

Package ‘SIPmg’

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Title Statistical Analysis to Identify Isotope Incorporating MAGs

Version 1.4.1

Description Statistical analysis as part of a stable isotope probing (SIP) metagenomics study to identify isotope incorporating taxa recovered as metagenome-assembled genomes (MAGs). Helpful reading and a vignette in bookdown format is provided on the package site <<https://zielslab.github.io/SIPmg.github.io/>>.

URL <https://zielslab.github.io/SIPmg.github.io/>

BugReports <https://github.com/ZielsLab/SIPmg>

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atomX	<i>Atom fraction excess table</i>
-------	-----------------------------------

Description

Data table generated from the "qSIP_atom_excess_MAGs" function

Usage

```
data(atomX)
```

Format

An object of class "list"

calc_atom_excess_MAGs *Calculate atom fraction excess*

Description

See Hungate et al., 2015 for more details

Usage

```
calc_atom_excess_MAGs(Mlab, Mlight, Mheavymax, isotope = "13C")
```

Arguments

Mlab	The molecular wight of labeled DNA
Mlight	The molecular wight of unlabeled DNA
Mheavymax	The theoretical maximum molecular weight of fully-labeled DNA
isotope	The isotope for which the DNA is labeled with ('13C' or '18O')

Value

numeric value: atom fraction excess (A)

calc_Mheavymax_MAGs *Calculate Mheavymax*

Description

This script was adapted from https://github.com/buckleylab/HTSSIP/blob/master/R/qSIP_atom_excess.R for use with genome-centric metagenomics. See Hungate et al., 2015 for more details

Usage

```
calc_Mheavymax_MAGs(Mlight, isotope = "13C", Gi = Gi)
```

Arguments

Mlight	The molecular wight of unlabeled DNA
isotope	The isotope for which the DNA is labeled with ('13C' or '18O')
Gi	The G+C content of unlabeled DNA

Value

numeric value: maximum molecular weight of fully-labeled DNA

coverage_normalization

Normalize feature coverages to estimate absolute abundance or relative coverage using MAG/contig coverage values with or without multiplying total DNA concentration of the fraction

Description

Normalize feature coverages to estimate absolute abundance or relative coverage using MAG/contig coverage values with or without multiplying total DNA concentration of the fraction

Usage

```
coverage_normalization(
  f_tibble,
  contig_coverage,
  sequencing_yield,
  fractions_df,
  approach = "relative_coverage"
)
```

Arguments

- | | |
|------------------|---|
| f_tibble | Can be either of (1) a tibble with first column "Feature" that contains bin IDs, and the rest of the columns represent samples with bins' coverage values. (2) a tibble as outputted by the program "checkm coverage" from the tool CheckM. Please check CheckM documentation - https://github.com/Ecogenomics/CheckM on the usage for "checkm coverage" program |
| contig_coverage | tibble with contig ID names ("Feature" column), sample columns with same sample names as in f_tibble containing coverage values of each contig, contig length in bp ("contig_length" column), and the MAG the contig is associated ("MAG" column) with same MAGs as in Feature column of f_tibble dataset. |
| sequencing_yield | tibble containing sample ID ("sample" column) with same sample names as in f_tibble and number of reads in bp recovered in that sample ("yield" column). |
| fractions_df | fractions data frame A fractions file with the following columns <ul style="list-style-type: none"> • Replicate: Depends on how many replicates the study has • Fractions: Typically in the range of 2-24 • Buoyant_density: As calculated from the refractometer for each fraction and replicate • Isotope: "12C", "13C", "14N", "15N" etc. • DNA_concentration • Sample: In the format "'isotope'rep#fraction#". For instance, "12C_rep_1_fraction_1" |

approach Please choose the method for coverage normalization as "relative_coverage", "greenlon", "starr" to estimate only relative coverage without multiplying DNA concentration of fraction, or as per methods in Greenlon et al. - <https://journals.asm.org/doi/full/10.1128/mSphere.00085-21> or Starr et al. - <https://journals.asm.org/doi/10.1128/mSphere.00085-21>

Value

tibble containing normalized coverage in required format with MAG name as first column and the normalized coverage values in each sample as the rest of the columns.

Examples

```
data(f_tibble)

rel.cov = coverage_normalization(f_tibble)
```

DESeq2_l2fc

Calculating log2 fold change for HTS-SIP data.

