

# Package ‘massiR’

June 16, 2019

**Type** Package

**Title** massiR: MicroArray Sample Sex Identifier

**Version** 1.20.0

**Date** 2014-09-15

**Author** Sam Buckberry

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**Description** Predicts the sex of samples in gene expression microarray datasets

**License** GPL-3

**Collate** 'massi\_y.R' 'massi\_y\_plot.R' 'massi\_select.R'  
'massi\_cluster.R' 'massi\_cluster\_plot.R' 'massi\_dip.R'

**LazyData** true

**Depends** cluster, gplots, diptest, Biobase, R (>= 3.0.2)

**Suggests** biomaRt, RUnit, BiocGenerics

**biocViews** Software, Microarray, GeneExpression, Clustering,  
Classification, QualityControl

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massi-package

*massiR: a microarray Gene Expression Sample Sex Identifier***Description**

massi uses y chromosome probe information to cluster samples and predict the sex of each sample in gene expression microarray datasets.

**Details**

Package: massi  
Type: Package  
Version: 0.99.0  
Date: 2014-01-27  
License: GPL-3

The massi analysis requires a typical normalized sample/probe values produced by a microarray experiment. The `massi_y` function will extract the y chromosome probe information and calculate y chromosome probe variance to allow the user to select the most informative probes. Using the `massi_select` function the user can select a probe variation threshold to reduce the number of probes used in the `massi_cluster` step. The `massi_cluster` function clusters samples into two clusters using the y chromosome probe values. Clustering is performed using the K-medoids method as implemented in the "fpc" package. There are two plotting functions, `massi_y_plot` and `massi_cluster_plot`, that allow the user to explore the data at various stages of the analysis. There is also a function, `massi_dip`, that can be used to test if there may be a sample sex-bias in the dataset.

**Author(s)**

Sam Buckberry

Maintainer: Sam Buckberry <sam.buckberry@adelaide.edu.au>

**References**

Christian Hennig (2013). fpc: Flexible procedures for clustering. R package version 2.1-6. <http://CRAN.R-project.org/package=fpc>

Martin Maechler (2013). diptest: Hartigan's dip test statistic for unimodality - corrected code. R package version 0.75-5. <http://CRAN.R-project.org/package=diptest>

Gregory R. Warnes, Ben Bolker, Lodewijk Bonebakker, Robert Gentleman, Wolfgang Huber, Andy Liaw, Thomas Lumley, Martin Maechler, Arni Magnusson, Steffen Moeller, Marc Schwartz and Bill Venables (2013). gplots: Various R programming tools for plotting data. R package version 2.12.1. <http://CRAN.R-project.org/package=gplots>

**See Also**

[massi\\_y](#), [massi\\_select](#), [massi\\_cluster](#), [massi\\_y\\_plot](#), [massi\\_dip](#), [massi\\_cluster\\_plot](#)

**Examples**

```

# load the test datasets
data(massi.test.dataset, massi.test.probes)

# use the massi.y function to calculate probe variation
massi_y_out <- massi.y(expression_data=massi.test.dataset, y_probes=massi.test.probes)

# plot probe variation to aid in deciding on the most informative subset of y chromosome probes
massi_y_plot(massi_y_out)

# Extract the informative probes for clustering
massi_select_out <- massi_select(massi.test.dataset, massi.test.probes, threshold=4)

# cluster samples to predict the sex for each sample
massi_cluster_out <- massi_cluster(massi_select_out)

# get the predicted sex for each sample
data.frame(massi_cluster_out[[2]])

```

---

massi.eset

*massi.eset*


---

**Description**

Object of ExpressionSet class containing the massiR test expression data.

