

Package ‘SQMtools’

May 19, 2021

Title Analyze results generated by the SqueezeMeta pipeline

Version 0.6.2

Description SqueezeMeta is a versatile pipeline for the automated analysis of metagenomics/metatranscriptomics data (<http://github.com/jtamames/SqueezeMeta>). This package provides functions loading SqueezeMeta results into R, filtering them based on different criteria, and visualizing the results using basic plots. The SqueezeMeta project (and any subsets of it generated by the different filtering functions) is parsed into a single object, whose different components (e.g. tables with the taxonomic or functional composition across samples, contig/gene abundance profiles) can be easily analyzed using other R packages such as *vegan* or *DESeq2*

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Depends R (>= 3.2.0)

Imports reshape2, ggplot2, pathview, data.table

Suggests vegan, DESeq2

License GPLv3

Encoding UTF-8

LazyData true

RoxygenNote 7.1.1

BugReports <https://github.com/jtamames/SqueezeMeta/issues>

URL <https://github.com/jtamames/SqueezeMeta>

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combineSQM	<i>Combine several SQM objects</i>
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Description

Combine an arbitrary number of SQM objects into a single SQM object. The input objects must be subsets of the same original SQM object (i.e. from the same SqueezeMeta run). For combining results from different runs please check [combineSQMlite](#).

Usage

```
combineSQM(  
  ...,  
  tax_source = "orfs",  
  trusted_functions_only = F,  
  ignore_unclassified_functions = F,  
  rescale_tpm = T,  
  rescale_copy_number = T  
)
```

Arguments

- `...` an arbitrary number of SQM objects
- `tax_source` character. Features used for calculating aggregated abundances at the different taxonomic ranks. Either "orfs" or "contigs" (default "orfs"). If the objects being combined contain a subset of taxa or bins, this parameter can be set to TRUE.
- `trusted_functions_only` logical. If TRUE, only highly trusted functional annotations (best hit + best average) will be considered when generating aggregated function tables. If FALSE, best hit annotations will be used (default FALSE).
- `ignore_unclassified_functions` logical. If FALSE, ORFs with no functional classification will be aggregated together into an "Unclassified" category. If TRUE, they will be ignored (default FALSE).
- `rescale_tpm` logical. If TRUE, TPMs for KEGGs, COGs, and PFAMs will be recalculated (so that the TPMs in the subset actually add up to 1 million). Otherwise, per-function TPMs will be calculated by aggregating the TPMs of the ORFs annotated with that function, and will thus keep the scaling present in the parent object (default TRUE).
- `rescale_copy_number` logical. If TRUE, copy numbers will be recalculated using the RecA/RadA coverages in the subset. Otherwise, RecA/RadA coverages will be taken from the parent object with the highest RecA/RadA coverages. By default it is set to TRUE, which means that the returned copy numbers will represent the average copy number per function *in the genomes of the selected bins or contigs*. If any SQM objects that are being combined contain a functional subset rather than a contig/bins subset, this parameter should be set to FALSE.

Value

A SQM object

See Also

[subsetFun](#), [subsetTax](#), [combineSQMlite](#)

Examples

```
data(Hadza)
# Select Carbohydrate metabolism ORFs in Bacteroidetes, and Amino acid metabolism ORFs in Proteobacteria
bact = subsetTax(Hadza, "phylum", "Bacteroidetes")
bact.carb = subsetFun(bact, "Carbohydrate metabolism")
proteo = subsetTax(Hadza, "phylum", "Proteobacteria")
proteo.amins = subsetFun(proteo, "Amino acid metabolism")
bact.carb_proteo.amins = combineSQM(bact.carb, proteo.amins, rescale_copy_number=F)
```

combineSQMLite	<i>Combine several SQM or SQMLite objects</i>
----------------	---

Description

Combine an arbitrary number of SQM or SQMLite objects into a single SQMLite object. This function accepts objects originating from different projects (i.e. different SqueezeMeta runs).

Usage

```
combineSQMLite(...)
```

Arguments

... an arbitrary number of SQM or SQMLite objects

Value

A SQMLite object

See Also

[subsetFun](#), [subsetTax](#), [combineSQM](#)

Examples

```
## Not run:
data(Hadza)
# Load data coming from a different run
other = loadSQMLite("/path/to/other/project/tables") # e.g. if the project was run using sqm_reads
# (We could also use loadSQM to load the data as long as the data comes from a SqueezeMeta run)
combined = combineSQMLite(Hadza, other)
plotTaxonomy(combined, 'family') # Now we can plot together the samples from Hadza and the second project.

## End(Not run)
```

exportKrona	<i>Export the taxonomy of a SQM object into a Krona Chart</i>
-------------	---

Description

Generate a krona chart containing the full taxonomy from a SQM object.

Usage

```
exportKrona(SQM, output_name = NA)
```

Arguments

SQM	A SQM or SQMlite object.
output_name	character. Name of the output file containing the Krona charts in html format (default "<project_name>.krona.html").

Details

Original code was kindly provided by Giuseppe D'Auria (dauria_giu@gva.es).

See Also

[plotTaxonomy](#) for plotting the most abundant taxa of a SQM object.

Examples

```
data(Hadza)
exportKrona(Hadza)
```

exportPathway

Export the functions of a SQM object into KEGG pathway maps

Description

This function is a wrapper for the pathview package (Luo *et al.*, 2017. *Nucleic acids research*, 45:W501-W508). It will generate annotated KEGG pathway maps showing which reactions are present in the different samples. It will also generate legends with the color scales for each sample in separate png files.

