

# Bioconductor mAPKL Package

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# 1 Introduction

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The mAPKL bioconductor R package implements a hybrid gene selection method, which is based on the hypothesis that among the statistically significant genes in a ranked list, there should be clusters of genes that share similar biological functions related to the investigated disease. Thus, instead of keeping a number of  $N$  top ranked genes, it would be more appropriate to define and keep a number of gene cluster exemplars.

The proposed methodology combines filtering and cluster analysis to select a small yet highly discriminatory set of non-redundant genes. Regarding the filtering step, a multiple hypothesis testing approach from *multtest* r-package (maxT) is employed to rank the genes of the training set according to their differential expression. Then the top  $N$  genes (e.g.  $N = 200$ ) are reserved for cluster analysis. First the index of Krzanowski and Lai as included in the *ClusterSim* r-package is applied on the disease samples of the training set to determine the number of clusters. The Krzanowski and Lai index is defined by  $DIFF(k) = (k - 1)^{\frac{2}{p}} W_{k-1} - k^{\frac{2}{p}} W_k$  when choosing the number of clusters ( $k$ ) to maximize the quantity  $KL(k) = \left| \frac{DIFF(k)}{DIFF(k+1)} \right|$ . The  $W_k$  denotes the within-cluster sum of squared errors.

Finally, cluster analysis is performed with the aid of Affinity Propagation (AP) clustering algorithm, which detects  $n$  ( $n = k$  the Krzanowski and Lai index) clusters among the top  $N$  genes, the so called exemplars. Those  $n$  exemplars are expected to form a classifier that shall discriminate between normal and disease samples (Sakellariou et al. 2012, *BMC Bioinformatics* **13**:270). This package implements the pre-mentioned methodology through a core function, the *mAPKL*. In the upcoming sections follows a guidance of the included functions and its functionality through differential expression analysis scenarios on a breast cancer dataset (GSE5764) which is part of the *mAPKLData* package.

## 2 Identification of differentially expressed genes

---

### 2.1 Breast cancer data

Throughout this tutorial we utilized a publicly available breast cancer dataset comprised of 30 samples, where 20 of them represent normal cases and the remaining 10 samples stand for tumor cases. We first load the package and then the breast cancer data. Then with the aid of the *sampling* function we create a separate training and validation sets where 60% of the samples will be used for training and the rest 40% of the samples will be used for evaluation purposes.

## Bioconductor mAPKL Package

```
library(mAPKL)
library(mAPKLData)
data(mAPKLData)
varLabels(mAPKLData)
breast <- sampling(Data=mAPKLData, valPercent=40, classLabels="type", seed=135)
```

The *sampling* function returns an S3 class (breast) with two eSet class objects that nest the relevant training and validation samples. Those two objects are used throughout the rest of the analysis.

```
breast

## $trainData
## ExpressionSet (storageMode: lockedEnvironment)
## assayData: 54675 features, 18 samples
##   element names: exprs
## protocolData: none
## phenoData
##   sampleNames: GSM134588 GSM134687 ... GSM134695 (18 total)
##   varLabels: title type
##   varMetadata: labelDescription
## featureData: none
## experimentData: use 'experimentData(object)'
##   pubMedIds: 17389037
## Annotation: hgu133plus2
##
## $testData
## ExpressionSet (storageMode: lockedEnvironment)
## assayData: 54675 features, 12 samples
##   element names: exprs
## protocolData: none
## phenoData
##   sampleNames: GSM134584 GSM134696 ... GSM134698 (12 total)
##   varLabels: title type
##   varMetadata: labelDescription
## featureData: none
## experimentData: use 'experimentData(object)'
```

```
##   pubMedIds: 17389037
## Annotation: hgu133plus2
```

## 2.2 Data normalization and transformation

We perform normalization to the expression values through the *preprocess* function.

```
normTrainData <- preprocess(breast$trainData)
normTestData  <- preprocess(breast$testData)
```

The *preprocess* function produces a list of several available normalization and transformation options. Besides density plots per method are produced and saved to current working directory to assist the user to decide upon which method to select before proceeding to mAPKL analysis.

```
attributes(normTrainData)

## $names
## [1] "rawdata"      "mc.normdata"  "z.normdata"   "q.normdata"
## [5] "cl.normdata"  "mcL2.normdata" "zL2.normdata" "qL2.normdata"
## [9] "cLL2.normdata"
```

The following graph presents the density plots of 8 possible normalization process with or without log2 transformation. The *preprocess* function applies all of them and it is up to the user, which one will engage for the rest of the analysis. In brief, the available approaches are mean-centering, z-score, quantile, and cyclic loess. During this case study we will proceed with the expression values following log2 transformation and cyclic loess normalization.