**Usage**

```
data(massi.eset)
```

**Format**

The format is: Formal class 'ExpressionSet' [package "Biobase"] with 7 slots ..@ experimentData :Formal class 'MIAME' [package "Biobase"] with 13 slots .. ..@ name : chr "" .. ..@ lab : chr "" .. ..@ contact : chr "" .. ..@ title : chr "" .. ..@ abstract : chr "" .. ..@ url : chr "" .. ..@ pubMedIds : chr "" .. ..@ samples : list() .. ..@ hybridizations : list() .. ..@ normControls : list() .. ..@ preprocessing : list() .. ..@ other : list() .. ..@ \_\_classVersion\_\_:Formal class 'Versions' [package "Biobase"] with 1 slots .. ..@ .Data:List of 2 .. ..@ assayData :<environment: 0x7fd91fda9d50> ..@ phenoData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots .. ..@ varMetadata :'data.frame': 0 obs. of 1 variable: .. ..@ labelDescription: chr(0) .. ..@ data :'data.frame': 60 obs. of 0 variables .. ..@ dimLabels : chr [1:2] "rowNames" "columnNames" .. ..@ \_\_classVersion\_\_:Formal class 'Versions' [package "Biobase"] with 1 slots .. ..@ .Data:List of 1 .. ..@ assayData :<environment: 0x7fd91fda9d50> ..@ featureData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots .. ..@ varMetadata :'data.frame': 0 obs. of 1 variable: .. ..@ labelDescription: chr(0) .. ..@ data :'data.frame': 1026 obs. of 0 variables .. ..@ dimLabels : chr [1:2] "featureNames" "featureColumns" .. ..@ \_\_classVersion\_\_:Formal class 'Versions' [package "Biobase"] with 1 slots .. ..@ .Data:List of 1 .. ..@ assayData :<environment: 0x7fd91fda9d50> ..@ annotation : chr(0) ..@ protocolData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots .. ..@ varMetadata :'data.frame': 0 obs. of 1 variable: .. ..@ labelDescription: chr(0) .. ..@ data :'data.frame': 0 obs. of 0 variables .. ..@ dimLabels : chr [1:2] "rowNames" "columnNames" .. ..@ \_\_classVersion\_\_:Formal class 'Versions' [package "Biobase"] with 1 slots .. ..@ .Data:List of 1 .. ..@ assayData :<environment: 0x7fd91fda9d50>

```
labelDescription: chr(0) .. .. ..@ data :'data.frame': 60 obs. of 0 variables .. ..@ dimLabels
: chr [1:2] "sampleNames" "sampleColumns" .. ..@ .__classVersion__:Formal class 'Versions'
[package "Biobase"] with 1 slots .. .. ..@ .Data:List of 1 .. .. .. ..$ : int [1:3] 1 1 0 ..@
.__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots .. .. ..@ .Data:List of 4
.. .. .. ..$ : int [1:3] 3 0 2 .. .. .. ..$ : int [1:3] 2 22 0 .. .. .. ..$ : int [1:3] 1 3 0 .. .. .. ..$ : int [1:3] 1 0
0
```

---

massi.test.dataset      *The massi test dataset*

---

## Description

This data.frame object contains expression data for 60 samples and 1026 probes

## Usage

```
data(massi.test.dataset)
```

## Format

A data frame with 1026 observations on the following 60 variables.

S1 a numeric vector  
 S2 a numeric vector  
 S3 a numeric vector  
 S4 a numeric vector  
 S5 a numeric vector  
 S6 a numeric vector  
 S7 a numeric vector  
 S8 a numeric vector  
 S9 a numeric vector  
 S10 a numeric vector  
 S11 a numeric vector  
 S12 a numeric vector  
 S13 a numeric vector  
 S14 a numeric vector  
 S15 a numeric vector  
 S16 a numeric vector  
 S17 a numeric vector  
 S18 a numeric vector  
 S19 a numeric vector  
 S20 a numeric vector  
 S21 a numeric vector  
 S22 a numeric vector  
 S23 a numeric vector

S24 a numeric vector  
S25 a numeric vector  
S26 a numeric vector  
S27 a numeric vector  
S28 a numeric vector  
S29 a numeric vector  
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S31 a numeric vector  
S32 a numeric vector  
S33 a numeric vector  
S34 a numeric vector  
S35 a numeric vector  
S36 a numeric vector  
S37 a numeric vector  
S38 a numeric vector  
S39 a numeric vector  
S40 a numeric vector  
S41 a numeric vector  
S42 a numeric vector  
S43 a numeric vector  
S44 a numeric vector  
S45 a numeric vector  
S46 a numeric vector  
S47 a numeric vector  
S48 a numeric vector  
S49 a numeric vector  
S50 a numeric vector  
S51 a numeric vector  
S52 a numeric vector  
S53 a numeric vector  
S54 a numeric vector  
S55 a numeric vector  
S56 a numeric vector  
S57 a numeric vector  
S58 a numeric vector  
S59 a numeric vector  
S60 a numeric vector

**Details**

This test dataset is in the `data.frame` format with sample names as column names and probe id's as `row.names`. This test dataset is a subset of an empirical dataset obtained from Illumina human-6 v2.0 expression beadchip arrays.

