

Package ‘mpra’

October 28, 2025

Version 1.31.0

Title Analyze massively parallel reporter assays

Description Tools for data management, count preprocessing, and differential analysis in massively parallel report assays (MPRA).

Depends R (>= 3.4.0), methods, BiocGenerics, SummarizedExperiment, limma

Suggests BiocStyle, knitr, rmarkdown, RUnit

Imports S4Vectors, scales, stats, graphics, statmod

Collate mpra_set.R utils.R fit.R

VignetteBuilder knitr

License Artistic-2.0

URL <https://github.com/hansenlab/mpra>

BugReports <https://github.com/hansenlab/mpra/issues>

biocViews Software, GeneRegulation, Sequencing, FunctionalGenomics

git_url <https://git.bioconductor.org/packages/mpra>

git_branch devel

git_last_commit 5f83e44

git_last_commit_date 2025-04-15

Repository Bioconductor 3.22

Date/Publication 2025-10-27

Author Leslie Myint [cre, aut],
Kasper D. Hansen [aut]

Maintainer Leslie Myint <leslie.myint@gmail.com>

Contents

mpra-package	2
compute_logratio	3
get_precision_weights	3
mpralm	4
MPRASet-class	6
mpraSetExample	7
normalize_counts	8

Index	10
--------------	-----------

mpira-package

Analyze massively parallel reporter assays

Description

Tools for data management, count preprocessing, and differential analysis in massively parallel report assays (MPRA).

Details

This package provides tools for the analysis of MPRA data. The primary purpose is to enable powerful differential analysis of activity measures, but the package can also be used to generate precision weights useful in regression analyses of activity scores on sequence features. The main workhorse is the `mpralm` function which draws on the previously proposed voom framework for RNA-seq analysis in the `limma` package.

Author(s)

Leslie Myint [cre, aut], Kasper D. Hansen [aut]

Maintainer: Leslie Myint <leslie.myint@gmail.com>

References

Myint, Leslie, Dimitrios G. Avramopoulos, Loyal A. Goff, and Kasper D. Hansen. *Linear models enable powerful differential activity analysis in massively parallel reporter assays*. BMC Genomics 2019, 209. doi:10.1186/s128640195556x.

Law, Charity W., Yunshun Chen, Wei Shi, and Gordon K. Smyth. *Voom: Precision Weights Unlock Linear Model Analysis Tools for RNA-Seq Read Counts*. Genome Biology 2014, 15:R29. doi:10.1186/gb2014152r29.

Smyth, Gordon K., Joelle Michaud, and Hamish S. Scott. *Use of within-Array Replicate Spots for Assessing Differential Expression in Microarray Experiments*. Bioinformatics 2005, 21 (9): 2067-75. doi:10.1093/bioinformatics/bti270.

Examples

```
data(mpraSetAggExample)
design <- data.frame(intcpt = 1,
                    episomal = grepl("MT", colnames(mpraSetAggExample)))
mpralm_fit <- mpralm(object = mpraSetAggExample, design = design,
                    aggregate = "none", normalize = TRUE,
                    model_type = "indep_groups", plot = FALSE)
toptab <- topTable(mpralm_fit, coef = 2, number = Inf)
head(toptab)
```

compute_logratio	<i>Compute activity measure (log-ratio) for each element.</i>
------------------	---

Description

Compute the log ratio of RNA counts to DNA counts using different methods. For "mean", uses the average of barcode-specific log ratios. For "sum", sums RNA and DNA counts over barcodes before forming the log ratio.

Usage

```
compute_logratio(object, aggregate = c("mean", "sum", "none"))
```

Arguments

object	An object of class MPRASets.
aggregate	Aggregation method over barcodes: "mean" to use the average of barcode-specific log ratios, "sum" to use the log ratio of summed RNA and DNA counts, "none" to perform no aggregation (counts have already been summarized over barcodes).