## 2.3 mAPKL gene selection

In this example we employ the expression values of log2 transformation and cyclic loess normalization to proceed with the *mAPKL* analysis.

```
exprs(breast$trainData) <- normTrainData$cLL2.normdata
exprs(breast$testData)  <- normTestData$cLL2.normdata
out.cLL2 <- mAPKL(trObj = breast$trainData, classLabels = "type",
                 valObj = breast$testData, dataType = 7)
```

## Bioconductor mAPKL Package

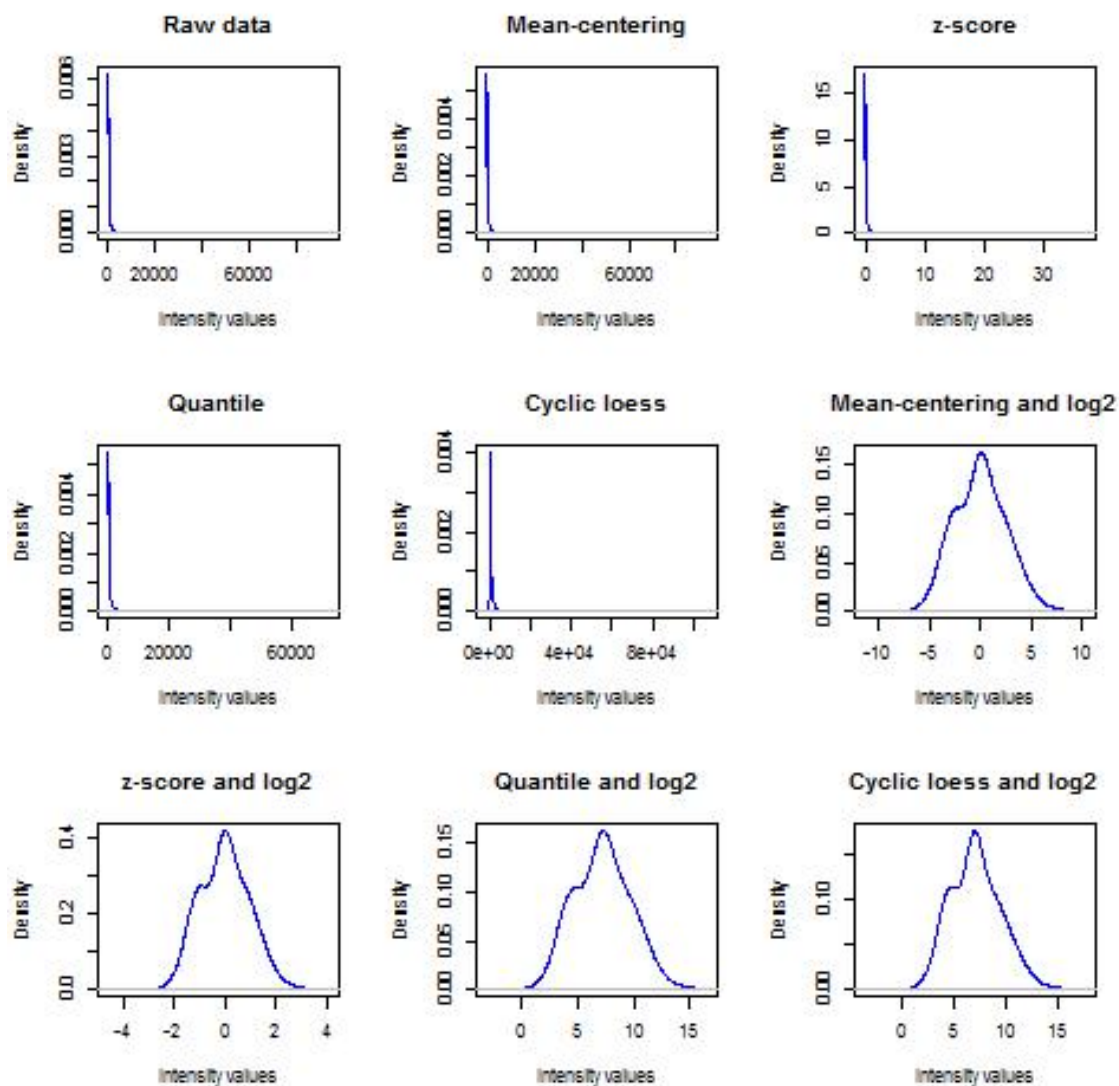


Figure 1: Density plots of normalized intensity values

```
## b=10 b=20 b=30 b=40 b=50 b=60 b=70 b=80 b=90 b=100
## b=110 b=120 b=130 b=140 b=150 b=160 b=170 b=180 b=190 b=200
## b=210 b=220 b=230 b=240 b=250 b=260 b=270 b=280 b=290 b=300
## b=310 b=320 b=330 b=340 b=350 b=360 b=370 b=380 b=390 b=400
## b=410 b=420 b=430 b=440 b=450 b=460 b=470 b=480 b=490 b=500
## b=510 b=520 b=530 b=540 b=550 b=560 b=570 b=580 b=590 b=600
## b=610 b=620 b=630 b=640 b=650 b=660 b=670 b=680 b=690 b=700
## b=710 b=720 b=730 b=740 b=750 b=760 b=770 b=780 b=790 b=800
```

## Bioconductor mAPKL Package

```
## b=810 b=820 b=830 b=840 b=850 b=860 b=870 b=880 b=890 b=900
## b=910 b=920 b=930 b=940 b=950 b=960 b=970 b=980 b=990 b=1000

## Please wait! The (KL) cluster indexing may take several minutes...

## Asking for 22 number of clusters

## Warning in .local(s, x, ...): algorithm did not converge; turn
on details
## and call plot() to monitor net similarity. Consider
## increasing 'maxits' and 'convits', and, if oscillations occur
## also increasing damping factor 'lam'.

## Warning in .local(s, x, ...): algorithm did not converge; turn
on details
## and call plot() to monitor net similarity. Consider
## increasing 'maxits' and 'convits', and, if oscillations occur
## also increasing damping factor 'lam'.

## Warning in .local(s, x, ...): algorithm did not converge; turn
on details
## and call plot() to monitor net similarity. Consider
## increasing 'maxits' and 'convits', and, if oscillations occur
## also increasing damping factor 'lam'.

## Warning in .local(s, x, ...): algorithm did not converge; turn
on details
## and call plot() to monitor net similarity. Consider
## increasing 'maxits' and 'convits', and, if oscillations occur
## also increasing damping factor 'lam'.

## fc according to limma
```