**Source**

Data were adapted from dataset GSE25906 <<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE25906>>

**Examples**

```
data(massi.test.dataset)
```

---

```
massi.test.probes      The massi test probes
```

---

**Description**

A data.frame containing 56 probes which correspond to y chromosome genes

**Usage**

```
data(massi.test.probes)
```

**Format**

data.frame

A data frame with 56 observations on the following 0 variables.

**Examples**

```
data(massi.test.probes)
```

---

```
massi_cluster      massi_cluster
```

---

**Description**

The massi\_cluster function predicts the sex of samples using k-medoids clustering.

**Usage**

```
massi_cluster(y_data)
```

**Arguments**

`y_data` the `y_data` object is the data.frame returned from the `massi_select` function. This is a data.frame with sample names as column names and probe id's as row.names.

**Details**

This function clusters samples into two clusters using y chromosome probe values. K-medoids clustering is performed using the partitioning around medoids (pam) method implemented in the "fpc" package. The cluster with the highest probe values is determined to be the cluster of male samples and the cluster the lowest values as female samples.

**Value**

cluster data	Contains all of the results from the k-medoids clustering.
massi.results	Contains the results for each sample, including sample id, predicted sex, sample z-score and mean probe expression.

**Author(s)**

Sam Buckberry

**References**

Christian Hennig (2013). fpc: Flexible procedures for clustering. R package version 2.1-6. <http://CRAN.R-project.org/package=fpc>

**See Also**

[massi\\_y](#), [massi\\_select](#), [massi\\_y\\_plot](#), [massi\\_dip](#), [massi\\_cluster\\_plot](#)

**Examples**

```
# load the test dataset
data(massi.test.dataset, massi.test.probes)

# select the y chromosome probes using massi_select
massi_select_out <-
massi_select(massi.test.dataset, massi.test.probes)

# cluster samples to predict sex using massi_cluster
massi_cluster_out <-
massi_cluster(massi_select_out)

# get the results in a data.frame format
data.frame(massi_cluster_out[[2]])
```

---

massi\_cluster\_plot     *massi\_cluster\_plot*

---

**Description**

This function produces three figures in a new graphics device to enable the exploration of the massi\_cluster and massi\_select results.

**Usage**

```
massi_cluster_plot(massi_select_data, massi_cluster_data)
```

## Arguments

massi\_select\_data

A data.frame containing the subset of y chromosome probe values for each sample. This is returned when running the massi\_select function.

massi\_cluster\_data

This is the list returned from the massi\_cluster function.

## Details

The first figure is a heatmap depicting probe values for each sample. The second figure is a bar plot showing the mean probe expression and standard deviation for each sample. The bars are colored with respect to the predicted sex. The third figure is a principal component plot which represents the distances between samples, with each cluster highlighted with ellipses.

## Value

Returns three plots in a new graphics device.

## Author(s)

Sam Buckberry

## References

Gregory R. Warnes, Ben Bolker, Lodewijk Bonebakker, Robert Gentleman, Wolfgang Huber, Andy Liaw, Thomas Lumley, Martin Maechler, Arni Magnusson, Steffen Moeller, Marc Schwartz and Bill Venables (2013). *gplots: Various R programming tools for plotting data*. R package version 2.12.1. <http://CRAN.R-project.org/package=gplots>

## See Also

[massi\\_cluster](#), [massi\\_select](#)

## Examples

```
# load the test dataset
data(massi.test.dataset, massi.test.probes)

# select the y chromosome probes using massi_select
massi_select_out <-
massi_select(massi.test.dataset, massi.test.probes)

# cluster samples to predict sex using massi_cluster
massi_cluster_out <-
massi_cluster(massi_select_out)

# produce plots using massi_cluster_plot
massi_cluster_plot(massi_select_out, massi_cluster_out)
```



---

`massi_dip`*massi\_dip*

---

## Description

The `massi_dip` function applies the dip test to the subset of y chromosome probe values returned from the `massi_select` function. This can be used to indicate if there may be either a male or female bias in the dataset. This function returns a message indicating if the dataset may have a sex bias. The results for `massi_dip` are not reliable for datasets with 10 or less samples.