Description

The 'use_geo_mean' parameter uses geometric means on all non-zero abundances for estimateSizeFactors instead of using the default log-transformed geometric means.

Usage

```
DESeq2_l2fc(
  physeq,
  density_min,
  density_max,
  design,
  l2fc_threshold = 0.25,
  sparsity_threshold = 0.25,
  sparsity_apply = "all",
  size_factors = "geoMean"
)
```

Arguments

physeq	Phyloseq object
density_min	Minimum buoyant density of the 'heavy' gradient fractions
density_max	Maximum buoyant density of the 'heavy' gradient fractions
design	design parameter used for DESeq2 analysis. See DESeq2::DESeq for more details.

- l2fc_threshold log2 fold change (l2fc) values must be significantly above this threshold in order to reject the hypothesis of equal counts.
- sparsity_threshold All OTUs observed in less than this portion (fraction: 0-1) of gradient fraction samples are pruned. A form of independent filtering, The sparsity cutoff with the most rejected hypotheses is used.
- sparsity_apply Apply sparsity threshold to all gradient fraction samples ('all') or just heavy fraction samples ('heavy')
- size_factors Method of estimating size factors. 'geoMean' is from (Pepe-Ranney et. al., 2016) and removes all zero-abundances from the calculation. 'default' is the default for estimateSizeFactors. 'iterate' is an alternative when every OTU has a zero in ≥ 1 sample.

Value

dataframe of HRSIP results

Examples

```
data(phylo.qSIP)
```

```
df_l2fc = DESeq2_l2fc(phylo.qSIP, density_min=1.71, density_max=1.75, design=~Isotope)
```

df_atomX_boot	<i>Bootstrapped atom fraction excess table</i>
---------------	--

Description

Data table generated from bootstrapping the AFE table using the "qSIP_bootstrap_fcr" function

Usage

```
data(df_atomX_boot)
```

Format

An object of class "data.frame"

filter_l2fc	<i>Filter l2fc table</i>
-------------	--------------------------

Description

filter_l2fc filters a l2fc table to 'best' sparsity cutoffs & bouyant density windows.

Usage

```
filter_l2fc(df_l2fc, padj_cutoff = 0.1)
```

Arguments

df_l2fc	data.frame of log2 fold change values
padj_cutoff	Adjusted p-value cutoff for rejecting the null hypothesis that l2fc values were not greater than the l2fc_threshold.

Value

filtered df_l2fc object

filter_na	<i>Remove MAGs with NAs from atomX table</i>
-----------	--

Description

This function enables removing NAs from the atomX table.

Usage

```
filter_na(atomX)
```

Arguments

atomX	A list object created by qSIP_atom_excess_MAGs()
-------	--

Value

A list of 2 data.frame objects without MAGs which have NAs. 'W' contains the weighted mean bouyant density (W) values for each OTU in each treatment/control. 'A' contains the atom fraction excess values for each OTU. For the 'A' table, the 'Z' column is bouyant density shift, and the 'A' column is atom fraction excess.

Examples

```
data(atomX)
```

```
### Remove NAs in atomX table  
atomx_no_na = filter_na(atomX)
```

fractions	<i>Fractions table</i>
-----------	------------------------

Description

Fractions data used for many functions in the package

Usage

```
data(fractions)
```

Format

An object of class "data.frame"

f_tibble	<i>Coverage table</i>
----------	-----------------------

Description

Coverage data used for many functions in the package

Usage

```
data(f_tibble)
```

Format

An object of class "data.frame"

GC_content	<i>GC_content table</i>
------------	-------------------------

Description

GC_content data

Usage

```
data(GC_content)
```

Format

An object of class "data.frame"

HRSIP	<i>(MW-)HR-SIP analysis</i>
-------	-----------------------------

Description

Conduct (multi-window) high resolution stable isotope probing (HR-SIP) analysis.

Usage

```
HRSIP(
  physeq,
  design,
  density_windows = data.frame(density_min = c(1.7), density_max = c(1.75)),
  sparsity_threshold = seq(0, 0.3, 0.1),
  sparsity_apply = "all",
  l2fc_threshold = 0.25,
  padj_method = "BH",
  padj_cutoff = NULL,
  parallel = FALSE
)
```

Arguments

physeq	Phyloseq object
design	design parameter used for DESeq2 analysis. This is usually used to differentiate labeled-treatment and unlabeled-control samples. See DESeq2::DESeq for more details on the option.
density_windows	The buoyant density window(s) used for calculating log2 fold change values. Input can be a vector (length 2) or a data.frame with a 'density_min' and a 'density_max' column (each row designates a density window).