## 2.4 Building and evaluating classification models

After having get the exemplars from *mAPKL* analysis we build an SVM classifier to test their discriminatory performance. Regarding the SVM setup, we utilize a linear kernel for which the cost attribute is inferred by the `tune.svm` function. however, the user may freely use another kernel and a different Cross Validation approach than 5-folds.

```
clasPred <- classification(out.clL2@exemplTrain, "type", out.clL2@exemplTest)

## The training set has 10 Negative and 8 Positive samples. Using
## k-fold=5 C-V
```

## Bioconductor mAPKL Package

```
## ##### THE BEST PARAMETERS TUNING STAGE #####

## ##### THE TRAINING STAGE #####

##
## Call:
## svm.default(x = train.mtx, y = lbls, scale = FALSE, type = "C-classification",
##   kernel = "linear", gamma = best_gamma, cost = best_cost, cross = k_fold)
##
##
## Parameters:
##   SVM-Type:  C-classification
##   SVM-Kernel: linear
##       cost:  2
##       gamma: 0.125
##
## Number of Support Vectors: 6

## ##### THE PREDICTION STAGE #####

##           Test Labels Prediction Labels
## GSM134584           0             1
## GSM134696           0             0
## GSM134705           0             0
## GSM134702           0             0
## GSM134709           0             0
## GSM134693           0             0
## GSM134708           0             0
## GSM134697           0             0
## GSM134703           0             1
## GSM134586           0             0
## GSM134710           1             1
## GSM134698           1             1

## Negative samples: 10
## Positive samples: 2
## TN=8
```

```
## FP=2
## TP=2
## FN=0
## AUC=0.90
## Accuracy=83.00
## MCC=0.63
## Specificity=0.80
## Sensitivity=1.00
```

The output of the *classification* inform us about the SVM set up, the number of Support Vectors and finally show the the predicted labels along with the initial. In this example there is a validation set different from the training set and therefore we may use the respective labels to obtain the performance characteristics. The relevant function *metrics* called inside the *classification* function, calculates five key measures: the Area Under the ROC curve AUC, the classification accuracy, the Matthews correlation coefficient MCC classification measure, the degree of true negative's identification Specificity, and finally the degree of true positive's identification Sensitivity.

