Maaslin 2 Analysis of GO Terms for COVIRT19

Lets install some R packages that we are gonna need to run this analysis

if(!requireNamespace("BiocManager", quietly = TRUE))

install.packages("BiocManager")

In [1]:
```
if (!requireNamespace("BiocManager", quietly = TRUE))
BiocManager::install(c("Maaslin2", "DESeq2",'phyloseq','microbiome','DirichletMultinomial','GenomicRanges'))
```

```
Updating HTML index of packages in '.Library'

Making 'packages.html' ...
 done
```

In [4]:
```
#install.packages("remotes")
#remotes::install_github("mikemc/speedyseq")
install.packages(c('circlize','ggpubr','viridis','mosaic'))
```

```
also installing the dependencies ‘corrplot’, ‘ggrepel’, ‘ggsci’, ‘cowplot’, ‘ggsignif’, ‘gridExtra’, ‘polynom’, ‘rstatix’


Updating HTML index of packages in '.Library'

Making 'packages.html' ...
 done

Updating HTML index of packages in '.Library'

Making 'packages.html' ...
 done

also installing the dependencies ‘tweenr’, ‘polyclip’, ‘ggforce’, ‘png’, ‘jpeg’, ‘raster’, ‘leaflet.providers’, ‘ggformul
a’, ‘mosaicData’, ‘mosaicCore’, ‘ggstance’, ‘ggridges’, ‘latticeExtra’, ‘ggdendro’, ‘leaflet’


Updating HTML index of packages in '.Library'

Making 'packages.html' ...
```

```
done
```

Now lets load our libraries and set out environment

In [5]:
```
library(tidyverse)
library(phyloseq)
library(microbiome)
library(DESeq2)
library(Maaslin2)
library(parallel)
library(DirichletMultinomial)
library(pheatmap)
library(ggpubr)
library(viridis)
library(mosaic)
```

```
microbiome R package (microbiome.github.com)



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    Sudarshan Shetty et al. <microbiome.github.io>



Attaching package: ‘microbiome’


The following object is masked from ‘package:ggplot2’:

    alpha


The following object is masked from ‘package:base’:

    transform


Loading required package: S4Vectors

Loading required package: stats4

Loading required package: BiocGenerics

Loading required package: parallel
```

```
Attaching package: ‘BiocGenerics’


The following objects are masked from ‘package:parallel’:

    clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
    clusterExport, clusterMap, parApply, parCapply, parLapply,
    parLapplyLB, parRapply, parSapply, parSapplyLB


The following objects are masked from ‘package:dplyr’:

    combine, intersect, setdiff, union


The following objects are masked from ‘package:stats’:

    IQR, mad, sd, var, xtabs


The following objects are masked from ‘package:base’:

    anyDuplicated, append, as.data.frame, basename, cbind, colnames,
    dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
    grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
    order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
    rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
    union, unique, unsplit, which.max, which.min



Attaching package: ‘S4Vectors’


The following objects are masked from ‘package:dplyr’:

    first, rename


The following object is masked from ‘package:tidyr’:

    expand


The following object is masked from ‘package:base’:

    expand.grid
```

```
Loading required package: IRanges

Attaching package: ‘IRanges’

The following object is masked from ‘package:microbiome’:

    coverage

The following object is masked from ‘package:phyloseq’:

    distance

The following objects are masked from ‘package:dplyr’:

    collapse, desc, slice

The following object is masked from ‘package:purrr’:

    reduce

Loading required package: GenomicRanges

Loading required package: GenomeInfoDb

Loading required package: SummarizedExperiment

Loading required package: MatrixGenerics

Loading required package: matrixStats

Attaching package: ‘matrixStats’

The following object is masked from ‘package:dplyr’:

    count


Attaching package: ‘MatrixGenerics’
```

```
The following objects are masked from ‘package:matrixStats’:

    colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
    colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
    colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
    colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
    colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
    colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
    colWeightedMeans, colWeightedMedians, colWeightedSds,
    colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
    rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
    rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
    rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
    rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
    rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
    rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
    rowWeightedSds, rowWeightedVars


Loading required package: Biobase

Welcome to Bioconductor

    Vignettes contain introductory material; view with
    'browseVignettes()'. To cite Bioconductor, see
    'citation("Biobase")', and for packages 'citation("pkgname")'.



Attaching package: ‘Biobase’


The following object is masked from ‘package:MatrixGenerics’:

    rowMedians


The following objects are masked from ‘package:matrixStats’:

    anyMissing, rowMedians


The following object is masked from ‘package:phyloseq’:

    sampleNames


Loading required package: viridisLite
```

```
Registered S3 method overwritten by 'mosaic':
  method                           from
  fortify.SpatialPolygonsDataFrame ggplot2


The 'mosaic' package masks several functions from core packages in order to add
additional features.  The original behavior of these functions should not be affected by this.


Attaching package: ‘mosaic’


The following object is masked from ‘package:Matrix’:

    mean


The following object is masked from ‘package:DESeq2’:

    counts


The following objects are masked from ‘package:matrixStats’:

    count, iqr


The following objects are masked from ‘package:IRanges’:

    cor, cov, IQR, mean, median, mid, quantile, sd, var


The following objects are masked from ‘package:S4Vectors’:

    cor, cov, sd, var


The following objects are masked from ‘package:BiocGenerics’:

    counts, IQR, mean, sd, var


The following objects are masked from ‘package:dplyr’:

    count, do, tally


The following object is masked from ‘package:purrr’:
```

```
    cross


The following object is masked from ‘package:ggplot2’:

    stat


The following objects are masked from ‘package:stats’:

    binom.test, cor, cor.test, cov, fivenum, IQR, median, prop.test,
    quantile, sd, t.test, var


The following objects are masked from ‘package:base’:

    max, mean, min, prod, range, sample, sum
```

In [ ]:
```
'/home/jovyan/work/Aagaard_Raid3/microbial/GO_term_analysis/R_Maaslin2'
```

In [7]:
```
getwd()
setwd('/home/jovyan/work/Aagaard_Raid3/microbial/GO_term_analysis')
```

'/home/jovyan/work/Aagaard_Raid3/microbial/GO_term_analysis'

In [8]:
```
raw<-as_tibble(read.table("Combined_BALF_GO_Terms_parent_propagated.tsv", sep = "\t", row.names = NULL, header = T, quote
```

A tibble: 47,233 x 2,020 # good so far now do a little regex and fix some stuff

In [9]:
```
colnames(raw)<-gsub("NA_tax","unclass", colnames(raw))%>%str_replace_all("NC1_SRR7796663", "NC1.SRR7796663")
```

Transform the raw table by type of count (euk, term, bac, arc)

In [10]:
```
df<-raw %>%
  select(GO_term,namespace,depth,name,ends_with("_counts"))%>%
  pivot_longer(cols = -c(GO_term,namespace,depth,name),
               names_to =  c("sample","type","abund"),#c("Total", "Archaea","Bacteria","Eukarya", "Viridae", "Unclassifie
               names_pattern = "(.*)_(.*)_(.*)")%>%
  select(-abund)%>%
  filter(value>1)%>%
  pivot_wider(names_from = sample, values_from=value, values_fill=0)
#SIDE NOTE:There are multiple processes and values for a single sample so you cant convert the sample to columns
```

Make individual tibbles for biological processes and molecular fxn

In [11]:
```
bio<-filter(df, namespace=="biological_process")
mol<-filter(df, namespace=="molecular_function")
```

make individual tibbles for each type (bac, euk, term, arc, vir, etc)

In [12]:
```
bio_bac<-bio%>%filter(type=="bac")%>%select(-type)
bio_term<-bio%>%filter(type=="term")%>%select(-type)
mol_bac<-mol%>%filter(type=="bac")%>%select(-type)
mol_term<-mol%>%filter(type=="term")%>%select(-type)
```

subselect tibbles for only the counts and go terminology

In [13]:
```
bio_bac_counts<-bio_bac%>%select(-c(namespace,depth,name))
bio_bac_tax<-bio_bac%>%select(GO_term,namespace,depth,name)
mol_bac_counts<-mol_bac%>%select(-c(namespace,depth,name))
mol_bac_tax<-mol_bac%>%select(GO_term,namespace,depth,name)
```

convert them to dataframes for downstream import to phylsoeq

In [14]:
```
bio_bac_counts<-data.frame(bio_bac_counts, row.names=1)
bio_bac_tax<-data.frame(bio_bac_tax, row.names=1)
mol_bac_counts<-data.frame(mol_bac_counts, row.names=1)
mol_bac_tax<-data.frame(mol_bac_tax, row.names=1)
```

convert the dataframes into phyloseq formats

In [15]:
```
bio_bac_counts_phy <- otu_table(bio_bac_counts, taxa_are_rows=TRUE)
bio_bac_tax_phy <- tax_table(as.matrix(bio_bac_tax), errorIfNULL=TRUE)
mol_bac_counts_phy<-otu_table(mol_bac_counts, taxa_are_rows = T)
mol_bac_tax_phy<-tax_table(as.matrix(mol_bac_tax), errorIfNULL = T)
```

import your metadata

In [17]:
```
bio_bac_sam<-as.data.frame(read.table("Combined_BALF_GO_Terms_metadata2.txt",header = T, sep = "\t",row.names = 1))
```

a little regex to fix the stupid filename

In [18]:
```
rownames(bio_bac_sam)<-rownames(bio_bac_sam)%>%str_replace_all("NC1_SRR7796663", "NC1.SRR7796663")
bio_bac_sam$accession<-rownames(bio_bac_sam)
```

I want to take a moment and thank the curators for all their hard work in annotation the metadata..... =)

In [19]:
```
bio_bac_sam$outcome<-bio_bac_sam$outcome%>%
```

```
str_replace_all("recovered", "Recovered")%>%
str_replace_all("deceased","Deceased")%>%
str_replace_all('stabilized','Stabilized")
###DONT FORGET TO DELETE THESE LINES LATER AFTER YOUR DONE PLAYING AROUND
#str_replace_all('Stabilized','Survived")%>%
#str_replace_all("Recovered", "Survived")
##############################################
```