<code>sparsity_threshold</code>	All OTUs observed in less than this portion (fraction: 0-1) of gradient fraction samples are pruned. This is a form of independent filtering. The sparsity cutoff with the most rejected hypotheses is used.
<code>sparsity_apply</code>	Apply sparsity threshold to all gradient fraction samples ('all') or just 'heavy' fraction samples ('heavy'), where 'heavy' samples are designated by the <code>density_windows</code> .
<code>l2fc_threshold</code>	log2 fold change (l2fc) values must be significantly above this threshold in order to reject the hypothesis of equal counts. See DESeq2 for more information.
<code>padj_method</code>	Method for global p-value adjustment (See <code>p.adjust()</code>).
<code>padj_cutoff</code>	Adjusted p-value cutoff for rejecting the null hypothesis that l2fc values were not greater than the <code>l2fc_threshold</code> . Set to NULL to skip filtering of results to the sparsity cutoff with most rejected hypotheses and filtering each OTU to the buoyant density window with the greatest log2 fold change.
<code>parallel</code>	Process each parameter combination in parallel. See <code>plyr::mapply()</code> for more information.

Details

The (MW-)HR-SIP workflow is as follows:

1. For each sparsity threshold & BD window: calculate log2 fold change values (with DESeq2) for each OTU
2. Globally adjust p-values with a user-defined method (see `p.adjust()`)
3. Select the sparsity cutoff with the most rejected hypotheses
4. For each OTU, select the BD window with the greatest log2 fold change value

Value

dataframe of HRSIP results

Examples

```
data(phylo.qSIP)

## HR-SIP
### Note: treatment-control samples differentiated with 'design=~Isotope'
df_l2fc = HRSIP(phylo.qSIP, design=~Isotope)
## Same, but multiple BD windows (MW-HR-SIP). For parallel processing change to parallel = TRUE
### Windows = 1.7-1.74, 1.72-1.75, and 1.73 - 1.76
windows = data.frame(density_min=c(1.71,1.72, 1.73), density_max=c(1.74,1.75,1.76))
df_l2fc = HRSIP(phylo.qSIP,
               design=~Isotope,
               density_windows = windows,
               padj_cutoff = 0.05,
               parallel=FALSE)
```

 incorporators_taxonomy

Isotope incorporator list with GTDB taxonomy

Description

This function provides a table with MAGs and their corresponding GTDB taxonomy as an output. This would be useful in identifying the taxa that have incorporation

Usage

```
incorporators_taxonomy(taxonomy, bootstrapped_AFE_table)
```

Arguments

taxonomy A taxonomy tibble obtained in the markdown. This taxonomy tibble is typically a concatenated list of archaeal and bacterial taxonomy from GTDB-Tk Please check GTDB-Tk documentation for running the tool

bootstrapped_AFE_table A data frame indicating bootstrapped atom fraction excess values

Value

A tibble with two columns, OTU and Taxonomy, with taxonomy of the incorporator MAGs

Examples

```
data(taxonomy_tibble, df_atomX_boot)
```

```
### Making incorporator taxonomy list
incorporator_list = incorporators_taxonomy(taxonomy = taxonomy_tibble,
                                           bootstrapped_AFE_table = df_atomX_boot)
```

 mag.table

MAG abundance table in phyloseq format

Description

MAG abundances in the format of phyloseq object to be used in the qSIP and (MW-)HR-SIP pipeline

Usage

```
data(mag.table)
```

Format

An object of class "phyloseq"

phylo.qSIP	<i>Master phyloseq object</i>
------------	-------------------------------

Description

Master phyloseq object

Usage

```
data(phylo.qSIP)
```

Format

An object of class "phyloseq"

phylo.table	<i>Master phyloseq object using the MAG phyloseq objects</i>
-------------	--

Description

Creates a phyloseq-style object using processed phyloseq objects for otu table (here, MAG table), taxa table, and sample table

