

Package ‘HTSSIP’

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Type Package

Title High Throughput Sequencing of Stable Isotope Probing Data Analysis

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Description Functions for analyzing high throughput sequencing stable isotope probing (HTS-SIP) data.
Analyses include high resolution stable isotope probing (HR-SIP), multi-window high resolution stable isotope probing (MW-HR-SIP), and quantitative stable isotope probing (q-SIP).

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LazyData TRUE

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as.Num	<i>conversion to numeric</i>
--------	------------------------------

Description

Conducts conversion: as.character → as.numeric

Usage

```
as.Num(x)
```

Arguments

x	single value
---	--------------

Value

numeric

BD_shift	<i>Assessing the magnitude of BD shifts with 16S rRNA community data by calculating the beta diversity between unlabeled control and labeled treatment gradient fraction communities.</i>
----------	---

Description

This function is meant to compare 16S rRNA sequence communities of gradient fractions from 2 gradients: a labeled treatment (eg., 13C-labeled DNA) and its corresponding unlabeled control. First, the beta-diversity (e.g, weighted-Unifrac) is calculated pairwise between fraction communities.

Usage

```
BD_shift(physeq, method = "unifrac", weighted = TRUE, fast = TRUE,
         normalized = FALSE, ex = "Substrate=='12C-Con'",
         perm_method = c("control", "treatment", "overlap", "adjacent"),
         nperm = 100, a = 0.1, parallel_perm = FALSE, parallel_dist = FALSE)
```

Arguments

physeq	phyloseq object
method	See phyloseq::distance
weighted	Weighted Unifrac (if calculating Unifrac)
fast	Fast calculation method
normalized	Normalized abundances

ex	Expression for selecting controls based on metadata
perm_method	"BD shift window" permutation method. See description.
nperm	Number of bootstrap permutations
a	The alpha for calculating confidence intervals
parallel_perm	Calculate bootstrap permutations in parallel
parallel_dist	Calculate beta-diveristy distances in parallel

Details

The `sample_data` table of the user-provided `phyloseq` object **MUST** contain the buoyant density (BD) of each sample (a "Buoyant_density" column in the `sample_data` table). The BD information is used to identify overlapping gradient fractions (gradient fractions usually only partially overlap in BD between gradients) between the labeled treatment gradient and the control gradient. Beta diversity between overlapping fractions is calculated. Then, to standardize the values relative to the unlabeled control (1 beta-diversity value for each control gradient fraction), the mean beta diversity of overlapping labeled treatment gradients is calculated for each unlabeled control, and the percent overlap of each labeled treatment fraction is used to weight the mean.

A permutation test is used to determine "BD shift windows". OTU abundances are permuted, and beta-diversity is calculated. The permutations are used to calculate confidence intervals. The possible permutation methods are:

- "control" = OTU abundances are permuted among all control samples, and these new samples are used as a null treatment. Thus, this provides a baseline beta-diversity distribution that would result from comparing the control fractions to a randomly shuffled version of themselves.
- "treatment" = OTU abundances are permuted among all treatment samples. Thus, a "homogenized" treatment gradient null model.
- "overlap" = OTU abundances are permuted among overlapping control & treatment fractions. Thus, is beta-diversity higher than if the overlapping treatment & control samples were homogenized. This method tends to be too permissive.
- "adjacent" = The null "treatment" communities are generated by permuting OTU abundances among adjacent control fractions. Thus, null model is local gradient region was homogenized.

Value

a `data.frame` object of weighted mean distances

Examples

```
data(physeq_S2D2)
## Not run:
# Subsetting phyloseq by Substrate and Day
params = get_treatment_params(physeq_S2D2, c('Substrate', 'Day'))
params = dplyr::filter(params, Substrate!='12C-Con')
ex = "(Substrate=='12C-Con' & Day=='${Day}') | (Substrate=='${Substrate}' & Day == '${Day}')"
physeq_S2D2_1 = phyloseq_subset(physeq_S2D2, params, ex)
```

```

# Calculating BD_shift on 1 subset (use lapply function to process full list)
wmean1 = BD_shift(physeq_S2D2_1[[1]], nperm=5)

ggplot(wmean1, aes(BD_min.x, wmean_dist)) +
  geom_point()

# Calculating BD_shift on all subsets; using just 5 permutations to speed up analysis
lapply(physeq_S2D2_1, BD_shift, nperm=5)

## End(Not run)

```

calc_atom_excess	<i>Calculate atom fraction excess</i>
------------------	---------------------------------------

Description

See Hungate et al., 2015 for more details

Usage

```
calc_atom_excess(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_Gi	<i>Calculate G+C from unlabeled buoyant density</i>
---------	---

Description

See Hungate et al., 2015 for more details

Usage

```
calc_Gi(Wlight)
```

Arguments

Wlight A vector with ≥ 1 weighted mean BD from 'light' gradient fractions

Value

numeric value (fractional G+C; Gi)

calc_Mheavymax	<i>Calculate the theoretical maximum molecular weight of fully-labeled DNA</i>
----------------	--

Description

See Hungate et al., 2015 for more details

Usage

