microbialPhenotypes: An R package that analyzes high-throughput microbial phenotype data

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Abstract

Various microbial high-throughput phenotyping techniques have been vastly conducted to infer functions of genes, generating large numbers of valuable datasets whose potential in providing insights to characterize genes hasn't been fully exploited. Therefore, computational tools that allow unbiased, systematic analysis of these data also have become vital. Here we describe a package that evaluates high-throughput microbial phenotype data by one or several sets of associated functional annotations are provided. In addition, some helper functions are provided to help clean high-throughput microbial phenotype data.

Introduction

Phenotypes play important roles in characterizing the functions of genes, particularly in microbiology, where the largest number of tests could be done much easier (Tohsato & Mori, 2008). With rising technologies (Kritikos et al., 2017; Nichols et al., 2011; Wetmore et al., 2015), high-throughput experimental approaches that measure large number of phenotypes under various conditions have flourished with complementary statistical methods in querying the behavior of gene products (Collins, Schuldiner, Krogan, & Weissman, 2006; Nichols et al., 2011; Price et al., 2018; Rishi et al., 2020). Although there are already many computational approaches written as R packages to analyze phenotype data (Deng, Gao, Wang, & Guo, 2015; Vaas et al., 2013; Vuckovic, Gasparini, Soranzo, & Iotchkova, 2015) (Vehkala, Shubin, Connor, Thomson, & Corander, 2015), software that quickly tests the potential of such high-throughput data in interpreting gene functions is lacking. Here we have wrapped the analytical pipeline of evaluating the phenotype data using annotation sets into a package named microbialPhenotypes. In this package, we provide 8 functions that are specific in dealing with high-throughput microbial phenotype data, as well as 10 functions that are meant to be more supplementary. A high-throughput *E. coli* phenotype dataset (Nichols et al., 2011) is used for the examples provided below. We note that there is room for significant improvement when the analytical pipeline is improved. Despite that the functions provided here started from a perspective of gaining biological insights for microbial phenotype data, the potential of using them as a tool for more general purposes should not be limited. If there are data from other

research domains that has a similar structure to the example described here, the utility of this package can be much more extended. For example, data from animal cells (Alonezi et al., 2016, 2017) . In addition, knowing that there are resources for complicated machine learning algorithms to do classification of functions, our work here does not aim to improve those methods. Rather, it tries to quickly assess the usefulness of the newly generated high-throughput phenotype data before implementing more advanced classification schema

Installation and functions

The **microPhenotypes** package can be downloaded from GitHub: <https://github.com/peterwu19881230/microbialPhenotypes>. To load the functions, source the .R files under R/.

Input data

This package assumes the input phenotypic profiles be in flat files (e.g., csv or tsv files) in a matrix format where each row represents a mutant strain and each column is a condition where the phenotypes of the mutant strains are assayed. The values in the columns represent the phenotypes.

Users can read their data into R using either the `read.csv()` or `read.table()` function with the appropriate arguments based on the format of the file.

For example:

```
> phenotype_data <- read.csv(file="my_phenotype_profile.csv", header = TRUE)
> head(phenotype_data)
```

	STREPTOMYCIN.0.05	SUCCINATE	SULFAMONOMETHOXINE.100	TAUROCHOLATE.0.1	TAUROCHOLATE.0.5
ECK3997-purD	-1.48	-12.30	3.33	-13.46	-13.79
ECK0516-purE	0.43	-6.75	2.63	-8.27	-9.81
ECK3763-ilvD	-1.41	-11.72	0.34	-0.69	0.32
ECK3766-ilvC	-0.58	-8.53	-0.81	0.06	0.51
ECK3762-ilvE	-0.01	-11.93	0.02	-0.68	-0.46
ECK3764-ilvA	-0.15	-11.55	-0.84	-0.04	-0.19

Discretize the input data

Quantitative data can be transformed into categorical data using either the `BinaryConvert()` or `TernaryConvert()` function, which produce output in a binary (0,1) or ternary (-1,0,1) form, respectively. The threshold argument is used to specify the phenotypic score cutoff used to select strains with a phenotype that is significantly different from that of the designated control, which is usually the phenotype of the wildtype.