## 3 Advanced usage of the package

---

### 3.1 Annotation analysis

For each contemporary chip technology, there is a relevant annotation file, in which the the user may drag several *genome oriented* information. Regarding the breast cancer microarray data, the gene expression values were stored on Affumetrix gene chips. Using the *annotate* function, the user may obtain several info related to probe id, gene symbol, Entrez id, ensembl id, and chromosomal location.

```
gene.info <- annotate(out.clL2@exemplars, "hgu133plus2.db")
gene.info@results
```

##	PROBEID	SYMBOL	ENTREZID	ENSEMBL	MAP
## 1	239492_at	SEC14L4	284904	ENSG00000133488	22q12.2
## 2	229947_at	PI15	51050	ENSG00000137558	8q21.13

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```
## 3 1556499_s_at COL1A1 1277 ENSG00000108821 17q21.33
## 4 201069_at MMP2 4313 ENSG00000087245 16q12.2
## 5 214598_at CLDN8 9073 ENSG00000156284 21q22.11
## 6 217127_at CTH 1491 ENSG00000116761 1p31.1
## 7 37892_at COL11A1 1301 ENSG00000060718 1p21.1
## 8 33768_at DMWD 1762 ENSG00000185800 19q13.32
## 9 217637_at KCNB1 3745 ENSG00000158445 20q13.13
## 10 1569828_at LOC101928107 101928107 ENSG00000226527 21q22.11
## 11 210170_at PDLIM3 27295 ENSG00000154553 4q35.1
## 12 212236_x_at JUP 3728 ENSG00000173801 17q21.2
## 13 212236_x_at KRT17 3872 ENSG00000128422 17q21.2
## 14 220932_at <NA> <NA> <NA> <NA>
## 15 225664_at COL12A1 1303 ENSG00000111799 6q13-q14.1
## 16 206391_at RARRES1 5918 ENSG00000118849 3q25.32
## 17 243177_at <NA> <NA> <NA> <NA>
## 18 1565733_at <NA> <NA> <NA> <NA>
## 19 1555926_a_at <NA> <NA> <NA> <NA>
## 20 201377_at UBAP2L 9898 ENSG00000143569 1q21.3
## 21 220033_at <NA> <NA> <NA> <NA>
## 22 205044_at GABRP 2568 ENSG00000094755 5q35.1
## 23 215131_at IQCK 124152 ENSG00000174628 16p12.3
```

We may exploit the output of the *annotate* function to extent our analysis. For instance, we may perform *pathway analysis* on the exemplars. For this purpose we will utilize the *probes2pathways* function that utilizes the *reactome.db* package. This function employs the probe ids to identify the relevant pathways.

```
probes2pathways(gene.info)
```

```
## R-HSA-109581
## "Homo sapiens: Hemostasis
## R-HSA-109581
## "Homo sapiens: Hemostasis
## R-HSA-114604
## "Homo sapiens: GPVI-mediated activation cascade
## R-HSA-128021
## "Homo sapiens: Adaptive Immune System
```

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```
## R-HSA-144249
## "Homo sapiens: Collagen degradation
## R-HSA-144249
## "Homo sapiens: Collagen degradation
## R-HSA-147422
## "Homo sapiens: Degradation of the extracellular matrix
## R-HSA-147422
## "Homo sapiens: Degradation of the extracellular matrix
## R-HSA-147424
## "Homo sapiens: Extracellular matrix organization
## R-HSA-147424
## "Homo sapiens: Extracellular matrix organization
## R-HSA-147429
## "Homo sapiens: Collagen formation
## R-HSA-16258
## "Homo sapiens: Signal Transduction
## R-HSA-165081
## "Homo sapiens: Collagen biosynthesis and modifying enzymes
## R-HSA-16825
## "Homo sapiens: Immune System
## R-HSA-19893
## "Homo sapiens: Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell
## R-HSA-202209
## "Homo sapiens: Assembly of collagen fibrils and other multimeric structures
## R-HSA-20273
## "Homo sapiens: Cell surface interactions at the vascular wall
## R-HSA-21243
## "Homo sapiens: Generic Transcription Pathway
## R-HSA-21608
## "Homo sapiens: Integrin cell surface interactions
## R-HSA-21608
## "Homo sapiens: Integrin cell surface interactions
## R-HSA-217378
## "Homo sapiens: Binding and Uptake of Ligands by Scavenger Receptors
## R-HSA-221432
```