Usage

```
exportPathway(
  SQM,
  pathway_id,
  count = "tpm",
  samples = NULL,
  split_samples = F,
  sample_colors = NULL,
  log_scale = F,
  fold_change_groups = NULL,
  fold_change_colors = NULL,
  max_scale_value = NULL,
  color_bins = 10,
  output_suffix = "pathview"
)
```

Arguments

<code>SQM</code>	A SQM or SQMLite object.
<code>pathway_id</code>	character. The five-number KEGG pathway identifier. A list of all pathway identifiers can be found in https://www.genome.jp/kegg/pathway.html .
<code>count</code>	character. Either "abund" for raw abundances, "percent" for percentages, "bases" for raw base counts, "tpm" for TPM normalized values or "copy_number" for copy numbers (default "tpm"). Note that a given count type might not available in this object (e.g. TPM or copy number in SQMLite objects originating from a SQM reads project).
<code>samples</code>	character. An optional vector with the names of the samples to export. If absent, all samples will be exported (default NULL).
<code>split_samples</code>	logical. Generate a different output file for each sample (default FALSE).
<code>sample_colors</code>	character. An optional vector with the plotting colors for each sample (default NULL).
<code>log_scale</code>	logical. Use a base 10 logarithmic transformation for the color scale. Will have no effect if <code>fold_change_groups</code> is provided (default FALSE).
<code>fold_change_groups</code>	list. An optional list containing two vectors of samples. If provided, the function will generate a single plot displaying the log2 fold-change between the average abundances of both groups of samples ($\log(\text{second group} / \text{first group})$) (default NULL).
<code>fold_change_colors</code>	character. An optional vector with the plotting colors of both groups in the fold-change plot. Will be ignored if <code>fold_change_group</code> is not provided.
<code>max_scale_value</code>	numeric. Maximum value to include in the color scale. By default it is the maximum value in the selected samples (if plotting abundances in samples) or the maximum absolute log2 fold-change (if plotting fold changes) (default NULL).
<code>color_bins</code>	numeric. Number of bins used to generate the gradient in the color scale (default 10).
<code>output_suffix</code>	character. Suffix to be added to the output files (default "pathview").

See Also

[plotFunctions](#) for plotting the most functions taxa of a SQM object.

Examples

```
data(Hadza)
exportPathway(Hadza, "00910", count = 'copy_number', output_suffix = "nitrogen_metabolism", sample_colors = c("red", "blue", "green", "yellow", "purple", "brown", "pink", "grey", "cyan", "magenta", "black", "white"))
exportPathway(Hadza, "00250", count = 'tpm', output_suffix = "ala_asp_glu_metabolism_FoldChange", fold_change_group = c("00910", "00250"))
```

exportTable	<i>Export results in tabular format</i>
-------------	---

Description

This function is a wrapper for R's write.table function.

Usage

```
exportTable(table, output_name)
```

Arguments

table	vector, matrix or data.frame. The table to be written.logical.
output_name	character. Name of the output file.

Examples

```
data(Hadza)
Hadza.iron = subsetFun(Hadza, "iron")
# Write the taxonomic distribution at the genus level of all the genes related to iron.
exportTable(Hadza.iron$taxa$genus$percent, "Hadza.ironGenes.genus.tsv")
# Now write the distribution of the different iron-related COGs (Clusters of Orthologous Groups) across samples.
exportTable(Hadza.iron$functions$COG$tpm, "Hadza.ironGenes.COG.tsv")
# Now write all the information contained in the ORF table.
exportTable(Hadza.iron$orfs$table, "Hadza.ironGenes.orftable.tsv")
```

Hadza	<i>Hadza hunter-gatherer gut metagenomes</i>
-------	--

Description

Subset of 5 bins (and the associated contigs and genes) generated by running SqueezeMeta on two gut metagenomic samples obtained from two hunter-gatherers of the Hadza ethnic group.

Usage

```
data(Hadza)
```

Format

A SQM object; see [loadSQM](#).

Source

[SRR1927149](#), [SRR1929485](#).

References

Rampelli *et al.*, 2015. Metagenome Sequencing of the Hadza Hunter-Gatherer Gut Microbiota. *Curr. biol.* **25**:1682-93 ([PubMed](#)).

Examples

```
data(Hadza)
plotTaxonomy(Hadza, "genus", rescale=T)
plotFunctions(Hadza, "COG")
```

loadSQM

Load a SqueezeMeta project into R

Description

This function takes the path to a project directory generated by **SqueezeMeta** (whose name is specified in the `-p` parameter of the `SqueezeMeta.pl` script) and parses the results into a SQM object.

Usage

```
loadSQM(
  project_path,
  tax_mode = "allfilter",
  trusted_functions_only = F,
  engine = "data.frame"
)
```

Arguments

<code>project_path</code>	character, project directory generated by SqueezeMeta.
<code>tax_mode</code>	character, which taxonomic classification should be loaded? SqueezeMeta applies the identity thresholds described in Luo <i>et al.</i>, 2014 . Use <code>allfilter</code> for applying the minimum identity threshold to all taxa (default), <code>prokfilter</code> for applying the threshold to Bacteria and Archaea, but not to Eukaryotes, and <code>nofilter</code> for applying no thresholds at all.
<code>trusted_functions_only</code>	logical. If TRUE, only highly trusted functional annotations (best hit + best average) will be considered when generating aggregated function tables. If FALSE, best hit annotations will be used (default FALSE). Will only have an effect if the <code>project_dir/results/tables</code> is not already present.
<code>engine</code>	character. Engine used to load the ORFs and contigs tables. Either <code>data.frame</code> (default) or <code>data.table</code> (significantly faster if your project is large).

Value

SQM object containing the parsed project.

Prerequisites

Run **SqueezeMeta**! An example call for running it would be:

```
/path/to/SqueezeMeta/scripts/SqueezeMeta.pl
-m coassembly -f fastq_dir -s samples_file -p project_dir
```

The SQM object structure

The SQM object is a nested list which contains the following information:

lvl1	lvl2	lvl3	type	rows/names	columns	data
\$orfs	\$table		<i>dataframe</i>	orfs	misc. data	misc. data
	\$abund		<i>numeric matrix</i>	orfs	samples	abundances
	\$bases		<i>numeric matrix</i>	orfs	samples	abundances
	\$tpm		<i>numeric matrix</i>	orfs	samples	tpm
	\$seqs		<i>character vector</i>	orfs	(n/a)	sequences
	\$tax		<i>character matrix</i>	orfs	tax. ranks	taxonomy
	\$contigs		<i>dataframe</i>	contigs	misc. data	misc. data
	\$abund		<i>numeric matrix</i>	contigs	samples	abundances
	\$tpm		<i>numeric matrix</i>	contigs	samples	tpm
	\$seqs		<i>character vector</i>	contigs	(n/a)	sequences
\$bins	\$tax		<i>character matrix</i>	contigs	tax. ranks	taxonomies
	\$bins		<i>character matrix</i>	contigs	bin. methods	bins
	\$table		<i>dataframe</i>	bins	misc. data	misc. data
	\$tpm		<i>numeric matrix</i>	bins	samples	tpm
	\$tax		<i>character matrix</i>	bins	tax. ranks	taxonomy
\$taxa	\$superkingdom	\$abund	<i>numeric matrix</i>	superkingdoms	samples	abundances
		\$percent	<i>numeric matrix</i>	superkingdoms	samples	percentages
	\$phylum	\$abund	<i>numeric matrix</i>	phyla	samples	abundances
		\$percent	<i>numeric matrix</i>	phyla	samples	percentages
	\$class	\$abund	<i>numeric matrix</i>	classes	samples	abundances
		\$percent	<i>numeric matrix</i>	classes	samples	percentages
	\$order	\$abund	<i>numeric matrix</i>	orders	samples	abundances
		\$percent	<i>numeric matrix</i>	orders	samples	percentages
	\$family	\$abund	<i>numeric matrix</i>	families	samples	abundances
		\$percent	<i>numeric matrix</i>	families	samples	percentages
	\$genus	\$abund	<i>numeric matrix</i>	genera	samples	abundances
		\$percent	<i>numeric matrix</i>	genera	samples	percentages
	\$species	\$abund	<i>numeric matrix</i>	species	samples	abundances
		\$percent	<i>numeric matrix</i>	species	samples	percentages
	\$functions	\$abund	<i>numeric matrix</i>	KEGG ids	samples	abundances
		\$bases	<i>numeric matrix</i>	KEGG ids	samples	abundances
		\$cov	<i>numeric matrix</i>	KEGG ids	samples	coverages
		\$tpm	<i>numeric matrix</i>	KEGG ids	samples	tpm
		\$copy_number	<i>numeric matrix</i>	KEGG ids	samples	avg. copies
		\$COG				
		\$abund	<i>numeric matrix</i>	COG ids	samples	abundances
		\$bases	<i>numeric matrix</i>	COG ids	samples	abundances
		\$cov	<i>numeric matrix</i>	COG ids	samples	coverages
		\$tpm	<i>numeric matrix</i>	COG ids	samples	tpm

		\$copy_number	<i>numeric matrix</i>	COG ids	samples	avg. copies
	\$PFAM	\$abund	<i>numeric matrix</i>	PFAM ids	samples	abundances
		\$bases	<i>numeric matrix</i>	PFAM ids	samples	abundances
		\$cov	<i>numeric matrix</i>	PFAM ids	samples	coverages
		\$tpm	<i>numeric matrix</i>	PFAM ids	samples	tpm
		\$copy_number	<i>numeric matrix</i>	PFAM ids	samples	avg. copies
\$total_reads			<i>numeric vector</i>	samples	(n/a)	total reads
\$misc	\$project_name		<i>character vector</i>	(empty)	(n/a)	project name
	\$samples		<i>character vector</i>	(empty)	(n/a)	samples
	\$tax_names_long	\$superkingdom	<i>character vector</i>	short names	(n/a)	full names
		\$phylum	<i>character vector</i>	short names	(n/a)	full names
		\$class	<i>character vector</i>	short names	(n/a)	full names
		\$order	<i>character vector</i>	short names	(n/a)	full names
		\$family	<i>character vector</i>	short names	(n/a)	full names
		\$genus	<i>character vector</i>	short names	(n/a)	full names
		\$species	<i>character vector</i>	short names	(n/a)	full names
	\$tax_names_short		<i>character vector</i>	full names	(n/a)	short names
	\$KEGG_names		<i>character vector</i>	KEGG ids	(n/a)	KEGG names
	\$KEGG_paths		<i>character vector</i>	KEGG ids	(n/a)	KEGG hiera
	\$COG_names		<i>character vector</i>	COG ids	(n/a)	COG names
	\$COG_paths		<i>character vector</i>	COG ids	(n/a)	COG hierar
	\$ext_annot_sources		<i>character vector</i>	COG ids	(n/a)	external data

If external databases for functional classification were provided to SqueezeMeta via the `-extdb` argument, the corresponding abundance (reads and bases), coverages, tpm and copy number profiles will be present in `SQM$functions` (e.g. results for the CAZy database would be present in `SQM$functions$CAZy`). Additionally, the extended names of the features present in the external database will be present in `SQM$misc` (e.g. `SQM$misc$CAZy_names`).

Examples

```
## Not run:
# (outside R)
/path/to/SqueezeMeta/scripts/SqueezeMeta.pl -p Hadza -f raw -m coassembly -s test.samples # Run SqueezeMeta on the
/path/to/SqueezeMeta/utis/sqm2tables.py Hadza Hadza/results/tables # Generate the tabular outputs! They must be p
# now go into R
R
library(SQMtools)
Hadza = loadSQM("Hadza") # Where Hadza is the path to the SqueezeMeta output directory

## End(Not run)

data(Hadza)
# Which are the ten most abundant KEGG IDs in our data?
topKEGG = sort(rowSums(Hadza$functions$KEGG$tpm), decreasing=T)[1:11]
topKEGG = topKEGG[names(topKEGG)!="Unclassified"]
# Which functions do those KEGG IDs represent?
Hadza$misc$KEGG_names[topKEGG]
What is the relative abundance of the Gammaproteobacteria class across samples?
```

```

Hadza$taxa$class$percent["Gammaproteobacteria",]
# Which information is stored in the orf, contig and bin tables?
colnames(Hadza$orfs$table)
colnames(Hadza$contigs$table)
colnames(Hadza$bins$table)
# What is the GC content distribution of my metagenome?
boxplot(Hadza$contigs$table[, "GC perc"]) # Not weighted by contig length or abundance!

```

loadSQMLite	<i>Load tables generated by sqm2tables.py, sqmreads2tables.py or combine-sqm-tables.py into R.</i>
-------------	--

Description

This function takes the path to the output directory generated by sqm2tables.py, sqmreads2tables.py or combine-sqm-tables.py a SQMLite object. The SQMLite object will contain taxonomic and functional profiles, but no detailed information on ORFs, contigs or bins. However, it will also have a much smaller memory footprint. A SQMLite object can be used for plotting and exporting, but it can not be subsetting.