For example:

```
> ter_phenotype <- ternary_convert(matrix=phenotype_data, threshold =0.5)
> head(ter_phenotype)
```

	STREPTOMYCIN.0.05	SUCCINATE	SULFAMONOMETHOXINE.100	TAUROCHOLATE.0.1	TAUROCHOLATE.0.5
ECK3997-purD	-1	-1	1	-1	-1
ECK0516-purE	0	-1	1	-1	-1
ECK3763-ilvD	-1	-1	0	-1	0
ECK3766-ilvC	-1	-1	-1	0	1
ECK3762-ilvE	0	-1	0	-1	0
ECK3764-ilvA	0	-1	-1	0	0

```
> binary_phenotype <- binary_convert (matrix=phenotype_data, threshold =0.5)
> head(binary_phenotype)
```

	STREPTOMYCIN.0.05	SUCCINATE	SULFAMONOMETHOXINE.100	TAUROCHOLATE.0.1	TAUROCHOLATE.0.5
ECK3997-purD	1	1	1	1	1
ECK0516-purE	0	1	1	1	1
ECK3763-ilvD	1	1	0	1	0
ECK3766-ilvC	1	1	1	0	1
ECK3762-ilvE	0	1	0	1	0
ECK3764-ilvA	0	1	1	0	0

Calculate similarities/distances between phenotypic profiles

The pairwise similarity/distance between phenotypic profiles can be calculated by a variety of functions, such as Hamming distance, Pearson Correlation Coefficient, Spearman Correlation Coefficient, Mutual Information. The method selected will depend on the type of assay used.

The function implemented in this package is hamming distance:

```
> hamming_dist=hamming_distance(head(ter_phenotype))
> hamming_dist
```

	ECK3997-purD	ECK0516-purE	ECK3763-ilvD	ECK3766-ilvC	ECK3762-ilvE	ECK3764-ilvA
ECK3997-purD	0	1	2	3	3	4
ECK0516-purE	1	0	3	4	2	3
ECK3763-ilvD	2	3	0	3	1	3
ECK3766-ilvC	3	4	3	0	4	2
ECK3762-ilvE	3	2	1	4	0	2
ECK3764-ilvA	4	3	3	2	2	0

Example for a self-defined function not included in this package. Pearson Correlation Coefficient was chosen because it's widely used in high-throughput microbial phenotype data:

```
> pearson_dist=1- cor(t(phenotype_data),method="pearson")
> pearson_dist
```

	ECK3997-purD	ECK0516-purE	ECK3763-ilvD	ECK3766-ilvC	ECK3762-ilvE	ECK3764-ilvA
ECK3997-purD	0.00000000	0.01130833	0.67443135	0.792573199	0.614310165	0.709983271
ECK0516-purE	0.01130833	0.00000000	0.78293109	0.891990335	0.706927257	0.800223580
ECK3763-ilvD	0.67443135	0.78293109	0.00000000	0.015107990	0.012529945	0.017520039
ECK3766-ilvC	0.79257320	0.89199034	0.01510799	0.000000000	0.017882605	0.006075217
ECK3762-ilvE	0.61431017	0.70692726	0.01252994	0.017882605	0.000000000	0.005903022
ECK3764-ilvA	0.70998327	0.80022358	0.01752004	0.006075217	0.005903022	0.000000000

Parse gene annotations

In order to link the functional annotations associated with a gene to the phenotypic profile of a strain where that gene is mutated, three functions, `attr_list()`, `one_attr()` and `generate_pairs_similarity_coannotation()`, are provided where:

`attr_list()` takes in a table that has mapping from mutant ids to their annotations and form a list from such mapping, for example, if starting from inputting the strain names and corresponding annotations:

`attr_list()` creates a list takes where each strain is associated with all the functional annotations and creates a list. To illustrate this, we will create a table with the name “name_attribute”:

```
> name_attribute = data.frame(id=c("ECK3762-ilvE", "ECK3762-ilvE", "ECK3762-ilvE",
"ECK3762-ilvE", "ECK3762-ilvE", "ECK3762-ilvE", "ECK3762-ilvE", "ECK3762-ilvE", "ECK3764-ilvA", "ECK3764-ilvA", "ECK3764-ilvA",
"ECK3997-purD", "ECK3997-purD", "ECK3997-purD", "ECK3997-purD", "ECK3997-
purD", "ECK0516-purE", "ECK0516-purE", "ECK0516-purE", "ECK3763-ilvD",
"ECK3763-ilvD", "ECK3763-ilvD", "ECK3766-ilvC", "ECK3766-ilvC",
"ECK3766-ilvC", "ECK3766-ilvC", "ECK3766-ilvC", "ECK3766-ilvC"),
annot=c("ALANINE-VALINESYN-PWY", "THREOCAT-PWY", "ALL-CHORISMATE-
PWY", "PWY0-1061", "PHESYN", "ILEUSYN-PWY", "LEUSYN-PWY", "VALSYN-
PWY", "COMPLETE-ARO-PWY", "BRANCHED-CHAIN-AA-SYN-PWY",
"THREOCAT-PWY", "ILEUSYN-PWY", "BRANCHED-CHAIN-AA-SYN-PWY", "PRPP-
PWY", "DENOVO PURINE2-PWY", "PWY-6121", "PWY-6122", "PWY-6277", "PRPP-
PWY", "PWY-6123", "DENOVO PURINE2-PWY", "THREOCAT-PWY", "ILEUSYN-
PWY", "VALSYN-PWY", "BRANCHED-CHAIN-AA-SYN-PWY", "THREOCAT-PWY",
"PANTO-PWY", "ILEUSYN-PWY", "VALSYN-PWY", "PANTOSYN-PWY",
"BRANCHED-CHAIN-AA-SYN-PWY"))
```

```
> name_attribute
```

ids	Pwy
1 ECK3762-ilvE	ALANINE-VALINESYN-PWY
2 ECK3762-ilvE	THREOCAT-PWY
3 ECK3762-ilvE	ALL-CHORISMATE-PWY
4 ECK3762-ilvE	PWY0-1061
5 ECK3762-ilvE	PHESYN
6 ECK3762-ilvE	ILEUSYN-PWY
7 ECK3762-ilvE	LEUSYN-PWY
8 ECK3762-ilvE	VALSYN-PWY
9 ECK3762-ilvE	COMPLETE-ARO-PWY
10 ECK3762-ilvE	BRANCHED-CHAIN-AA-SYN-PWY
11 ECK3764-ilvA	THREOCAT-PWY
12 ECK3764-ilvA	ILEUSYN-PWY
13 ECK3764-ilvA	BRANCHED-CHAIN-AA-SYN-PWY
14 ECK3997-purD	PRPP-PWY
15 ECK3997-purD	DENOVO PURINE2-PWY
16 ECK3997-purD	PWY-6121
17 ECK3997-purD	PWY-6122
18 ECK3997-purD	PWY-6277
19 ECK0516-purE	PRPP-PWY
20 ECK0516-purE	PWY-6123
21 ECK0516-purE	DENOVO PURINE2-PWY
22 ECK3763-ilvD	THREOCAT-PWY
23 ECK3763-ilvD	ILEUSYN-PWY
24 ECK3763-ilvD	VALSYN-PWY

```

25 ECK3763-ilvD BRANCHED-CHAIN-AA-SYN-PWY
26 ECK3766-ilvC THREOCAT-PWY
27 ECK3766-ilvC PANTO-PWY
28 ECK3766-ilvC ILEUSYN-PWY
29 ECK3766-ilvC VALSYN-PWY
30 ECK3766-ilvC PANTOSYN-PWY
31 ECK3766-ilvC BRANCHED-CHAIN-AA-SYN-PWY