```
calc_Mheavymax(Mlight, isotope = "13C", Gi = NA)
```

Arguments

Mlight The molecular weight 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

data-physeq_rep3	<i>(Data) A simulated HTS-SIP dataset</i>
------------------	---

Description

6 gradients:

- *12C-control, replicate 1
- *12C-control, replicate 2
- *12C-control, replicate 3
- *13C-treatment, replicate 1
- *13C-treatment, replicate 2
- *13C-treatment, replicate 3

data-physeq_rep3_qPCR (Data) qPCR data associated with the physeq_rep3 HTS-SIP dataset

Description

The dataset contains simulated 16S rRNA copies for each gradient fraction of each gradient (measured via qPCR).

See Also

physeq_rep3

data-physeq_S2D1 (Data) A subset of full HTS-SIP dataset (Substrates=2, Days=1)

Description

1 of the 2 'substrates' is the 12C-control; the other is the 13C-labeled substrate

data-physeq_S2D1_1 (Data) A subset of full HTS-SIP dataset (Substrates=2, Days=1)

Description

1 of the 2 'substrates' is the 12C-control; the other is the 13C-labeled substrate.

Details

The dataset has been parsed into a list of corresponding 13C-treatment vs 12C-control comparisons. Each comparison is of all gradient fraction samples of the treatment vs the gradient fraction samples of the control.

data-physeq_S2D2 (Data) A subset of full HTS-SIP dataset (Substrates=2, Days=2)

Description

1 of the 2 'substrates' is the 12C-control; the other is the 13C-labeled substrate.

data-physeq_S2D2_1 *(Data) A subset of full HTS-SIP dataset (Substrates=2, Days=2)*

Description

1 of the 2 'substrates' is the 12C-control; the other is the 13C-labeled substrate.

Details

The dataset has been parsed into a list of corresponding 13C-treatment vs 12C-control comparisons. Each comparison is of all gradient fraction samples of the treatment vs the gradient fraction samples of the control.

delta_BD *delta_BD calculation*

Description

Calculate delta_BD as described in [Pepe-Ranney et al., 2016](#).

Usage

```
delta_BD(physeq, control_expr, n = 20, BD_min = NULL, BD_max = NULL)
```

Arguments

physeq	Phyloseq object
control_expr	An expression for identifying unlabeled control samples in the phyloseq object (eg., "Substrate=='12C-Con'")
n	How many evenly-spaced buoyant density values to use for linear interpolation of abundances.
BD_min	The minimum BD value of the BD range used for OTU abundance interpolation. If NULL, then BD_min will be the minimum of all BD values in the phyloseq object.
BD_max	The maximum BD value of the BD range used for OTU abundance interpolation. If NULL, then BD_max will be the maximum of all BD values in the phyloseq object.

Details

Basically, the abundance of each OTU is interpolated at specific BD values in order to have abundance values at consistent points across gradients (gradient fraction BDs normally vary from gradient to gradient). The center of mass (CM) is calculated from these interpolated values, which is the weighted mean BD with interpolated OTU abundances used as weights (ie., where in the density gradient contains the 'center' of the OTU abundance distribution). Delta_BD is then calculated by subtracting the CM for the unlabeled control gradient from the labeled treatment gradient.

The delta_BD calculation will be a comparison between unlabeled control and labeled treatment samples. These samples are distinguished from each other with the 'control_expr' parameter. NOTE: if multiple gradients fall into the control or treatment category, they will be treated as one gradient (which may be OK if you want to combine replicate gradients).

NaN values may occur due low abundances.

The BD range used for interpolation is set by the min/max of all buoyant density values in the phyloseq object (standardize across).

Value

data.frame with delta_BD values for each OTU. 'CM' stands for 'center of mass'.

Examples

```
data(physeq_S2D2_1)
# just selecting 1 treatment-control comparison
physeq = physeq_S2D2_1[[1]]

## Not run:
# calculating delta_BD
df = delta_BD(physeq, control_expr='Substrate=="12C-Con"')
head(df)

# In this example, the replicate gradients will be combined for treatments/controls
data(physeq_rep3)
df = delta_BD(physeq_rep3, control_expr='Treatment=="12C-Con"')
head(df)

## End(Not run)
```

Description

The phyloseq object will be filtered to 1) just OTUs that pass the sparsity cutoff 2) just samples in the user-defined 'heavy' fractions. The log2 fold change (l2fc) is calculated between labeled treatment and control gradients.

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.

Details

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

Value

dataframe of HRSIP results

Examples

```
data(physeq_S2D2)
## Not run:
df_l2fc = DESeq2_l2fc(physeq_S2D2, density_min=1.71, density_max=1.75, design=~Substrate)
head(df_l2fc)

## End(Not run)
```

evaluate_matches	<i>Evaluate String Interpolation Matches</i>
------------------	--

Description

The expression part of string interpolation matches are evaluated in a specified environment and formatted for replacement in the original string.

Usage

```
evaluate_matches(matches, env)
```

Arguments

matches	Match data
env	The environment in which to evaluate the expressions.

Value

A character vector of replacement strings.

expr_param_extract	<i>Extract all quoted values in the expression used for phyloseq subsetting.</i>
--------------------	--

Description

This can be useful for creating custom (shorter) labels for each subset relative to using the entire subsetting expression.

Usage

```
expr_param_extract(ex, collapse = NULL)
```

Arguments

ex	Expression for subsetting the phyloseq object
collapse	Similar to the collapse parameter in base: :paste

Value

If `length(ex) == 1`, then a vector of quoted values in the input string. If `length(ex) > 1`, then a matrix or list of quote values, 1 column/index per input string.

Examples