Usage

```
phylo.table(mag, taxa, samples)
```

Arguments

mag	phyloseq-styled MAG table
taxa	phyloseq-styled taxa table
samples	sample information table

Value

phyloseq object for MAGs

Examples

```
data(mag.table, taxonomy.object, samples.object, fractions, taxonomy_tibble)
###Making phyloseq table from fractions metadata
samples.object = sample.table(fractions)
taxonomy.object = tax.table(taxonomy_tibble)

### Making master phyloseq table from scaled MAG data, taxa and fractions phyloseq data
phylo.qSIP = phylo.table(mag.table, taxonomy.object, samples.object)
```

qSIP_atom_excess_format_MAGs

Reformat a phyloseq object of qSIP_atom_excess_MAGs analysis

Description

Reformat a phyloseq object of qSIP_atom_excess_MAGs analysis

Usage

```
qSIP_atom_excess_format_MAGs(physeq, control_expr, treatment_rep)
```

Arguments

physeq	A phyloseq object
control_expr	An expression for identifying unlabeled control samples in the phyloseq object (eg., "Substrate=='12C-Con'")
treatment_rep	Which column in the phyloseq sample data designates replicate treatments

Value

numeric value: atom fraction excess (A)

qSIP_atom_excess_MAGs *Calculate atom fraction excess using q-SIP method*

Description

Calculate atom fraction excess using q-SIP method

Usage

```
qSIP_atom_excess_MAGs(
  physeq,
  control_expr,
  treatment_rep = NULL,
  isotope = "13C",
  df_OTU_W = NULL,
  Gi
)
```

Arguments

physeq	A phyloseq object
control_expr	Expression used to identify control samples based on sample_data.
treatment_rep	Which column in the phyloseq sample data designates replicate treatments
isotope	The isotope for which the DNA is labeled with ('13C' or '18O')
df_OTU_W	Keep NULL
Gi	GC content of the MAG

Value

A list of 2 data.frame objects. 'W' contains the weighted mean buoyant density (W) values for each OTU in each treatment/control. 'A' contains the atom fraction excess values for each OTU. For the 'A' table, the 'Z' column is buoyant density shift, and the 'A' column is atom fraction excess.

Examples

```
data(phylo.qSIP,GC_content)
### Making atomx table

## Not run::
### BD shift (Z) & atom excess (A)
atomX = qSIP_atom_excess_MAGs(phylo.qSIP,
                              control_expr='Isotope=="12C"',
                              treatment_rep='Replicate',
                              Gi = GC_content)
```

qSIP_bootstrap_fcr	<i>Calculate adjusted bootstrap CI after for multiple testing for atom fraction excess using q-SIP method. Multiple hypothesis tests are corrected by</i>
--------------------	---

Description

Calculate adjusted bootstrap CI after for multiple testing for atom fraction excess using q-SIP method. Multiple hypothesis tests are corrected by

Usage

```
qSIP_bootstrap_fcr(
  atomX,
  isotope = "13C",
  n_sample = c(3, 3),
  ci_adjust_method = "fcr",
  n_boot = 10,
  parallel = FALSE,
  a = 0.1
)
```

Arguments

atomX	A list object created by qSIP_atom_excess_MAGs()
isotope	The isotope for which the DNA is labeled with ('13C' or '18O')
n_sample	A vector of length 2. The sample size for data resampling (with replacement) for 1) control samples and 2) treatment samples.
ci_adjust_method	Confidence interval adjustment method. Please choose 'FCR', 'Bonferroni', or 'none' (if no adjustment is needed). Default is FCR and also provides unadjusted CI.
n_boot	Number of bootstrap replicates.
parallel	Parallel processing. See .parallel option in dplyr::mdply() for more details.
a	A numeric value. The alpha for calculating confidence intervals.

Value

A data.frame of atom fraction excess values (A) and atom fraction excess confidence intervals adjusted for multiple testing.

Examples

```

data(phylo.qSIP,GC_content)

### BD shift (Z) & atom excess (A)
atomX = qSIP_atom_excess_MAGs(phylo.qSIP,
                              control_expr='Isotope=="12C"',
                              treatment_rep='Replicate', Gi = GC_content)

### Add doParallel::registerDoParallel(num_cores) if parallel bootstrapping is to be done
df_atomX_boot = qSIP_bootstrap_fcr(atomX, n_boot=5, parallel = FALSE)

```

sample.table	<i>phyloseq-styled sample table</i>
--------------	-------------------------------------

Description

Creates a phyloseq-styled sample table from fractions metadata containing data on fraction number, number of replicates, buoyant density calculated from a refractometer, type of isotope, and DNA concentration of each fraction, and isotope type. See below for information on "fractions" file.

