = 5, seed = 1789,
  plot = FALSE
)
```

The function returns a S4 object of class `runClassifiers.obj` with:

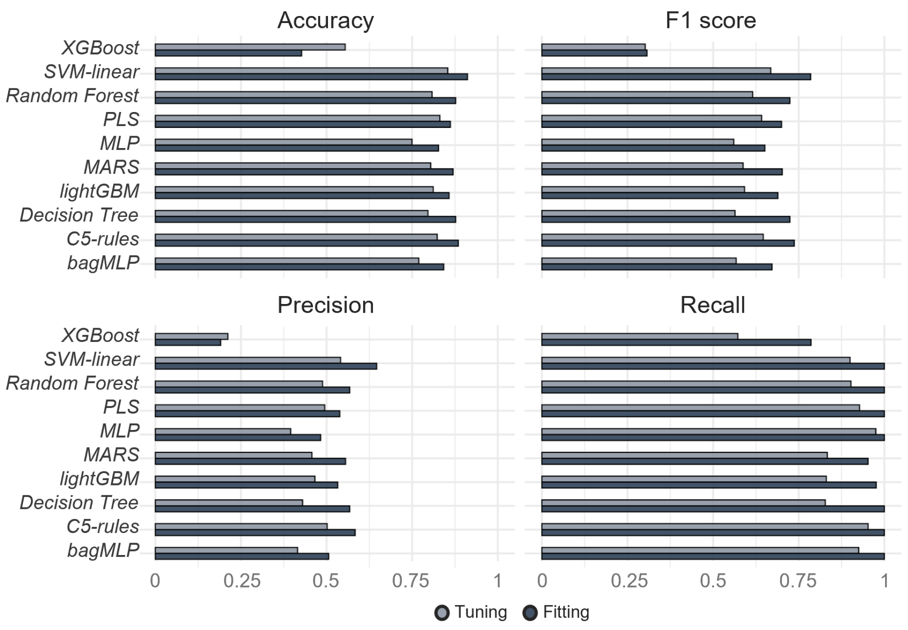
- `data`: adjusted data and metadata used for modeling.
- `models.info`: finalized workflows per model (recipes + model + tuned parameters).
- `model.features`: variable importance tables for each model.
- `performances`: tuning curves and final metrics for each model.
- `predictions`: out-of-fold predictions and associated probabilities, when available.

3.2.1 Visualizing model performance and feature overlap

After training, **TENTACLES** provides dedicated visualization utilities to interpret model performance and feature-selection overlap. Three main plotting utilities are available for objects returned by `runClassifiers()`.

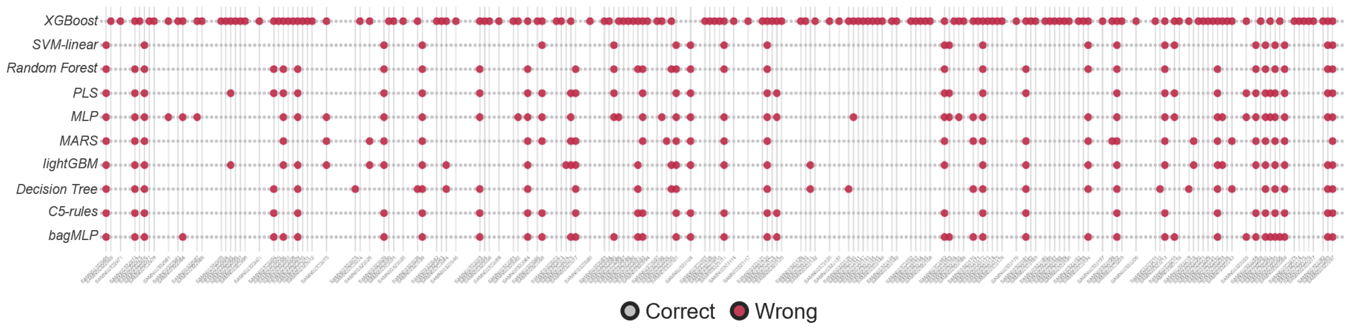
The `performances.plot()` function visualizes the cross-validation and final metrics for each trained model. It takes as input the `runClassifiers.obj` object and returns a grouped bar plot summarizing **Accuracy**, **F1-score**, **Precision**, and **Recall**.
Light-colored bars represent performance during resampling (cross-validation), whereas dark bars correspond to the final model fitted on the entire training set:

```
performances.plot(rc)
```



To examine how individual samples are classified by each model, the `wrong.preds.plot()` function can be used. It takes the `@predictions` slot of the `runClassifiers.obj` object and produces a concordance plot where each column represents a sample and each row a classifier.
Red dots indicate misclassified samples, and grey dots denote correct predictions.
This visualization highlights samples that are consistently difficult to classify across multiple algorithms:

```
wrong.preds.plot(rc@predictions) +
  theme( axis.text.x = element_text( angle = 45, hjust = 1, vjust = 1,
                                     margin = margin(t = 2),
                                     size = 6,
                                     color = "#7c7b7b"),
  plot.margin = margin(t = 10, r = 10, b = 5, l = 10)
  )
```

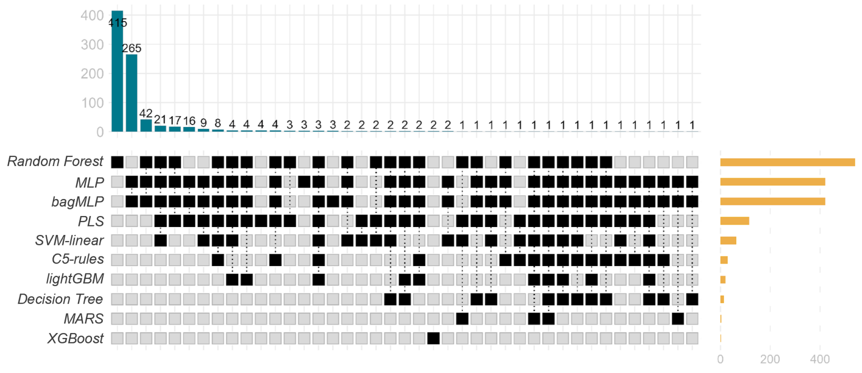


Note: Because these plots are built on **ggplot2**, they can be fully customized with standard `theme()` or aesthetic arguments, as shown above (e.g., adjusting text size or margins to better fit in documents).

The `upset.plot()` function visualizes the intersection of important genes across models.
It takes the `runClassifiers()` object and generates an UpSet plot showing how feature sets selected by different classifiers intersect.

Each horizontal row represents a model, and each vertical bar shows the size of an intersection (number of genes selected by that specific combination of models).
This provides a concise overview of shared vs. model-specific gene selections, helping identify robust features contributing to the consensus signature:

```
upset.plot(rc)
```



3.3 Identification of consensus gene-signature through ensemble learning (`getConsensus`)

After training individual models, it is possible to derive a consensus set of features repeatedly appearing across high performing classifiers derived and computed in the training part through the `runClassifiers()` function. To do this we use the `getConsensus()` function which takes as input a `runClassifiers.obj` or a binary data frame and supports 3 possible approaches:

- **Threshold-based selection:** assign values to `n_min` and `exclude` parameters. In this way we can exclude algorithms with poor performances and pick as consensus genes, those that appeared in at least a number of `n_min` algorithms.
- **Group-wise logic (single group):** leave as `NULL` these 2 parameters and specify instead `group1` and `meth1` parameters. In this way we can take the `'union'` or the `'intersection'` of the algorithms listed in `group1`.

- **Group-wise logic (two groups):** leave as `NULL` `n_min` and `exclude` while specifying `group1`, `group2`, `meth1`, `meth2` and `meth_comb` parameters all together. In this way we can take genes based on the `meth1` of `group1`, `meth_comb` with the `meth2` of `group2`. e.g. take the union (`meth1 = "union"`) of genes in `group1`, the union (`meth2 = "union"`) of genes in `group2` and pick the intersection (`meth_comb = "intersect"`) of these two groups.

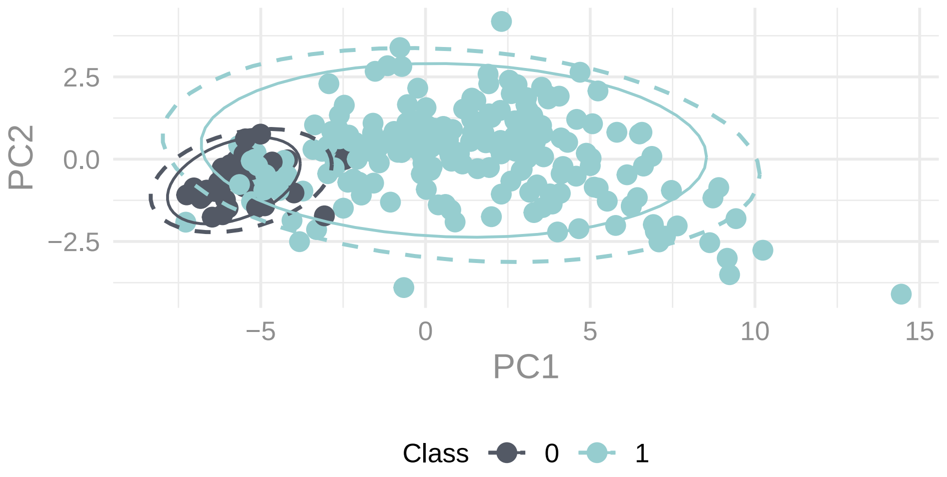
In this analysis, we used the **threshold-based approach** and retrieved genes that were appearing in at least six algorithms (`n.min = 6`) excluding XGBoost (`exclude = c(‘’xgboost_base’’)`):

```r
# getConsensus()
gc <- getConsensus(
  rc,
  n.min = 6,
  exclude = c("xgboost_base"),
  plot = FALSE
)
```

To analyze and visualize the discriminative performance of the genes selected by `getConsensus()`, the output object provides access to three different plots, each representing a complementary analysis of the consensus gene set.
These visualizations assess both multivariate and univariate behavior of the selected genes on the dataset used as input to `getConsensus()`.

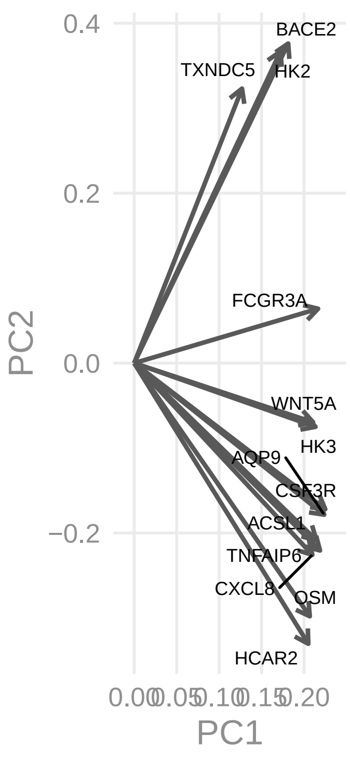
- `getConsensus.obj$test_consensus@pca$plots$pca_plot` displays a **Principal Component Analysis (PCA)** of the samples based on the consensus genes, showing overall class separation.

```r
gc$test_consensus@pca$plots$pca_plot
```



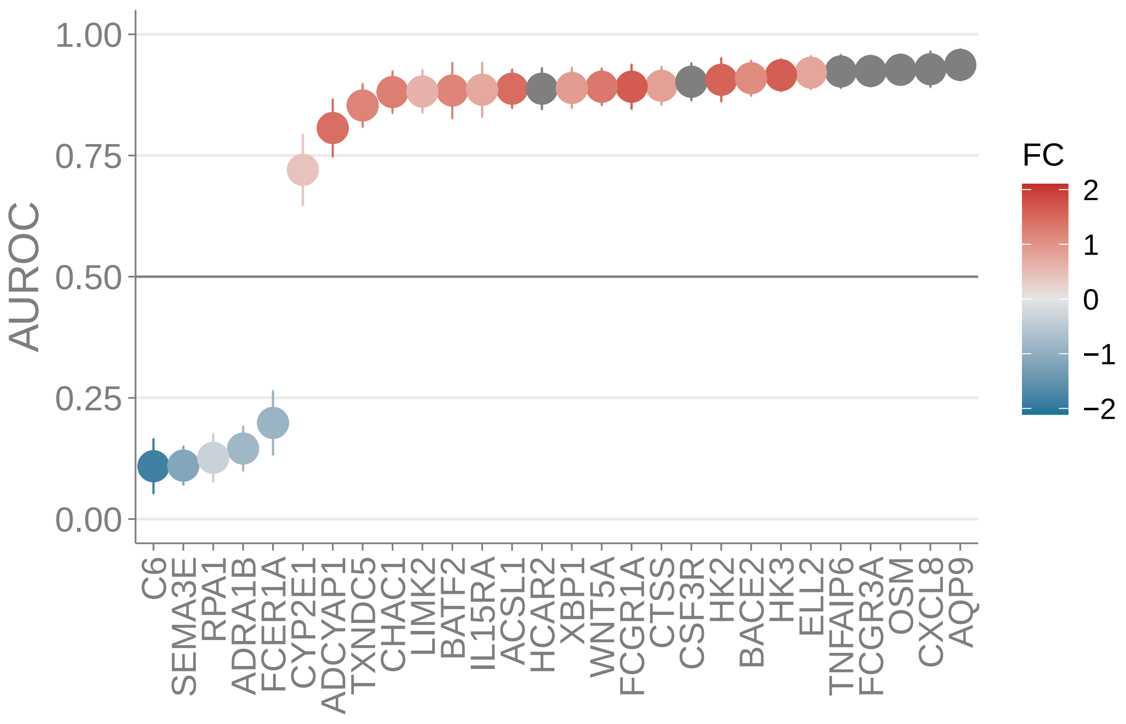
- `getConsensus.obj$test_consensus@pca$plots$loadings_plot` shows the **PCA loadings**, highlighting the contribution of each gene to the principal components and its recurrence across models.