Usage

```
loadSQMLite(tables_path, tax_mode = "allfilter")
```

Arguments

tables_path	character, tables directory generated by sqm2table.py, sqmreads2tables.py or combine-sqm-tables.py.
tax_mode	character, which taxonomic classification should be loaded? SqueezeMeta applies the identity thresholds described in Luo <i>et al.</i>, 2014 . Use allfilter for applying the minimum identity threshold to all taxa (default), prokfilter for applying the threshold to Bacteria and Archaea, but not to Eukaryotes, and nofilter for applying no thresholds at all.

Value

SQMLite object containing the parsed tables.

The SQMLite object structure

The SQMLite object is a nested list which contains the following information:

lvl1	lvl2	lvl3	type	rows/names	columns	data
\$taxa	\$superkingdom	\$abund	<i>numeric matrix</i>	superkingdoms	samples	abundances
		\$percent	<i>numeric matrix</i>	superkingdoms	samples	percentages
	\$phylum	\$abund	<i>numeric matrix</i>	phyla	samples	abundances
		\$percent	<i>numeric matrix</i>	phyla	samples	percentages
	\$class	\$abund	<i>numeric matrix</i>	classes	samples	abundances

		\$percent	<i>numeric matrix</i>	classes	samples	percentages
	\$order	\$abund	<i>numeric matrix</i>	orders	samples	abundances
		\$percent	<i>numeric matrix</i>	orders	samples	percentages
	\$family	\$abund	<i>numeric matrix</i>	families	samples	abundances
		\$percent	<i>numeric matrix</i>	families	samples	percentages
	\$genus	\$abund	<i>numeric matrix</i>	genera	samples	abundances
		\$percent	<i>numeric matrix</i>	genera	samples	percentages
	\$species	\$abund	<i>numeric matrix</i>	species	samples	abundances
		\$percent	<i>numeric matrix</i>	species	samples	percentages
\$functions	\$KEGG	\$abund	<i>numeric matrix</i>	KEGG ids	samples	abundances (read)
		\$bases	<i>numeric matrix</i>	KEGG ids	samples	abundances (base)
		\$tpm	<i>numeric matrix</i>	KEGG ids	samples	tpm
	\$COG	\$copy_number	<i>numeric matrix</i>	KEGG ids	samples	avg. copies
		\$abund	<i>numeric matrix</i>	COG ids	samples	abundances (read)
		\$bases	<i>numeric matrix</i>	COG ids	samples	abundances (base)
	\$PFAM	\$tpm	<i>numeric matrix</i>	COG ids	samples	tpm
		\$copy_number	<i>numeric matrix</i>	COG ids	samples	avg. copies
		\$abund	<i>numeric matrix</i>	PFAM ids	samples	abundances (read)
		\$bases	<i>numeric matrix</i>	PFAM ids	samples	abundances (base)
		\$tpm	<i>numeric matrix</i>	PFAM ids	samples	tpm
		\$copy_number	<i>numeric matrix</i>	PFAM ids	samples	avg. copies
\$total_reads			<i>numeric vector</i>	samples	(n/a)	total reads
\$misc	\$project_name		<i>character vector</i>	(empty)	(n/a)	project name
	\$samples		<i>character vector</i>	(empty)	(n/a)	samples
	\$tax_names_long	\$superkingdom	<i>character vector</i>	short names	(n/a)	full names
		\$phylum	<i>character vector</i>	short names	(n/a)	full names
		\$class	<i>character vector</i>	short names	(n/a)	full names
		\$order	<i>character vector</i>	short names	(n/a)	full names
		\$family	<i>character vector</i>	short names	(n/a)	full names
		\$genus	<i>character vector</i>	short names	(n/a)	full names
		\$species	<i>character vector</i>	short names	(n/a)	full names
		\$tax_names_short	<i>character vector</i>	full names	(n/a)	short names
	\$KEGG_names		<i>character vector</i>	KEGG ids	(n/a)	KEGG names
	\$KEGG_paths		<i>character vector</i>	KEGG ids	(n/a)	KEGG hierarchy
	\$COG_names		<i>character vector</i>	COG ids	(n/a)	COG names
	\$COG_paths		<i>character vector</i>	COG ids	(n/a)	COG hierarchy
	\$ext_annot_sources		<i>character vector</i>	(empty)	(n/a)	external database

If external databases for functional classification were provided to SqueezeMeta or SqueezeMeta_reads via the `-extdb` argument, the corresponding abundance, tpm and copy number profiles will be present in `SQM$functions` (e.g. results for the CAZy database would be present in `SQM$functions$CAZy`). Additionally, the extended names of the features present in the external database will be present in `SQM$misc` (e.g. `SQM$misc$CAZy_names`). Note that results generated by SqueezeMeta_reads will contain only read abundances, but not bases, tpm or copy number estimations.

See Also

[plotBars](#) and [plotFunctions](#) will plot the most abundant taxa and functions in a SQMLite object.

[exportKrona](#) will generate Krona charts reporting the taxonomy in a SQMLite object.

Examples

```
## Not run:
# (outside R)
/path/to/SqueezeMeta/scripts/SqueezeMeta.pl -p Hadza -f raw -m coassembly -s test.samples # Run SqueezeMeta on the
/path/to/SqueezeMeta/utis/sqm2tables.py Hadza Hadza/results/tables # Generate the tabular outputs! They must be p
# now go into R
R
library(SQMtools)
Hadza = loadSQMLite("Hadza/results/tables") # Where Hadza is the path to the SqueezeMeta output directory
# Note that this is not the whole SQM project, just the directory containing the tables.
# It would also work with tables generated by sqmreads2tables.py, or combine-sqm-tables.py
# plotTaxonomy(Hadza)
# plotFunctions(Hadza)
# exportKrona(Hadza, 'myKronaTest.html')

## End(Not run)
```

MGKOs

Single Copy Phylogenetic Marker Genes from Sunagawa's group (KOs)

Description

Lists of Single Copy Phylogenetic Marker Genes. These are useful for transforming coverages or tpms into copy numbers. This is an alternative way of normalizing data in order to be able to compare functional profiles in samples with different sequencing depths.

Usage

```
data(MGKOs)
```

Format

Character vector with the KEGG identifiers for 10 Single Copy Phylogenetic Marker Genes.

