Package: geneClassifiers (Version 1.0.0)

R.Kuiper

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Combining gene expression profiling data with survival data has led to the development of robust outcome predictors (gene classifiers).This package provides a method for running gene classifiers generating patient specific predictive outcomes. This package is intended to support and enable research. The workflow is illustrated in Figure 1. The raw gene expression data obtained by microarray experiments is normalized using existing techniques (independent of this package). The choice of normalization method is dictated by the classifier. Some classifiers were developed using MAS5.0. In that case, the data to be classified should be normalized using MAS5.0. Normalization is followed by preprocessing (this package) and generating scores/classifications (this package). This package is suitable only for datasets of at least 20 patients.

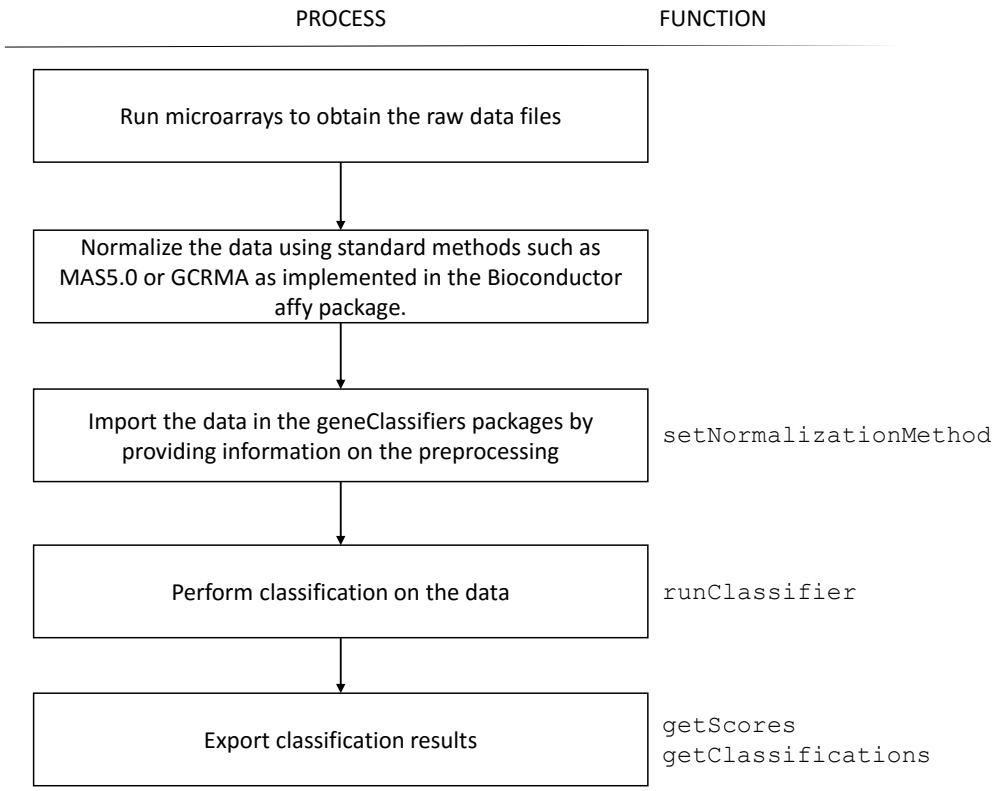


Figure 1: The workflow of the geneClassifiers package. The raw gene expression data is normalized. This normalized data is used as input in the geneClassifiers package. The processes with their relevant functions are shown.

1 Classifiers

The currently implemented list of classifiers can be obtained with the command:

```
> showClassifierList()
```

```
     name            normalizationMethod
[1,] "EMC92"         "MAS5.0"
[2,] "UAMS70"        "MAS5.0"
[3,] "UAMS17"        "MAS5.0"
[4,] "UAMS80"        "MAS5.0"
[5,] "HM19"          "GCRMA"
[6,] "IFM15"         "MAS5.0"
[7,] "MRCIX6"        "MAS5.0"
[8,] "MILLENNIUM100" "MAS5.0"
     description
[1,] "A risk classifier for multiple myeloma"
[2,] "A risk classifier for multiple myeloma"
[3,] "A risk classifier for multiple myeloma"
[4,] "A risk classifier for multiple myeloma"
[5,] "A risk classifier for multiple myeloma"
[6,] "A risk classifier for multiple myeloma"
[7,] "A risk classifier for multiple myeloma"
[8,] "A risk classifier for multiple myeloma based on Affy HG-U133 A/B chip"
```