## Usage

```
massi_dip(y_subset_values)
```

## Arguments

`y_subset_values`

A data.frame containing the subset of y chromosome probe values for each sample, as returned from the `massi_select` function.

## Details

This function calculates z-scores for the y.chromosome probe values returned from the `massi_select` function and then checks if the average z-scores for each sample show a unimodal or multi-modal distribution by applying the dip test. If the proportion of male and female samples in the dataset is relatively balanced, the distribution of average z-scores should be bi-modal. If the distribution looks unimodal, the dataset likely contains a high proportion of one sex. By testing with empirical datasets and randomly generating data subsets with different male/female proportions, guideline values were developed to provide an indication if there is a potential sex bias in the dataset. If the dip statistic is  $> 0.08$  then the dataset is highly likely to have a proportions of male and female samples that will allow the `massi_cluster` function to predict the sex of samples with a high degree of accuracy. The results of this test should only be used as a guide and the results should be interpreted in light of the `massi_cluster` results. For more details see the `massi` package vignette.

## Value

This function returns a list containing

`dip.statistics` The results from the dip test

`sample.mean.z.score`

The mean of the probe z-scores for each sample used to calculate the dip statistics

`density`

Density values for the z-scores. Can be informative to plot these results

## Author(s)

Sam Buckberry

## References

Martin Maechler (2013). `diptest`: Hartigan's dip test statistic for unimodality - corrected code. R package version 0.75-5. <http://CRAN.R-project.org/package=diptest>

**See Also**

[massi\\_y](#), [massi\\_select](#), [massi\\_cluster](#), [massi\\_y\\_plot](#), [massi\\_cluster\\_plot](#)

**Examples**

```
# load the test dataset
data(massi.test.dataset, massi.test.probes)

massi_select_out <- massi_select(expression_data=massi.test.dataset, y_probes=massi.test.probes, threshold=4)

# Use the list returned from massi.select to calculate dip statistics and z-scores.
massi_dip_out <- massi_dip(y_subset_values=massi_select_out)

# view a density plot
plot(massi_dip_out[[3]])

# view a histogram of z-scores
hist(x=massi_dip_out[[2]])
```

---

massi\_select

*massi\_select*

---

**Description**

This function selects the y chromosome probe data for each sample.

**Usage**

```
massi_select(expression_data, y_probes, threshold = 3)
```

**Arguments**

expression\_data

The expression.data item contains normalized array expression data for all samples. This can be a data.frame with sample names as columns and probe id's as row names. This argument also allows the specification of an ExpressionSet object.

y\_probes

A data.frame of probe id's in one column that match y chromosome genes for the array platform. massiR includes probes for several Illumina and Affymetrix platforms. Details on using these probes are included in the vignette and the [y.probes](#) manual.

threshold

The threshold value corresponds to probe variation quantiles. This option allows the selection of the most variable probes. Deciding on a probe threshold value should be informed by viewing the plot generated by the [massi\\_y\\_plot](#) function. Threshold must be an integer "1", "2", "3", or "4". A threshold of "1" will select all y chromosome probes matching the id's in y.probes, Thresholds of "2", "3" and "4" will select probes with a CV in the top 75%, 50% and 25% respectively. The aim here is to remove probes with little to no variance across the samples. Default = 3.

**Value**

A data.frame containing the subset of y chromosome probe values for each sample.

**Author(s)**

Sam Buckberry

**See Also**

[massi\\_y](#), [massi\\_cluster](#), [massi\\_y\\_plot](#), [massi\\_dip](#), [massi\\_cluster\\_plot](#)

**Examples**

```
data(massi.test.dataset, massi.test.probes)
```

```
massi_select(expression_data=massi.test.dataset, y_probes=massi.test.probes)
```

---

massi_y	<i>massi_y</i>
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---

**Description**

The `massi_y` function extracts the y chromosome probe values for each sample and calculates the coefficient of variation (CV) for each probe. The returned list contains CV values (%) for each probe and quantile data. The probe variation data can be visualized using the `massi_y_plot` function.