```r
gc$test_consensus@pca$plots$loadings_plot
```



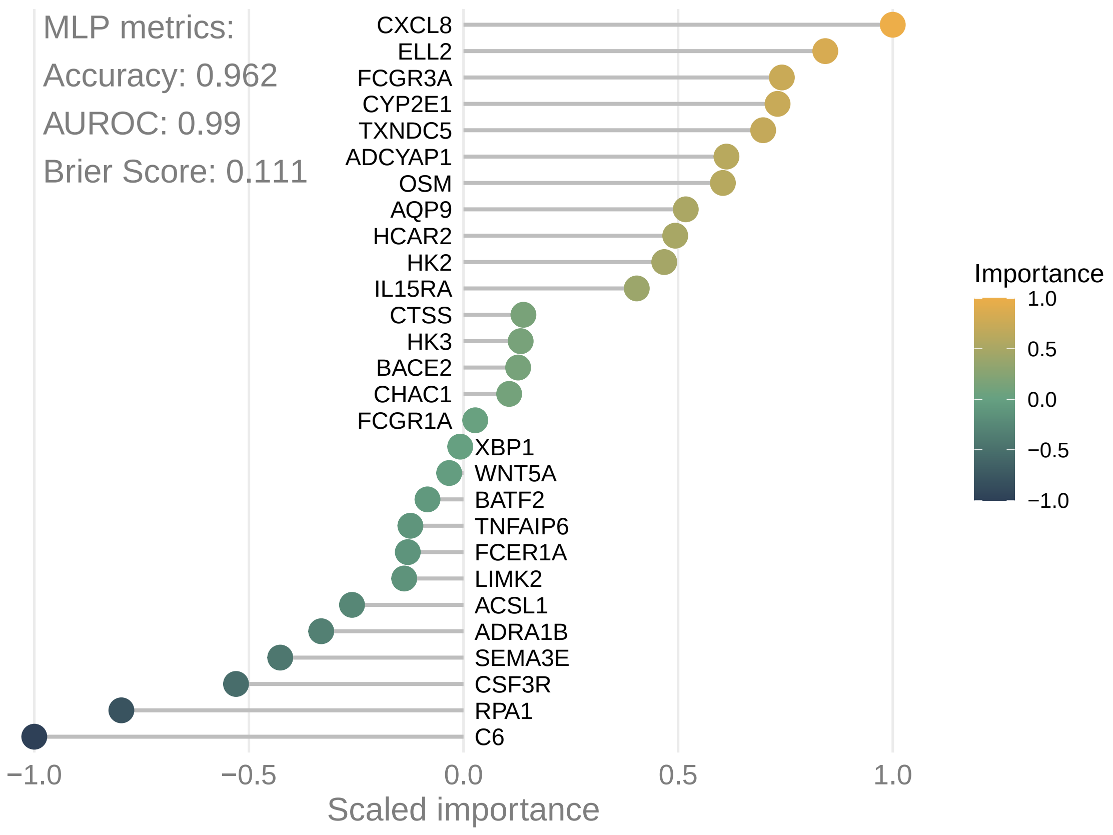
- `getConsensus.obj$test_consensus@auroc_fc$plot` summarizes **univariate performance** by plotting the AUROC of each gene, with colors indicating log2 fold change between classes.

```
gc$test_consensus@auroc_fc$plot
```



- `getConsensus.obj$test_consensus@mlp$plot` reports the performance of a **multivariate MLP classifier** trained only on the consensus genes, including classification metrics and variable importance.

```
gc$test_consensus@mlp$plot
```



3.4 Differential gene expression analysis (DEG)

To complement the workflow, we conducted a classical **differential expression (DEG)** analysis on the training cohort (**Dataset 1 – PRJNA248469**). The goal was to identify genes significantly modulated between CD and HC, to later compare their discriminative performance against the **TENTACLES consensus signature**.

Raw counts were filtered for low-coverage samples (total reads < 500,000), normalized with **DESeq2**, and modeled with the formula `~ class`. Genes were considered differentially expressed if **padj < 0.05** and **|log2FC| >= 1**.
The resulting DEG list (hereafter `genes_dds_d1`) served as a baseline reference for benchmarking:

```
# Transpose the gene count matrix and align sample names with clinical data
x <- t(count_d1)
desc <- desc_d1
m <- match(desc$ID, colnames(x))

# Filter the count table to include only samples present in clinical data
x <- x[, m]
```

```r
# Remove samples with low total counts (<500,000) and update clinical data
keepsamples <- colSums(x) > 500000
x <- x[, keepsamples]
desc <- desc[keepsamples, ]
# Clean and prepare class and batch columns in clinical data
desc$class <- as.factor(desc$class)
desc$class <- relevel(desc$class, ref = "Not IBD")  # Set "Not IBD" as reference
desc$batch2 <- as.factor(paste(desc$batch, desc$internal.batch))  # Combine batch info
desc$Group <- as.factor(paste(desc$batch, desc$class))  # Create group column

# ---------------------------- DESeq2 Analysis ----------------------------
# Create DESeq2 dataset object using count data and clinical metadata
dds_d1 <- DESeqDataSetFromMatrix(
  countData = x,
  colData = desc,
  design = ~ class  # Design formula: batch and class as factors
)

keep <- rownames(dds_d1) %in% colnames(pp_d1@processed$adjusted.data)

# Subset the DESeq2 dataset to retain only highly expressed genes
dds_d1 <- dds_d1[keep, ]

# Perform DESeq2 normalization and differential expression analysis
dds_d1 <- DESeq(dds_d1)

# Clean up unnecessary objects from the environment
rm(x, m, keep, keepsamples, desc)
```

3.5 Benchmarking via `testConsensus`

To assess the discriminative performance of the **differentially expressed genes** identified and to compare it with the discriminative performance of the consensus gene-signature, we use the `testConsensus()` function. This function performs a comprehensive analysis of the input genes on the dataset passed as input, combining univariate and multivariate evaluations.

Specifically, `testConsensus()` includes the following components:

- **Principal Component Analysis (PCA):**
  Dimensionality reduction based on the consensus genes.
  The top `top_loadings` genes (by absolute loading) are identified, and the PCA scores and loadings are returned showing class separation.
- **AUROC and Fold Change analysis:**
  For each gene, the function computes the **Area Under the ROC Curve (AUROC)** and the **log2 fold change** between classes. These metrics are summarized in a data frame and visualized in a joint plot.
- **Heatmap with hierarchical clustering (HC):**
  A normalized expression heatmap of the consensus genes, grouped by hierarchical clustering, to visualize co-expression and class-specific patterns.
- **Multi-Layer Perceptron (MLP):**
  A multivariate model is trained on the consensus gene set.
  The resulting output includes the **model's performance metrics** and **feature importance** scores.

The output is an S4 object of class `testConsensus.obj`, containing all intermediate data structures and ggplot2 objects for full customization and reuse.