```
ex = '(Substrate=="12C-Con" & Day=="14")'
expr_param_extract(ex)

ex = '(Substrate=="12C-Con" & Day=="14") | (Substrate=="13C-Cel" & Day == "14")'
expr_param_extract(ex)

# returns a matrix
ex = c('(Substrate=="12C-Con" & Day=="14")',
      '(Substrate=="13C-Cel" & Day == "14")')
expr_param_extract(ex)

# returns a list
ex = c('(Substrate=="12C-Con" & Day=="14")',
      '(Substrate=="13C-Cel" & Day == "14")',
      '(Substrate=="13C-Cel")')
expr_param_extract(ex)
```

extract_expressions *Extract Expression Objects from String Interpolation Matches*

Description

An interpolation match object will contain both its wrapping `{ }` part and possibly a format. This extracts the expression parts and parses them to prepare them for evaluation.

Usage

```
extract_expressions(matches)
```

Arguments

matches Match data

Value

list of R expressions

extract_formats	<i>Extract String Interpolation Formats from Matched Placeholders</i>
-----------------	---

Description

An expression placeholder for string interpolation may optionally contain a format valid for `sprintf`. This function will extract such or default to "s" the format for strings.

Usage

```
extract_formats(matches)
```

Arguments

matches	Match data
---------	------------

Value

A character vector of format specifiers.

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

format_metadata	<i>Format phyloseq metadata for calculating BD range overlaps.</i>
-----------------	--

Description

Format phyloseq metadata for calculating BD range overlaps.

Usage

```
format_metadata(physeq, ex = "Substrate=='12C-Con'", rep = "Replicate")
```

Arguments

physeq	Phyloseq object
ex	Expression for selecting the control samples to compare to the non-control samples.
rep	Column specifying gradient replicates. If the column is not present, then all are considered "Replicate=1"

Value

a data.frame object of formatted metadata

Examples

```
## Not run:  
data(physeq_S2D1)  
ex = "Substrate=='12C-Con'"  
metadata = HTSSIP::format_metadata(physeq_S2D1, ex)  
  
## End(Not run)
```

fraction_overlap	<i>Calculate the BD range overlap of gradient fractions</i>
------------------	---

Description

Calculate the BD range overlap of gradient fractions

Usage

```
fraction_overlap(metadata)
```

Arguments

metadata Metadata data.frame object. See format_metadata().

Value

a data.frame object of metadata with fraction BD overlaps

Examples

```
## Not run:
data(physeq_S2D2)
ex = "Substrate=='12C-Con'"
metadata = HTSSIP::format_metadata(physeq_S2D2, ex)
m = HTSSIP::fraction_overlap(metadata)
head(m)

## End(Not run)
```

get_treatment_params *Get parameters for subsetting the phyloseq dataset*

Description

This function is needed if you want to make multiple subsets of the phyloseq object in order to make specific comparisons between isotopically labeled-treatments and

Usage

```
get_treatment_params(physeq, exp_params, treatment = NULL)
```

Arguments

physeq Phyloseq object

exp_params a vector listing the columns in the phyloseq sample_data table that can subset the phyloseq dataset in order to make the specific labeled-treatment vs labeled-control comparisons that you would like to make.

treatment This is an expression used to filter out the control-specific parameters (if needed).

Details

their corresponding controls (eg., from the same time point).

Makes a data.frame of all of the parameter values that differ among the treatment-control comparisons.

For example, if you want to compare the gradient fractions from each labeled-treatment to its corresponding unlabeled-Control (both from the same time point).

Examples

```

data(physeq_S2D2)
# Here, the treatment/controls (12C & 13C) are listed in substrate,
# and should be matched by 'Day'. The 13C-treatments can be identified by
# the expression: "Substrate != '12C-Con'"
get_treatment_params(physeq_S2D2, c('Substrate', 'Day'), "Substrate != '12C-Con'")

```

gradient_sim

Simulate HTS-SIP communities for 1 density gradient

Description

Simulate HTS-SIP communities for 1 density gradient

Usage

```

gradient_sim(locs, params, responseModel = "gaussian",
             countModel = "poisson", ...)

```

Arguments

locs	Buoyant densities of each gradient fraction
params	A matrix of parameters for <code>coenocliner::coenocline()</code> . See that function's documentation for more details.
responseModel	See <code>coenocliner::coenocline()</code>
countModel	See <code>coenocliner::coenocline()</code>
...	Other parameters passed to <code>coenocliner::coenocline()</code>

Value

A data.frame of OTU counts.

Examples

```

# setting parameters
set.seed(2)
M = 10 # number of species (OTUs)
ming = 1.67 # gradient minimum...
maxg = 1.78 # ...and maximum
nfrac = 24 # number of gradient fractions
locs = seq(ming, maxg, length=nfrac) # gradient fraction BD values
tol = rep(0.005, M) # species tolerances
h = ceiling(rlnorm(M, meanlog=11)) # max abundances
opt = rnorm(M, mean=1.7, sd=0.005) # species optima
params = cbind(opt=opt, tol=tol, h=h) # put in a matrix
# simulate the OTU abundances

```



```
df_OTU = gradient_sim(locs, params)
head(df_OTU)
```

heavy_SIP

Heavy-SIP analysis

Description

Compare taxon abundances in 'heavy' fractions versus specific controls.

Usage