References

Salazar, G *et al.* (2019). Gene Expression Changes and Community Turnover Differentially Shape the Global Ocean Metatranscriptome *Cell* **179**:1068-1083. ([PubMed](#)).

See Also

[MGOGs](#) for an equivalent list using OGs instead of KOs; [USiCGs](#) for an alternative set of single copy genes, and for examples on how to generate copy numbers.

MGOGs	<i>Single Copy Phylogenetic Marker Genes from Sunagawa's group (OGs)</i>
-------	--

Description

Lists of Single Copy Phylogenetic Marker Genes. These are useful for transforming coverages or tpms into copy numbers. This is an alternative way of normalizing data in order to be able to compare functional profiles in samples with different sequencing depths.

Usage

```
data(MGOGs)
```

Format

Character vector with the COG identifiers for 10 Single Copy Phylogenetic Marker Genes.

References

Salazar, G *et al.* (2019). Gene Expression Changes and Community Turnover Differentially Shape the Global Ocean Metatranscriptome *Cell* **179**:1068-1083. ([PubMed](#)).

See Also

[MGKOs](#) for an equivalent list using KOs instead of OGs; [USiCGs](#) for an alternative set of single copy genes, and for examples on how to generate copy numbers.

mostAbundant	<i>Get the N most abundant rows from a numeric table</i>
--------------	--

Description

Return a subset of an input matrix or data frame, containing only the N most abundant rows, sorted. Alternatively, a custom set of rows can be returned.

Usage

```
mostAbundant(data, N = 10, items = NULL, others = F, rescale = F)
```

Arguments

<code>data</code>	numeric matrix or data frame
<code>N</code>	integer Number of rows to return (default 10).
<code>items</code>	Character vector. Custom row names to return. If provided, it will override <code>N</code> (default NULL).
<code>others</code>	logical. If TRUE, an extra row will be returned containing the aggregated abundances of the elements not selected with <code>N</code> or <code>items</code> (default FALSE).
<code>rescale</code>	logical. Scale result to percentages column-wise (default FALSE).

Value

A matrix or data frame (same as input) with the selected rows.

Examples

```
data(Hadza)
Hadza.carb = subsetFun(Hadza, "Carbohydrate metabolism")
# Which are the 20 most abundant KEGG functions in the ORFs related to carbohydrate metabolism?
topCarb = mostAbundant(Hadza.carb$functions$KEGG$tpm, N=20)
# Now print them with nice names
rownames(topCarb) = paste(rownames(topCarb), Hadza.carb$misc$KEGG_names[rownames(topCarb)], sep="; ")
topCarb
We can pass this to any R function
heatmap(topCarb)
But for convenience we provide wrappers for plotting ggplot2 heatmaps and barplots
plotHeatmap(topCarb, label_y="TPM")
plotBars(topCarb, label_y="TPM")
```

plotBars

Plot a barplot using ggplot2

Description

Plot a ggplot2 barplot from a matrix or data frame. The data should be in tabular format (e.g. features in rows and samples in columns).

Usage

```
plotBars(
  data,
  label_x = "Samples",
  label_y = "Abundances",
  label_fill = "Features",
  color = NULL,
  base_size = 11,
  max_scale_value = NULL
)
```

Arguments

<code>data</code>	Numeric matrix or data frame.
<code>label_x</code>	character Label for the x axis (default "Samples").
<code>label_y</code>	character Label for the y axis (default "Abundances").
<code>label_fill</code>	character Label for color categories (default "Features").
<code>color</code>	Vector with custom colors for the different features. If empty, the default ggplot2 palette will be used (default NULL).
<code>base_size</code>	numeric. Base font size (default 11).
<code>max_scale_value</code>	numeric. Maximum value to include in the y axis. By default it is handled automatically by ggplot2 (default NULL).

Value

a ggplot2 plot object.

See Also

[plotTaxonomy](#) for plotting the most abundant taxa of a SQM object; [plotHeatmap](#) for plotting a heatmap with arbitrary data; [mostAbundant](#) for selecting the most abundant rows in a dataframe or matrix.

Examples

```
data(Hadza)
sk = Hadza$taxa$superkingdom$abund
plotBars(sk, label_y = "Raw reads", label_fill = "Superkingdom")
```

plotFunctions

Heatmap of the most abundant functions in a SQM object

Description

This function selects the most abundant functions across all samples in a SQM object and represents their abundances in a heatmap. Alternatively, a custom set of functions can be represented.

Usage

```
plotFunctions(
  SQM,
  fun_level = "KEGG",
  count = "tpm",
  N = 25,
  fun = NULL,
  samples = NULL,
  ignore_unmapped = T,
```



```

    ignore_unclassified = T,
    gradient_col = c("ghostwhite", "dodgerblue4"),
    base_size = 11
  )

```

Arguments

SQM	A SQM or SQMLite object.
fun_level	character. Either "KEGG", "COG", "PFAM" or any other custom database used for annotation (default "KEGG").
count	character. Either "abund" for raw abundances, "percent" for percentages, "bases" for raw base counts, "tpm" for TPM normalized values or "copy_number" for copy numbers (default "tpm"). Note that a given count type might not available in this object (e.g. TPM or copy number in SQMLite objects originating from a SQM reads project).
N	integer Plot the N most abundant functions (default 25).
fun	character. Custom functions to plot. If provided, it will override N (default NULL).
samples	character. Character vector with the names of the samples to include in the plot. Can also be used to plot the samples in a custom order. If not provided, all samples will be plotted (default NULL).
ignore_unmapped	logical. Don't include unmapped ORFs in the plot (default TRUE).
ignore_unclassified	logical. Don't include unclassified ORFs in the plot (default TRUE).
gradient_col	A vector of two colors representing the low and high ends of the color gradient (default c("ghostwhite", "dodgerblue4")).
base_size	numeric. Base font size (default 11).

Value

a ggplot2 plot object.

See Also

[plotTaxonomy](#) for plotting the most abundant taxa of a SQM object; [plotBars](#) and [plotHeatmap](#) for plotting barplots or heatmaps with arbitrary data.

Examples

```

data(Hadza)
plotFunctions(Hadza)

```

plotHeatmap

Plot a heatmap using ggplot2

Description

Plot a ggplot2 heatmap from a matrix or data frame. The data should be in tabular format (e.g. features in rows and samples in columns).