```

> attr_list(name_attribute)

```

$`ECK3762-ilvE`
[1] "ALANINE-VALINESYN-PWY" "THREOCAT-PWY" "ALL-CHORISMATE-PWY" "PWY0-1061" "PHESYN"
"ILEUSYN-PWY"
[7] "LEUSYN-PWY" "VALSYN-PWY" "COMPLETE-ARO-PWY" "BRANCHED-CHAIN-AA-SYN-PWY"

$`ECK3764-ilvA`
[1] "THREOCAT-PWY" "ILEUSYN-PWY" "BRANCHED-CHAIN-AA-SYN-PWY"

$`ECK3997-purD`
[1] "PRPP-PWY" "DENOVO-PURINE2-PWY" "PWY-6121" "PWY-6122" "PWY-6277"

$`ECK0516-purE`
[1] "PRPP-PWY" "PWY-6123" "DENOVO-PURINE2-PWY"

$`ECK3763-ilvD`
[1] "THREOCAT-PWY" "ILEUSYN-PWY" "VALSYN-PWY" "BRANCHED-CHAIN-AA-SYN-PWY"

$`ECK3766-ilvC`
[1] "THREOCAT-PWY" "PANTO-PWY" "ILEUSYN-PWY" "VALSYN-PWY" "PANTOSYN-
PWY" "BRANCHED-CHAIN-AA-SYN-PWY"

```

one_attr() takes the output from attr_list() as the input. It generates a table that contains all possible combination of mutants and whether they share annotations (0 stands for not having any same annotations, 1 for having at least 1 same annotation). For example:

> attribute_list=attr_list(name_attribute)

> one_attr(attribute_list)

	mutant1	mutant2	sameORnot
1	ECK3762-ilvE	ECK3764-ilvA	1
2	ECK3762-ilvE	ECK3997-purD	0
3	ECK3762-ilvE	ECK0516-purE	0
4	ECK3762-ilvE	ECK3763-ilvD	1
5	ECK3762-ilvE	ECK3766-ilvC	1
6	ECK3764-ilvA	ECK3997-purD	0
7	ECK3764-ilvA	ECK0516-purE	0
8	ECK3764-ilvA	ECK3763-ilvD	1
9	ECK3764-ilvA	ECK3766-ilvC	1
10	ECK3997-purD	ECK0516-purE	1
11	ECK3997-purD	ECK3763-ilvD	0
12	ECK3997-purD	ECK3766-ilvC	0
13	ECK0516-purE	ECK3763-ilvD	0
14	ECK0516-purE	ECK3766-ilvC	0
15	ECK3763-ilvD	ECK3766-ilvC	1

generate_pairs_similarity_coannotation() generates the pairwise similarity table that contains the strain pairs, similarity/distance value and a Boolean column of whether the corresponding strain pairs share the same annotation(s). It takes phenotype data, the result from attr_list() and a function as an argument to specify the similarity metric. For example:

> attribute_list<-one_attr(name_attribute)

> names(attribute_list)<-rownames(phenotype_data) #(Must do) Synchronize the strain names

> generate_pairs_similarity_coannotation(data=phenotype_data,attribute_list=attribute_list,
+ dist_metric=pcc_dist)

mutant1	mutant2	similarity	same_annot	
1	ECK3762-ilvE	ECK3764-ilvA	0.005903022	1
2	ECK3766-ilvC	ECK3764-ilvA	0.006075217	1
3	ECK3997-purD	ECK0516-purE	0.011308326	1
4	ECK3763-ilvD	ECK3762-ilvE	0.012529945	1
5	ECK3763-ilvD	ECK3766-ilvC	0.015107990	1
6	ECK3763-ilvD	ECK3764-ilvA	0.017520039	1
7	ECK3766-ilvC	ECK3762-ilvE	0.017882605	1
8	ECK3997-purD	ECK3762-ilvE	0.614310165	0
9	ECK3997-purD	ECK3763-ilvD	0.674431348	0
10	ECK0516-purE	ECK3762-ilvE	0.706927257	0
11	ECK3997-purD	ECK3764-ilvA	0.709983271	0
12	ECK0516-purE	ECK3763-ilvD	0.782931085	0
13	ECK3997-purD	ECK3766-ilvC	0.792573199	0
14	ECK0516-purE	ECK3764-ilvA	0.800223580	0
15	ECK0516-purE	ECK3766-ilvC	0.891990335	0