```
heavy_SIP(physeq, ex = "Substrate=='12C-Con'", rep = "Replicate",
  light_window = c(1.68, 1.7), heavy_window = c(1.73, 1.75),
  comparison = c("H", "H-v-L", "H-v-H"), hypo_test = c("binary", "t-test",
  "wilcox"), alternative = c("greater", "two.sided", "less"),
  sparsity_threshold = 0.1, sparsity_apply = c("all", "heavy"),
  padj_method = "BH")
```

Arguments

physeq	A phyloseq object of just treatment vs control. If you have a more complicated experimental design, subset the phyloseq object into a list of treatment vs control comparisons.
ex	Expression for selecting controls based on metadata
rep	Column specifying gradient replicates. If the column does not exist, then the column will be created, and all will be considered "replicate=1"
light_window	A vector designating the "light" BD window start and stop
heavy_window	A vector designating the "heavy" BD window start and stop
comparison	Which light/heavy BD windows to compare (see the description)?
hypo_test	Which hypothesis test to run on each OTU? Note that "binary" isn't really a hypothesis test, but just qualitative.
alternative	The "alternative" option for the hypothesis test functions. Note that "two.sided" doesn't work for the "binary" test.
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')
padj_method	Multiple hypothesis correction method. See 'p.adjust()' for more details.

Details

'Heavy-SIP' encompasses the analyses often used in SIP studies prior to new HTS-SIP methodologies. These methods all consisted of identifying 'heavy' gradient fractions. This was often done by comparing the distribution of DNA conc. or gene copies across gradient fractions in labeled treatments versus unlabeled controls. Sometimes, the unlabeled control was left out, and "heavy" gradients were identified based on comparisons with theoretic distributions of unlabeled DNA.

Although hypothesis testing was often used to assess increased taxon abundances in "heavy" gradients of labeled treatments (eg., one-tailed t-tests), the hypothesis testing usually did not account for the compositional nature of sequence data (relative abundances).

Here, "heavy-SIP" can define incorporators as either:

- "H" = Any taxa IN the "heavy" fractions of the labeled treatment gradients
- "H-v-L" = Any taxa IN the "heavy" fractions of the labeled treatment and NOT present in the "heavy" fractions of the control
- "H-v-H" = Any taxa IN the "heavy" fractions of the labeled treatment and NOT present in the "light" fractions of the labeled treatment

Instead of binary comparisons (presence/absence), one-tailed t-tests or Wilcoxon Rank Sum tests can be used to assess differential abundance between "heavy" and controls. The hypothesis testing methods require multiple replicate controls, will use the mean taxon abundance in the "heavy" (and "light") window.

Value

a data.frame object of hypothesis test results

Examples

```
data(physeq_S2D2)
data(physeq_rep3)
## Not run:
# Calculating 'binary' for unreplicated experiment
## Subsetting phyloseq by Substrate and Day
params = get_treatment_params(physeq_S2D2, c('Substrate', 'Day'))
params = dplyr::filter(params, Substrate!='12C-Con')
ex = "(Substrate=='12C-Con' & Day=='${Day}') | (Substrate=='${Substrate}' & Day == '${Day}')"
physeq_S2D2_1 = phyloseq_subset(physeq_S2D2, params, ex)

## Calculating heavy-SIP on 1 subset (use lapply function to process full list)
incorps = heavy_SIP(physeq_S2D2_1[[1]])

# Calculating wilcox test on replicated design
## (comparing heavy-treatment versus heavy-control)
incorps = heavy_SIP(physeq_rep3, ex="Treatment=='12C-Con'", comparison='H-v-H', hypo_test='wilcox')

## End(Not run)
```

HRSIP *(MW-)HR-SIP analysis*

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 log ₂ 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).
sparsity_threshold	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.
sparsity_apply	Apply sparsity threshold to all gradient fraction samples ('all') or just 'heavy' fraction samples ('heavy'), where 'heavy' samples are designated by the density_windows.
l2fc_threshold	log ₂ fold change (l2fc) values must be significantly above this threshold in order to reject the hypothesis of equal counts. See DESeq2 for more information.
padj_method	Method for global p-value adjustment (See p.adjust()).
padj_cutoff	Adjusted p-value cutoff for rejecting the null hypothesis that l2fc values were not greater than the l2fc_threshold. 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 log ₂ fold change.
parallel	Process each parameter combination in parallel. See plyr::mapply() 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(physeq_S2D2_1)

## Not run:
# HR-SIP on just 1 treatment-control comparison
## 1st item in list of phyloseq objects
physeq = physeq_S2D2_1[[1]]
## HR-SIP
### Note: treatment-control samples differentiated with 'design=~Substrate'
df_l2fc = HRSIP(physeq, design=~Substrate)
head(df_l2fc)

## Same, but multiple BD windows (MW-HR-SIP) & run in parallel
### Windows = 1.7-1.73 & 1.72-1.75
doParallel::registerDoParallel(2)
dw = data.frame(density_min=c(1.7, 1.72), density_max=c(1.73, 1.75))
df_l2fc = HRSIP(physeq_S2D1_1[[1]],
               design=~Substrate,
               density_windows=dw,
               parallel=TRUE)

head(df_l2fc)

## End(Not run)
```

HTSSIP

HTSSIP: analyzing high throughput sequence data from nucleotide stable isotope probing experiments

Description

HTSSIP provides a toolset for reproducibly analyzing HTS-SIP data. HTS-SIP data is the combination of nucleotide stable isotope probing (DNA- & RNA-SIP) and high throughput sequence data (e.g., MiSeq of 16S rRNA amplicons).