To find more information on a specific classifier (e.g. EMC92),the classifier parameters can be obtained by

```
> EMC92Classifier<-getClassifier("EMC92")
> EMC92Classifier


--------------
Classifier:  EMC92
Description:  A risk classifier for multiple myeloma
Based on n =  92  probe sets
Number of risk groups: n =  2
To be used on  MAS5.0  normalized data
R.Kuiper, A.Broyl, Y.de Knegt, et.al.; A gene expression signature for high-risk
 multiple myeloma; Leukemia (2012) 26, 2046-2413; doi:10.1038/leu.2012.127

> HM19Classifier<-getClassifier("HM19")
> HM19Classifier


--------------
Classifier:  HM19
Description:  A risk classifier for multiple myeloma
Based on n =  19  probe sets
Number of risk groups: n =  2
To be used on  GCRMA  normalized data
Reme T, Hose D, Theillet C, Klein B. Modeling risk stratification in human cance
r. Bioinformatics. 2013;29(9):1149-1157
```

This is an object of class 'ClassifierParameters' which stores classifier related information, such as probe-sets used and their weights, means, standard deviations and covariance structure as observed in the classifiers' training data, and the description of the procedure on how to preprocess new data prior to application of the classifier.

Further information can be obtained from this object e.g. obtaining the weights used in a classifier:

```
> getWeights(EMC92Classifier)[1:10]

204379_s_at 210334_x_at   201795_at    38158_at   201307_at   205046_at
0.059427338 0.017489792 0.006685847 0.042315407 0.016492508 0.008662337
204026_s_at   238662_at   220351_at 202542_s_at
0.004558196 0.048955859 0.042045299 0.087027609
```

or the decision boundaries used to decide which class a sample score belongs to

```
> getDecisionBoundaries(HM19Classifier)

[1] 28.4 54.6
```

or the 'eventChain' which gives information on preprocessing:

```
> getEventChain(EMC92Classifier)

$targetValue
[1] 500

$truncate
[1] -Inf

$allow.reweighted
[1] TRUE

$to.log
[1] 2

$to.meancentering
[1] TRUE

$to.unitvariance
[1] TRUE
```

2 Data to be classified

The input data for the 'geneClassifiers' package is a Bioconductor ExpressionSet which has been prenormalized using existing methods such as MAS5.0 or GCRMA. For more information on these methods see the Bioconductor 'affy' package. The 'geneClassifiers' package contains an example dataset of MAS5.0 normalized (target value = 500) gene expression data of 25 multiple myeloma patients from the HOVON65/GMMG-HD4 trial (Pieter Sonneveld et al., J Clin Oncol, 2012)

```
> library(Biobase)
> data(exampleMAS5)
> class(exampleMAS5) #an object of class ExpressionSet

[1] "ExpressionSet"
attr(,"package")
[1] "Biobase"

> dim(exampleMAS5)

Features  Samples
     374       25

> preproc(experimentData(exampleMAS5))

[[1]]
[1] "MAS5.0"

$targetValue
[1] 500
```

To import this data set into the 'geneClassifiers' package, the setNormalization function is used:

```
> fixedData <- setNormalizationMethod( exampleMAS5, method="MAS5.0", targetValue = 500 )
> fixedData
```

```
--------------------
Fixed expression set

Normalization method:  MAS5.0
Number of samples   :  25
Number of features  :  374
```

Nb the targetValue = 500 is only required in the example (see below).

To get reliable results in the classification, the function depends on unmanipulated output from the normalization methods, i.e. read in CEL files into affy functions, obtain ExpressionSet, and use this set (without modification) for obtaining classifier scores. The function can detect deviations such as subsets of data sets or log transformed data, but detection of deviations is not guaranteed. When providing an ExpressionSet with all probe-sets still included, the 'targetValue=500' argument is not necessary because the function is able to extract the value from the data. In the example the number of probe-sets was reduced due to space considerations, the MAS5.0 target value cannot be obtained from the data so that the argument has to be provided. See '?setNormalizationMethod' for more details.