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```
##                                "Homo sapiens: Anchoring fibril formation
##                                R-HSA-224391
##                                "Homo sapiens: Crosslinking of collagen fibrils
##                                R-HSA-300017
##                                "Homo sapiens: Syndecan interactions
##                                R-HSA-300017
##                                "Homo sapiens: Non-integrin membrane-ECM interactions
##                                R-HSA-300017
##                                "Homo sapiens: Non-integrin membrane-ECM interactions
##                                R-HSA-300017
##                                "Homo sapiens: ECM proteoglycans
##                                R-HSA-300017
##                                "Homo sapiens: ECM proteoglycans
##                                R-HSA-300048
##                                "Homo sapiens: Scavenging by Class A Receptors
##                                R-HSA-43011
##                                "Homo sapiens: GP1b-IX-V activation signalling
##                                R-HSA-565365
##                                "Homo sapiens: Vesicle-mediated transport
##                                R-HSA-680683
##                                "Homo sapiens: Signaling by MET
##                                R-HSA-7385
##                                "Homo sapiens: RNA Polymerase II Transcription
##                                R-HSA-7416
##                                "Homo sapiens: Gene expression (Transcription)
##                                R-HSA-7589
##                                "Homo sapiens: Platelet Adhesion to exposed collagen
##                                R-HSA-7589
##                                "Homo sapiens: Platelet Adhesion to exposed collagen
##                                R-HSA-7600
##                                "Homo sapiens: Platelet activation, signaling and aggregation
##                                R-HSA-7600
##                                "Homo sapiens: Platelet Aggregation (Plug Formation)
##                                R-HSA-887408
##                                "Homo sapiens: MET activates PTK2 signaling
```

```
## R-HSA-887587
## "Homo sapiens: MET promotes cell motility"
## R-HSA-887816
## "Homo sapiens: Transcriptional regulation by RUNX2"
## R-HSA-894097
## "Homo sapiens: RUNX2 regulates osteoblast differentiation"
## R-HSA-894132
## "Homo sapiens: RUNX2 regulates bone development"
## R-HSA-894821
## "Homo sapiens: Collagen chain trimerization"
## R-HSA-900693
## "Homo sapiens: Signaling by Receptor Tyrosine Kinases"
```

### 3.2 Network characteristics

Regarding the network characteristics, we compute through the *netwAttr* function three different types of centralities (degree, closeness, betweenness) and a measure for clustering coefficient called transitivity. The degree centrality of a node refers to the number of connections or edges of that node to other nodes. The closeness centrality describes the reciprocal accumulated shortest length distance from a node to all other connected nodes. The betweenness centrality depicts the number of times a node intervenes along the shortest path of two other nodes. Transitivity measures the degree of nodes to create clusters within a network. For all four network measures we provide both global and local values. Furthermore, we compose an edge list (Node1-Node2-weight) based on the *N* top ranked genes. We may exploit that measures to depict the exemplars' network characteristics

```
net.attr <- netwAttr(out.clL2)
wDegreeL <- net.attr@degree$wdegreeL[out.clL2@exemplars]
wClosenessL <- net.attr@closeness$wclosenessL[out.clL2@exemplars]
wBetweennessL <- net.attr@betweenness$wbetweennessL[out.clL2@exemplars]
wTransitivityL <- net.attr@transitivity$wtransitivityL[out.clL2@exemplars]
```

```
Global.val <- c(net.attr@degree$wdegreeG, net.attr@closeness$wclosenessG,
               net.attr@betweenness$wbetweennessG, net.attr@transitivity$wtransitivityG)
```

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```
Global.val <- round(Global.val, 2)
exempl.netattr <- rbind(wDegreeL, wClosenessL, wBetweenessL, wTransitivityL)
```

```
netAttr <- cbind(Global.val, exempl.netattr)
netAttr <- t(netAttr)
netAttr
```