Details

To learn more about HTSSIP, start with the vignettes: `browseVignettes(package = "HTSSIP")`

HTSSIP_sim

*Simulate a HTS-SIP dataset***Description**

This is a simple method for simulating high throughput sequencing stable isotope probing datasets and is mainly used for package testing purposes. See SIPSim for more detailed and simulation pipeline.

Usage

```
HTSSIP_sim(locs, params, responseModel = "gaussian", countModel = "poisson",
  meta = NULL, sim_tree = FALSE, parallel = FALSE, ...)
```

Arguments

locs	Buoyant densities of each gradient fraction
params	A matrix of parameters for <code>coenocliner::coenocline()</code> . See that function's documentation for more details.
responseModel	See <code>coenocliner::coenocline()</code>
countModel	See <code>coenocliner::coenocline()</code>
meta	Data.frame object of metadata to add to <code>sample_data</code> table. The data.frame object must have a 'Gradient' column, which is used for joining with <code>dplyr::left_join()</code> .
sim_tree	Simulate a tree?
parallel	Parallel processing. See <code>.parallel</code> option in <code>dplyr::mdply()</code> for more details.
...	Other parameters passed to <code>coenocliner::coenocline()</code>

Value

A phyloseq object

Examples

```
# setting parameters for tests
set.seed(2)
M = 10 # number of species
ming = 1.67 # gradient minimum...
maxg = 1.78 # ...and maximum
nfrac = 24 # number of gradient fractions
locs = seq(ming, maxg, length=nfrac) # gradient locations
tol = rep(0.005, M) # species tolerances
h = ceiling(rlnorm(M, meanlog=11)) # max abundances
## creating parameter matrices for each density gradient
opt1 = rnorm(M, mean=1.7, sd=0.005) # species optima
params1 = cbind(opt=opt1, tol=tol, h=h) # put in a matrix
opt2 = rnorm(M, mean=1.7, sd=0.005) # species optima
```

```

params2 = cbind(opt=opt2, tol=tol, h=h) # put in a matrix
param_l = list(
  '12C-Con_rep1' = params1,
  '13C-Cel_rep1' = params2
)
## Not run:
# simulating phyloseq object
physeq = HTSSIP_sim(locs, param_l)
physeq

## End(Not run)

```

match_brace

Utility Function for Matching a Closing Brace

Description

Given positions of opening and closing braces match_brace identifies the closing brace matching the first opening brace.

Usage

```
match_brace(opening, closing)
```

Arguments

opening integer: Vector with positions of opening braces.
closing integer: Vector with positions of closing braces.

Value

Integer with the position of the matching brace.

match_placeholders

Match Expression Placeholders for String Interpolation

Description

Given a character string a set of expression placeholders are matched. They are of the form `${...}` or optionally `$(f){...}` where `f` is a valid format for `sprintf`.

Usage

```
match_placeholders(string)
```

Arguments

string character: The string to be interpolated.

Value

list containing indices (regex match data) and matches, the string representations of matched expressions.

max_BD_range	<i>Adjusting BD range size if negative.</i>
--------------	---

Description

If BD (buoyant density) range size is negative, use BD_to_set value to set new BD_max. The BD_to_set determines the BD_max if BD range is negative

Usage

```
max_BD_range(BD_range, BD_min, BD_max, BD_to_set)
```

Arguments

BD_range	BD range size
BD_min	Minimum BD value
BD_max	Maximum BD value
BD_to_set	Value added to BD_min to set new BD_max

Value

New max BD value

OTU_qPCR_trans	<i>Transform OTU counts based on qPCR data</i>
----------------	--

Description

OTU counts in the phyloseq otu_table object will be normalized to sample totals (total sum scaling), then multiplied by the qPCR value associated with each sample. Thus, the qPCR table should have ONE value matching the OTU count table. Value matching between the OTU table & qPCR value table to set by sample_idx().

Usage

```
OTU_qPCR_trans(physeq, qPCR, sample_idx = "Sample",
  value_idx = "qPCR_tech_rep_mean")
```

Arguments

physeq	A phyloseq object
qPCR	Either a list or a data.frame of qPCR data. If a list, the list should include a 'summary' tag as is produced from qPCR_sim(). If a data.frame, the table should be formatted as the 'summary' table produced from qPCR_sim().
sample_idx	The qPCR table column index for matching to otu table samples.
value_idx	The qPCR table column index for qPCR values.

Details

Note: only the 'summa

Value

A phyloseq object with transformed OTU counts

Examples

```
# qPCR data simulation
data(physeq_rep3)
data(physeq_rep3_qPCR)
physeq_rep3_t = OTU_qPCR_trans(physeq_rep3, physeq_rep3_qPCR)
```

overlap_wmean_dist	<i>Calculating weighted mean beta-diversities of overlapping gradient fractions.</i>
--------------------	--

Description

Calculating weighted mean beta-diversities of overlapping gradient fractions.