In [20]:

```
bio_bac_sam$sex<-bio_bac_sam$sex%>%
str_replace_all("M", "male")%>%
str_replace_all("F", "female")%>%
str_replace_all("na", "<NA>")
tally(~sex,bio_bac_sam)
```

```
sex
  <NA> female   male   <NA>
    22     54     61     30
```

In [21]:

```
tally(~case,bio_bac_sam,format = "data.frame")
tally(~sample_type,bio_bac_sam,format = "data.frame")
tally(~outcome,bio_bac_sam, format = "data.frame")
```

A data.frame: 6 × 2

case	Freq
<fct>	<int>
Community_acquired_pneumonia	25
Control_Healthy	32
Control_Neg	5
Control_Sick	36
Control_Unknown	21
COVID19	48

A data.frame: 13 × 2

sample_type	Freq
<fct>	<int>
Asthma	7
Asthma_Ex_smoker	3

sample_type	Freq
<fct>	<int>
Asthma_Smoker	2
Community_acquired_pneumonia	25
COVID_19	48
Healthy	32
neg_control	5
Obese	6
Obese_Asthma	9
Obese_Asthma_Smoker	1
Obese_Smoker	5
Smoker	3
Unknown	21

A data.frame: 4 × 2

outcome	Freq
<fct>	<int>
Deceased	20
Recovered	11
Stabilized	8
NA	128

making physeq object

In [22]:
```
bio_bac_pseq <- phyloseq(bio_bac_counts_phy, bio_bac_tax_phy, sample_data(bio_bac_sam))
mol_bac_pseq<-phyloseq(mol_bac_counts_phy,mol_bac_tax_phy, sample_data(bio_bac_sam))
bac_pseq<-merge_phyloseq(bio_bac_pseq,mol_bac_pseq)
bac_pseq
```

```
phyloseq-class experiment-level object
otu_table()   OTU Table:         [ 13846 taxa and 167 samples ]
```

```
sample_data() Sample Data:       [ 167 samples by 71 sample variables ]
tax_table()   Taxonomy Table:    [ 13846 taxa by 3 taxonomic ranks ]
```

Filter out the top level depth 0 GO Terms "molecular function" and "biological process"

In [23]:
```
filtme<-c("GO:0003674")
bac_pseq <- prune_taxa(taxa=taxa_names(bac_pseq)!=filtme, bac_pseq)
filtme<-c("GO:0008150")
bac_pseq <- prune_taxa(taxa=taxa_names(bac_pseq)!=filtme, bac_pseq)
bac_pseq
```

```
phyloseq-class experiment-level object
otu_table()   OTU Table:         [ 13844 taxa and 167 samples ]
sample_data() Sample Data:       [ 167 samples by 71 sample variables ]
tax_table()   Taxonomy Table:    [ 13844 taxa by 3 taxonomic ranks ]
```

filter out the negative control and unknown samples

In [24]:
```
bac_pseq_no_neg<-subset_samples(bac_pseq, sample_type!="neg_control")
bac_pseq_no_neg# [ 13846 taxa and 162 samples ]:
bac_pseq_no_neg<-subset_samples(bac_pseq_no_neg, sample_type!="Unknown")
bac_pseq_no_neg# [ 13846 taxa and 141 samples ]:
```

```
phyloseq-class experiment-level object
otu_table()   OTU Table:         [ 13844 taxa and 162 samples ]
sample_data() Sample Data:       [ 162 samples by 71 sample variables ]
tax_table()   Taxonomy Table:    [ 13844 taxa by 3 taxonomic ranks ]
phyloseq-class experiment-level object
otu_table()   OTU Table:         [ 13844 taxa and 141 samples ]
sample_data() Sample Data:       [ 141 samples by 71 sample variables ]
tax_table()   Taxonomy Table:    [ 13844 taxa by 3 taxonomic ranks ]
```

Lets change the names of the Go Terms so we can understand the description as well as the tag

This code was causing mismatches with name and GO TAG and has since been resolved 19 NOV 2020

In [25]:
```
#names<-paste(taxa_names(bac_pseq_no_neg),get_taxa_unique(bac_pseq_no_neg,taxonomic.rank = "name" ),sep = "-")
#taxa_names(bac_pseq_no_neg)<-names
```

NEW and improved code

In [26]:
```
tax<-data.frame(tax_table(bac_pseq_no_neg))
names<-paste(rownames(tax),tax$name,sep="-")
length(names)
taxa_names(bac_pseq_no_neg)<-names
```

13844

DESeq2 VST transformation

In [27]:
```
sample_info_tab<-sample_data(bac_pseq_no_neg)
sample_info_tab_phy <- sample_data(sample_info_tab)
deseq_counts<-phyloseq_to_deseq2(physeq = bac_pseq_no_neg,design = ~ 1)
deseq_counts_vst <- estimateSizeFactors(deseq_counts, type = "poscounts")
vst_trans_count_tab <- assay(deseq_counts_vst)
```

```
converting counts to integer mode
```

YAAAAAAAAAAAAAAAASSSSSSSSSSSS THANK YOU LIMMMA

Dont worry about the limma batch effect correction step, I think I found a better way by including it in the multivariate model

In [28]:
```
#vst_trans_count_tab2 <- limma::removeBatchEffect(vst_trans_count_tab, sample_info_tab$publication)
```

IT FIXED THE BATCH EFFECT!

convert the normalized counts to a phyloseq object and transform into relative abundances

In [29]:
```
vst_count_phy <- otu_table(vst_trans_count_tab, taxa_are_rows=T)
vst_tax_phy <- tax_table(bac_pseq_no_neg)
vst_physeq <- phyloseq(vst_count_phy, vst_tax_phy,sample_data(bac_pseq_no_neg))
vst_physeq_comp<-microbiome::transform(x = vst_physeq,transform = "compositional")
```

MaAsLIN2

In [30]:
```
#dir.create("R_Maaslin2") # Create a new directory
#setwd("/home/jovyan/work/Jochum_3/jupyter_lab/GO_term_analysis/R_Maaslin2/") # Change the current working directory
getwd() #check if directory has been successfully changed
```

'/home/jovyan/work/Aagaard_Raid3/microbial/GO_term_analysis'

In [31]:
```
df_input_data2<-data.frame(t(otu_table(vst_physeq_comp)))
df_input_metadata2<-data.frame(sample_data(vst_physeq_comp))
```

In [32]:
```
#class(df_input_metadata2$case)
#df_input_metadata2$age<-as.factor(df_input_metadata2$age)
#df_input_metadata2$temp_degC<-as.factor(df_input_metadata2$temp_degC)
```

```
#df_input_metadata2$days.after.onset<-as.factor(df_input_metadata2$days.after.onset)
#df_input_metadata2$case<-factor(x = df_input_metadata2$case, levels = c("COVID19","Community_acquired_pneumonia","Contro
#df_input_metadata2$outcome<-factor(x = df_input_metadata2$outcome, levels = c("Deceased","Stabilized","Recovered"))
#class(df_input_metadata2$case)
#df_input_metadata2$case
```

ok so here are the parameters you want to manipulate:

min abundance= the min rel abund hits (1%) #filters out XXXX GO_terms \ min prevalence = Min samples required with min abundance for a feature not to be filtered (0.1=10%=14.1000 samples) \ max_significance = the maximinum p adjusted value to be significant \

This will filter out 13770 GO TERMS \

*normalization = CLR transformation *

CORRECTION dont normalize here, just use the VST transformed counts

correction = the mutliple test correction method to be done (BH=Benjamini-Hochberg)