**Usage**

```
massi_y(expression_data, y_probes)
```

**Arguments**

`expression_data`

The `expression.data` item contains normalized array expression data for all samples. This can be a data.frame with sample names as columns and probe id's as row names. This argument also allows the specification of an ExpressionSet object.

`y_probes`

A data.frame of probe id's in one column that match y chromosome genes for the array platform. `massiR` includes probes for several Illumina and Affymetrix platforms. Details on using these probes are included in the vignette and the [y.probes](#) manual.

**Details**

The `expression.data` must be as a data.frame with sample names as column names and probe id's as row.names. ExpressionSet objects can be input and with expression data will be extracted from the ExpressionSet and the returned list would be the same as if data as entered in data.frame format.

**Value**

The `massi_y` function returns a list containing probe id's, probe cv and quantiles.

<code>id</code>	Probe id's
<code>cv</code>	Probe cv values
<code>quantiles</code>	Quantiles of cv values data

**Author(s)**

Sam Buckberry

**See Also**

[massi\\_select](#), [massi\\_cluster](#), [massi\\_y\\_plot](#), [massi\\_dip](#), [massi\\_cluster\\_plot](#)

**Examples**

```
data(massi.test.dataset, massi.test.probes)
massi_y(massi.test.dataset, massi.test.probes)
```

---

`massi_y_plot`*massi\_y\_plot*

---

**Description**

The `massi_y_plot` function plots the data output from `massi.y` function.

**Usage**

```
massi_y_plot(massi_y_out)
```

**Arguments**

`massi_y_out` This object is the list returned from [massi\\_y](#) function.

**Details**

This function produces a bar plot of the coefficient of variation (CV) for each probe in the dataset. This allows the user to identify the most variable probes that are likely to be the most informative in the sex prediction step. The 25%, 50% and 75% quantiles are represented as horizontal lines and represent the threshold values that can be specified for the [massi\\_select](#) function.

**Value**

This function produces a bar plot in a new graphics device.

**Note**

See vignette for more details.

**Author(s)**

Sam Buckberry

**See Also**[massi\\_y](#), [massi\\_select](#), [massi\\_cluster](#), [massi\\_dip](#), [massi\\_cluster\\_plot](#)**Examples**

```
data(massi.test.dataset, massi.test.probes)

massi_y_out <-
  massi_y(expression_data=massi.test.dataset, y_probes=massi.test.probes)

massi_y_plot(massi_y_out)
```

---

`y.probes`*Y chromosome probe list*

---

**Description**

A list containing probes corresponding to y chromosome genes for Illumina and Affymetrix platforms. Each item in the list is a data.frame of y chromosome probes that can be used in the massi analysis. The names of each item in the list correspond to the ensembl biomart attribute names.

**Usage**

```
data(y.probes)
```

**Format**

The format is: List of 6 \$ illumina\_humanwg\_6\_v1:'data.frame': 58 obs. of 0 variables \$ illumina\_humanwg\_6\_v2:'data.frame': 74 obs. of 0 variables \$ illumina\_humanwg\_6\_v1:'data.frame': 112 obs. of 0 variables \$ illumina\_humanht\_12:'data.frame': 112 obs. of 0 variables \$ affy\_hugene\_1\_0\_st\_v1:'data.frame': 138 obs. of 0 variables \$ affy\_hg\_u133\_plus\_2:'data.frame': 94 obs. of 0 variables

**Details**

The y chromosome probes for each platform were downloaded from Ensembl biomart using the 'biomaRt' package. For more details on the methods of selecting the probes and how to obtain probes for other platform, see the vignette for the massiR package.

**References**

Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. Steffen Durinck, Paul T. Spellman, Ewan Birney and Wolfgang Huber, Nature Protocols 4, 1184-1191 (2009).

BioMart and Bioconductor: a powerful link between biological databases and microarray data analysis. Steffen Durinck, Yves Moreau, Arek Kasprzyk, Sean Davis, Bart De Moor, Alvis Brazma and Wolfgang Huber, Bioinformatics 21, 3439-3440 (2005).

**Examples**

```
# load the probes list
data(y.probes)
# look at the platform names
names(y.probes)
# extract the probes using the platform name
probe.list <- y.probes[["illumina_humanwg_6_v2"]]
```

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