Usage

```
overlap_wmean_dist(df_dist)
```

Arguments

df_dist	Filtered distance matrix in data.frame format. See parse_dist()
---------	---

Value

a data.frame object of weighted mean distances

parse_dist	<i>Filtering out non-relevant distances in distance matrix</i>
------------	--

Description

Filtering out non-relevant distances in distance matrix

Usage

```
parse_dist(d)
```

Arguments

d a distance matrix object

Value

a data.frame object of metadata with fraction BD overlaps

Examples

```
## Not run:
data(physeq_S2D2)
physeq_S2D2_d = phyloseq::distance(physeq_S2D2,
                                  method='unifrac',
                                  weighted=TRUE,
                                  fast=TRUE,
                                  normalized=FALSE)
physeq_S2D2_d = HTSSIP:::parse_dist(physeq_S2D2_d)
head(physeq_S2D2_d)

## End(Not run)
```

perc_overlap	<i>Calculate the percent overlap between two ranges (x & y).</i>
--------------	--

Description

The fraction of overlap is relative to Range X (see examples).

Usage

```
perc_overlap(x.start, x.end, y.start, y.end)
```

Arguments

x.start	The start value for Range X
x.end	The end value for Range X
y.start	The start value for Range Y
y.end	The end value for Range Y

Value

the percent overlap of the ranges

Examples

```
## Not run:  
x = HTSSIP::perc_overlap(0, 1, 0, 0.5)  
stopifnot(x == 50)  
x = HTSSIP::perc_overlap(0, 0.5, 0, 1)  
stopifnot(x == 100)  
  
## End(Not run)
```

phyloseq2df

phyloseq data object conversion to data.frame

Description

Conducts conversion of 1 of the data objects in a phyloseq object (eg., tax_table) to a dataframe

Usage

```
phyloseq2df(physeq, table_func)
```

Arguments

physeq	Phyloseq object
table_func	See <code>Phyloseq::phyloseq-class</code> for options

Value

data.frame

Examples

```
data(physeq_S2D1)
df_otu = phyloseq2df(physeq_S2D1, table_func=phyloseq::otu_table)
head(df_otu)

df_sample = phyloseq2df(physeq_S2D1, table_func=phyloseq::sample_data)
head(df_sample)
```

phyloseq2table	<i>Phyloseq conversion to a ggplot-formatted table</i>
----------------	--

Description

Convert the OTU table (+ metadata) to a format that can be easily plotted with phyloseq

Usage

```
phyloseq2table(physeq, include_sample_data = FALSE, sample_col_keep = NULL,
  include_tax_table = FALSE, tax_col_keep = NULL, control_expr = NULL)
```

Arguments

physeq	Phyloseq object
include_sample_data	Include sample_table information?
sample_col_keep	Which columns in the sample_data table to keep? Use NULL to keep all columns.
include_tax_table	Include tax_table information?
tax_col_keep	A vector for column names to keep. Use NULL to keep all columns.
control_expr	An expression for identifying which samples are controls. Control/non-control identification will be in the 'IS_CONTROL' column of the returned data.frame object.

Value

data.frame

Examples

```
data(physeq_S2D1)
# Including some columns from sample metadata
df_OTU = phyloseq2table(physeq_S2D1,
  include_sample_data=TRUE,
  sample_col_keep=c('Buoyant_density', 'Substrate', 'Day'))
head(df_OTU)
```

```
## Not run:
# Including some columns from sample metadata & taxonomy
df_OTU = phyloseq2table(physeq_S2D1,
                        include_sample_data=TRUE,
                        sample_col_keep=c('Buoyant_density', 'Substrate', 'Day'),
                        include_tax_table=TRUE)

head(df_OTU)

## End(Not run)
```

phyloseq_list_ord_dfs *Converting ordination objects to data.frames*

Description

For each ordination object in a list, converts to a data.frame for easy plotting with ggplot

Usage

```
phyloseq_list_ord_dfs(physeq_l, physeq_l_ords, parallel = FALSE)
```

Arguments

physeq_l	A list of phyloseq objects
physeq_l_ords	A list of ordination objects
parallel	Parallel processing. See <code>plyr::adply</code> for more information.

Value

List of data.frame objects

Examples

```
data(physeq_S2D2_1)
## Not run:
# make a list of beta diversity distance matrix objects
physeq_S2D2_1_d = physeq_list_betaDiv(physeq_S2D2_1)
# make a list of ordinations
physeq_S2D2_1_d_ord = physeq_list_ord(physeq_S2D2_1, physeq_S2D2_1_d)
# convert ordination information to data.frame objects
physeq_S2D2_1_d_ord_df = phyloseq_list_ord_dfs(physeq_S2D2_1, physeq_S2D2_1_d_ord)

## End(Not run)
```

phyloseq_ord_plot *Plotting beta diversity ordination*

Description

For each data.frame object in a list (converted from ordination objects), creates a ggplot figure.