, where `pcc_dist = 1- cor(t(your_data),method="pearson")`

Get metrics derived of the confusion matrix

After the similarity and co-annotation columns are computed as above, `get_confusionMatrix()` can be used to get the similarity-based confusion matrices and the derived metrics: sensitivity, specificity, precision, and accuracy. `get_confusionMatrix()` also permute the co-annotation column and calculate the confusion matrices and other derived metrics as negative controls. For example:

```
> new <- generate_pairs_similarity_coannotation(data= phenotype_data,
attribute_list=attribute_list, dist_metric=pcc_dist)
```

```
> get_confusionMatrix_and_metrics (df=new, annot="same_annot",similarity="similarity")
```

TP	FP	TN	FN	sensitivity	specificity	precision	accuracy	random_TP	random_FP	random_TN	random_FN
random_sensitivity	random_specificity	random_precision	random_accuracy								
1	1	0	8	6	0.1428571	1.000	1.0000000	0.6000000	0	1	7
7					0.0000000	0.875	0.0000000	0.4666667			
2	2	0	8	5	0.2857143	1.000	1.0000000	0.6666667	0	2	6
7					0.0000000	0.750	0.0000000	0.4000000			
3	3	0	8	4	0.4285714	1.000	1.0000000	0.7333333	0	3	5
7					0.0000000	0.625	0.0000000	0.3333333			
4	4	0	8	3	0.5714286	1.000	1.0000000	0.8000000	0	4	4
7					0.0000000	0.500	0.0000000	0.2666667			
5	5	0	8	2	0.7142857	1.000	1.0000000	0.8666667	0	5	3
7					0.0000000	0.375	0.0000000	0.2000000			
6	6	0	8	1	0.8571429	1.000	1.0000000	0.9333333	1	5	3
6					0.1428571	0.375	0.1666667	0.2666667			
7	7	0	8	0	1.0000000	1.000	1.0000000	1.0000000	2	5	3
5					0.2857143	0.375	0.2857143	0.3333333			
8	7	1	7	0	1.0000000	0.875	0.8750000	0.9333333	3	5	3
4					0.4285714	0.375	0.3750000	0.4000000			
9	7	2	6	0	1.0000000	0.750	0.7777778	0.8666667	4	5	3
3					0.5714286	0.375	0.4444444	0.4666667			
10	7	3	5	0	1.0000000	0.625	0.7000000	0.8000000	4	6	2
3					0.5714286	0.250	0.4000000	0.4000000			
11	7	4	4	0	1.0000000	0.500	0.6363636	0.7333333	4	7	1
3					0.5714286	0.125	0.3636364	0.3333333			
12	7	5	3	0	1.0000000	0.375	0.5833333	0.6666667	5	7	1
2					0.7142857	0.125	0.4166667	0.4000000			
13	7	6	2	0	1.0000000	0.250	0.5384615	0.6000000	5	8	0
2					0.7142857	0.000	0.3846154	0.3333333			
14	7	7	1	0	1.0000000	0.125	0.5000000	0.5333333	6	8	0
1					0.8571429	0.000	0.4285714	0.4000000			
15	7	8	0	0	1.0000000	0.000	0.4666667	0.4666667	7	8	0
0					1.0000000	0.000	0.4666667	0.4666667			

Plot the results

`graph_corr_annot()` takes the output from `get_confusionMatrix()` to plot the final result, where precisions were plotted against the ranked pairs of mutants. Enrichment for sensitivity, specificity, precision, and accuracy could be compared with the dotted line, which is the negative control. For example:

```
> confusionMatrix_obj <- get_confusionMatrix_and_metrics(
  new,"same_annot","similarity",seed=103)
> metric="precision"; similarity="pcc"; subset=dim(confusionMatrix_obj)[1]; cols="blue";
  ylim=c(0,1); lwd=1 # set graphing parameters
> graph_corr_annot(confusionMatrix_obj, metric, similarity, subset, cols, x_lab="",
  ylim=c(0,1.05), lwd)
```

```
> metric="sensitivity" # use another metric for the y axis
> graph_corr_annot(confusionMatrix_obj, metric, similarity, subset, cols, x_lab="",
  ylim=c(0,1.05), lwd)
```