Value

A matrix with the same dimension as object, containing element- and sample-specific log ratios.

Examples

```
data(mpraSetAggExample)
logr <- compute_logratio(mpraSetAggExample, aggregate = "sum")
```

get_precision_weights	<i>Get precision weights from the copy number-variance relationship.</i>
-----------------------	--

Description

Estimates the variability of the supplied log-ratios across samples as a function of copy number (DNA count levels).

Usage

```
get_precision_weights(logr, design, log_dna, span = 0.4, plot = TRUE, ...)
```

Arguments

<code>logr</code>	Matrix of outcome measures: log2 ratio of RNA counts to DNA counts.
<code>design</code>	Design matrix specifying comparisons of interest.
<code>log_dna</code>	Matrix of log2 aggregated DNA counts of the same dimension as <code>logr</code> .
<code>span</code>	The smoothing span for lowess in estimating the copy number-variance relationship. Default: 0.4.
<code>plot</code>	If TRUE, plot the copy number-variance relationship.
<code>...</code>	Further arguments to be passed to <code>lmFit</code> for obtaining residual standard deviations used in estimating the copy number-variance relationship.

Details

Residual standard deviations are computed using the supplied outcomes and design matrix. The square root of the the residual standard deviations are modeled as a function of the average log2 aggregated DNA counts to estimate the copy number-variance relationship.

Value

A matrix of precision weights of the same dimension as `logr` and `log_dna`.

References

Law, Charity W., Yunshun Chen, Wei Shi, and Gordon K. Smyth. *Voom: Precision Weights Unlock Linear Model Analysis Tools for RNA-Seq Read Counts*. *Genome Biology* 2014, 15:R29. [doi:10.1186/gb2014152r29](https://doi.org/10.1186/gb2014152r29).

Examples

```
data(mpraSetAggExample)
design <- data.frame(intcpt = 1,
                    episomal = grepl("MT", colnames(mpraSetAggExample)))
logr <- compute_logratio(mpraSetAggExample, aggregate = "none")
log_dna <- log2(getDNA(mpraSetAggExample, aggregate = FALSE) + 1)
w <- get_precision_weights(logr = logr, design = design,
                           log_dna = log_dna, plot = FALSE)
```

mpralm

Linear models for differential analysis of MPRA data

Description

Fits weighted linear models to test for differential activity in MPRA data.

Usage

```
mpralm(object, design, aggregate = c("mean", "sum", "none"),
        normalize = TRUE, normalizeSize = 10e6,
        block = NULL, model_type = c("indep_groups", "corr_groups"),
        plot = TRUE, endomorphic = FALSE, ...)
```

Arguments

object	An object of class MPRASet.
design	Design matrix specifying comparisons of interest. The number of rows of this matrix should equal the number of columns in object. The number of columns in this design matrix has no constraints and should correspond to the experimental design.
aggregate	Aggregation method over barcodes: "mean" to use the average of barcode-specific log ratios, "sum" to use the log ratio of summed RNA and DNA counts, "none" to perform no aggregation (counts have already been summarized over barcodes).
normalize	If TRUE, perform total count normalization before model fitting.
normalizeSize	If normalizing, the target library size (default is 10e6).
block	A vector giving the sample designations of the columns of object. The default, NULL, indicates that all columns are separate samples.
model_type	Indicates whether an unpaired model fit ("indep_groups") or a paired mixed-model fit ("corr_groups") should be used.
plot	If TRUE, plot the mean-variance relationship.
endomorph	If TRUE, return the same class as the input, i.e. an object of class MPRASet.
...	Further arguments to be passed to <code>lmFit</code> for obtaining residual standard deviations used in estimating the mean-variance relationship.

Details

Using `method_type = "corr_groups"` use the `duplicateCorrelation` function from the `limma` package to estimate the intra-replicate correlation of log-ratio values.

Value

An object of class `MArrayLM` resulting from the `eBayes` function.