In [33]:

```
case<-Maaslin2(
  input_data = df_input_data2,
  input_metadata = df_input_metadata2,
  output="./case",
  min_abundance = 0.01,
  min_prevalence = 0.01,
  normalization = "NONE",
  transform = "NONE",
  analysis_method = "LM",
  max_significance = 0.25,
  #random_effects = c("sample_name","publication","collection_location","sequence_type"),
  random_effects = c("sample_name","publication"),
  fixed_effects = c("case"),
  correction="BH",
  standardize = TRUE,
  cores = 48,
  plot_heatmap = TRUE,
  plot_scatter = TRUE,
  heatmap_first_n = 100,
  reference=c("case,COVID19"))
```

```
[1] "Creating output folder"
```

```
[1] "Creating output figures folder"
2020-11-23 18:59:15 INFO::Writing function arguments to log file
2020-11-23 18:59:15 INFO::Verifying options selected are valid
2020-11-23 18:59:15 INFO::Determining format of input files
2020-11-23 18:59:15 INFO::Input format is data samples as rows and metadata samples as rows
2020-11-23 18:59:15 INFO::Formula for random effects: expr ~ (1 | sample_name) + (1 | publication)
2020-11-23 18:59:15 INFO::Formula for fixed effects: expr ~  case
2020-11-23 18:59:15 INFO::Filter data based on min abundance and min prevalence
2020-11-23 18:59:15 INFO::Total samples in data: 141
2020-11-23 18:59:15 INFO::Min samples required with min abundance for a feature not to be filtered: 1.410000
2020-11-23 18:59:16 INFO::Total filtered features: 13715
2020-11-23 18:59:16 INFO::Filtered feature names from abundance and prevalence filtering: GO.0045212.obsolete.neurotransm
itter.receptor.biosynthetic.process, GO.0001319.obsolete.inheritance.of.oxidatively.modified.proteins.involved.in.replica
tive.cell.aging, GO.1900008.obsolete.negative.regulation.of.extrachromosomal.rDNA.circle.accumulation.involved.in.replica
tive.cell.aging, GO.0001302.obsolete.replicative.cell.aging, GO.0051704.multi.organism.process, GO.0051703.intraspecies.i
nteraction.between.organisms, GO.0110148.biomineralization, GO.0043473.pigmentation, GO.0002376.immune.system.process, G
O.0048511.rhythmic.process, GO.0023052.signaling, GO.0007610.behavior, GO.0015976.carbon.utilization, GO.0040011.locomoti
on, GO.0032502.developmental.process, GO.0032501.multicellular.organismal.process, GO.0040007.growth, GO.0009758.carbohyd
rate.utilization, GO.0022610.biological.adhesion, GO.0022414.reproductive.process, GO.0050896.response.to.stimulus, GO.00
06791.sulfur.utilization, GO.0098754.detoxification, GO.0000003.reproduction, GO.0019740.nitrogen.utilization, GO.000834
0.determination.of.adult.lifespan, GO.0019748.secondary.metabolic.process, GO.0060384.innervation, GO.0022413.reproductiv
e.process.in.single.celled.organism, GO.0009628.response.to.abiotic.stimulus, GO.0055114.oxidation.reduction.process, GO.
0009791.post.embryonic.development, GO.0007585.respiratory.gaseous.exchange.by.respiratory.system, GO.0008037.cell.recogn
ition, GO.0009605.response.to.external.stimulus, GO.0009846.pollen.germination, GO.0043335.protein.unfolding, GO.0060033.
anatomical.structure.regression, GO.0071722.detoxification.of.arsenic.containing.substance, GO.0032504.multicellular.orga
nism.reproduction, GO.0000920.septum.digestion.after.cytokinesis, GO.0003006.developmental.process.involved.in.reproducti
on, GO.0045494.photoreceptor.cell.maintenance, GO.0001503.ossification, GO.0007568.aging, GO.0030537.larval.behavior, GO.
0097194.execution.phase.of.apoptosis, GO.0001906.cell.killing, GO.0071840.cellular.component.organization.or.biogenesis,
GO.0007017.microtubule.based.process, GO.0051702.interaction.with.symbiont, GO.0098727.maintenance.of.cell.number, GO.000
2532.production.of.molecular.mediator.involved.in.inflammatory.response, GO.0010312.detoxification.of.zinc.ion, GO.001485
4.response.to.inactivity, GO.0022611.dormancy.process, GO.0009653.anatomical.structure.morphogenesis, GO.0009607.respons
e.to.biotic.stimulus, GO.0007566.embryo.implantation, GO.0009566.fertilization, GO.0051775.response.to.redox.state, GO.00
36268.swimming, GO.0009847.spore.germination, GO.0016049.cell.growth, GO.0001775.cell.activation, GO.0033036.macromolecul
e.localization, GO.0045103.intermediate.filament.based.process, GO.0019674.NAD.metabolic.process, GO.0002252.immune.effec
tor.process, GO.0071684.organism.emergence.from.protective.structure, GO.0045730.respiratory.burst, GO.0021700.developmen
tal.maturation, GO.0098743.cell.aggregation, GO.0010073.meristem.maintenance, GO.0051301.cell.division, GO.0048532.anatom
ical.structure.arrangement, GO.0008283.cell.population.proliferation, GO.0042221.response.to.chemical, GO.0061919.proces
s.utilizing.autophagic.mechanism, GO.0022412.cellular.process.involved.in.reproduction.in.multicellular.organism, GO.0007
155.cell.adhesion, GO.0043934.sporulation, GO.0035640.exploration.behavior, GO.0051606.detection.of.stimulus, GO.0048646.
anatomical.structure.formation.involved.in.morphogenesis, GO.0007631.feeding.behavior, GO.0048589.developmental.growth, G
O.0007154.cell.communication, GO.0007049.cell.cycle, GO.0006955.immune.response, GO.0007624.ultradian.rhythm, GO.0007163.
establishment.or.maintenance.of.cell.polarity, GO.0022404.molting.cycle.process, GO.0045058.T.cell.selection, GO.0006739.
NADP.metabolic.process, GO.0044110.growth.involved.in.symbiotic.interaction, GO.0042303.molting.cycle, GO.0032259.methyla
tion, GO.0070085.glycosylation, GO.0007635.chemosensory.behavior, GO.0061842.microtubule.organizing.center.localization,
GO.0080190.lateral.growth, GO.0030534.adult.behavior, GO.0140253.cell.cell.fusion, GO.0030447.filamentous.growth, GO.0035
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vity, GO.0036042.long.chain.fatty.acyl.CoA.binding, GO.0005300.high.affinity.tryptophan.transmembrane.transporter.activit
y, GO.0008900.potassium.proton.exchanging.ATPase.activity, GO.0015408.ATPase.coupled.ferric.iron.transmembrane.transporte
r.activity, GO.0005242.inward.rectifier.potassium.channel.activity, GO.0015366.malate.proton.symporter.activity, GO.00344
59.3.5.RNA.helicase.activity, GO.0015661.L.lysine.efflux.transmembrane.transporter.activity, GO.0034458.3.5.RNA.helicase.
activity, GO.0005223.intracellular.cGMP.activated.cation.channel.activity, GO.0016286.small.conductance.calcium.activate
d.potassium.channel.activity, GO.0070888.E.box.binding, GO.0043858.arginine.ornithine.antiporter.activity, GO.0001003.RN
A.polymerase.III.type.2.promoter.sequence.specific.DNA.binding, GO.0008551.cadmium.transmembrane.transporter.activity..ph
osphorylative.mechanism, GO.0009378.four.way.junction.helicase.activity, GO.0043995.histone.acetyltransferase.activity..H
4.K5.specific., GO.0015655.alanine.sodium.symporter.activity, GO.0005298.proline.sodium.symporter.activity, GO.0008508.bi

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le.acid.sodium.symporter.activity, GO.0043996.histone.acetyltransferase.activity..H4.K8.specific., GO.0015517.galactose.p
roton.symporter.activity, GO.0048763.calcium.induced.calcium.release.activity, GO.0005219.ryanodine.sensitive.calcium.rel
ease.channel.activity, GO.0005295.neutral.amino.acid.sodium.symporter.activity, GO.0044668.sodium.malonate.symporter.acti
vity, GO.0005220.inositol.1.4.5.trisphosphate.sensitive.calcium.release.channel.activity, GO.0046972.histone.acetyltransf
erase.activity..H4.K16.specific., GO.0008511.sodium.potassium.chloride.symporter.activity, GO.0015501.glutamate.sodium.sy
mporter.activity, GO.0000102.L.methionine.secondary.active.transmembrane.transporter.activity, GO.0022848.acetylcholine.g
ated.cation.selective.channel.activity, GO.0015535.fucose.proton.symporter.activity, GO.0016934.extracellularly.glycine.g
ated.chloride.channel.activity
2020-11-23 18:59:16 INFO::Running selected normalization method: NONE
2020-11-23 18:59:16 INFO::Total filtered features with variance filtering: 0
2020-11-23 18:59:16 INFO::Filtered feature names from variance filtering:
2020-11-23 18:59:16 INFO::Applying z-score to standardize continuous metadata
2020-11-23 18:59:16 INFO::Running selected transform method: NONE
2020-11-23 18:59:16 INFO::Running selected analysis method: LM
2020-11-23 18:59:16 INFO::Creating cluster of 48 R processes
2020-11-23 19:00:26 INFO::Counting total values for each feature
2020-11-23 19:00:26 INFO::Writing residuals to file ./case/residuals.rds
2020-11-23 19:00:27 INFO::Writing all results to file (ordered by increasing q-values): ./case/all_results.tsv
2020-11-23 19:00:27 INFO::Writing the significant results (those which are less than or equal to the threshold of 0.25000
0 ) to file (ordered by increasing q-values): ./case/significant_results.tsv
2020-11-23 19:00:27 INFO::Writing heatmap of significant results to file: ./case/heatmap.pdf
2020-11-23 19:00:30 INFO::Writing association plots (one for each significant association) to output folder: ./case
2020-11-23 19:00:30 INFO::Plotting associations from most to least significant, grouped by metadata
2020-11-23 19:00:30 INFO::Plotting data for metadata number 1, case
2020-11-23 19:00:30 INFO::Creating boxplot for categorical data, case vs GO.0044271.cellular.nitrogen.compound.biosynthet
ic.process
2020-11-23 19:00:30 INFO::Creating boxplot for categorical data, case vs GO.0003677.DNA.binding
2020-11-23 19:00:30 INFO::Creating boxplot for categorical data, case vs GO.0043169.cation.binding
2020-11-23 19:00:31 INFO::Creating boxplot for categorical data, case vs GO.0046872.metal.ion.binding
2020-11-23 19:00:31 INFO::Creating boxplot for categorical data, case vs GO.0016740.transferase.activity
2020-11-23 19:00:31 INFO::Creating boxplot for categorical data, case vs GO.0018130.heterocycle.biosynthetic.process
2020-11-23 19:00:31 INFO::Creating boxplot for categorical data, case vs GO.0034660.ncRNA.metabolic.process
2020-11-23 19:00:31 INFO::Creating boxplot for categorical data, case vs GO.0140098.catalytic.activity..acting.on.RNA
2020-11-23 19:00:32 INFO::Creating boxplot for categorical data, case vs GO.0043170.macromolecule.metabolic.process
2020-11-23 19:00:32 INFO::Creating boxplot for categorical data, case vs GO.0019538.protein.metabolic.process
2020-11-23 19:00:32 INFO::Creating boxplot for categorical data, case vs GO.0016070.RNA.metabolic.process
2020-11-23 19:00:32 INFO::Creating boxplot for categorical data, case vs GO.0043168.anion.binding
2020-11-23 19:00:32 INFO::Creating boxplot for categorical data, case vs GO.1901362.organic.cyclic.compound.biosynthetic.
process
2020-11-23 19:00:32 INFO::Creating boxplot for categorical data, case vs GO.0016772.transferase.activity..transferring.ph
osphorus.containing.groups
2020-11-23 19:00:33 INFO::Creating boxplot for categorical data, case vs GO.0065007.biological.regulation
2020-11-23 19:00:33 INFO::Creating boxplot for categorical data, case vs GO.0050794.regulation.of.cellular.process
2020-11-23 19:00:33 INFO::Creating boxplot for categorical data, case vs GO.0016051.carbohydrate.biosynthetic.process
2020-11-23 19:00:33 INFO::Creating boxplot for categorical data, case vs GO.0019438.aromatic.compound.biosynthetic.proces
s
2020-11-23 19:00:33 INFO::Creating boxplot for categorical data, case vs GO.0044260.cellular.macromolecule.metabolic.proc
ess
2020-11-23 19:00:33 INFO::Creating boxplot for categorical data, case vs GO.0005976.polysaccharide.metabolic.process
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2020-11-23 19:00:34 INFO::Creating boxplot for categorical data, case vs GO.0044264.cellular.polysaccharide.metabolic.pro
cess
2020-11-23 19:00:34 INFO::Creating boxplot for categorical data, case vs GO.0000271.polysaccharide.biosynthetic.process
2020-11-23 19:00:34 INFO::Creating boxplot for categorical data, case vs GO.0090304.nucleic.acid.metabolic.process
2020-11-23 19:00:34 INFO::Creating boxplot for categorical data, case vs GO.0034637.cellular.carbohydrate.biosynthetic.pr
ocess
2020-11-23 19:00:34 INFO::Creating boxplot for categorical data, case vs GO.0033692.cellular.polysaccharide.biosynthetic.
process
2020-11-23 19:00:34 INFO::Creating boxplot for categorical data, case vs GO.0046379.extracellular.polysaccharide.metaboli
c.process
2020-11-23 19:00:35 INFO::Creating boxplot for categorical data, case vs GO.0045226.extracellular.polysaccharide.biosynth
etic.process
2020-11-23 19:00:35 INFO::Creating boxplot for categorical data, case vs GO.0016491.oxidoreductase.activity
2020-11-23 19:00:35 INFO::Creating boxplot for categorical data, case vs GO.0016829.lyase.activity
2020-11-23 19:00:35 INFO::Creating boxplot for categorical data, case vs GO.0016779.nucleotidyltransferase.activity
2020-11-23 19:00:35 INFO::Creating boxplot for categorical data, case vs GO.0016301.kinase.activity
2020-11-23 19:00:36 INFO::Creating boxplot for categorical data, case vs GO.0008879.glucose.1.phosphate.thymidylyltransfe
rase.activity
2020-11-23 19:00:36 INFO::Creating boxplot for categorical data, case vs GO.0050789.regulation.of.biological.process
2020-11-23 19:00:36 INFO::Creating boxplot for categorical data, case vs GO.0016740.transferase.activity
2020-11-23 19:00:36 INFO::Creating boxplot for categorical data, case vs GO.0044262.cellular.carbohydrate.metabolic.proce
ss
2020-11-23 19:00:36 INFO::Creating boxplot for categorical data, case vs GO.0071704.organic.substance.metabolic.process
2020-11-23 19:00:36 INFO::Creating boxplot for categorical data, case vs GO.0034645.cellular.macromolecule.biosynthetic.p
rocess
2020-11-23 19:00:36 INFO::Creating boxplot for categorical data, case vs GO.0009987.cellular.process
2020-11-23 19:00:37 INFO::Creating boxplot for categorical data, case vs GO.0009059.macromolecule.biosynthetic.process
2020-11-23 19:00:37 INFO::Creating boxplot for categorical data, case vs GO.0006793.phosphorus.metabolic.process
2020-11-23 19:00:37 INFO::Creating boxplot for categorical data, case vs GO.0044260.cellular.macromolecule.metabolic.proc
ess
2020-11-23 19:00:37 INFO::Creating boxplot for categorical data, case vs GO.0044281.small.molecule.metabolic.process
2020-11-23 19:00:37 INFO::Creating boxplot for categorical data, case vs GO.0006796.phosphate.containing.compound.metabol
ic.process
2020-11-23 19:00:37 INFO::Creating boxplot for categorical data, case vs GO.1901566.organonitrogen.compound.biosynthetic.
process
2020-11-23 19:00:37 INFO::Creating boxplot for categorical data, case vs GO.0044283.small.molecule.biosynthetic.process
2020-11-23 19:00:38 INFO::Creating boxplot for categorical data, case vs GO.0019438.aromatic.compound.biosynthetic.proces
s
2020-11-23 19:00:38 INFO::Creating boxplot for categorical data, case vs GO.0003676.nucleic.acid.binding
2020-11-23 19:00:38 INFO::Creating boxplot for categorical data, case vs GO.0009987.cellular.process
2020-11-23 19:00:38 INFO::Creating boxplot for categorical data, case vs GO.0006082.organic.acid.metabolic.process
2020-11-23 19:00:38 INFO::Creating boxplot for categorical data, case vs GO.0006139.nucleobase.containing.compound.metabo
lic.process
2020-11-23 19:00:38 INFO::Creating boxplot for categorical data, case vs GO.0019752.carboxylic.acid.metabolic.process
2020-11-23 19:00:39 INFO::Creating boxplot for categorical data, case vs GO.0043436.oxoacid.metabolic.process
2020-11-23 19:00:39 INFO::Creating boxplot for categorical data, case vs GO.0005975.carbohydrate.metabolic.process
2020-11-23 19:00:39 INFO::Creating boxplot for categorical data, case vs GO.0044249.cellular.biosynthetic.process
2020-11-23 19:00:39 INFO::Creating boxplot for categorical data, case vs GO.0008652.cellular.amino.acid.biosynthetic.proc
ess
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2020-11-23 19:00:39 INFO::Creating boxplot for categorical data, case vs GO.0003677.DNA.binding
2020-11-23 19:00:39 INFO::Creating boxplot for categorical data, case vs GO.1901576.organic.substance.biosynthetic.proces
s
2020-11-23 19:00:40 INFO::Creating boxplot for categorical data, case vs GO.0016053.organic.acid.biosynthetic.process
2020-11-23 19:00:40 INFO::Creating boxplot for categorical data, case vs GO.0046394.carboxylic.acid.biosynthetic.process
2020-11-23 19:00:40 INFO::Creating boxplot for categorical data, case vs GO.0036094.small.molecule.binding
2020-11-23 19:00:40 INFO::Creating boxplot for categorical data, case vs GO.1901265.nucleoside.phosphate.binding