Usage

```
plotHeatmap(
  data,
  label_x = "Samples",
  label_y = "Features",
  label_fill = "Abundance",
  gradient_col = c("ghostwhite", "dodgerblue4"),
  base_size = 11
)
```

Arguments

data	numeric matrix or data frame.
label_x	character Label for the x axis (default "Samples").
label_y	character Label for the y axis (default "Features").
label_fill	character Label for color scale (default "Abundance").
gradient_col	A vector of two colors representing the low and high ends of the color gradient (default c("ghostwhite", "dodgerblue4")).
base_size	numeric. Base font size (default 11).

Value

A ggplot2 plot object.

See Also

[plotFunctions](#) for plotting the top functional categories of a SQM object; [plotBars](#) for plotting a barplot with arbitrary data; [mostAbundant](#) for selecting the most abundant rows in a dataframe or matrix.

Examples

```
data(Hadza)
topPFAM = mostAbundant(Hadza$functions$PFAM$tpm)
topPFAM = topPFAM[rownames(topPFAM) != "Unclassified",] # Take out the Unclassified ORFs.
plotHeatmap(topPFAM, label_x = "Samples", label_y = "PFAMs", label_fill = "TPM")
```

plotTaxonomy

*Barplot of the most abundant taxa in a SQM object***Description**

This function selects the most abundant taxa across all samples in a SQM object and represents their abundances in a barplot. Alternatively, a custom set of taxa can be represented.

Usage

```
plotTaxonomy(
  SQM,
  rank = "phylum",
  count = "percent",
  N = 15,
  tax = NULL,
  others = T,
  samples = NULL,
  nocds = "treat_separately",
  ignore_unmapped = F,
  ignore_unclassified = F,
  no_partial_classifications = F,
  rescale = F,
  color = NULL,
  base_size = 11,
  max_scale_value = NULL
)
```

Arguments

SQM	A SQM or a SQMLite object.
rank	Taxonomic rank to plot (default phylum).
count	character. Either "percent" for percentages, or "abund" for raw abundances (default "percent").
N	integer Plot the N most abundant taxa (default 15).
tax	character. Custom taxa to plot. If provided, it will override N (default NULL).
others	logical. Collapse the abundances of least abundant taxa, and include the result in the plot (default TRUE).
samples	character. Character vector with the names of the samples to include in the plot. Can also be used to plot the samples in a custom order. If not provided, all samples will be plotted (default NULL).
nocds	character. Either "treat_separately" to treat reads annotated as No CDS separately, "treat_as_unclassified" to treat them as Unclassified or "ignore" to ignore them in the plot (default "treat_separately").

ignore_unmapped	logical. Don't include unmapped reads in the plot (default FALSE).
ignore_unclassified	logical. Don't include unclassified reads in the plot (default FALSE).
no_partial_classifications	logical. Treat reads not fully classified at the requested level (e.g. "Unclassified bacteroidetes" at the class level or below) as fully unclassified. This takes effect before ignore_unclassified, so if both are TRUE the plot will only contain fully classified contigs (default FALSE).
rescale	logical. Re-scale results to percentages (default FALSE).
color	Vector with custom colors for the different features. If empty, we will use our own hand-picked palette if N<=15, and the default ggplot2 palette otherwise (default NULL).
base_size	numeric. Base font size (default 11).
max_scale_value	numeric. Maximum value to include in the y axis. By default it is handled automatically by ggplot2 (default NULL).

Value

a ggplot2 plot object.

See Also

[plotFunctions](#) for plotting the most abundant functions of a SQM object; [plotBars](#) and [plotHeatmap](#) for plotting barplots or heatmaps with arbitrary data.

Examples

```
data(Hadza)
Hadza.amin = subsetFun(Hadza, "Amino acid metabolism")
# Taxonomic distribution of amino acid metabolism ORFs at the family level.
plotTaxonomy(Hadza.amin, "family")
```

RecA	<i>RecA/RadA recombinase</i>
------	------------------------------

Description

The recombination protein RecA/RadA is essential for the repair and maintenance of DNA, and has homologs in every bacteria and archaea. By dividing the coverage of functions by the coverage of RecA, abundances can be transformed into copy numbers, which can be used to compare functional profiles in samples with different sequencing depths. RecA-derived copy numbers are available in the SQM object (SQM\$functions\$<annotation_type>\$copy_number).

Usage

```
data(RecA)
```

Format

Character vector with the COG identifier for RecA/RadA.

Source

[EggNOG Database](#).

Examples

```
data(Hadza)
data(RecA)
### Let's calculate the average copy number of each function in our samples.
# We do it for COG annotations here, but we could also do it for KEGG or PFAMs.
COG.coverage = SQMtools::aggregate.fun(Hadza, "COG", trusted_functions_only=T,
                                       ignore_unclassified_functions=F)$cov
COG.copynumber = t(t(COG.coverage) / COG.coverage[RecA,]) # Sample-wise division by RecA coverage.
```

rowMaxs

Return a vector with the row-wise maxima of a matrix or dataframe.

Description

Return a vector with the row-wise maxima of a matrix or dataframe.

Usage

```
rowMaxs(table)
```

rowMins

Return a vector with the row-wise minima of a matrix or dataframe.

Description

Return a vector with the row-wise minima of a matrix or dataframe.

Usage

```
rowMins(table)
```

subsetBins	Create a SQM object containing only the requested bins, and the contigs and ORFs contained in them.
------------	---

Description

Create a SQM object containing only the requested bins, and the contigs and ORFs contained in them.

Usage

```
subsetBins(
  SQM,
  bins,
  trusted_functions_only = F,
  ignore_unclassified_functions = F,
  rescale_tpm = T,
  rescale_copy_number = T
)
```

Arguments

SQM	SQM object to be subsetted.
bins	character. Vector of bins to be selected.
trusted_functions_only	logical. If TRUE, only highly trusted functional annotations (best hit + best average) will be considered when generating aggregated function tables. If FALSE, best hit annotations will be used (default FALSE).
ignore_unclassified_functions	logical. If FALSE, ORFs with no functional classification will be aggregated together into an "Unclassified" category. If TRUE, they will be ignored (default FALSE).
rescale_tpm	logical. If TRUE, TPMs for KEGGs, COGs, and PFAMs will be recalculated (so that the TPMs in the subset actually add up to 1 million). Otherwise, per-function TPMs will be calculated by aggregating the TPMs of the ORFs annotated with that function, and will thus keep the scaling present in the parent object. By default it is set to TRUE, which means that the returned TPMs will be scaled <i>by million of reads of the selected bins</i> .
rescale_copy_number	logical. If TRUE, copy numbers will be recalculated using the RecA/RadA coverages in the subset. Otherwise, RecA/RadA coverages will be taken from the parent object. By default it is set to TRUE, which means that the returned copy numbers for each function will represent the average copy number of that function <i>per genome of the selected bins</i> .