```r
# Retrieve DEG results
res_dds_d1 <- results(dds_d1, alpha = 0.05)
# Subset DEGs
res_dds_d1 <- subset(res_dds_d1,
                     padj < 0.05 &
                       abs(log2FoldChange) >= 1)
# Define the final DEGs list
genes_dds_d1 <- rownames(res_dds_d1)
```

```r
# Apply testConsensus() to DEGs
tc_dds_d1_d1 <- testConsensus(df.count = pp_d1@processed$adjusted.data,
                              gene.list = genes_dds_d1,
                              class = pp_d1@metadata$class)
```

4 Cross-cohort validation on Dataset 2 (`testConsensus`)

To evaluate the **cross-cohort generalizability** of the consensus gene-signature identified in the training dataset (Dataset 1), we use an independent test set (**Dataset 2**) composed of two additional studies: **PRJNA565216** and **PRJNA985602**.

These datasets are first **merged** to obtain a more heterogeneous cohort and then processed using the `preProcess()` function to perform normalization and batch-effect correction.

We then apply `testConsensus()` to this combined dataset to evaluate the performance of the consensus gene-signature—both at the univariate and multivariate level.
Finally, we compare these results to those obtained using differentially expressed genes (DEGs) from **Dataset 1**, providing a benchmark for assessing the added value of the ensemble-derived signature.

4.1 Preprocess of Dataset 2 composed by PRJNA565216 and PRJNA985602

Since the two studies originate from different experiments, they introduce **batch effects** that must be addressed before downstream analysis.
To handle this, we create a new variable—`batch_new`—and we pass this variable to the `batch` argument of `preProcess()` function:

```r
# Create Dataset 2 by combining PRJNA565216 and PRJNA985602
tmp <- filter_study_dataset(ibd.clin, ibd.count, c("PRJNA565216", "PRJNA985602"))
desc_g23 <- tmp$desc
count_g23 <- tmp$count
# Create batch_new to account for different studies
desc_g23$batch_new <- as.factor(paste0(desc_g23$batch, desc_g23$internal.batch))
# preprocess Dataset 2
pp_g23 <- preProcess(df.count = count_g23, df.clin = desc_g23, batch = "batch_new",
                     class = "class", case.label = "CD", plot = FALSE)
rm(tmp)
```

4.2 External Validation of the consensus gene-signature

The first external validation of the consensus gene-signature is performed using the `testConsensus()` function, applied to the merged Dataset 2 (Studies 2 and 3):

```r
# External validation via testConsensus()
tc <- testConsensus(df.count = pp_g23@processed$adjusted.data,
                    gene.list = gc$consensusGenes,
                    class = pp_g23@metadata$class,
                    plot = FALSE)
```

The output of `testConsensus()` is a structured S4 object of class `testConsensus.obj`, which includes:

- **`data`**: a list with the input expression matrix (`df.count`), gene list (`gene.list`), and class labels (`class`).
- **`pca`**: PCA results, including sample scores, gene loadings, variance explained, and the corresponding PCA plot.
- **`auroc_fc`**: per-gene **AUROC** and **log2 fold change** values, alongside a summary plot.

- **heatmap**: a heatmap with **hierarchical clustering** of the consensus genes.
- **mlp**: a multivariate **MLP classifier**, with model performance metrics, variable importances, and an associated plot.

The resulting visualizations can be accessed directly from the object using:

```
tc@pca$plots$pca_plot
tc@pca$plots$loadings_plot
tc@auroc_fc$plot
tc@mlp$plot
```

4.3 Comparison with classical DEG Analysis

To provide a direct benchmark, we applied the same evaluation procedure to the set of **DEGs** identified in **Dataset 1** via DESeq2. Specifically, we use the `testConsensus()` function to assess the **discriminative performance** of the DEG panel when applied to **Dataset 2**, following the same approach used for the TENTACLES-derived signature:

```
tc_dds_d1_g23 <- testConsensus(df.count = pp_g23@processed$adjusted.data,
                               gene.list = genes_dds_d1,
                               class = pp_g23@metadata$class)
```

The resulting testConsensus.obj is again an S4 object of five elements containing all results, informations and plots accessible as previously shown.

```
str(tc_dds_d1_g23@data, max.level = 4, vec.len = 2, list.len = 5)
#> List of 3
#>  $ df.count :'data.frame':   179 obs. of  8367 variables:
#>   ..$ TNFRSF4      : num [1:179] 1 2.54 ...
#>   ..$ TNFRSF18     : num [1:179] 2.32 3.1 ...
#>   ..$ PRDM16       : num [1:179] 2.44 2.17 ...
#>   ..$ PRKCZ        : num [1:179] 0.775 0.775 ...
#>   ..$ ACAP3        : num [1:179] 3.81 2.35 ...
#>   .. [list output truncated]
#>  $ gene.list: chr [1:582] "MYOM3" "AJAP1" ...
#>  $ class    : Factor w/ 2 levels "0","1": 2 2 2 2 2 ...
```

```
str(tc_dds_d1_g23@pca, max.level = 2, vec.len = 2, list.len = 5)
#> List of 5
#>  $ scores             :'data.frame':  179 obs. of  180 variables:
#>   ..$ sample: chr [1:179] "SAMN12736816" "SAMN12736818" ...
#>   ..$ PC1   : num [1:179] -9.53 6.38 ...
#>   ..$ PC2   : num [1:179] -4.664 -0.208 ...
#>   ..$ PC3   : num [1:179] 3.48 2.32 ...
#>   ..$ PC4   : num [1:179] -6.4 -1.81 ...
#>   .. [list output truncated]
#>  $ loadings           :'data.frame':  541 obs. of  180 variables:
#>   ..$ gene : chr [1:541] "MYOM3" "AJAP1" ...
#>   ..$ PC1  : num [1:541] -0.0309 0.0125 ...
#>   ..$ PC2  : num [1:541] 0.1053 0.0229 ...
#>   ..$ PC3  : num [1:541] -0.0689 0.0333 ...
#>   ..$ PC4  : num [1:541] 0.01335 0.00527 ...
#>   .. [list output truncated]
#>  $ explained_variance:'data.frame':  179 obs. of  4 variables:
#>   ..$ PC      : int [1:179] 1 2 3 4 5 ...
#>   ..$ variance: num [1:179] 160.2 31.1 ...
#>   ..$ pct     : num [1:179] 29.61 5.75 ...
#>   ..$ pct_cum : num [1:179] 29.6 35.4 ...
#>  $ top_loadings      :'data.frame':  15 obs. of  3 variables:
#>   ..$ gene: chr [1:15] "MYOM3" "AADAC" ...
#>   ..$ PC1 : num [1:15] -0.0309 -0.0491 ...
#>   ..$ PC2 : num [1:15] 0.105 0.106 ...
#>  $ plots             :List of 2
#>   ..$ pca_plot     :List of 11
#>   .. ..- attr(*, "class")= chr [1:2] "gg" "ggplot"
#>   ..$ loadings_plot:List of 11
#>   .. ..- attr(*, "class")= chr [1:2] "gg" "ggplot"
```