2020-11-23 19:00:40 INFO::Creating boxplot for categorical data, case vs GO.0000166.nucleotide.binding
2020-11-23 19:00:40 INFO::Creating boxplot for categorical data, case vs GO.0034641.cellular.nitrogen.compound.metabolic.
process
2020-11-23 19:00:41 INFO::Creating boxplot for categorical data, case vs GO.0043168.anion.binding
2020-11-23 19:00:41 INFO::Creating boxplot for categorical data, case vs GO.0043170.macromolecule.metabolic.process
2020-11-23 19:00:41 INFO::Creating boxplot for categorical data, case vs GO.0016310.phosphorylation
2020-11-23 19:00:41 INFO::Creating boxplot for categorical data, case vs GO.0034660.ncRNA.metabolic.process
2020-11-23 19:00:41 INFO::Creating boxplot for categorical data, case vs GO.0006520.cellular.amino.acid.metabolic.process
2020-11-23 19:00:41 INFO::Creating boxplot for categorical data, case vs GO.0044877.protein.containing.complex.binding
2020-11-23 19:00:41 INFO::Creating boxplot for categorical data, case vs GO.0018130.heterocycle.biosynthetic.process
2020-11-23 19:00:42 INFO::Creating boxplot for categorical data, case vs GO.1901265.nucleoside.phosphate.binding
2020-11-23 19:00:42 INFO::Creating boxplot for categorical data, case vs GO.0000166.nucleotide.binding
2020-11-23 19:00:42 INFO::Creating boxplot for categorical data, case vs GO.0008652.cellular.amino.acid.biosynthetic.proc
ess
2020-11-23 19:00:42 INFO::Creating boxplot for categorical data, case vs GO.0044238.primary.metabolic.process
2020-11-23 19:00:42 INFO::Creating boxplot for categorical data, case vs GO.0019538.protein.metabolic.process
2020-11-23 19:00:43 INFO::Creating boxplot for categorical data, case vs GO.0016053.organic.acid.biosynthetic.process
2020-11-23 19:00:43 INFO::Creating boxplot for categorical data, case vs GO.0046394.carboxylic.acid.biosynthetic.process
2020-11-23 19:00:43 INFO::Creating boxplot for categorical data, case vs GO.0046417.chorismate.metabolic.process
2020-11-23 19:00:43 INFO::Creating boxplot for categorical data, case vs GO.0009423.chorismate.biosynthetic.process
2020-11-23 19:00:43 INFO::Creating boxplot for categorical data, case vs GO.0003677.DNA.binding
2020-11-23 19:00:43 INFO::Creating boxplot for categorical data, case vs GO.0003855.3.dehydroquinate.dehydratase.activity
2020-11-23 19:00:43 INFO::Creating boxplot for categorical data, case vs GO.0043650.dicarboxylic.acid.biosynthetic.proces
s
2020-11-23 19:00:44 INFO::Creating boxplot for categorical data, case vs GO.0016835.carbon.oxygen.lyase.activity
2020-11-23 19:00:44 INFO::Creating boxplot for categorical data, case vs GO.0019438.aromatic.compound.biosynthetic.proces
s
2020-11-23 19:00:44 INFO::Creating boxplot for categorical data, case vs GO.0036094.small.molecule.binding
2020-11-23 19:00:44 INFO::Creating boxplot for categorical data, case vs GO.0009073.aromatic.amino.acid.family.biosynthet
ic.process
2020-11-23 19:00:44 INFO::Creating boxplot for categorical data, case vs GO.0016835.carbon.oxygen.lyase.activity
2020-11-23 19:00:44 INFO::Creating boxplot for categorical data, case vs GO.0016836.hydro.lyase.activity
2020-11-23 19:00:45 INFO::Creating boxplot for categorical data, case vs GO.0009423.chorismate.biosynthetic.process
2020-11-23 19:00:45 INFO::Creating boxplot for categorical data, case vs GO.0043168.anion.binding
2020-11-23 19:00:45 INFO::Creating boxplot for categorical data, case vs GO.0016836.hydro.lyase.activity
2020-11-23 19:00:45 INFO::Creating boxplot for categorical data, case vs GO.0043648.dicarboxylic.acid.metabolic.process
2020-11-23 19:00:45 INFO::Creating boxplot for categorical data, case vs GO.0009072.aromatic.amino.acid.family.metabolic.
process
2020-11-23 19:00:45 INFO::Creating boxplot for categorical data, case vs GO.0043650.dicarboxylic.acid.biosynthetic.proces
s
2020-11-23 19:00:46 INFO::Creating boxplot for categorical data, case vs GO.0003855.3.dehydroquinate.dehydratase.activity
2020-11-23 19:00:46 INFO::Creating boxplot for categorical data, case vs GO.0006082.organic.acid.metabolic.process
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2020-11-23 19:00:46 INFO::Creating boxplot for categorical data, case vs GO.0043436.oxoacid.metabolic.process
2020-11-23 19:00:46 INFO::Creating boxplot for categorical data, case vs GO.0016874.ligase.activity
2020-11-23 19:00:46 INFO::Creating boxplot for categorical data, case vs GO.0097367.carbohydrate.derivative.binding
2020-11-23 19:00:46 INFO::Creating boxplot for categorical data, case vs GO.0019752.carboxylic.acid.metabolic.process
2020-11-23 19:00:46 INFO::Creating boxplot for categorical data, case vs GO.0006807.nitrogen.compound.metabolic.process
2020-11-23 19:00:47 INFO::Creating boxplot for categorical data, case vs GO.0009058.biosynthetic.process
2020-11-23 19:00:47 INFO::Creating boxplot for categorical data, case vs GO.0046417.chorismate.metabolic.process
2020-11-23 19:00:47 INFO::Creating boxplot for categorical data, case vs GO.0046483.heterocycle.metabolic.process
2020-11-23 19:00:47 INFO::Creating boxplot for categorical data, case vs GO.0044271.cellular.nitrogen.compound.biosynthet
ic.process
2020-11-23 19:00:47 INFO::Creating boxplot for categorical data, case vs GO.0019538.protein.metabolic.process
2020-11-23 19:00:47 INFO::Creating boxplot for categorical data, case vs GO.0018130.heterocycle.biosynthetic.process
2020-11-23 19:00:48 INFO::Creating boxplot for categorical data, case vs GO.1901362.organic.cyclic.compound.biosynthetic.
process
2020-11-23 19:00:48 INFO::Creating boxplot for categorical data, case vs GO.0032553.ribonucleotide.binding
2020-11-23 19:00:48 INFO::Creating boxplot for categorical data, case vs GO.0043648.dicarboxylic.acid.metabolic.process
2020-11-23 19:00:48 INFO::Creating boxplot for categorical data, case vs GO.0044281.small.molecule.metabolic.process
2020-11-23 19:00:48 INFO::Creating boxplot for categorical data, case vs GO.0044877.protein.containing.complex.binding
2020-11-23 19:00:48 INFO::Creating boxplot for categorical data, case vs GO.0017076.purine.nucleotide.binding
2020-11-23 19:00:48 INFO::Creating boxplot for categorical data, case vs GO.0003676.nucleic.acid.binding
2020-11-23 19:00:49 INFO::Creating boxplot for categorical data, case vs GO.0032555.purine.ribonucleotide.binding
2020-11-23 19:00:49 INFO::Creating boxplot for categorical data, case vs GO.0097367.carbohydrate.derivative.binding
2020-11-23 19:00:49 INFO::Creating boxplot for categorical data, case vs GO.0016301.kinase.activity
2020-11-23 19:00:49 INFO::Creating boxplot for categorical data, case vs GO.0016070.RNA.metabolic.process
2020-11-23 19:00:49 INFO::Creating boxplot for categorical data, case vs GO.0035639.purine.ribonucleoside.triphosphate.bi
nding
2020-11-23 19:00:49 INFO::Creating boxplot for categorical data, case vs GO.0044283.small.molecule.biosynthetic.process
2020-11-23 19:00:50 INFO::Creating boxplot for categorical data, case vs GO.0032553.ribonucleotide.binding
2020-11-23 19:00:50 INFO::Creating boxplot for categorical data, case vs GO.1901566.organonitrogen.compound.biosynthetic.
process
2020-11-23 19:00:50 INFO::Creating boxplot for categorical data, case vs GO.0044249.cellular.biosynthetic.process
2020-11-23 19:00:50 INFO::Creating boxplot for categorical data, case vs GO.1901576.organic.substance.biosynthetic.proces
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2020-11-23 19:00:50 INFO::Creating boxplot for categorical data, case vs GO.0032555.purine.ribonucleotide.binding
2020-11-23 19:00:50 INFO::Creating boxplot for categorical data, case vs GO.0140098.catalytic.activity..acting.on.RNA
2020-11-23 19:00:51 INFO::Creating boxplot for categorical data, case vs GO.0009072.aromatic.amino.acid.family.metabolic.
process
2020-11-23 19:00:51 INFO::Creating boxplot for categorical data, case vs GO.0017076.purine.nucleotide.binding
2020-11-23 19:00:51 INFO::Creating boxplot for categorical data, case vs GO.0004518.nuclease.activity
2020-11-23 19:00:51 INFO::Creating boxplot for categorical data, case vs GO.0009073.aromatic.amino.acid.family.biosynthet
ic.process
2020-11-23 19:00:51 INFO::Creating boxplot for categorical data, case vs GO.0035639.purine.ribonucleoside.triphosphate.bi
nding
2020-11-23 19:00:51 INFO::Creating boxplot for categorical data, case vs GO.0009058.biosynthetic.process
2020-11-23 19:00:51 INFO::Creating boxplot for categorical data, case vs GO.0050794.regulation.of.cellular.process
2020-11-23 19:00:52 INFO::Creating boxplot for categorical data, case vs GO.0044281.small.molecule.metabolic.process
2020-11-23 19:00:52 INFO::Creating boxplot for categorical data, case vs GO.0065007.biological.regulation
2020-11-23 19:00:52 INFO::Creating boxplot for categorical data, case vs GO.0016829.lyase.activity
2020-11-23 19:00:52 INFO::Creating boxplot for categorical data, case vs GO.0003824.catalytic.activity
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2020-11-23 19:00:52 INFO::Creating boxplot for categorical data, case vs GO.0034641.cellular.nitrogen.compound.metabolic.
process
2020-11-23 19:00:52 INFO::Creating boxplot for categorical data, case vs GO.0050789.regulation.of.biological.process
2020-11-23 19:00:53 INFO::Creating boxplot for categorical data, case vs GO.0006520.cellular.amino.acid.metabolic.process
2020-11-23 19:00:53 INFO::Creating boxplot for categorical data, case vs GO.0046483.heterocycle.metabolic.process
2020-11-23 19:00:53 INFO::Creating boxplot for categorical data, case vs GO.0044271.cellular.nitrogen.compound.biosynthet
ic.process
2020-11-23 19:00:53 INFO::Creating boxplot for categorical data, case vs GO.0006811.ion.transport
2020-11-23 19:00:53 INFO::Creating boxplot for categorical data, case vs GO.0051540.metal.cluster.binding
2020-11-23 19:00:53 INFO::Creating boxplot for categorical data, case vs GO.0051536.iron.sulfur.cluster.binding
2020-11-23 19:00:54 INFO::Creating boxplot for categorical data, case vs GO.0044877.protein.containing.complex.binding
2020-11-23 19:00:54 INFO::Creating boxplot for categorical data, case vs GO.0036094.small.molecule.binding
2020-11-23 19:00:54 INFO::Creating boxplot for categorical data, case vs GO.0044419.interspecies.interaction.between.orga
nisms
2020-11-23 19:00:54 INFO::Creating boxplot for categorical data, case vs GO.0044403.symbiotic.process
2020-11-23 19:00:54 INFO::Creating boxplot for categorical data, case vs GO.1901265.nucleoside.phosphate.binding
2020-11-23 19:00:54 INFO::Creating boxplot for categorical data, case vs GO.0051539.4.iron..4.sulfur.cluster.binding
2020-11-23 19:00:54 INFO::Creating boxplot for categorical data, case vs GO.0000166.nucleotide.binding
2020-11-23 19:00:55 INFO::Creating boxplot for categorical data, case vs GO.0051540.metal.cluster.binding
2020-11-23 19:00:55 INFO::Creating boxplot for categorical data, case vs GO.0044403.symbiotic.process
2020-11-23 19:00:55 INFO::Creating boxplot for categorical data, case vs GO.0044419.interspecies.interaction.between.orga
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2020-11-23 19:00:55 INFO::Creating boxplot for categorical data, case vs GO.0006811.ion.transport
2020-11-23 19:00:55 INFO::Creating boxplot for categorical data, case vs GO.0051536.iron.sulfur.cluster.binding
2020-11-23 19:00:56 INFO::Creating boxplot for categorical data, case vs GO.0006810.transport
2020-11-23 19:00:56 INFO::Creating boxplot for categorical data, case vs GO.0051539.4.iron..4.sulfur.cluster.binding
2020-11-23 19:00:56 INFO::Creating boxplot for categorical data, case vs GO.0055086.nucleobase.containing.small.molecule.
metabolic.process
2020-11-23 19:00:56 INFO::Creating boxplot for categorical data, case vs GO.0016787.hydrolase.activity
2020-11-23 19:00:56 INFO::Creating boxplot for categorical data, case vs GO.0044283.small.molecule.biosynthetic.process
2020-11-23 19:00:56 INFO::Creating boxplot for categorical data, case vs GO.0071941.nitrogen.cycle.metabolic.process
2020-11-23 19:00:57 INFO::Creating boxplot for categorical data, case vs GO.0009399.nitrogen.fixation
2020-11-23 19:00:57 INFO::Creating boxplot for categorical data, case vs GO.0051179.localization
2020-11-23 19:00:57 INFO::Creating boxplot for categorical data, case vs GO.0051234.establishment.of.localization
2020-11-23 19:00:57 INFO::Creating boxplot for categorical data, case vs GO.0019637.organophosphate.metabolic.process
2020-11-23 19:00:57 INFO::Creating boxplot for categorical data, case vs GO.0051234.establishment.of.localization
2020-11-23 19:00:57 INFO::Creating boxplot for categorical data, case vs GO.0003824.catalytic.activity
2020-11-23 19:00:58 INFO::Creating boxplot for categorical data, case vs GO.0051179.localization
2020-11-23 19:00:58 INFO::Creating boxplot for categorical data, case vs GO.0006810.transport
2020-11-23 19:00:58 INFO::Creating boxplot for categorical data, case vs GO.0051540.metal.cluster.binding
2020-11-23 19:00:58 INFO::Creating boxplot for categorical data, case vs GO.0006725.cellular.aromatic.compound.metabolic.
process
2020-11-23 19:00:58 INFO::Creating boxplot for categorical data, case vs GO.0009399.nitrogen.fixation
2020-11-23 19:00:58 INFO::Creating boxplot for categorical data, case vs GO.0051536.iron.sulfur.cluster.binding
2020-11-23 19:00:58 INFO::Creating boxplot for categorical data, case vs GO.0003676.nucleic.acid.binding
2020-11-23 19:00:59 INFO::Creating boxplot for categorical data, case vs GO.0071941.nitrogen.cycle.metabolic.process
2020-11-23 19:00:59 INFO::Creating boxplot for categorical data, case vs GO.0051539.4.iron..4.sulfur.cluster.binding
2020-11-23 19:00:59 INFO::Creating boxplot for categorical data, case vs GO.0006725.cellular.aromatic.compound.metabolic.
process
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2020-11-23 19:00:59 INFO::Creating boxplot for categorical data, case vs GO.0016310.phosphorylation
2020-11-23 19:00:59 INFO::Creating boxplot for categorical data, case vs GO.0097367.carbohydrate.derivative.binding
2020-11-23 19:00:59 INFO::Creating boxplot for categorical data, case vs GO.0016788.hydrolase.activity..acting.on.ester.b
onds
2020-11-23 19:01:00 INFO::Creating boxplot for categorical data, case vs GO.0022857.transmembrane.transporter.activity
2020-11-23 19:01:00 INFO::Creating boxplot for categorical data, case vs GO.0016817.hydrolase.activity..acting.on.acid.an
hydrides
2020-11-23 19:01:00 INFO::Creating boxplot for categorical data, case vs GO.1901566.organonitrogen.compound.biosynthetic.
process
2020-11-23 19:01:00 INFO::Creating boxplot for categorical data, case vs GO.0006753.nucleoside.phosphate.metabolic.proces
s
2020-11-23 19:01:00 INFO::Creating boxplot for categorical data, case vs GO.0016818.hydrolase.activity..acting.on.acid.an
hydrides..in.phosphorus.containing.anhydrides
2020-11-23 19:01:00 INFO::Creating boxplot for categorical data, case vs GO.0009117.nucleotide.metabolic.process
2020-11-23 19:01:01 INFO::Creating boxplot for categorical data, case vs GO.0003824.catalytic.activity
2020-11-23 19:01:01 INFO::Creating boxplot for categorical data, case vs GO.0032553.ribonucleotide.binding
2020-11-23 19:01:01 INFO::Creating boxplot for categorical data, case vs GO.0005215.transporter.activity
2020-11-23 19:01:01 INFO::Creating boxplot for categorical data, case vs GO.0008233.peptidase.activity
2020-11-23 19:01:01 INFO::Creating boxplot for categorical data, case vs GO.0043170.macromolecule.metabolic.process
2020-11-23 19:01:01 INFO::Creating boxplot for categorical data, case vs GO.1901564.organonitrogen.compound.metabolic.pro
cess
2020-11-23 19:01:01 INFO::Creating boxplot for categorical data, case vs GO.0016835.carbon.oxygen.lyase.activity
2020-11-23 19:01:02 INFO::Creating boxplot for categorical data, case vs GO.0017076.purine.nucleotide.binding
2020-11-23 19:01:02 INFO::Creating boxplot for categorical data, case vs GO.0016462.pyrophosphatase.activity
2020-11-23 19:01:02 INFO::Creating boxplot for categorical data, case vs GO.0032555.purine.ribonucleotide.binding
2020-11-23 19:01:02 INFO::Creating boxplot for categorical data, case vs GO.0016772.transferase.activity..transferring.ph
osphorus.containing.groups
2020-11-23 19:01:02 INFO::Creating boxplot for categorical data, case vs GO.0035639.purine.ribonucleoside.triphosphate.bi
nding
```