Helper functions

The following 10 functions are intended to speed up the phenotype data curation process:

`any_incomplete()` checks if the input matrix, dataframe or datatable contains any NA, NAN, NULL or "" (empty string). For example:

```
> incomplete_phenotype_data<- phenotype_data
> incomplete_phenotype_data[1:2, 1:2]<- NA #introduce some NA
> incomplete_phenotype_data
```

	STREPTOMYCIN.0.05	SUCCINATE	SULFAMONOMETHOXINE.100	TAUROCHOLATE.0.1	TAUROCHOLATE.0.5	
ECK3997-purD	NA	NA	3.33	-13.46	-	
13.79						
ECK0516-purE	NA	NA	2.63	-8.27	-	
9.81						
ECK3763-ilvD	-1.41	-11.72	0.34	-0.69	0.32	
ECK3766-ilvC	-0.58	-8.53	-0.81	0.06	0.51	
ECK3762-ilvE	-0.01	-11.93	0.02	-0.68	-	
0.46						
ECK3764-ilvA	-0.15	-11.55	-0.84	-0.04	-	
0.19						

```
> any_incomplete(incomplete_phenotype_data)
```

```
$dimension
[1] "Dimension: 6 rows * 5 columns"
```

```
$na
STREPTOMYCIN.0.05      SUCCINATE
                2                2
```

```
$null
named integer(0)
```

```
$nan
named integer(0)
```



```
$empty
named integer(0)

$completeness
[1] "4 of NA, NAN, NULL, or empty character is found from 30 data points. They constitute
13.333333333333333%"
```

`filter_table()` filters the input matrix, dataframe or datatable so that all rows and columns with NA/NAN/NULL/"" are removed. For example:

```
> filter_table(incomplete_phenotype_data)
```

	SULFAMONOMETHOXINE.100	TAUROCHOLATE.0.1	TAUROCHOLATE.0.5
ECK3997-purD	3.33	-13.46	-13.79
ECK0516-purE	2.63	-8.27	-9.81
ECK3763-ilvD	0.34	-0.69	0.32
ECK3766-ilvC	-0.81	0.06	0.51
ECK3762-ilvE	0.02	-0.68	-0.46
ECK3764-ilvA	-0.84	-0.04	-0.19

`graph_table()` takes a matrix, dataframe or a table as the input and represents it using a heatmap. It also deals with continuous/categorical/mixed variables. For example:

```
> graph_table(phenotype_data)
```

```
> graph_table(ter_phenotype)
```

`checkDuplicates_vect()` checks if an input vector has duplicates. If so, it will return the frequency table. Otherwise, the text “Everything in this vector is unique” will be returned. For example:

```
> my_vector <- c(1,1,2,2,3)
> checkDuplicates_vect(my_vector)
[1] "Some duplicates are found:"
vect
1 2 3
2 2 1

> my_vector <- c(1,2,3)
> checkDuplicates_vect(my_vector)
[1] "Everything in this vector is unique"
```

`change_names ()` changes row names or column names of a matrix, dataframe or datatable based on another matrix/dataframe/datatable. For example:

```
> new_col_names <-c("0.05 µg/ml streptomycin",
"0.3% succinate",
"100 µg/ml sulfamonomethoxine","0.1% taurocholate","0.5% taurocholate ")

> change_names(rowOrCol="col", phenotype_data,
matrix(c(colnames(phenotype_data), new_col_names), ncol=2, byrow=FALSE))
```

	0.05 µg/ml streptomycin	0.3% succinate	100 µg/ml sulfamonomethoxine	0.1% taurocholate	0.5% taurocholate
ECK3997-purD	-1.48	-12.30	3.33	-13.46	-13.79
ECK0516-purE	0.43	-6.75	2.63	-8.27	-9.81
ECK3763-ilvD	-1.41	-11.72	0.34	-0.69	0.32
ECK3766-ilvC	-0.58	-8.53	-0.81	0.06	0.51
ECK3762-ilvE	-0.01	-11.93	0.02	-0.68	-0.46
ECK3764-ilvA	-0.15	-11.55	-0.84	-0.04	-0.19