Usage

```
phyloseq_ord_plot(physeq_ord_df, title = NULL,
  point_size = "Buoyant_density", point_fill = "Substrate",
  point_alpha = 0.5, point_shape = NULL)
```

Arguments

physeq_ord_df	A list of data.frame objects (see phyloseq_list_ord_dfs)
title	Plot title
point_size	The data.frame column determining point size
point_fill	The data.frame column determining point fill color
point_alpha	The data.frame column (or just a single value) determining point alpha
point_shape	The data.frame column (or just a single value) determining point shape

Value

ggplot2 object

Examples

```
data(physeq_S2D2_1)
## Not run:
# make a list of beta diversity distance matrix objects
physeq_S2D2_1_d = physeq_list_betaDiv(physeq_S2D2_1)
# make a list of ordinations
physeq_S2D2_1_d_ord = physeq_list_ord(physeq_S2D2_1, physeq_S2D2_1_d)
# convert ordination information to data.frame objects
physeq_S2D2_1_d_ord_df = phyloseq_list_ord_dfs(physeq_S2D2_1, physeq_S2D2_1_d_ord)
# make ordination plots with ggplot2
phyloseq_ord_plot(physeq_S2D2_1_d_ord_df)

## End(Not run)
```

phyloseq_subset	<i>Make a list of phyloseq object subsets</i>
-----------------	---

Description

Create a list of phyloseq object subsets based on phyloseq sample data parameters (e.g., a phyloseq subset for each treatment)

Usage

```
phyloseq_subset(physeq, params, ex)
```

Arguments

physeq	Phyloseq object
params	data.frame of parameters supplies to ex
ex	Expression for subsetting the phyloseq object

Value

A list of Phyloseq objects

Examples

```
data(physeq_S2D2)
# making subsets by substrate and time point
params = get_treatment_params(physeq_S2D2, c('Substrate', 'Day'))
# filtering out controls
params = dplyr::filter(params, Substrate!='12C-Con')
# making expression for subsetting labeled-unlabeled gradient comparisons
ex = "(Substrate=='12C-Con' & Day=='${Day}') | (Substrate=='${Substrate}' & Day == '${Day}')"
physeq_l = phyloseq_subset(physeq_S2D2, params, ex)
physeq_l
```

physeq_format	<i>Checking format of phyloseq object for HTSSIP compatibility</i>
---------------	--

Description

Checking format of phyloseq object for HTSSIP compatibility

Usage

```
physeq_format(physeq)
```

Arguments

physeq Phyloseq object

Value

phyloseq object

Examples

```
# this data should be formatted for HTSSIP
data(physeq_S2D2)
physeq_format(physeq_S2D2)

# this data should NOT be correctly formatted for HTSSIP
## Not run:
library(phyloseq)
data(GlobalPatterns)
tryCatch(
  physeq_format(GlobalPatterns),
  function(e) e
)

## End(Not run)
```

physeq_list_betaDiv *calculating beta diversity for a list of phyloseq objects*

Description

For each phyloseq object in a list, calculates beta-diversity between all samples using the phyloseq::distance function.

Usage

```
physeq_list_betaDiv(physeq_l, method = "unifrac", weighted = TRUE,
  fast = TRUE, normalized = TRUE, parallel = FALSE)
```

Arguments

physeq_l	A list of phyloseq objects
method	See phyloseq::distance
weighted	Weighted Unifrac (if calculating Unifrac)
fast	Fast calculation method
normalized	Normalized abundances
parallel	Calculate in parallel

Details

Note: for calculating Unifrac values, phyloseq will select a root at random if the input phylogeny is not rooted.

Value

List of dist objects

Examples

```
data(physeq_S2D2_1)
## Not run:
physeq_S2D2_1_d = physeq_list_betaDiv(physeq_S2D2_1)

## End(Not run)
```

physeq_list_ord	<i>calculating ordinations from a list of distance matrices</i>
-----------------	---

Description

For each dist object in a provided list, the function calculates an ordination with the phyloseq::ordinate function.

Usage

```
physeq_list_ord(physeq_l, physeq_l_d, ord_method = "NMDS")
```

Arguments

physeq_l	A list of phyloseq objects
physeq_l_d	A list of dist objects
ord_method	See phyloseq::ordinate

Value

List of ordination objects

Examples

```
data(physeq_S2D2_1)
## Not run:
# make a list of beta diversity distance matrix objects
physeq_S2D2_1_d = physeq_list_betaDiv(physeq_S2D2_1)
# make a list of ordinations
physeq_S2D2_1_d_ord = physeq_list_ord(physeq_S2D2_1, physeq_S2D2_1_d)

## End(Not run)
```


qPCR_sim

*Simulate qPCR values***Description**

qPCR values will be simulated for each sample in the provided phyloseq object. The error distribution for each sample is drawn from a Gaussian distribution, where the mean and standard deviation of the Gaussian distribution are set by user-defined functions. The user-defined functions that take buoyant density as input and returns a numeric value (see examples), which allows the qPCR values to increase in mean & variance at certain buoyant densities.

Usage

```
qPCR_sim(physeq, control_mean_fun, control_sd_fun, treat_mean_fun, treat_sd_fun,
         n_tech_rep = 3, control_expr = NULL)
```

Arguments

physeq	Object of class "phyloseq"
control_mean_fun	Function used for simulating the qPCR normal distribution mean for control samples.
control_sd_fun	Function used for simulating the qPCR normal distribution standard deviation for control samples.
treat_mean_fun	Function used for simulating the qPCR normal distribution mean for treatment samples.
treat_sd_fun	Function used for simulating the qPCR normal distribution standard deviation for treatment samples.
n_tech_rep	Number of technical replicates.
control_expr	Expression used to identify control samples based on sample_data.