In [34]:

```
outcome<-Maaslin2(
  input_data = df_input_data2,
  input_metadata = df_input_metadata2,
  output="./outcome",
  min_abundance = 0.001,
  min_prevalence = 0.001,
  normalization = "NONE",
  transform = "NONE",
  analysis_method = "LM",
  max_significance = 0.77,
  #random_effects = c("sample_name","publication","collection_location","sequence_type"),
  random_effects = c("sample_name","publication"),
  fixed_effects = c("outcome"),
  correction="BH",
  standardize = TRUE,
  cores = 48,
  plot_heatmap = TRUE,
  plot_scatter = TRUE,
```

```
  heatmap_first_n = 100,
  reference=c("outcome,Deceased"))
```

```
[1] "Creating output folder"
[1] "Creating output figures folder"
2020-11-23 19:01:04 INFO::Writing function arguments to log file
2020-11-23 19:01:04 INFO::Verifying options selected are valid
2020-11-23 19:01:04 INFO::Determining format of input files
2020-11-23 19:01:04 INFO::Input format is data samples as rows and metadata samples as rows
2020-11-23 19:01:05 INFO::Formula for random effects: expr ~ (1 | sample_name) + (1 | publication)
2020-11-23 19:01:05 INFO::Formula for fixed effects: expr ~  outcome
2020-11-23 19:01:05 INFO::Filter data based on min abundance and min prevalence
2020-11-23 19:01:05 INFO::Total samples in data: 141
2020-11-23 19:01:05 INFO::Min samples required with min abundance for a feature not to be filtered: 0.141000
2020-11-23 19:01:05 INFO::Total filtered features: 13051
2020-11-23 19:01:05 INFO::Filtered feature names from abundance and prevalence filtering: GO.0045212.obsolete.neurotransm
itter.receptor.biosynthetic.process, GO.0001319.obsolete.inheritance.of.oxidatively.modified.proteins.involved.in.replica
tive.cell.aging, GO.1900008.obsolete.negative.regulation.of.extrachromosomal.rDNA.circle.accumulation.involved.in.replica
tive.cell.aging, GO.0001302.obsolete.replicative.cell.aging, GO.0051704.multi.organism.process, GO.0051703.intraspecies.i
nteraction.between.organisms, GO.0110148.biomineralization, GO.0043473.pigmentation, GO.0002376.immune.system.process, G
O.0048511.rhythmic.process, GO.0023052.signaling, GO.0007610.behavior, GO.0015976.carbon.utilization, GO.0040007.growth,
GO.0009758.carbohydrate.utilization, GO.0022414.reproductive.process, GO.0006791.sulfur.utilization, GO.0098754.detoxific
ation, GO.0000003.reproduction, GO.0019740.nitrogen.utilization, GO.0008340.determination.of.adult.lifespan, GO.0019748.s
econdary.metabolic.process, GO.0060384.innervation, GO.0022413.reproductive.process.in.single.celled.organism, GO.000962
8.response.to.abiotic.stimulus, GO.0009791.post.embryonic.development, GO.0007585.respiratory.gaseous.exchange.by.respira
tory.system, GO.0008037.cell.recognition, GO.0009846.pollen.germination, GO.0043335.protein.unfolding, GO.0060033.anatomi
cal.structure.regression, GO.0071722.detoxification.of.arsenic.containing.substance, GO.0032504.multicellular.organism.re
production, GO.0000920.septum.digestion.after.cytokinesis, GO.0003006.developmental.process.involved.in.reproduction, GO.
0045494.photoreceptor.cell.maintenance, GO.0001503.ossification, GO.0007568.aging, GO.0030537.larval.behavior, GO.009719
4.execution.phase.of.apoptosis, GO.0001906.cell.killing, GO.0007017.microtubule.based.process, GO.0051702.interaction.wit
h.symbiont, GO.0098727.maintenance.of.cell.number, GO.0002532.production.of.molecular.mediator.involved.in.inflammatory.r
esponse, GO.0010312.detoxification.of.zinc.ion, GO.0014854.response.to.inactivity, GO.0022611.dormancy.process, GO.000756
6.embryo.implantation, GO.0009566.fertilization, GO.0051775.response.to.redox.state, GO.0036268.swimming, GO.0009847.spor
e.germination, GO.0016049.cell.growth, GO.0001775.cell.activation, GO.0045103.intermediate.filament.based.process, GO.001
9674.NAD.metabolic.process, GO.0002252.immune.effector.process, GO.0071684.organism.emergence.from.protective.structure,
GO.0045730.respiratory.burst, GO.0021700.developmental.maturation, GO.0098743.cell.aggregation, GO.0010073.meristem.maint
enance, GO.0051301.cell.division, GO.0048532.anatomical.structure.arrangement, GO.0008283.cell.population.proliferation,
GO.0061919.process.utilizing.autophagic.mechanism, GO.0022412.cellular.process.involved.in.reproduction.in.multicellular.
organism, GO.0043934.sporulation, GO.0035640.exploration.behavior, GO.0051606.detection.of.stimulus, GO.0048646.anatomica
l.structure.formation.involved.in.morphogenesis, GO.0007631.feeding.behavior, GO.0048589.developmental.growth, GO.000704
9.cell.cycle, GO.0006955.immune.response, GO.0007624.ultradian.rhythm, GO.0007163.establishment.or.maintenance.of.cell.po
larity, GO.0022404.molting.cycle.process, GO.0045058.T.cell.selection, GO.0006739.NADP.metabolic.process, GO.0044110.grow
th.involved.in.symbiotic.interaction, GO.0042303.molting.cycle, GO.0070085.glycosylation, GO.0007635.chemosensory.behavio
r, GO.0061842.microtubule.organizing.center.localization, GO.0080190.lateral.growth, GO.0030534.adult.behavior, GO.014025
3.cell.cell.fusion, GO.0030447.filamentous.growth, GO.0035176.social.behavior, GO.0048869.cellular.developmental.process,
GO.0007638.mechanosensory.behavior, GO.0032505.reproduction.of.a.single.celled.organism, GO.0050817.coagulation, GO.00300
29.actin.filament.based.process, GO.0006276.plasmid.maintenance, GO.0006734.NADH.metabolic.process, GO.0014823.response.t
o.activity, GO.0032963.collagen.metabolic.process, GO.0009719.response.to.endogenous.stimulus, GO.0019954.asexual.reprodu
ction, GO.0002440.production.of.molecular.mediator.of.immune.response, GO.0009405.pathogenesis, GO.0007059.chromosome.seg
```