```
str(tc_dds_d1_g23@auroc_fc, max.level = 1, vec.len = 2, list.len = 5)
#> List of 2
#>  $ data:'data.frame':    541 obs. of  5 variables:
#>  $ plot:List of 11
#>   ..- attr(*, "class")= chr [1:2] "gg" "ggplot"
```

```
str(tc_dds_d1_g23@heatmap, max.level = 1, vec.len = 2, list.len = 5)
#> List of 5
#>  $ matrix     :'data.frame': 541 obs. of  179 variables:
#>  $ hc_rows    :List of 7
#>   ..- attr(*, "class")= chr "hclust"
#>  $ hc_cols    :List of 7
#>   ..- attr(*, "class")= chr "hclust"
#>  $ side_colors:'data.frame': 179 obs. of  1 variable:
#>  $ plot       :List of 8
#>   ..- attr(*, "class")= chr [1:2] "plotly" "htmlwidget"
#>   ..- attr(*, "package")= chr "plotly"
```

```
str(tc_dds_d1_g23@mlp, max.level = 1, vec.len = 2, list.len = 5)
#> List of 4
#>  $ models.info     :List of 4
#>   ..- attr(*, "class")= chr "workflow"
#>  $ importances     :'data.frame':    534 obs. of  4 variables:
#>   ..- attr(*, "type")= chr "olden"
#>  $ test_performance: tibble [3 x 4] (S3: tbl_df/tbl/data.frame)
#>  $ plot            :List of 11
#>   ..- attr(*, "class")= chr [1:2] "gg" "ggplot"
```

4.4 Independent DEG Analysis on Dataset 2

Finally, we also performed an **independent DEG analysis** on the merged **Dataset 2**.
The following code reproduces this step, filtering low-count samples and including both `class` and `batch2` as factors in the model design:

```r
x <- t(count_g23)
desc <- desc_g23
m <- match(desc$ID, colnames(x))

x <- x[, m]
desc$ID == colnames(x)

keepsamples <- colSums(x) > 500000
x <- x[, keepsamples]
desc <- desc[keepsamples, ]

desc$class <- as.factor(desc$class)
desc$class <- relevel(desc$class, ref = "Not IBD")
desc$batch2 <- as.factor(paste(desc$batch, desc$internal.batch))
desc$Group <- as.factor(paste(desc$batch, desc$class))

dds_g23 <- DESeqDataSetFromMatrix(
  countData = x,
  colData = desc,
  design = ~ class + batch2
)

keep <- rownames(dds_g23) %in% colnames(pp_g23@processed$adjusted.data)
dds_g23 <- dds_g23[keep, ]
dds_g23 <- DESeq(dds_g23)

res_dds_g23 <- results(dds_g23, alpha = 0.05)

res_dds_g23 <- subset(res_dds_g23,
                      padj < 0.05 &
                        abs(log2FoldChange) >= 1)

genes_dds_g23 <- rownames(res_dds_g23)

rm(x, m, keep, keepsamples, desc)
```

And to assess intersection among consensus gene-signature, DEGs derived genes from Dataset 1 and DEGs derived genes from Dataset 2 we used the following:

```r
# Define dds_d1 and genes_dds_g23 intersection
intersection_deseqs <- intersect(genes_dds_d1, genes_dds_g23)
# Find overlap with TENTACLES consensus gene-signature
intersect(intersection_deseqs, gc$consensusGenes)
#>  [1] "FCGR3A"  "CSF3R"   "HK2"     "TNFAIP6" "WNT5A"   "CXCL8"   "ACSL1"
#>  [8] "HK3"     "ADRA1B"  "C6"      "TXNDC5"  "SEMA3E"  "BATF2"   "HCAR2"
#> [15] "AQP9"    "OSM"     "BACE2"
```

5 Unsupervised external validation of the consensus-gene signature on Dataset 3 (`valConsensus`)

To further evaluate the **cross-cohort generalizability** of the consensus gene-signature identified in the training dataset (Dataset 1) and previously tested on an independent test set (Dataset 2), we used a third dataset for **external unsupervised validation**: **PRJNA702434** (referred to here as **Dataset 3**).

For this purpose, we first preprocess the dataset anf then apply the `valConsensus()` function, which assesses the **discriminative capacity** of a gene set using multiple unsupervised clustering methods and ranks combinations based on internal clustering metrics.

5.1 Preprocess of Dataset 3: PRJNA702434

```r
# Helper Function to get Dataset 3
tmp <- filter_study_dataset(ibd.clin, ibd.count, "PRJNA702434")
desc_d4 <- tmp$desc
count_d4 <- tmp$count
# Preprocess Dataset 3
pp_d4 <- preProcess(df.count = count_d4, df.clin = desc_d4,
                    class = "class", case.label = "CD")
rm(tmp)
```

5.2 Validation of the consensus gene-signature

The `valConsensus()` function provides an **unsupervised validation framework** for assessing the **discriminative power** of a gene signature on a new dataset, without fitting supervised models.

Given an expression matrix (`df.count`) and a set of input genes (`gene.list`), the function evaluates the **clustering performance** of multiple combinations of genes across a panel of unsupervised algorithms.

Specifically, the following clustering methods are implemented:

- **K-Means**
- **Gaussian Mixture Models (GMM)**
- **Hierarchical Clustering**
- **K-Means on PCA-reduced dimensions**
- **K-Means on t-SNE-reduced dimensions**
- **K-Means on UMAP-reduced dimensions**

For each method and gene combination, the function computes standard classification metrics by comparing cluster assignments to the known class labels (`labels` vector parameter), including Accuracy, Precision, Recall and F1 Score (default metric for ranking).

The user can also specify:

- `N`: the number of top-performing gene combinations to retain (default = 10),
- `metric`: the evaluation criterion to prioritize combinations (`"Accuracy"`, `"Precision"`, `"Recall"`, or `"FScore"`).

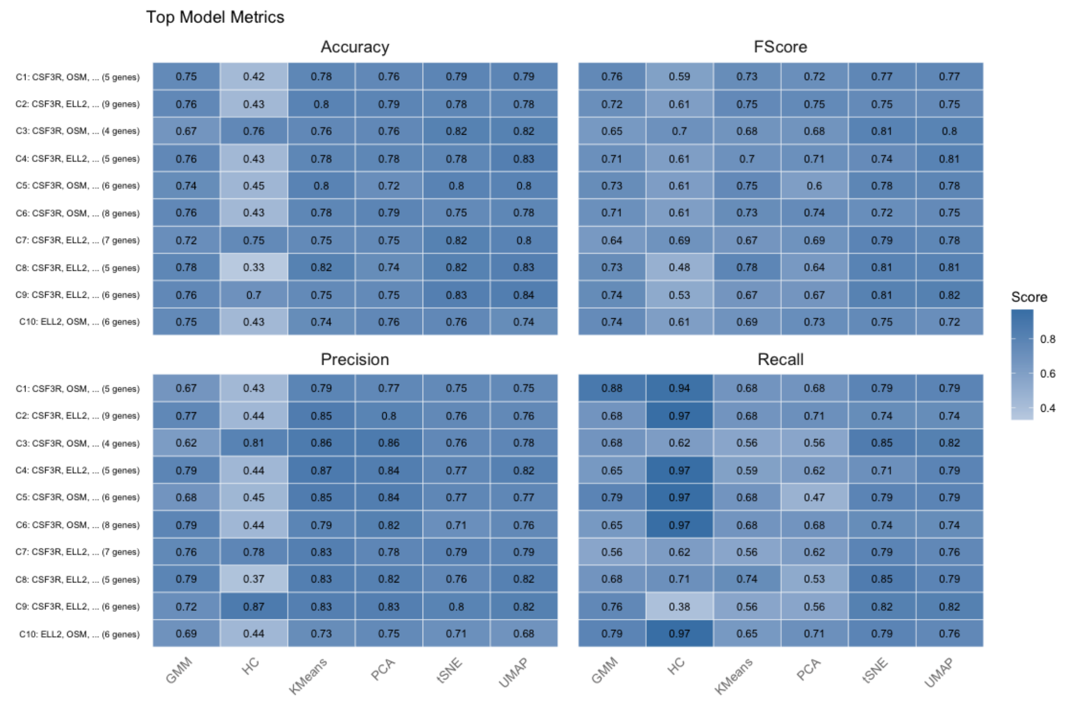
In the manuscript, we applied `valConsensus()` using a refined subset of genes that **preserved the direction of importance**(i.e., same sign of contribution) in both the training phase on **Dataset 1** and the cross-cohort testing on **Dataset 2**:

```
#Analysis----
# Joining the importance from d1 and g23-----
importances_d1g23 <- full_join(

  gc$test_consensus@mlp$importances %>%
    mutate(d1_signal = ifelse(Importance > 0, "Positive", "Negative")) %>%
    select(Variable, Importance_D1 = Importance, d1_signal)
  ,

  tc@mlp$importances %>%
    mutate(g23_signal = ifelse(Importance > 0, "Positive", "Negative")) %>%
    select(Variable, Importance_g23 = Importance, g23_signal)

) %>%
  mutate(same_signal = d1_signal == g23_signal,
         sum_importance = abs(Importance_D1 + Importance_g23)) %>%
  arrange(desc(same_signal), desc(sum_importance))

# Pull genes that have the same importance direction
genes_for_valcons <- importances_d1g23 %>%
  filter(same_signal) %>%
  pull(Variable)
```

```
# valConsensus
vc <- valConsensus(
  df.count = pp_d4@processed$adjusted.data,
  gene.list = genes_for_valcons,
  class = pp_d4@metadata$class
)
```

The output is a list containing clustering results, evaluation metrics, and ranked gene combinations, which can be visualized using the `plotTopMetrics()` function that creates a heatmap to visualize the performance of different clustering and dimensionality reduction models across multiple evaluation metrics, for each gene combination. Each combination is labeled with a concise and readable gene summary on the y-axis, prefixed by a combination number (C1, C2, . . .):