`melt_similarity()` takes a similarity matrix or a distance object as the input and converts it to a long form dataframe, with an option to sort the molten dataframe by the 3rd column (the numeric column). For example:

```
> dist(phenotype_data) # the distance object of interest
```

	ECK3997-purD	ECK0516-purE	ECK3763-ilvD	ECK3766-ilvC	ECK3762-ilvE
ECK0516-purE	8.815730				
ECK3763-ilvD	19.272945	13.906973			
ECK3766-ilvC	20.480305	13.853281	3.575766		
ECK3762-ilvE	18.822093	13.374180	1.647726	3.749973	
ECK3764-ilvA	19.615726	13.989089	1.921328	3.131485	1.177327

```
> melt_similarity(dist(phenotype_data))
```

object_1	object_2	value
2	ECK3997-purD ECK0516-purE	8.815730
3	ECK3997-purD ECK3763-ilvD	19.272945
4	ECK3997-purD ECK3766-ilvC	20.480305
5	ECK3997-purD ECK3762-ilvE	18.822093
6	ECK3997-purD ECK3764-ilvA	19.615726
9	ECK0516-purE ECK3763-ilvD	13.906973
10	ECK0516-purE ECK3766-ilvC	13.853281
11	ECK0516-purE ECK3762-ilvE	13.374180

```
12 ECK0516-purE ECK3764-ilvA 13.989089
16 ECK3763-ilvD ECK3766-ilvC 3.575766
17 ECK3763-ilvD ECK3762-ilvE 1.647726
18 ECK3763-ilvD ECK3764-ilvA 1.921328
23 ECK3766-ilvC ECK3762-ilvE 3.749973
24 ECK3766-ilvC ECK3764-ilvA 3.131485
30 ECK3762-ilvE ECK3764-ilvA 1.177327
```

When compared with `reshape2::melt()`, the differences are: 1. `melt_similarity()` Can take a distance object as the main input 2. When a matrix is used as the main input, it has to be a similarity matrix 3. `melt_similarity()` remove the diagonal elements and the duplicated pairs that share the same similarity.

`convert_table()` takes a matrix, dataframe or a datatable as the input and converts the types of elements to a designated type. For example:

```
> str(phenotype_data)

'data.frame': 6 obs. of 5 variables:
 $ STREPTOMYCIN.0.05 : num -1.48 0.43 -1.41 -0.58 -0.01 -0.15
 $ SUCCINATE : num -12.3 -6.75 -11.72 -8.53 -11.93 ...
 $ SULFAMONOMETHOXINE.100: num 3.33 2.63 0.34 -0.81 0.02 -0.84
 $ TAUROCHOLATE.0.1 : num -13.46 -8.27 -0.69 0.06 -0.68 ...
 $ TAUROCHOLATE.0.5 : num -13.79 -9.81 0.32 0.51 -0.46 ...

> phenotype_data_chr <- convert_table(phenotype_data, as.character)
> str(phenotype_data_chr)

'data.frame': 6 obs. of 5 variables:
 $ STREPTOMYCIN.0.05 : chr "-1.48" "0.43" "-1.41" "-0.58" ...
 $ SUCCINATE : chr "-12.3" "-6.75" "-11.72" "-8.53" ...
 $ SULFAMONOMETHOXINE.100: chr "3.33" "2.63" "0.34" "-0.81" ...
 $ TAUROCHOLATE.0.1 : chr "-13.46" "-8.27" "-0.69" "0.06" ...
 $ TAUROCHOLATE.0.5 : chr "-13.79" "-9.81" "0.32" "0.51" ...
```

`remove_NA()` removes NA from an R vector object. For example:

```
> remove_NA(c(1,2,3,4,NA,NA))
[1] 1 2 3 4
```

Summary

The `microbialPhenotypes` package has implemented a pipeline to systematically parse and analyze high-throughput phenotype screens, with many functions as well as an example using a published *E. coli* dataset (Nichols et al., 2011). Although the motivation for this software is to process microbial phenotype data, we expect its usability to be easily extended to multivariate dataset with distinct annotation sets.

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