Usage

```
sample.table(fractions_df)
```

Arguments

`fractions_df` fractions data frame A fractions file with the following columns

- Replicate: Depends on how many replicates the study has
- Fractions: Typically in the range of 2-24
- Buoyant_density: As calculated from the refractometer for each fraction and replicate
- Isotope: "12C", "13C", "14N", "15N" etc.
- DNA_concentration
- Sample: In the format "'isotope'rep#fraction#". For instance, "12C_rep_1_fraction_1"

Value

data frame: phyloseq-style sample table

Examples

```
data(fractions)

### Making phyloseq table from fractions metadata
samples.object = sample.table(fractions)
```

samples.object	<i>Fractions table in phyloseq format</i>
----------------	---

Description

Fractions metadata in the format of phyloseq object to be used in the qSIP and (MW-)HR-SIP pipeline

Usage

```
data(samples.object)
```

Format

An object of class "phyloseq"

scale_features_lm	<i>Scale feature coverage values to estimate their absolute abundance</i>
-------------------	---

Description

Calculates global scaling factors for features (contigs or bins), based on linear regression of sequin coverage. Options include log-transformations of coverage, as well as filtering features based on limit of detection. This function must be called first, before the feature abundance table, feature detection table, and plots are retrieved.

Usage

```
scale_features_lm(
  f_tibble,
  sequin_meta,
  seq_dilution,
  log_trans = TRUE,
  coe_of_variation = 250,
  lod_limit = 0,
  save_plots = TRUE,
  plot_dir = tempdir(),
  cook_filtering = TRUE
)
```

Arguments

f_tibble	Can be either of (1) a tibble with first column "Feature" that contains bin IDs, and the rest of the columns represent samples with bins' coverage values. (2) a tibble as outputted by the program "checkm coverage" from the tool CheckM. Please check CheckM documentation - https://github.com/Ecogenomics/CheckM on the usage for "checkm coverage" program
sequin_meta	tibble containing sequin names ("Feature column") and concentrations in attamoles/uL ("Concentration") column.
seq_dilution	tibble with first column "Sample" with same sample names as in f_tibble , and a second column "Dilution" showing ratio of sequins added to final sample volume (e.g. a value of 0.01 for a dilution of 1 volume sequin to 99 volumes sample)
log_trans	Boolean (TRUE or FALSE), should coverages and sequin concentrations be log-scaled?
coe_of_variation	Acceptable coefficient of variation for coverage and detection (eg. 20 - for 20 % threshold of coefficient of variation). Coverages above the threshold value will be flagged in the plots.
lod_limit	(Decimal range 0-1) Threshold for the percentage of minimum detected sequins per concentration group. Default = 0
save_plots	Boolean (TRUE or FALSE), should sequin scaling be saved? Default = TRUE
plot_dir	Directory where plots are to be saved. Will create a directory "sequin_scaling_plots_lm" if it does not exist.
cook_filtering	Boolean (TRUE or FALSE), should data points be filtered based on Cook's distance metric. Cooks distance can be useful in detecting influential outliers in an ordinary least square's regression model, which can negatively influence the model. A threshold of Cooks distance of $4/n$ (where n is the sample size) is chosen, and any data point with Cooks distance $> 4/n$ is filtered out. It is typical to choose $4/n$ as the threshold in detecting the outliers in the data. Default = TRUE

Value

a list of tibbles containing

- mag_tab: a tibble with first column "Feature" that contains bin (or contig IDs), and the rest of the columns represent samples with features' scaled abundances (attamoles/uL)
- mag_det: a tibble with first column "Feature" that contains bin (or contig IDs),
- plots: linear regression plots for scaling MAG coverage values to absolute abundance
- scale_fac: a master tibble with all of the intermediate values in above calculations

Examples

```
data(f_tibble, sequins, seq_dil)
```

```
### scaling sequins from coverage values
scaled_features_lm = scale_features_lm(f_tibble, sequin_meta, seq_dil)
```

scale_features_rlm *Scale feature coverage values to estimate their absolute abundance*

Description

Calculates global scaling factors for features (contigs or bins), based on linear regression of sequin coverage. Options include log-transformations of coverage, as well as filtering features based on limit of detection. This function must be called first, before the feature abundance table, feature detection table, and plots are retrieved.

