

# Package ‘RNAetc’

August 3, 2021

**Type** Package

**Title** What the Package Does (Title Case)

**Version** 0.1.0

**Author** Kim Dill-McFarland

**Maintainer** Kim Dill-McFarland <kadm@uw.edu>

**Description** More about what it does (maybe more than one line)

Use four spaces when indenting paragraphs within the Description.

**License** MIT + file LICENSE

**Encoding** UTF-8

**LazyData** true

**RoxygenNote** 7.1.1

**Imports** dplyr, edgeR, forcats, ggplot2, limma, magrittr, readr, tibble, tidyr, WGCNA

**Depends** R (>= 2.10)

## R topics documented:

align_metrics . . . . .	1
example.dat . . . . .	2
example.kin . . . . .	3
example.voom . . . . .	4
filter_rare . . . . .	5
make_modules . . . . .	6
<b>Index</b>	<b>7</b>

---

align_metrics	<i>Extract and format cleaning and alignment metrics</i>
---------------	--

---

## Description

Extract data from FastQC trim settings, Picard, samtools flagstat, and featureCounts output by the RNA-seq fastq pipeline

**Usage**

```
align_metrics(
  data.dir = NULL,
  trim = TRUE,
  bam = TRUE,
  picard = TRUE,
  bam.filter = TRUE,
  count = TRUE
)
```

**Arguments**

<code>data.dir</code>	Character string of directory containing all associated files
<code>trim</code>	Logical if should include FastQC trim settings
<code>bam</code>	Logical if should include samtools flagstat for raw alignments
<code>picard</code>	Logical if should include Picard for raw alignments
<code>bam.filter</code>	Logical if should include samtools flagstat for filtered alignments
<code>count</code>	Logical if should include featureCounts total reads in genes

**Value**

Data frame cleaning and alignment metrics for all libraries

---

example.dat	<i>kimma example DGEList.</i>
-------------	-------------------------------

---

**Description**

An edgeR DGEList data set containing unnormalized RNA-seq counts. RNA-seq of human dendritic cells cultured with and without virus. Samples from 3 donors and a random subset of 1000 genes were selected. Counts are unnormalized.

**Usage**

```
example.dat
```

**Format**

Formal class 'DGEList' [package "edgeR"] with 1 slot:

1. **counts** A matrix with 1000 rows and 6 columns
  - rownames** character. ENSEMBL gene ID.
  - lib1** integer. Counts in library 1.
  - lib2** integer. Counts in library 2.
  - lib3** integer. Counts in library 3.
  - lib4** integer. Counts in library 4.
  - lib5** integer. Counts in library 5.
  - lib6** integer. Counts in library 6.

2. **samples** A data frame with 6 rows and 7 columns
  - group** factor. No grouping was provided. All = 1.
  - lib.size** numeric. Total library size for this 1000 gene subset.
  - norm.factors** numeric. Normalization factors. No normalization was completed. All = 1.
  - libID** character. Unique library ID. Matches column names in counts.
  - donorID** character. Donor ID.
  - median\_cv\_coverage** numeric. Median coefficient of variation of coverage. Quality metric for sequencing libraries calculated from original full data set.
  - virus** character. A for media samples with no virus. B for virus-infected samples.
3. **genes** A data frame with 1000 rows and 5 columns
  - hgnc\_symbol** character. Current approved HGNC symbol.
  - Previous symbols** character. Previous HGNC symbols.
  - Alias symbols** character. Alias HGNC symbols.
  - gene\_biotype** character. Gene product type. All = protein-coding.
  - geneName** character. ENSEMBL gene ID. Matches row names in counts.

### Source

[https://github.com/altman-lab/P259\\_pDC\\_public](https://github.com/altman-lab/P259_pDC_public)

### References

Dill-McFarland et al. 2021. Eosinophil-mediated suppression and Anti-IL-5 enhancement of plasmacytoid dendritic cell interferon responses in asthma. J Allergy Clin Immunol. In revision

---

example.kin	<i>kinma example kinship.</i>
-------------	-------------------------------

---

### Description

Matrix of pairwise kinship values between donor 1,2,3. Values are dummy data with 1 for self comparison, 0.5 for siblings, and 0.1 for unrelated.

### Usage

```
example.kin
```

### Format

A matrix with 3 rows and 3 variables:

**rowname** Donor ID. Same as column names  
**donor1** numeric kinship (0-1) with donor 1  
**donor2** numeric kinship (0-1) with donor 2  
**donor3** numeric kinship (0-1) with donor 3

---

example.voom

*limma example EList.*


---

## Description

A limma EList data set containing normalized log2 RNA-seq counts. RNA-seq of human dendritic cells cultured with and without virus. Samples from 3 donors and a random subset of 1000 genes were selected. Counts are TMM normalized log2 counts per million (CPM).