If `endomorph = TRUE`, then an `MPRASet` is returned, with the output of `topTable` added to the `rowData`, and the `MArrayLM` results added as an attribute `"MArrayLM"`.

References

- Myint, Leslie, Dimitrios G. Avramopoulos, Loyal A. Goff, and Kasper D. Hansen. *Linear models enable powerful differential activity analysis in massively parallel reporter assays*. BMC Genomics 2019, 209. doi:10.1186/s128640195556x.
- Law, Charity W., Yunshun Chen, Wei Shi, and Gordon K. Smyth. *Voom: Precision Weights Unlock Linear Model Analysis Tools for RNA-Seq Read Counts*. Genome Biology 2014, 15:R29. doi:10.1186/gb2014152r29.
- Smyth, Gordon K., Joelle Michaud, and Hamish S. Scott. *Use of within-Array Replicate Spots for Assessing Differential Expression in Microarray Experiments*. Bioinformatics 2005, 21 (9): 2067-75. doi:10.1093/bioinformatics/bti270.

Examples

```
data(mpraSetAggExample)
design <- data.frame(intcpt = 1,
                    episomal = grepl("MT", colnames(mpraSetAggExample)))
```

```

mpralm_fit <- mpralm(object = mpraSetAggExample, design = design,
                    aggregate = "none", normalize = TRUE,
                    model_type = "indep_groups", plot = FALSE)
toptab <- topTable(mpralm_fit, coef = 2, number = Inf)
head(toptab)

```

MPRASET-class

Class "MPRASET"

Description

A container for data from massively parallel reporter assays (MPRA). Builds on the SummarizedExperiment class.

Usage

```

## Constructor
MPRASET(DNA = new("matrix"), RNA = new("matrix"),
        barcode = new("character"), eid = new("character"),
        eseq = new("character"), ...)

## Accessors
getRNA(object, aggregate = FALSE)
getDNA(object, aggregate = FALSE)
getBarcode(object)
getEid(object)
getEseq(object)

```

Arguments

object	A MPRASET object.
aggregate	A logical indicating if data should be aggregated to the element level (by summing across barcodes).
DNA	A matrix of DNA counts where rows correspond to elements or individual barcodes and columns to samples of conditions being compared.
RNA	A matrix of RNA counts where rows correspond to elements or individual barcodes and columns to samples of conditions being compared.
barcode	If barcodes are supplied, a character vector of length equal to the number of rows in DNA and RNA containing the barcode sequences or identifiers. NULL otherwise.
eid	A character vector of length equal to the number of rows in DNA and RNA containing the enhancer identifiers corresponding to each row.
eseq	If supplied, a character vector of length equal to the number of rows in DNA and RNA containing the enhancer sequences corresponding to the regulatory elements in each row. NULL otherwise.
...	Further arguments to be passed to SummarizedExperiment.

Value

The constructor function MPRASET returns an object of class "MPRASET".

Slots

Slots are as described in a `SummarizedExperiment`. Of particular interest are `colData` which describes the phenotype data. The assay slot holds the assay data, with specific assay names RNA and DNA (accessed by `getRNA` and `getDNA`). Element and barcode data are accessible in the `rowData` slot. We have chosen to store barcode and element as character to be flexible, although they are often DNA sequences (and could therefore be considered `DNAStringSet` (from package `Biostrings`)).

Extends

Class "`SummarizedExperiment`", directly.

Accessors

`getDNA`: Gets the DNA channel data.
`getRNA`: Gets the RNA channel data.
`getBarcode`: Gets the barcode, if present.
`getEid`: Gets the element ID
`getEseq`: Gets the element sequence, if present.

See Also

`SummarizedExperiment` for the basic class that is used as a building block.

Examples

```
showClass("MPRASET")
```

`mpraSetExample`

Example data for the mpra package.