Usage

```
scale_features_rlm(
  f_tibble,
  sequin_meta,
  seq_dilution,
  log_trans = TRUE,
  coe_of_variation = 250,
  lod_limit = 0,
  save_plots = TRUE,
  plot_dir = tempdir()
)
```

Arguments

f_tibble	Can be either of (1) a tibble with first column "Feature" that contains bin IDs, and the rest of the columns represent samples with bins' coverage values. (2) a tibble as outputted by the program "checkm coverage" from the tool CheckM. Please check CheckM documentation - https://github.com/Ecogenomics/CheckM on the usage for "checkm coverage" program
sequin_meta	tibble containing sequin names ("Feature column") and concentrations in attamoles/uL ("Concentration") column.
seq_dilution	tibble with first column "Sample" with same sample names as in f_tibble , and a second column "Dilution" showing ratio of sequins added to final sample volume (e.g. a value of 0.01 for a dilution of 1 volume sequin to 99 volumes sample)
log_trans	Boolean (TRUE or FALSE), should coverages and sequin concentrations be log-scaled? Default = TRUE
coe_of_variation	Acceptable coefficient of variation for coverage and detection (eg. 20 - for 20 % threshold of coefficient of variation). Coverages above the threshold value will be flagged in the plots. Default = 250

<code>lod_limit</code>	(Decimal range 0-1) Threshold for the percentage of minimum detected sequins per concentration group. Default = 0
<code>save_plots</code>	Boolean (TRUE or FALSE), should sequin scaling be saved? Default = TRUE
<code>plot_dir</code>	Directory where plots are to be saved. Will create a directory "sequin_scaling_plots_rlm" if it does not exist.

Value

a list of tibbles containing

- `mag_tab`: a tibble with first column "Feature" that contains bin (or contig IDs), and the rest of the columns represent samples with features' scaled abundances (attamoles/uL)
- `mag_det`: a tibble with first column "Feature" that contains bin (or contig IDs),
- `plots`: linear regression plots for scaling MAG coverage values to absolute abundance (optional)
- `scale_fac`: a master tibble with all of the intermediate values in above calculations

Examples

```
data(f_tibble, sequins, seq_dil)
```

```
### scaling sequins from coverage values
scaled_features_rlm = scale_features_rlm(f_tibble,sequins, seq_dil)
```

`sequins`

Sequins table

Description

Sequins metadata

Usage

```
data(sequins)
```

Format

An object of class "data.frame"

seq_dil	<i>Sequins dilution table</i>
---------	-------------------------------

Description

Sequins dilution data

Usage

```
data(seq_dil)
```

Format

An object of class "data.frame"

tax.table	<i>phyloseq taxa table from GTDB taxonomy input</i>
-----------	---

Description

A MAG table, similar to OTU table in phyloseq, will be generated from a concatenated GTDB taxa table for bacteria and archaea

Usage

```
tax.table(taxonomy)
```

Arguments

taxonomy	GTDB taxonomy data frame. A taxonomy file in the GTDB output format. Load the bacteria and archaea taxonomy outputs separately. The markdown requires loading the standard output files from GTDB-Tk separately for bacteria and archaea
----------	--

Value

phyloseq-style taxonomy table, but for MAGs

Examples

```
data(taxonomy_tibble)
```

```
### Making phyloseq table from taxonomy metadata
taxonomy.object = tax.table(taxonomy_tibble)
```

taxonomy.object	<i>Taxonomy table in phyloseq format</i>
-----------------	--

Description

Taxonomy table in the format of phyloseq object to be used in the qSIP and (MW-)HR-SIP pipeline

Usage

```
data(taxonomy.object)
```

Format

An object of class "phyloseq"

taxonomy_tibble	<i>Taxonomy table</i>
-----------------	-----------------------

Description

Taxonomy table from GTDB-Tk output - combining both bacterial and archaeal taxonomy

Usage

```
data(taxonomy_tibble)
```

Format

An object of class "data.frame"

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