## Usage

```
example.voom
```

## Format

Formal class 'EList' [package "limma"] with 1 slot:

1. **genes** A data frame with 1000 rows and 5 columns
  - hgnc\_symbol** character. Current approved HGNC symbol.
  - Previous symbols** character. Previous HGNC symbols.
  - Alias symbols** character. Alias HGNC symbols.
  - gene\_biotype** character. Gene product type. All = protein-coding.
  - geneName** character. ENSEMBL gene ID. Matches row names in E.
2. **targets** A data frame with 6 rows and 7 columns
  - group** factor. No grouping was provided. All = 1.
  - lib.size** numeric. Total library size for this 1000 gene subset.
  - norm.factors** numeric. TMM normalization factors.
  - libID** character. Unique library ID. Matches column names in E.
  - donorID** character. Donor ID.
  - median\_cv\_coverage** numeric. Median coefficient of variation of coverage. Quality metric for sequencing libraries calculated from original full data set.
  - virus** character. A for media samples with no virus. B for virus-infected samples.
3. **E** A matrix with 1000 rows and 6 columns
  - rownames** character. ENSEMBL gene ID.
  - lib1** integer. log2 CPM in library 1.
  - lib2** integer. log2 CPM in library 2.
  - lib3** integer. log2 CPM in library 3.
  - lib4** integer. log2 CPM in library 4.
  - lib5** integer. log2 CPM in library 5.
  - lib6** integer. log2 CPM in library 6.
4. **weights** A matrix with 1000 rows and 6 columns
  - 1** numeric. limma gene weights for library 1.
  - 2** numeric. limma gene weights for library 2.
  - 3** numeric. limma gene weights for library 3.
  - 4** numeric. limma gene weights for library 4.
  - 5** numeric. limma gene weights for library 5.
  - 6** numeric. limma gene weights for library 6.
5. **design** A matrix with 6 rows and 1 column
  - GrandMean** numeric. limma default design matrix.

**Source**

[https://github.com/altman-lab/P259\\_pDC\\_public](https://github.com/altman-lab/P259_pDC_public)

**References**

Dill-McFarland et al. 2021. Eosinophil-mediated suppression and Anti-IL-5 enhancement of plasmacytoid dendritic cell interferon responses in asthma. J Allergy Clin Immunol. In revision

---

filter_rare	<i>Filter rare and low abundance genes</i>
-------------	--

---

**Description**

Filter genes at a minimum counts per million (CPM) in a minimum number or percent of total samples.

**Usage**

```
filter_rare(
  dat,
  min.CPM,
  gene.var = "geneName",
  min.sample = NULL,
  min.pct = NULL,
  plot = FALSE
)
```

**Arguments**

dat	DGEList output by edgeR::DEGList()
min.CPM	numeric minimum counts per million (CPM)
gene.var	character name for column with gene names in dat\$genes that matches names in expression data dat\$E. Default "geneName"
min.sample	numeric minimum number of samples
min.pct	numeric minimum percent of samples (0-100)
plot	logical if should plot mean variance trends

**Value**

DGEList object filtered to not rare genes

**Examples**

```
dat.filter <- filter_rare(dat = example.dat, min.CPM = 0.1, min.sample = 3)
dat.filter <- filter_rare(dat = example.dat, min.CPM = 0.1, min.pct = 10, plot = TRUE)
```

---

make\_modules

Construct WGCNA modules and associated data

---

## Description

Make WGCNA modules from gene expression data with dynamic soft threshold selection. Also outputs mean module expression and DAVID formatted gene lists

## Usage

```
make_modules(
  dat,
  genes = NULL,
  Rsq.min = NULL,
  sft.value = NULL,
  minModuleSize = 20,
  deepSplit = 3,
  nThread = 2
)
```

## Arguments

dat	limma EList output by voom( )
genes	Character vector of genes to used in module building. Must match rownames in dat. If not set, all genes in dat are used
Rsq.min	Numeric minimum R-squared for soft threshold selection. If set, sft.value is not used
sft.value	Numeric soft threshold. Set when minimum R-squared is no used
minModuleSize	Numeric minimum module size
deepSplit	Integer value between 0 and 4. Provides a simplified control over how sensitive module detection should be to module splitting, with 0 least and 4 most sensitive
nThread	Integer for number of threads to use

## Value

List including:

- genes Character vector of genes used in module building
- sft Data frame with soft thresholding selected for module building. Includes power, minimum R-squared, and connectivity
- sft.plot ggplot object of soft thresholding topology and connectivity
- mods Data frame of genes in modules
- mods.voom Data frame of mean module expression in each library
- david DAVID formatted data frame of genes in modules

## Examples

```
dat.mods <- make_modules(dat = example.voom, sft.value = 1)
```

# Index

## **\* datasets**

- example.dat, [2](#)
- example.kin, [3](#)
- example.voom, [4](#)

align\_metrics, [1](#)

example.dat, [2](#)  
example.kin, [3](#)  
example.voom, [4](#)

filter\_rare, [5](#)

make\_modules, [6](#)