Value

data.frame of qPCR values

Examples

```
# making functions for simulating values
## 'x' will be Buoyant_density as defined in the phyloseq object sample_data
control_mean_fun = function(x) dnorm(x, mean=1.70, sd=0.01) * 1e8
## This will set sd to scale with the mean
control_sd_fun = function(x) control_mean_fun(x) / 3
## This will 'shift' the gene copy distribution to 'heavier' BDs
treat_mean_fun = function(x) dnorm(x, mean=1.75, sd=0.01) * 1e8
treat_sd_fun = function(x) treat_mean_fun(x) / 3
# simulating qPCR values
```

```

df_qPCR = qPCR_sim(physeq_S2D2,
                    control_expr='Substrate=="12C-Con"',
                    control_mean_fun=control_mean_fun,
                    control_sd_fun=control_sd_fun,
                    treat_mean_fun=treat_mean_fun,
                    treat_sd_fun=treat_sd_fun)

# using the Cauchy distribution instead of normal distributions
control_mean_fun = function(x) dcauchy(x, location=1.70, scale=0.01) * 1e8
control_sd_fun = function(x) control_mean_fun(x) / 3
treat_mean_fun = function(x) dcauchy(x, location=1.74, scale=0.01) * 1e8
treat_sd_fun = function(x) treat_mean_fun(x) / 3
# simulating qPCR values
df_qPCR = qPCR_sim(physeq_S2D2,
                    control_expr='Substrate=="12C-Con"',
                    control_mean_fun=control_mean_fun,
                    control_sd_fun=control_sd_fun,
                    treat_mean_fun=treat_mean_fun,
                    treat_sd_fun=treat_sd_fun)

```

qSIP_atom_excess	<i>Calculate atom fraction excess using q-SIP method</i>
------------------	--

Description

Calculate atom fraction excess using q-SIP method

Usage

```

qSIP_atom_excess(physeq, control_expr, treatment_rep = NULL,
                 isotope = "13C", df_OTU_W = NULL)

```

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

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

```
# tranforming values
physeq_rep3_t = OTU_qPCR_trans(physeq_rep3, physeq_rep3_qPCR)

## Not run:
# BD shift (Z) & atom excess (A)
atomX = qSIP_atom_excess(physeq_rep3_t,
                          control_expr='Treatment=="12C-Control"',
                          treatment_rep='Replicate')

## End(Not run)
```

qSIP_atom_excess_format

Reformat a phyloseq object of qSIP_atom_excess analysis

Description

Reformat a phyloseq object of qSIP_atom_excess analysis

Usage

```
qSIP_atom_excess_format(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_bootstrap	<i>Calculate bootstrap CI for atom fraction excess using q-SIP method</i>
----------------	---

Description

Calculate bootstrap CI for atom fraction excess using q-SIP method

Usage

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

Arguments

atomX	A list object created by qSIP_atom_excess()
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.
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.

Examples

```
# transforming values
physeq_rep3_t = OTU_qPCR_trans(physeq_rep3, physeq_rep3_qPCR)

## Not run:
# BD shift (Z) & atom excess (A)
atomX = qSIP_atom_excess(physeq_rep3_t,
  control_expr='Treatment=="12C-Con"',
  treatment_rep='Replicate')

# bootstrapping in parallel
doParallel::registerDoParallel(2)
df_atomX_boot = qSIP_bootstrap(atomX, parallel=TRUE)
head(df_atomX_boot)

## End(Not run)
```

SIP_betaDiv_ord	<i>Calculating & plotting beta diversity for a list of phyloseq objects</i>
-----------------	---

Description

For each phyloseq object in a list, calculates beta-diversity between all samples using the phyloseq::distance function.

Usage

```
SIP_betaDiv_ord(physeq_l, method = "unifrac", weighted = TRUE,  
fast = TRUE, normalized = TRUE, parallel = FALSE, plot = FALSE)
```

Arguments

physeq_l	A list of phyloseq objects
method	See phyloseq::distance
weighted	Weighted Unifrac (if calculating Unifrac)
fast	Fast calculation method
normalized	Normalized abundances
parallel	Calculate in parallel
plot	Return a plot (instead of a data.frame of ordination data)

Value

If plot==FALSE, a data.frame object of beta-diversity values. If plot==TRUE, a glob object for plotting.

Examples

```
data(physeq_S2D2_1)  
## Not run:  
physeq_S2D2_1_df = SIP_betaDiv_ord(physeq_S2D2_1)  
head(physeq_S2D2_1_df, n=3)  
  
## End(Not run)
```

stringterpolate *String Interpolation*

Description

String interpolation is a useful way of specifying a character string which depends on values in a certain environment. It allows for string creation which is easier to read and write when compared to using e.g. `paste` or `sprintf`. The (template) string can include expression placeholders of the form `${expression}` or `#[format]{expression}`, where expressions are valid R expressions that can be evaluated in the given environment, and `format` is a format specification valid for use with `sprintf`.

Usage

```
stringterpolate(string, env = parent.frame())
```

Arguments

<code>string</code>	A template character string.
<code>env</code>	The environment in which to evaluate the expressions.

Value

An interpolated character string.

tss *Total sum scaling*

Description

Total sum scaling

Usage

```
tss(x, MARGIN = 2, na.rm = FALSE)
```

Arguments

<code>x</code>	data.frame of numeric values
<code>MARGIN</code>	table margin (1=rows, 2=columns)
<code>na.rm</code>	remove NAs?

Value

data.frame of qPCR values

Examples

```
# making functions for simulating values
df = data.frame(1:5, 5:9)
df_t = tss(df)
apply(df_t, 2, sum)
```

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