##	wDegreeL	wClosenessL	wBetweenessL	wTransitivityL
## Global.val	350.90	0.02	1542.12	0.53
## 239492_at	387.54	0.02	0.00	0.13
## 229947_at	320.55	0.02	1342.00	0.13
## 1556499_s_at	363.44	0.02	0.00	0.13
## 201069_at	294.55	0.02	17550.00	0.13
## 214598_at	400.74	0.02	1564.00	0.13
## 217127_at	301.88	0.02	2.00	0.13
## 37892_at	384.02	0.02	0.00	0.13
## 33768_at	295.85	0.02	1286.00	0.13
## 217637_at	310.87	0.02	2.00	0.13
## 1569828_at	344.24	0.02	0.00	0.13
## 210170_at	310.57	0.02	0.00	0.12
## 212236_x_at	412.59	0.02	1176.00	0.14
## 220932_at	321.35	0.02	0.00	0.13
## 225664_at	366.51	0.02	764.00	0.13
## 206391_at	343.50	0.02	524.00	0.13
## 243177_at	364.64	0.02	4.00	0.13
## 1565733_at	390.27	0.02	470.00	0.13
## 1555926_a_at	352.14	0.02	3360.00	0.13
## 201377_at	499.64	0.02	397.00	0.14
## 220033_at	460.89	0.02	396.00	0.14
## 205044_at	310.78	0.02	0.00	0.12
## 215131_at	308.69	0.02	0.00	0.14

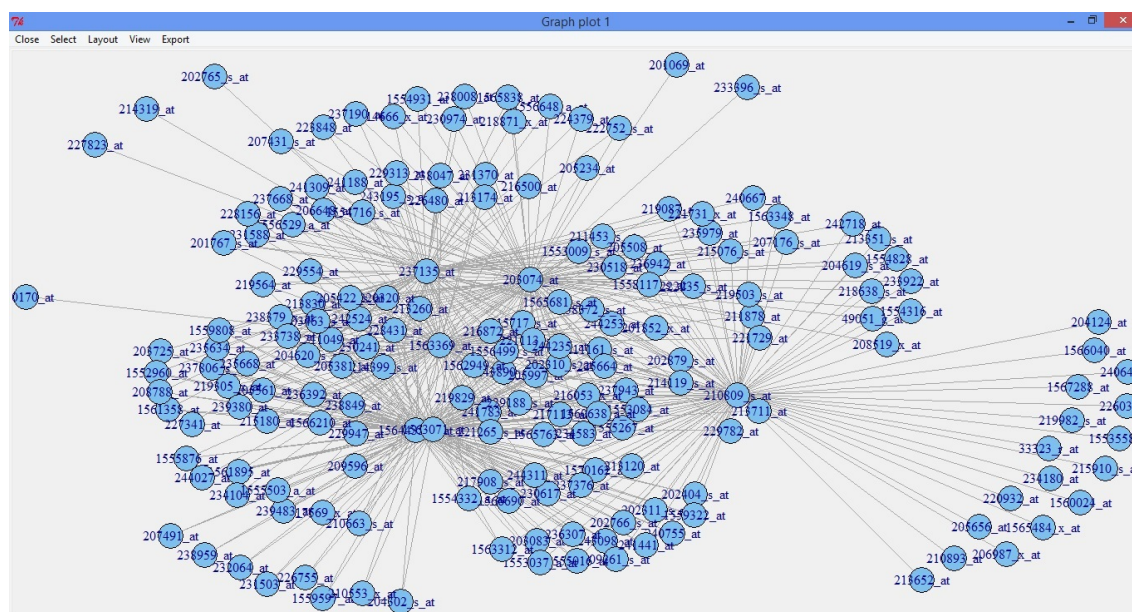
and identify potential hubs. The calculations of this example are based on the "clr" network reconstruction method. However, there are for the time being two more options, including the "aracne.a" and "aracne.m".