Value

SQM object containing only the requested bins.

See Also

[subsetContigs](#), [subsetORFs](#)

Examples

```
data(Hadza)
# Which are the two most complete bins?
topBinNames = rownames(Hadza$bins$table)[order(Hadza$bins$table[, "Completeness"], decreasing=T)][1:2]
topBins = subsetBins(Hadza, topBinNames)
```

subsetContigs	<i>Select contigs</i>
---------------	-----------------------

Description

Create a SQM object containing only the requested contigs, the ORFs contained in them and the bins that contain them.

Usage

```
subsetContigs(
  SQM,
  contigs,
  trusted_functions_only = F,
  ignore_unclassified_functions = F,
  rescale_tpm = F,
  rescale_copy_number = F
)
```

Arguments

SQM	SQM object to be subsetted.
contigs	character. Vector of contigs to be selected.
trusted_functions_only	logical. If TRUE, only highly trusted functional annotations (best hit + best average) will be considered when generating aggregated function tables. If FALSE, best hit annotations will be used (default FALSE).
ignore_unclassified_functions	logical. If FALSE, ORFs with no functional classification will be aggregated together into an "Unclassified" category. If TRUE, they will be ignored (default FALSE).

rescale_tpm logical. If TRUE, TPMs for KEGGs, COGs, and PFAMs will be recalculated (so that the TPMs in the subset actually add up to 1 million). Otherwise, per-function TPMs will be calculated by aggregating the TPMs of the ORFs annotated with that function, and will thus keep the scaling present in the parent object (default FALSE).

rescale_copy_number logical. If TRUE, copy numbers will be recalculated using the RecA/RadA coverages in the subset. Otherwise, RecA/RadA coverages will be taken from the parent object. By default it is set to FALSE, which means that the returned copy numbers for each function will represent the average copy number of that function per genome in the parent object.

Value

SQM object containing only the selected contigs.

See Also

[subsetORFs](#)

Examples

```
data(Hadza)
# Which contigs have a GC content below 40?
lowGCcontigNames = rownames(Hadza$contigs$table[Hadza$contigs$table[, "GC perc"] < 40,])
lowGCcontigs = subsetContigs(Hadza, lowGCcontigNames)
hist(lowGCcontigs$contigs$table[, "GC perc"])
```

subsetFun

Filter results by function

Description

Create a SQM object containing only the ORFs with a given function, and the contigs and bins that contain them.

Usage

```
subsetFun(
  SQM,
  fun,
  columns = NULL,
  ignore_case = T,
  fixed = F,
  trusted_functions_only = F,
  ignore_unclassified_functions = F,
  rescale_tpm = F,
  rescale_copy_number = F
)
```


Arguments

SQM	SQM object to be subsetted.
fun	character. Pattern to search for in the different functional classifications.
columns	character. Restrict the search to the provided column names from <code>SQM\$orfs\$table</code> . If not provided the search will be performed in all the columns containing functional information (default NULL).
ignore_case	logical. Make pattern matching case-insensitive (default TRUE).
fixed	logical. If TRUE, pattern is a string to be matched as is. If FALSE the pattern is treated as a regular expression (default FALSE).
trusted_functions_only	logical. If TRUE, only highly trusted functional annotations (best hit + best average) will be considered when generating aggregated function tables. If FALSE, best hit annotations will be used (default FALSE).
ignore_unclassified_functions	logical. If FALSE, ORFs with no functional classification will be aggregated together into an "Unclassified" category. If TRUE, they will be ignored (default FALSE).
rescale_tpm	logical. If TRUE, TPMs for KEGGs, COGs, and PFAMs will be recalculated (so that the TPMs in the subset actually add up to 1 million). Otherwise, per-function TPMs will be calculated by aggregating the TPMs of the ORFs annotated with that function, and will thus keep the scaling present in the parent object (default FALSE).
rescale_copy_number	logical. If TRUE, copy numbers will be recalculated using the RecA/RadA coverages in the subset. Otherwise, RecA/RadA coverages will be taken from the parent object. By default it is set to FALSE, which means that the returned copy numbers for each function will represent the average copy number of that function per genome in the parent object.

Value

SQM object containing only the requested function.

See Also

[subsetTax](#), [subsetORFs](#), [combineSQM](#). The most abundant items of a particular table contained in a SQM object can be selected with [mostAbundant](#).

Examples

```
data(Hadza)
Hadza.iron = subsetFun(Hadza, "iron")
Hadza.carb = subsetFun(Hadza, "Carbohydrate metabolism")
# Search for multiple patterns using regular expressions
Hadza.twoKOs = subsetFun(Hadza, "K00812|K00813", fixed=F)
```

subsetORFs

*Select ORFs***Description**

Create a SQM object containing only the requested ORFs, and the contigs and bins that contain them. Internally, all the other subset functions in this package end up calling subsetORFs to do the work for them.

Usage

```
subsetORFs(
  SQM,
  orfs,
  tax_source = "orfs",
  trusted_functions_only = F,
  ignore_unclassified_functions = F,
  rescale_tpm = F,
  rescale_copy_number = F,
  contigs_override = NULL
)
```

Arguments

SQM	SQM object to be subsetted.
orfs	character. Vector of ORFs to be selected.
tax_source	character. Features used for calculating aggregated abundances at the different taxonomic ranks. Either "orfs" or "contigs" (default "orfs").
trusted_functions_only	logical. If TRUE, only highly trusted functional annotations (best hit + best average) will be considered when generating aggregated function tables. If FALSE, best hit annotations will be used (default FALSE).
ignore_unclassified_functions	logical. If FALSE, ORFs with no functional classification will be aggregated together into an "Unclassified" category. If TRUE, they will be ignored (default FALSE).
rescale_tpm	logical. If TRUE, TPMs for KEGGs, COGs, and PFAMs will be recalculated (so that the TPMs in the subset actually add up to 1 million). Otherwise, per-function TPMs will be calculated by aggregating the TPMs of the ORFs annotated with that function, and will thus keep the scaling present in the parent object (default FALSE).
rescale_copy_number	logical. If TRUE, copy numbers will be recalculated using the RecA/RadA coverages in the subset. Otherwise, RecA/RadA coverages will be taken from the parent object. By default it is set to FALSE, which means that the returned copy numbers for each function will represent the average copy number of that function per genome in the parent object.