3 Performing classifications

To perform the classification using a classifier described in section 1 on the data described in section 2, the 'runClassifier' function is called using both arguments:

```
> resultsEMC92  <- runClassifier( "EMC92" , fixedData )
> resultsUAMS70 <- runClassifier( "UAMS70", fixedData )
> resultsEMC92

Note: Research use only
Classifier: EMC92
         > 0.827 : Risk-II
classifications
 Risk-I Risk-II
     19       6

        Batch corrected   : yes
        weighting type      : complete
----------------

> resultsUAMS70

Note: Research use only
Classifier: UAMS70
         > 0.66 : Risk-II
classifications
 Risk-I Risk-II
     23       2

        Batch corrected   : yes
        weighting type      : complete
----------------
```

The scores and classifications can be extracted using the 'getScores' and 'getClassifications' function

```
> data.frame(
+     "score_EMC92"  = getScores( resultsEMC92 ),
+     "class_EMC92"  = getClassifications( resultsEMC92 ),
+     "score_UAMS70" = getScores( resultsUAMS70 ),
+     "class_UAMS70" = getClassifications( resultsUAMS70 )
+ )
```

```
          score_EMC92 class_EMC92 score_UAMS70 class_UAMS70
GSM493958 -0.71624019      Risk-I  -0.52762419       Risk-I
GSM493959 -0.27585287      Risk-I  -0.33862140       Risk-I
GSM493960 -0.78728255      Risk-I  -0.30964906       Risk-I
GSM493961 -1.50733627      Risk-I  -0.71023469       Risk-I
GSM493962 -1.36569438      Risk-I  -0.59723884       Risk-I
GSM493963 -0.99382605      Risk-I  -0.35502398       Risk-I
GSM493964  1.20345559     Risk-II   0.87469597      Risk-II
GSM493965 -0.23111232      Risk-I   0.69126041      Risk-II
GSM493966  0.90148041     Risk-II   0.06231283       Risk-I
GSM493967  0.05209633      Risk-I  -0.12333754       Risk-I
GSM493968  0.33663874      Risk-I   0.07822597       Risk-I
GSM493969 -0.18411181      Risk-I  -0.75836587       Risk-I
GSM493970 -0.77179375      Risk-I  -0.58808060       Risk-I
GSM493971  1.18945257     Risk-II  -0.03323022       Risk-I
GSM493972  1.34454785     Risk-II   0.28384630       Risk-I
GSM493973 -1.02682845      Risk-I   0.10755853       Risk-I
GSM493974  0.67574560      Risk-I   0.07488403       Risk-I
GSM493975 -1.73924311      Risk-I  -0.52809629       Risk-I
GSM493976  1.23116416     Risk-II   0.58564808       Risk-I
GSM493977  0.43139670      Risk-I  -0.97249674       Risk-I
GSM493978  0.60432989      Risk-I   0.58399765       Risk-I
GSM493979  0.63122523      Risk-I   0.10798259       Risk-I
GSM493980 -0.88162933      Risk-I   0.05694285       Risk-I
GSM493981  0.73216640      Risk-I   0.28518870       Risk-I
GSM493982  1.14725160     Risk-II   0.25455012       Risk-I
```

4 Caution: non standard situations

The geneClassifiers package performs a batch correction by applying a linear transformation of the probe-set means and standard deviations to the values observed in the classifiers' training set. In order to accurately do this the data must contain a sufficient number of samples (n>=20) to estimate the means and standard deviations. If less samples are available, the 'runClassifier' function will give a warning and suggest to consider setting 'do.batchcorrection = FALSE'. Please note this will most likely result in invalid classifications (or certainly different classifications).