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```
# For local degree > global + standard deviation
sdev <- sd(net.attr@degree$WdegreeL)
msd <- net.attr@degree$WdegreeG + sdev
hubs <- wDegreeL[which(wDegreeL > msd)]
hubs

##      214598_at 212236_x_at   201377_at   220033_at
##      400.74    412.59      499.64      460.89
```

Finally, we may plot the network for those nodes that their local weighted degree is greater than Global weighted degree plus 2 times the standard deviation. We set this rule for both significance and illustration purposes (that edge list has dimension 604 x 3).



**Figure 2: Degree centrality network**

```
sdev <- sd(net.attr@degree$WdegreeL)
ms2d <- net.attr@degree$WdegreeG + 2 * sdev
net <- net.attr@degree$WdegreeL[which(net.attr@degree$WdegreeL >
  ms2d)]
idx <- which(net.attr@edgelist[, 1] %in% names(net))
new.edgeList <- net.attr@edgelist[idx, ]
dim(new.edgeList)
```

```
## [1] 428 3

require(igraph)
g = graph.data.frame(new.edgeList, directed = FALSE)
# tkplot(g, layout=layout.fruchterman.reingold)
```

## 4 Reporting

---

The overall analysis is summarized in an **html** report produced by the *report* function. It covers the dataset representation depicting the samples' names and their respective class labels, the exemplars section where statistical results and network characteristics are included. The classification performance section illustrates the performance metrics achieved in either cross-validation or hold-out validation. Finally, several annotation info are presented if an annotation analysis has occurred.

## 5 Session info

---

```
sessionInfo()

## R version 3.6.0 Patched (2019-05-02 r76456)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 17134)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=C LC_CTYPE=Greek_Greece.1253
## [3] LC_MONETARY=Greek_Greece.1253 LC_NUMERIC=C
## [5] LC_TIME=Greek_Greece.1253
##
## attached base packages:
## [1] stats4 parallel stats graphics grDevices utils datasets
## [8] methods base
##
## other attached packages:
```

## Bioconductor mAPKL Package