Value

SQM object containing the requested ORFs.

A note on contig/bins subsetting

While this function selects the contigs and bins that contain the desired orfs, it DOES NOT recalculate contig/bin abundance and statistics based on the selected ORFs only. This means that the abundances presented in tables such as `SQM$contig$abund` or `SQM$bins$tpm` will still refer to the complete contigs and bins, regardless of whether only a fraction of their ORFs are actually present in the returned SQM object. This is also true for the statistics presented in `SQM$contigs$table` and `SQM$bins$table`.

Examples

```
data(Hadza)
# Select the 100 most abundant ORFs in our dataset.
mostAbundantORFnames = names(sort(rowSums(Hadza$orfs$tpm), decreasing=T))[1:100]
mostAbundantORFs = subsetORFs(Hadza, mostAbundantORFnames)
```

subsetRand	Select random ORFs
------------	--------------------

Description

Create a random subset of a SQM object.

Usage

```
subsetRand(SQM, N)
```

Arguments

SQM	SQM object to be subsetted.
N	numeric. number of random ORFs to select.

Value

SQM object containing a random subset of ORFs.

See Also

[subsetORFs](#)

subsetTax	<i>Filter results by taxonomy</i>
-----------	-----------------------------------

Description

Create a SQM object containing only the contigs with a given consensus taxonomy, the ORFs contained in them and the bins that contain them.

Usage

```
subsetTax(
  SQM,
  rank,
  tax,
  trusted_functions_only = F,
  ignore_unclassified_functions = F,
  rescale_tpm = T,
  rescale_copy_number = T
)
```

Arguments

SQM	SQM object to be subsetted.
rank	character. The taxonomic rank from which to select the desired taxa (superkingdom, phylum, class, order, family, genus, species)
tax	character. The taxon to select.
trusted_functions_only	logical. If TRUE, only highly trusted functional annotations (best hit + best average) will be considered when generating aggregated function tables. If FALSE, best hit annotations will be used (default FALSE).
ignore_unclassified_functions	logical. If FALSE, ORFs with no functional classification will be aggregated together into an "Unclassified" category. If TRUE, they will be ignored (default FALSE).
rescale_tpm	logical. If TRUE, TPMs for KEGGs, COGs, and PFAMs will be recalculated (so that the TPMs in the subset actually add up to 1 million). Otherwise, per-function TPMs will be calculated by aggregating the TPMs of the ORFs annotated with that function, and will thus keep the scaling present in the parent object. By default it is set to TRUE, which means that the returned TPMs will be scaled <i>by million of reads of the selected taxon</i> .
rescale_copy_number	logical. If TRUE, copy numbers will be recalculated using the RecA/RadA coverages in the subset. Otherwise, RecA/RadA coverages will be taken from the parent object. By default it is set to TRUE, which means that the returned copy numbers for each function will represent the average copy number of that function <i>per genome of the selected taxon</i> .

Value

SQM object containing only the requested taxon.

See Also

[subsetFun](#), [subsetContigs](#), [combineSQM](#). The most abundant items of a particular table contained in a SQM object can be eslected with [mostAbundant](#).

Examples

```
data(Hadza)
Hadza.Escherichia = subsetTax(Hadza, "genus", "Escherichia")
Hadza.Bacteroidetes = subsetTax(Hadza, "phylum", "Bacteroidetes")
```

summary.SQM	<i>summary method for class SQM</i>
-------------	-------------------------------------

Description

Computes different statistics of the data contained in the SQM object.

Usage

```
## S3 method for class 'SQM'
summary(SQM)
```

Value

A list of summary statistics.

summary.SQMLite	<i>summary method for class SQMLite</i>
-----------------	---

Description

Computes different statistics of the data contained in the SQMLite object.

Usage

```
## S3 method for class 'SQMLite'
summary(SQM)
```

Value

A list of summary statistics.

USiCGs

*Universal Single-Copy Genes***Description**

Lists of Universal Single Copy Genes for Bacteria and Archaea. These are useful for transforming coverages or tpms into copy numbers. This is an alternative way of normalizing data in order to be able to compare functional profiles in samples with different sequencing depths.

Usage

```
data(USiCGs)
```

Format

Character vector with the KEGG identifiers for 15 Universal Single Copy Genes.

Source

[Carr *et al.*, 2013. Table S1.](#)

References

Carr, Shen-Orr & Borenstein (2013). Reconstructing the Genomic Content of Microbiome Taxa through Shotgun Metagenomic Deconvolution *PLoS Comput. Biol.* **9**:e1003292. ([PubMed](#)).

Examples

```
data(Hadza)
data(USiCGs)
### Let's look at the Universal Single Copy Gene distribution in our samples.
KEGG.tpm = Hadza$functions$KEGG$tpm
all(USiCGs %in% rownames(KEGG.tpm)) # Are all the USiCGs present in our dataset?
# Plot a boxplot of USiCGs tpms and calculate median USiCGs tpm.
# This looks weird in the test dataset because it contains only a small subset of the metagenomes.
# In a set of complete metagenomes USiCGs should have fairly similar TPM averages
# and low dispersion across samples.
boxplot(t(KEGG.tpm[USiCGs,]), names=USiCGs, ylab="TPM", col="slateblue2")

### Now let's calculate the average copy numbers of each function.
# We do it for KEGG annotations here, but we could also do it for COGs or PFAMs.
USiCGs.cov = apply(Hadza$functions$KEGG$cov[USiCGs,], 2, median)
# Sample-wise division by the median USiCG coverage.
KEGG.copynumber = t(t(Hadza$functions$KEGG$cov) / USiCGs.cov)
```

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