regation, GO.0007389.pattern.specification.process, GO.0030431.sleep, GO.0097242.amyloid.beta.clearance, GO.0051189.prosthetic.group.metabolic.process, GO.0016203.muscle.attachment, GO.0140029.exocytic.process, GO.0044703.multi.organism.reproductive.process, GO.0007272.ensheathment.of.neurons, GO.0090713.immunological.memory.process, GO.0070988.demethylation, GO.0048771.tissue.remodeling, GO.0007340.acrosome.reaction, GO.1990748.cellular.detoxification, GO.0022602.ovulation.cycle.process, GO.0071554.cell.wall.organization.or.biogenesis, GO.0032196.transposition, GO.0016037.light.absorption, GO.0019882.antigen.processing.and.presentation, GO.0002200.somatic.diversification.of.immune.receptors, GO.0022402.cell.cycle.process, GO.0061687.detoxification.of.inorganic.compound, GO.0010022.meristem.determinacy, GO.0035637.multicellular.organismal.signaling, GO.0022406.membrane.docking, GO.1902579.multi.organism.localization, GO.0048609.multicellular.organismal.reproductive.process, GO.0044706.multi.multicellular.organism.process, GO.0007622.rhythmic.behavior, GO.0008219.cell.death, GO.0048066.developmental.pigmentation, GO.0002118.aggressive.behavior, GO.0007626.locomotory.behavior, GO.0010127.mycothiol.dependent.detoxification, GO.0044764.multi.organism.cellular.process, GO.0014874.response.to.stimulus.involved.in.regulation.of.muscle.adaptation, GO.0003008.system.process, GO.0042440.pigment.metabolic.process, GO.0010118.stomatal.movement, GO.0019835.cytolysis, GO.0042330.taxis, GO.0009838.abscission, GO.0007623.circadian.rhythm, GO.0009845.seed.germination, GO.0018933.nicotine.metabolic.process, GO.0010817.regulation.of.hormone.levels, GO.0048148.behavioral.response.to.cocaine, GO.0045196.establishment.or.maintenance.of.neuroblast.polarity, GO.0046677.response.to.antibiotic, GO.0001709.cell.fate.determination, GO.0006403.RNA.localization, GO.0072593.reactive.oxygen.species.metabolic.process, GO.0003002.regionalization, GO.0035878.nail.development, GO.0120252.hydrocarbon.metabolic.process, GO.0003360.brainstem.development, GO.0048002.antigen.processing.and.presentation.of.peptide.antigen, GO.0009292.genetic.transfer, GO.0008356.asymmetric.cell.division, GO.0021670.lateral.ventricle.development, GO.0030901.midbrain.development, GO.0048286.lung.alveolus.development, GO.0035107.appendage.morphogenesis, GO.0009877.nodulation, GO.0021508.floor.plate.formation, GO.0035902.response.to.immobilization.stress, GO.0018996.molting.cycle..collagen.and.cuticulin.based.cuticle, GO.0070314.G1.to.G0.transition, GO.0006081.cellular.aldehyde.metabolic.process, GO.0098869.cellular.oxidant.detoxification, GO.0036342.post.anal.tail.morphogenesis, GO.0030010.establishment.of.cell.polarity, GO.0098609.cell.cell.adhesion, GO.0061077.chaperone.mediated.protein.folding, GO.0001505.regulation.of.neurotransmitter.levels, GO.0050877.nervous.system.process, GO.0099402.plant.organ.development, GO.0071216.cellular.response.to.biotic.stimulus, GO.0090659.walking.behavior, GO.0021794.thalamus.development, GO.0019883.antigen.processing.and.presentation.of.endogenous.antigen, GO.0007569.cell.aging, GO.0001708.cell.fate.specification, GO.0090693.plant.organ.senescence, GO.0009581.detection.of.external.stimulus, GO.0097502.mannosylation, GO.0044406.adhesion.of.symbiont.to.host, GO.0097485.neuron.projection.guidance, GO.0071852.fungal.type.cell.wall.organization.or.biogenesis, GO.0022600.digestive.system.process, GO.0043900.regulation.of.multi.organism.process, GO.0021984.adenohypophysis.development, GO.0001966.thigmotaxis, GO.0010228.vegetative.to.reproductive.phase.transition.of.meristem, GO.0061299.retina.vasculature.morphogenesis.in.camera.type.eye, GO.0051304.chromosome.separation, GO.0061339.establishment.or.maintenance.of.monopolar.cell.polarity, GO.0032964.collagen.biosynthetic.process, GO.0000320.re.entry.into.mitotic.cell.cycle, GO.0035239.tube.morphogenesis, GO.0032409.regulation.of.transporter.activity, GO.0009988.cell.cell.recognition, GO.0031640.killing.of.cells.of.other.organism, GO.0085030.symbiotic.process.benefiting.host, GO.0071495.cellular.response.to.endogenous.stimulus, GO.0048149.behavioral.response.to.ethanol, GO.0060135.maternal.process.involved.in.female.pregnancy, GO.0048070.regulation.of.developmental.pigmentation, GO.0009900.dehiscence, GO.0042755.eating.behavior, GO.0051236.establishment.of.RNA.localization, GO.0007023.post.chaperonin.tubulin.folding.pathway, GO.0120254.olefinic.compound.metabolic.process, GO.0003157.endocardium.development, GO.0042620.poly.3.hydroxyalkanoate..metabolic.process, GO.0046148.pigment.biosynthetic.process, GO.0072089.stem.cell.proliferation, GO.0006949.syncytium.formation, GO.0009453.energy.taxis, GO.0048653.anther.development, GO.0007379.segment.specification, GO.0021604.cranial.nerve.structural.organization, GO.0046844.chorion.micropyle.formation, GO.0046149.pigment.catabolic.process, GO.0003151.outflow.tract.morphogenesis, GO.0035152.regulation.of.tube.architecture..open.tracheal.system, GO.0006458.de.novo.protein.folding, GO.0019608.nicotine.catabolic.process, GO.0097164.ammonium.ion.metabolic.process, GO.0098813.nuclear.chromosome.segregation, GO.0042698.ovulation.cycle, GO.0009611.response.to.wounding, GO.0008344.adult.locomotory.behavior, GO.0070265.necrotic.cell.death, GO.0007343.egg.activation, GO.0032879.regulation.of.localization, GO.0046843.dorsal.appendage.formation, GO.0000910.cytokinesis, GO.0007618.mating, GO.0019953.sexual.reproduction, GO.0007530.sex.determination, GO.0016318.ommatidial.rotation, GO.0007487.analia.development, GO.0040005.chitin.based.cuticle.attachment.to.epithelium, GO.0018990.ecdysis..chitin.based.cuticle, GO.0002566.somatic.diversification.of.immune.receptors.via.somatic.mutation, GO.0034293.sexual.sporulation, GO.0007114.cell.budding, GO.0097354.prenylation, GO.0070482.response.to.oxygen.levels, GO.0050878.regulation.of.body.fluid.levels, GO.0090598.male.anatomic