```
## [1] igraph_1.2.4.1      hgu133plus2.db_3.2.3 org.Hs.eg.db_3.8.2
## [4] AnnotationDbi_1.46.0 IRanges_2.18.0      S4Vectors_0.22.0
## [7] mAPKLData_1.15.0     mAPKL_1.15.1        Biobase_2.44.0
## [10] BiocGenerics_0.30.0 knitr_1.22
##
## loaded via a namespace (and not attached):
## [1] bit64_0.9-7          jsonlite_1.6         splines_3.6.0
## [4] modeest_2.3.3        shiny_1.3.2          BiocManager_1.30.4
## [7] highr_0.8            blob_1.1.1           yaml_2.2.0
## [10] RSQLite_2.1.1        lattice_0.20-38      limma_3.40.0
## [13] rmutil_1.1.3         digest_0.6.18        manipulateWidget_0.10.0
## [16] promises_1.0.1       R2HTML_2.3.2         htmltools_0.3.6
## [19] httpuv_1.5.1         Matrix_1.2-17        pkgconfig_2.0.2
## [22] timeDate_3043.102    XML_3.98-1.19        genefilter_1.66.0
## [25] reactome.db_1.68.0   xtable_1.8-4         webshot_0.5.1
## [28] later_0.8.0          stable_1.1.4         annotate_1.62.0
## [31] spatial_7.3-11       survival_2.44-1.1    magrittr_1.5
## [34] mime_0.6             memoise_1.1.0        evaluate_0.13
## [37] apcluster_1.4.7      MASS_7.3-51.4        class_7.3-15
## [40] tools_3.6.0          BiocStyle_2.12.0     formatR_1.6
## [43] stringr_1.4.0        clusterSim_0.47-3    bazar_1.0.11
## [46] cluster_2.0.8        stabledist_0.7-1     kimisc_0.4
## [49] ade4_1.7-13          compiler_3.6.0       e1071_1.7-1
## [52] timeSeries_3042.102  grid_3.6.0           RCurl_1.95-4.12
## [55] htmlwidgets_1.3      crosstalk_1.0.0      miniUI_0.1.1.1
## [58] bitops_1.0-6         rmarkdown_1.12       multtest_2.40.0
## [61] DBI_1.0.0            statip_0.2.0         R6_2.4.0
## [64] bit_1.1-14           parmigene_1.0.2       clue_0.3-57
## [67] fBasics_3042.89      stringi_1.4.3        Rcpp_1.0.1
## [70] rpart_4.1-15         rgl_0.100.19         xfun_0.6
```

## 6 Reference

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Sakellariou, A., D. Sanoudou, and G. Spyrou. "Combining Multiple Hypothesis Testing and Affinity Propagation Clustering Leads to Accurate, Robust and Sample Size Independent Classification on Gene Expression Data. " BMC Bioinformatics 13 (2012): 270.

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5764>

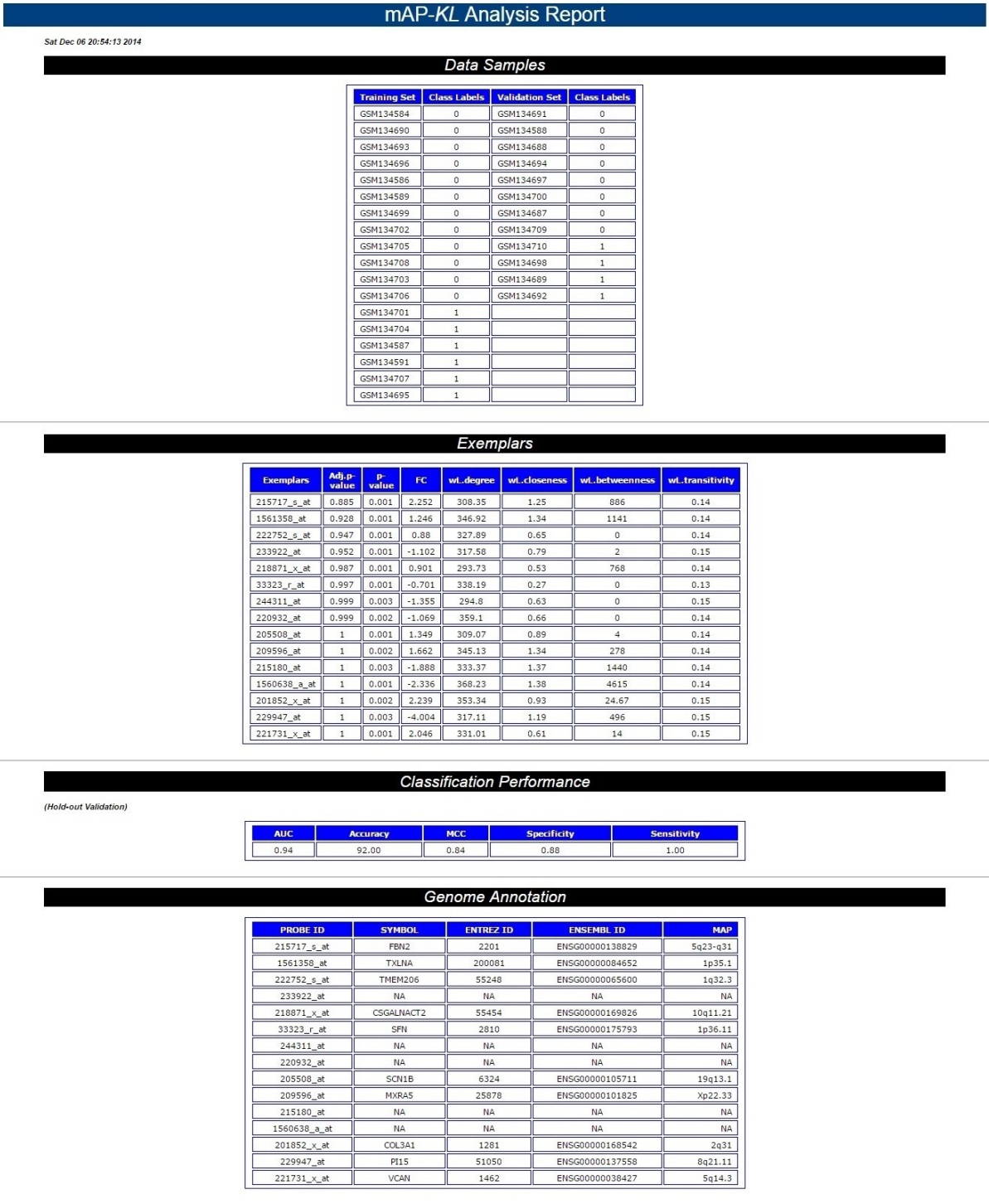


Figure 3: mAPKL analysis report