```r
plotTopMetrics(vc$topCombinations)
```



6 Session Info

```
devtools::session_info()
#> Warning in system2("quarto", "-V", stdout = TRUE, env = paste0("TMPDIR=", :
#> running command '"quarto"
#> TMPDIR=C:/Users/gabri/AppData/Local/Temp/RtmpSQvQup/filea1bc50b55154 -V' had
#> status 1
#> - Session info ---------------------------------------------------------------
#>  setting  value
#>  version  R version 4.4.3 (2025-02-28 ucrt)
#>  os       Windows 11 x64 (build 26200)
#>  system   x86_64, mingw32
#>  ui       RTerm
#>  language en
#>  collate  Portuguese_Brazil.utf8
#>  ctype    Portuguese_Brazil.utf8
#>  tz       America/Sao_Paulo
#>  date     2025-11-02
#>  pandoc   3.2 @ C:/Program Files/RStudio/resources/app/bin/quarto/bin/tools/ (via rmarkdown)
#>  quarto   NA @ C:\\PROGRA~1\\RStudio\\RESOUR~1\\app\\bin\\quarto\\bin\\quarto.exe
#>
#> - Packages -------------------------------------------------------------------
#>  package              * version    date (UTC) lib source
#>  abind                  1.4-8      2024-09-12 [1] CRAN (R 4.4.1)
#>  annotate               1.84.0     2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  AnnotationDbi          1.68.0     2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  assertthat             0.2.1      2019-03-21 [1] CRAN (R 4.4.3)
#>  baguette               1.1.0      2025-01-28 [1] CRAN (R 4.4.3)
#>  Biobase              * 2.66.0     2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  BiocGenerics         * 0.52.0     2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  BiocParallel           1.40.2     2025-06-04 [1] Bioconductor
#>  Biostrings             2.74.1     2024-12-16 [1] Bioconductor 3.20 (R 4.4.2)
#>  bit                    4.6.0      2025-03-06 [1] CRAN (R 4.4.3)
#>  bit64                  4.6.0-1    2025-01-16 [1] CRAN (R 4.4.2)
#>  blob                   1.2.4      2023-03-17 [1] CRAN (R 4.4.1)
#>  bonsai                 0.4.0      2025-06-25 [1] CRAN (R 4.4.3)
#>  Boruta                 9.0.0      2025-07-22 [1] CRAN (R 4.4.3)
#>  C50                    0.2.0      2025-04-03 [1] CRAN (R 4.4.3)
#>  ca                     0.71.1     2020-01-24 [1] CRAN (R 4.4.3)
#>  cachem                 1.1.0      2024-05-16 [1] CRAN (R 4.4.1)
#>  class                  7.3-23     2025-01-01 [2] CRAN (R 4.4.3)
#>  cli                    3.6.5      2025-04-23 [1] CRAN (R 4.4.3)
#>  codetools              0.2-20     2024-03-31 [2] CRAN (R 4.4.3)
#>  colino                 0.0.1      2025-04-28 [1] Github (stevenpawley/colino@9a786ce)
#>  colorspace             2.1-1      2024-07-26 [1] CRAN (R 4.4.1)
#>  corpcor                1.6.10     2021-09-16 [1] CRAN (R 4.4.0)
#>  cowplot              * 1.2.0      2025-07-07 [1] CRAN (R 4.4.3)
#>  crayon                 1.5.3      2024-06-20 [1] CRAN (R 4.4.1)
#>  Cubist                 0.5.0      2025-04-03 [1] CRAN (R 4.4.3)
#>  data.table             1.17.8     2025-07-10 [1] CRAN (R 4.4.3)
#>  DBI                    1.2.3      2024-06-02 [1] CRAN (R 4.4.1)
#>  DelayedArray           0.32.0     2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  dendextend             1.19.1     2025-07-15 [1] CRAN (R 4.4.3)
#>  DESeq2              * 1.46.0    2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  devtools              2.4.5     2022-10-11 [1] CRAN (R 4.4.1)
#>  dials                 1.4.2     2025-09-04 [1] CRAN (R 4.4.3)
#>  DiceDesign            1.10      2023-12-07 [1] CRAN (R 4.4.3)
#>  dichromat             2.0-0.1   2022-05-02 [1] CRAN (R 4.4.0)
#>  digest                0.6.37    2024-08-19 [1] CRAN (R 4.4.1)
#>  dplyr               * 1.1.4     2023-11-17 [1] CRAN (R 4.4.3)
#>  edgeR                 4.4.2     2025-01-27 [1] Bioconductor 3.20 (R 4.4.2)
#>  ellipse               0.5.0     2023-07-20 [1] CRAN (R 4.4.2)
#>  ellipsis              0.3.2     2021-04-29 [1] CRAN (R 4.4.1)
#>  evaluate              1.0.5     2025-08-27 [1] CRAN (R 4.4.3)
#>  farver                2.1.2     2024-05-13 [1] CRAN (R 4.4.1)
#>  fastmap               1.2.0     2024-05-15 [1] CRAN (R 4.4.1)
#>  forcats             * 1.0.0     2023-01-29 [1] CRAN (R 4.4.1)
#>  foreach               1.5.2     2022-02-02 [1] CRAN (R 4.4.1)
#>  Formula               1.2-5     2023-02-24 [1] CRAN (R 4.4.0)
#>  fs                    1.6.6     2025-04-12 [1] CRAN (R 4.4.3)
#>  FSelectorRcpp         0.3.13    2024-10-02 [1] CRAN (R 4.4.3)
#>  furrr                 0.3.1     2022-08-15 [1] CRAN (R 4.4.2)
#>  future                1.67.0    2025-07-29 [1] CRAN (R 4.4.3)
#>  future.apply          1.20.0    2025-06-06 [1] CRAN (R 4.4.3)
#>  genefilter            1.88.0    2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  generics              0.1.4     2025-05-09 [1] CRAN (R 4.4.3)
#>  GenomeInfoDb        * 1.42.3    2025-01-27 [1] Bioconductor 3.20 (R 4.4.2)
#>  GenomeInfoDbData      1.2.13    2024-12-03 [1] Bioconductor
#>  GenomicRanges       * 1.58.0    2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  ggplot2             * 3.5.2     2025-04-09 [1] CRAN (R 4.4.3)
#>  ggrepel             * 0.9.6     2024-09-07 [1] CRAN (R 4.4.1)
#>  globals               0.18.0    2025-05-08 [1] CRAN (R 4.4.3)
#>  glue                  1.8.0     2024-09-30 [1] CRAN (R 4.4.1)
#>  gower                 1.0.2     2024-12-17 [1] CRAN (R 4.4.2)
#>  GPfit                 1.0-9     2025-04-12 [1] CRAN (R 4.4.3)
#>  gridExtra             2.3       2017-09-09 [1] CRAN (R 4.4.1)
#>  gtable                0.3.6     2024-10-25 [1] CRAN (R 4.4.2)
#>  hardhat               1.4.2     2025-08-20 [1] CRAN (R 4.4.3)
#>  heatmaply             1.6.0     2025-07-12 [1] CRAN (R 4.4.3)
#>  hms                   1.1.3     2023-03-21 [1] CRAN (R 4.4.1)
#>  htmltools             0.5.8.1   2024-04-04 [1] CRAN (R 4.4.1)
#>  htmlwidgets           1.6.4     2023-12-06 [1] CRAN (R 4.4.1)
#>  httpuv                1.6.16    2025-04-16 [1] CRAN (R 4.4.3)
#>  httr                  1.4.7     2023-08-15 [1] CRAN (R 4.4.1)
#>  igraph                2.1.4     2025-01-23 [1] CRAN (R 4.4.2)
#>  inum                  1.0-5     2023-03-09 [1] CRAN (R 4.4.3)
#>  ipred                 0.9-15    2024-07-18 [1] CRAN (R 4.4.2)
#>  IRanges             * 2.40.1    2024-12-05 [1] Bioconductor 3.20 (R 4.4.2)
#>  iterators             1.0.14    2022-02-05 [1] CRAN (R 4.4.1)
#>  jsonlite              2.0.0     2025-03-27 [1] CRAN (R 4.4.3)
#>  KEGGREST              1.46.0    2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  knitr                 1.50      2025-03-16 [1] CRAN (R 4.4.3)
#>  labeling              0.4.3     2023-08-29 [1] CRAN (R 4.4.0)
#>  later                 1.4.4     2025-08-27 [1] CRAN (R 4.4.3)
#>  lattice               0.22-7    2025-04-02 [2] CRAN (R 4.4.3)
#>  lava                  1.8.1     2025-01-12 [1] CRAN (R 4.4.2)