al.structure.morphogenesis, GO.0002931.response.to.ischemia, GO.0061245.establishment.or.maintenance.of.bipolar.cell.pola
rity, GO.0048859.formation.of.anatomical.boundary, GO.0014009.glial.cell.proliferation, GO.0090715.immunological.memory.f
ormation.process, GO.0051321.meiotic.cell.cycle, GO.0035172.hemocyte.proliferation, GO.0035803.egg.coat.formation, GO.004
8868.pollen.tube.development, GO.0015977.carbon.fixation, GO.0003170.heart.valve.development, GO.0033037.polysaccharide.l
ocalization, GO.0051098.regulation.of.binding, GO.0071695.anatomical.structure.maturation, GO.0044182.filamentous.growth.
of.a.population.of.unicellular.organisms, GO.0046950.cellular.ketone.body.metabolic.process, GO.0021756.striatum.developm
ent, GO.0071669.plant.type.cell.wall.organization.or.biogenesis, GO.0002475.antigen.processing.and.presentation.via.MHC.c
lass.Ib, GO.1901615.organic.hydroxy.compound.metabolic.process, GO.1901440.poly.hydroxyalkanoate..metabolic.process, GO.0
048839.inner.ear.development, GO.0060325.face.morphogenesis, GO.0071838.cell.proliferation.in.bone.marrow, GO.0021987.cer
ebral.cortex.development, GO.0007349.cellularization, GO.0050787.detoxification.of.mercury.ion, GO.0010582.floral.meriste
m.determinacy, GO.0042395.ecdysis..collagen.and.cuticulin.based.cuticle, GO.0006910.phagocytosis..recognition, GO.005123
9.regulation.of.multicellular.organismal.process, GO.0043931.ossification.involved.in.bone.maturation, GO.0030902.hindbra
in.development, GO.0003207.cardiac.chamber.formation, GO.0031018.endocrine.pancreas.development, GO.0050906.detection.of.
stimulus.involved.in.sensory.perception, GO.0048857.neural.nucleus.development, GO.0034405.response.to.fluid.shear.stres
s, GO.0021587.cerebellum.morphogenesis, GO.0060322.head.development, GO.0030154.cell.differentiation, GO.1902074.respons
e.to.salt, GO.0008608.attachment.of.spindle.microtubules.to.kinetochore, GO.0051666.actin.cortical.patch.localization, G
O.0051640.organelle.localization, GO.0008217.regulation.of.blood.pressure, GO.0003407.neural.retina.development, GO.00019
46.lymphangiogenesis, GO.0043609.regulation.of.carbon.utilization, GO.0060042.retina.morphogenesis.in.camera.type.eye, G
O.1900619.acetate.ester.metabolic.process, GO.0001887.selenium.compound.metabolic.process, GO.0060021.roof.of.mouth.devel
opment, GO.0009593.detection.of.chemical.stimulus, GO.0110095.cellular.detoxification.of.aldehyde, GO.0010191.mucilage.me
tabolic.process, GO.0098630.aggregation.of.unicellular.organisms, GO.0030436.asexual.sporulation, GO.0032506.cytokinetic.
process, GO.0042756.drinking.behavior, GO.0006808.regulation.of.nitrogen.utilization, GO.0016334.establishment.or.mainten
ance.of.polarity.of.follicular.epithelium, GO.0010842.retina.layer.formation, GO.0070887.cellular.response.to.chemical.st
imulus, GO.0035910.ascending.aorta.morphogenesis, GO.0044550.secondary.metabolite.biosynthetic.process, GO.0050795.regula
tion.of.behavior, GO.0017144.drug.metabolic.process, GO.0034975.protein.folding.in.endoplasmic.reticulum, GO.0050673.epit
helial.cell.proliferation, GO.0040012.regulation.of.locomotion, GO.0009314.response.to.radiation, GO.0021546.rhombomere.d
evelopment, GO.0009266.response.to.temperature.stimulus, GO.0061032.visceral.serous.pericardium.development, GO.0006959.h
umoral.immune.response, GO.0042026.protein.refolding, GO.0009567.double.fertilization.forming.a.zygote.and.endosperm, GO.
0009886.post.embryonic.animal.morphogenesis, GO.0000902.cell.morphogenesis, GO.1905392.plant.organ.morphogenesis, GO.0006
099.tricarboxylic.acid.cycle, GO.0090647.modulation.of.age.related.behavioral.decline, GO.0009880.embryonic.pattern.speci
fication, GO.0051191.prosthetic.group.biosynthetic.process, GO.0015979.photosynthesis, GO.0002679.respiratory.burst.invol
ved.in.defense.response, GO.0030900.forebrain.development, GO.0007164.establishment.of.tissue.polarity, GO.0002089.lens.m
orphogenesis.in.camera.type.eye, GO.1905393.plant.organ.formation, GO.0060788.ectodermal.placode.formation, GO.0044770.ce
ll.cycle.phase.transition, GO.0002250.adaptive.immune.response, GO.0008366.axon.ensheathment, GO.0009887.animal.organ.mor
phogenesis, GO.0018899.1.2.dichloroethane.metabolic.process, GO.0048544.recognition.of.pollen, GO.0048729.tissue.morphoge
nesis, GO.0017145.stem.cell.division, GO.0030917.midbrain.hindbrain.boundary.development, GO.0070661.leukocyte.proliferat
ion, GO.0048598.embryonic.morphogenesis, GO.1903047.mitotic.cell.cycle.process, GO.0021549.cerebellum.development, GO.002
1766.hippocampus.development, GO.0007275.multicellular.organism.development, GO.0010273.detoxification.of.copper.ion, GO.
0042335.cuticle.development, GO.0042048.olfactory.behavior, GO.0050803.regulation.of.synapse.structure.or.activity, GO.00
03012.muscle.system.process, GO.0006979.response.to.oxidative.stress, GO.0008038.neuron.recognition, GO.0000278.mitotic.c
ell.cycle, GO.0070276.halogen.metabolic.process, GO.0070995.NADPH.oxidation, GO.0003188.heart.valve.formation, GO.008018
6.developmental.vegetative.growth, GO.0002121.inter.male.aggressive.behavior, GO.0051298.centrosome.duplication, GO.00216
75.nerve.development, GO.0001568.blood.vessel.development, GO.0043474.pigment.metabolic.process.involved.in.pigmentation,
GO.0035264.multicellular.organism.growth, GO.0048731.system.development, GO.0007018.microtubule.based.movement, GO.005123
5.maintenance.of.location, GO.0030541.plasmid.partitioning, GO.0036302.atrioventricular.canal.development, GO.0030638.pol
yketide.metabolic.process, GO.0007548.sex.differentiation, GO.0061383.trabecula.morphogenesis, GO.0060004.reflex, GO.0060
411.cardiac.septum.morphogenesis, GO.0001825.blastocyst.formation, GO.0018904.ether.metabolic.process, GO.0035188.hatchin
g, GO.0008345.larval.locomotory.behavior, GO.0032835.glomerulus.development, GO.0019098.reproductive.behavior, GO.004885
5.adenohypophysis.morphogenesis, GO.0061380.superior.colliculus.development, GO.0035094.response.to.nicotine, GO.0048067.