Description

Example data for the MPRA package. `mpraSetExample` and `mpraSetAggExample` come from a study by Inoue et al that compares episomal and lentiviral MPRA. The former contains data at the barcode level and the latter contains data aggregated over barcodes. `mpraSetAllelicExample` come from a study by Tewhey et al that looks at regulatory activity of allelic versions of thousands of SNPs to follow up on prior eQTL results.

Usage

```
data("mpraSetExample")
data("mpraSetAggExample")
data("mpraSetAllelicExample")
```

Format

An `MPRASET`.

Details

`mpraSetExample` contains barcode level information for the study by Inoue et al. `mpraSetAggExample` contains count information from `mpraSetExample` where the counts have been summed over barcodes for each element. `mpraSetAllelicExample` contains count information for the Tewhey et al study. The counts have been summed over barcodes for each element.

Source

A script for creating the three datasets is supplied in the `scripts` folder of the package. The data are taken from the GEO submission associated with the paper (see references), specifically GSE83894 and GSE75661.

References

Inoue, Fumitaka, Martin Kircher, Beth Martin, Gregory M. Cooper, Daniela M. Witten, Michael T. McManus, Nadav Ahituv, and Jay Shendure. *A Systematic Comparison Reveals Substantial Differences in Chromosomal versus Episomal Encoding of Enhancer Activity*. Genome Research 2017, 27(1):38-52. doi:10.1101/gr.212092.116.

Tewhey R, Kotliar D, Park DS, Liu B, Winnicki S, Reilly SK, Andersen KG, Mikkelsen TS, Lander ES, Schaffner SF, Sabeti PC. *Direct Identification of Hundreds of Expression-Modulating Variants using a Multiplexed Reporter Assay*. Cell 2016, 165:1519-1529. doi:10.1016/j.cell.2016.04.027.

Examples

```
data(mpraSetAggExample)
```

<code>normalize_counts</code>	<i>Total count normalization of DNA and RNA counts</i>
-------------------------------	--

Description

Total count normalization of DNA and RNA counts.

Usage

```
normalize_counts(object, normalizeSize = 10e6, block = NULL)
```

Arguments

<code>object</code>	An object of class <code>MPRASet</code> .
<code>normalizeSize</code>	If normalizing, the target library size (default is 10e6).
<code>block</code>	A vector giving the sample designations of the columns of <code>object</code> . The default, <code>NULL</code> , indicates that all columns are separate samples.

Details

`block` is a vector that is used when the columns of the `MPRASet` object are paired. This often is the case when comparing allelic versions of an element. In this case, the first `$$` columns of `object` give the counts for the reference allele in `$$` samples. The second `$$` columns give the counts for the alternative allele measured in the same `$$` samples. With 3 samples, `block` would be `c(1, 2, 3, 1, 2, 3)`. All columns are scaled to have 10 million counts.

Value

An object of class `MPRASET` with the total count-normalized DNA and RNA counts.

Examples

```
data(mpraSetAggExample)
mpraSetAggExample <- normalize_counts(mpraSetAggExample)
```

Index

- * **classes**
 - MPRASET-class, [6](#)
- * **datasets**
 - mpraSetExample, [7](#)
- * **package**
 - mpra-package, [2](#)

compute_logratio, [3](#)

get_precision_weights, [3](#)
getBarcode (MPRASET-class), [6](#)
getDNA (MPRASET-class), [6](#)
getEid (MPRASET-class), [6](#)
getEseq (MPRASET-class), [6](#)
getRNA (MPRASET-class), [6](#)

mpa (mpa-package), [2](#)
mpa-package, [2](#)
mpalm, [4](#)
MPRASET (MPRASET-class), [6](#)
MPRASET-class, [6](#)
mpaSetAggExample (mpaSetExample), [7](#)
mpaSetAllelicExample (mpaSetExample),
[7](#)
mpaSetExample, [7](#)

normalize_counts, [8](#)

show,MPRASET-method (MPRASET-class), [6](#)
SummarizedExperiment, [7](#)