#>  lazyeval             0.2.2      2019-03-15 [1] CRAN (R 4.4.1)
#>  lhs                  1.2.0      2024-06-30 [1] CRAN (R 4.4.3)
#>  libcoin              1.0-10     2023-09-27 [1] CRAN (R 4.4.3)
#>  lifecycle            1.0.4      2023-11-07 [1] CRAN (R 4.4.1)
#>  limma                3.62.2     2025-01-09 [1] Bioconductor 3.20 (R 4.4.2)
#>  listenv              0.9.1      2024-01-29 [1] CRAN (R 4.4.2)
#>  locfit               1.5-9.12   2025-03-05 [1] CRAN (R 4.4.3)
#>  lubridate          * 1.9.4      2024-12-08 [1] CRAN (R 4.4.2)
#>  magrittr             2.0.3      2022-03-30 [1] CRAN (R 4.4.1)
#>  MASS                 7.3-65     2025-02-28 [2] CRAN (R 4.4.3)
#>  Matrix               1.7-3      2025-03-11 [2] CRAN (R 4.4.3)
#>  MatrixGenerics     * 1.18.1     2025-01-09 [1] Bioconductor 3.20 (R 4.4.2)
#>  matrixStats        * 1.5.0      2025-01-07 [1] CRAN (R 4.4.2)
#>  mclust               6.1.1      2024-04-29 [1] CRAN (R 4.4.3)
#>  memoise              2.0.1      2021-11-26 [1] CRAN (R 4.4.1)
#>  mgcv                 1.9-3      2025-04-04 [1] CRAN (R 4.4.3)
#>  mime                 0.13       2025-03-17 [1] CRAN (R 4.4.3)
#>  miniUI               0.1.2      2025-04-17 [1] CRAN (R 4.4.3)
#>  mixOmics             6.30.0     2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  mvtnorm              1.3-3      2025-01-10 [1] CRAN (R 4.4.2)
#>  NeuralNetTools       1.5.3      2022-01-06 [1] CRAN (R 4.4.3)
#>  nlme                 3.1-168    2025-03-31 [2] CRAN (R 4.4.3)
#>  nnet                 7.3-20     2025-01-01 [1] CRAN (R 4.4.2)
#>  parallelly           1.45.1     2025-07-24 [1] CRAN (R 4.4.3)
#>  parsnip              1.3.3      2025-08-31 [1] CRAN (R 4.4.3)
#>  partykit             1.2-24     2025-05-02 [1] CRAN (R 4.4.3)
#>  pillar               1.11.0     2025-07-04 [1] CRAN (R 4.4.3)
#>  pkgbuild             1.4.8      2025-05-26 [1] CRAN (R 4.4.3)
#>  pkgconfig            2.0.3      2019-09-22 [1] CRAN (R 4.4.1)
#>  pkgload              1.4.0      2024-06-28 [1] CRAN (R 4.4.1)
#>  plotly               4.11.0     2025-06-19 [1] CRAN (R 4.4.3)
#>  plsmod               1.0.0      2022-09-06 [1] CRAN (R 4.4.3)
#>  plyr                 1.8.9      2023-10-02 [1] CRAN (R 4.4.1)
#>  png                  0.1-8      2022-11-29 [1] CRAN (R 4.4.0)
#>  praznik              11.0.0     2022-05-20 [1] CRAN (R 4.4.3)
#>  prodlim              2025.04.28 2025-04-28 [1] CRAN (R 4.4.3)
#>  profvis              0.4.0      2024-09-20 [1] CRAN (R 4.4.1)
#>  promises             1.3.3      2025-05-29 [1] CRAN (R 4.4.3)
#>  purrr              * 1.1.0      2025-07-10 [1] CRAN (R 4.4.3)
#>  R6                   2.6.1      2025-02-15 [1] CRAN (R 4.4.3)
#>  rARPACK              0.11-0     2016-03-10 [1] CRAN (R 4.4.3)
#>  RColorBrewer         1.1-3      2022-04-03 [1] CRAN (R 4.4.0)
#>  Rcpp                 1.1.0      2025-07-02 [1] CRAN (R 4.4.3)
#>  readr              * 2.1.5      2024-01-10 [1] CRAN (R 4.4.1)
#>  recipes              1.3.1      2025-05-21 [1] CRAN (R 4.4.3)
#>  registry             0.5-1      2019-03-05 [1] CRAN (R 4.4.0)
#>  remotes              2.5.0      2024-03-17 [1] CRAN (R 4.4.1)
#>  reshape2             1.4.4      2020-04-09 [1] CRAN (R 4.4.1)
#>  rlang                1.1.6      2025-04-11 [1] CRAN (R 4.4.3)
#>  rmarkdown            2.29       2024-11-04 [1] CRAN (R 4.4.2)
#>  rpart                4.1.24     2025-01-07 [2] CRAN (R 4.4.3)
#>  rsample              1.3.1      2025-07-29 [1] CRAN (R 4.4.3)
#>  RSpectra             0.16-2     2024-07-18 [1] CRAN (R 4.4.2)
#>  RSQLite                 2.4.3      2025-08-20 [1] CRAN (R 4.4.3)
#>  rstudioapi              0.17.1     2024-10-22 [1] CRAN (R 4.4.2)
#>  rules                   1.0.2      2023-03-08 [1] CRAN (R 4.4.3)
#>  S4Arrays                1.6.0      2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  S4Vectors             * 0.44.0     2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  scales                * 1.4.0      2025-04-24 [1] CRAN (R 4.4.3)
#>  seriation               1.5.8      2025-08-20 [1] CRAN (R 4.4.3)
#>  sessioninfo             1.2.3      2025-02-05 [1] CRAN (R 4.4.3)
#>  shiny                   1.11.1     2025-07-03 [1] CRAN (R 4.4.3)
#>  SparseArray             1.6.2      2025-02-20 [1] Bioconductor 3.20 (R 4.4.2)
#>  statmod                 1.5.0      2023-01-06 [1] CRAN (R 4.4.2)
#>  stringi                 1.8.7      2025-03-27 [1] CRAN (R 4.4.3)
#>  stringr               * 1.5.1      2023-11-14 [1] CRAN (R 4.4.1)
#>  SummarizedExperiment * 1.36.0     2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  survival                3.8-3      2024-12-17 [1] CRAN (R 4.4.2)
#>  sva                     3.54.0     2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  TENTACLES             * 0.1.0      2025-10-30 [1] Github (Giomu/TENTACLES@199e3f7)
#>  tibble                * 3.3.0      2025-06-08 [1] CRAN (R 4.4.3)
#>  tidyr                 * 1.3.1      2024-01-24 [1] CRAN (R 4.4.1)
#>  tidyselect              1.2.1      2024-03-11 [1] CRAN (R 4.4.1)
#>  tidyverse             * 2.0.0      2023-02-22 [1] CRAN (R 4.4.1)
#>  timechange              0.3.0      2024-01-18 [1] CRAN (R 4.4.1)
#>  timeDate                4041.110   2024-09-22 [1] CRAN (R 4.4.1)
#>  TSP                     1.2-5      2025-05-27 [1] CRAN (R 4.4.3)
#>  tune                    2.0.0      2025-09-01 [1] CRAN (R 4.4.3)
#>  tzdb                    0.5.0      2025-03-15 [1] CRAN (R 4.4.3)
#>  UCSC.utils              1.2.0      2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  urlchecker              1.0.1      2021-11-30 [1] CRAN (R 4.4.1)
#>  usethis                 3.2.0      2025-08-28 [1] CRAN (R 4.4.3)
#>  vctrs                   0.6.5      2023-12-01 [1] CRAN (R 4.4.1)
#>  viridis                 0.6.5      2024-01-29 [1] CRAN (R 4.4.1)
#>  viridisLite             0.4.2      2023-05-02 [1] CRAN (R 4.4.1)
#>  webshot                 0.5.5      2023-06-26 [1] CRAN (R 4.4.3)
#>  withr                   3.0.2      2024-10-28 [1] CRAN (R 4.4.2)
#>  workflows               1.3.0      2025-08-27 [1] CRAN (R 4.4.3)
#>  xfun                    0.53       2025-08-19 [1] CRAN (R 4.4.3)
#>  XML                     3.99-0.19  2025-08-22 [1] CRAN (R 4.4.3)
#>  xtable                  1.8-4      2019-04-21 [1] CRAN (R 4.4.1)
#>  XVector                 0.46.0     2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>  yaml                    2.3.10     2024-07-26 [1] CRAN (R 4.4.1)
#>  yardstick               1.3.2      2025-01-22 [1] CRAN (R 4.4.3)
#>  zlibbioc                1.52.0     2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
#>
#>  [1] C:/Users/gabri/AppData/Local/R/win-library/4.4
#>  [2] C:/Program Files/R/R-4.4.3/library
#>  * -- Packages attached to the search path.
#>
#> ------------------------------------------------------------------------------
```