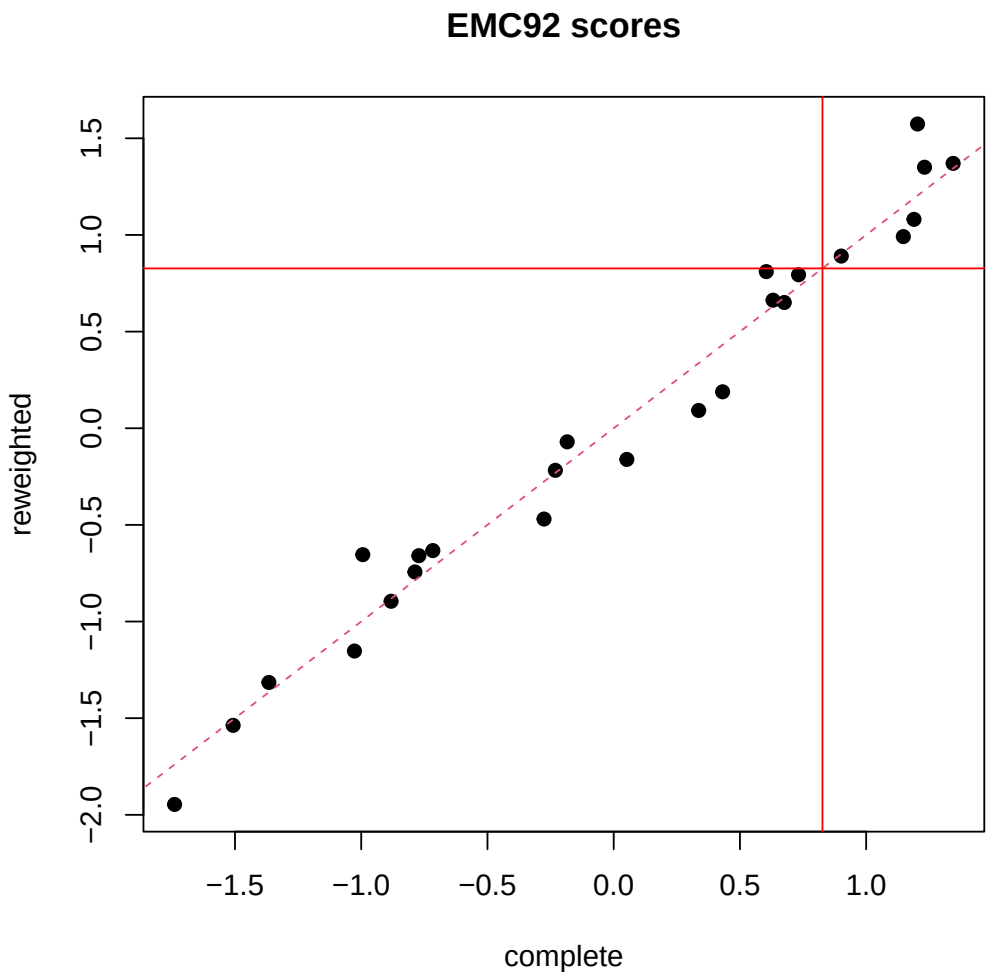
Besides the requirements of a matching normalization method between data and classifier and sufficient samples, the assumption is that the probe-sets needed for classification are present in the data. If this is not true, simply ignoring the missing probe-set may heavily bias the results. Therefore, when detecting missing probe-sets, the 'run-classifier' function will give an error message and suggest to consider using the argument 'allow.reweighted = TRUE'. This will reweight the weightings for the probe-sets which are present, based on the covariance structure of the classifiers' trainings data. See the vignette 'MissingCovariates' for more information. Please note this is not how the classifiers are intended and consequentially will result in different classifications.

```
> resultsEMC92.reWeighted <- runClassifier(
+     "EMC92" ,
+     fixedData[1:70,] ,
+     allow.reweighted=TRUE
+ )
> resultsEMC92.reWeighted

Note: Research use only
Classifier: EMC92_reweighted
         > 0.827 : Risk-II
classifications
 Risk-I Risk-II
     19       6
```

```
        Batch corrected   : yes
        weighting type      : reweighted
----------------
```

```
> plot(
+     x = getScores(resultsEMC92),
+     y = getScores(resultsEMC92.reWeighted),
+     xlab = "complete",
+     ylab = "reweighted",
+     main = "EMC92 scores",
+     pch = 21,
+     bg  ='black'
+ )
> lines(c(-10,10),c(-10,10),col=2,lty=2)
> abline(
+     v = getDecisionBoundaries( getClassifier( resultsEMC92           )),
+     h = getDecisionBoundaries( getClassifier( resultsEMC92.reWeighted)),
+     col='red'
+ )
```




```
> sessionInfo()

R version 4.3.0 RC (2023-04-13 r84269 ucrt)
Platform: x86_64-w64-mingw32/x64 (64-bit)
```

```
Running under: Windows Server 2022 x64 (build 20348)

Matrix products: default


locale:
[1] LC_COLLATE=C
[2] LC_CTYPE=English_United States.utf8
[3] LC_MONETARY=English_United States.utf8
[4] LC_NUMERIC=C
[5] LC_TIME=English_United States.utf8

time zone: America/New_York
tzcode source: internal

attached base packages:
[1] stats     graphics  grDevices utils     datasets  methods   base

other attached packages:
[1] Biobase_2.60.0         BiocGenerics_0.46.0    geneClassifiers_1.24.0

loaded via a namespace (and not attached):
[1] compiler_4.3.0 tools_4.3.0
```