cuticle.pigmentation, GO.0010035.response.to.inorganic.substance, GO.0007276.gamete.generation, GO.0007338.single.fertili
zation, GO.0018942.organometal.metabolic.process, GO.0104004.cellular.response.to.environmental.stimulus, GO.0010227.flor
al.organ.abscission, GO.0035148.tube.formation, GO.0007565.female.pregnancy, GO.0021680.cerebellar.Purkinje.cell.layer.de
velopment, GO.0071981.exit.from.diapause, GO.0060032.notochord.regression, GO.0060274.maintenance.of.stationary.phase, G
O.0009799.specification.of.symmetry, GO.0016445.somatic.diversification.of.immunoglobulins, GO.0006116.NADH.oxidation, G
O.0001554.luteolysis, GO.0007370.ventral.furrow.formation, GO.0007412.axon.target.recognition, GO.0036473.cell.death.in.r
esponse.to.oxidative.stress, GO.0050793.regulation.of.developmental.process, GO.2001057.reactive.nitrogen.species.metabol
ic.process, GO.0003016.respiratory.system.process, GO.0090066.regulation.of.anatomical.structure.size, GO.0009404.toxin.m
etabolic.process, GO.0003014.renal.system.process, GO.0061635.regulation.of.protein.complex.stability, GO.0019884.antige
n.processing.and.presentation.of.exogenous.antigen, GO.0009606.tropism, GO.0045165.cell.fate.commitment, GO.0003179.hear
t.valve.morphogenesis, GO.0030574.collagen.catabolic.process, GO.0009933.meristem.structural.organization, GO.0042752.reg
ulation.of.circadian.rhythm, GO.0006935.chemotaxis, GO.0061034.olfactory.bulb.mitral.cell.layer.development, GO.0043476.p
igment.accumulation, GO.0009798.axis.specification, GO.0036075.replacement.ossification, GO.0060560.developmental.growth.
involved.in.morphogenesis, GO.0007442.hindgut.morphogenesis, GO.0070121.Kupffers.vesicle.development, GO.0009812.flavonoi
d.metabolic.process, GO.0003279.cardiac.septum.development, GO.0035265.organ.growth, GO.0030168.platelet.activation, GO.0
060465.pharynx.development, GO.0048736.appendage.development, GO.0040008.regulation.of.growth, GO.0002263.cell.activatio
n.involved.in.immune.response, GO.0055115.entry.into.diapause, GO.0080167.response.to.karrikin, GO.0051673.membrane.disru
ption.in.other.organism, GO.0021603.cranial.nerve.formation, GO.0048588.developmental.cell.growth, GO.0090559.regulation.
of.membrane.permeability, GO.0006792.regulation.of.sulfur.utilization, GO.0007588.excretion, GO.0042391.regulation.of.mem
brane.potential, GO.0014850.response.to.muscle.activity, GO.0031288.sorocarp.morphogenesis, GO.0006970.response.to.osmoti
c.stress, GO.0010358.leaf.shaping, GO.0009595.detection.of.biotic.stimulus, GO.0048069.eye.pigmentation, GO.0001525.angio
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GO.0051786.all.trans.retinol.13.14.reductase.activity, GO.0005126.cytokine.receptor.binding, GO.0015204.urea.transmembrane.transporter.activity, GO.0016663.oxidoreductase.activity..acting.on.other.nitrogenous.compounds.as.donors..oxygen.as.acceptor, GO.0005345.purine.nucleobase.transmembrane.transporter.activity, GO.0004478.methionine.adenosyltransferase.activity, GO.0050030.L.pipecolate.dehydrogenase.activity, GO.0061547.glycogen.synthase.activity..transferring.glucose.1.phosphate, GO.0048030.disaccharide.binding, GO.0008111.alpha.methylacyl.CoA.racemase.activity, GO.0018537.coenzyme.F420.dependent.N5.N10.methenyltetrahydromethanopterin.reductase.activity, GO.0004012.ATPase.coupled.intramembrane.lipid.transporter.activity, GO.0016615.malate.dehydrogenase.activity, GO.0046978.TAP1.binding, GO.0047470..1.4..alpha.D.glucan.1.alpha.D.glucosylmutase.activity, GO.0046507.UDPsulfoquinovose.synthase.activity, GO.0032050.clathrin.heavy.chain.binding, 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GO.0033558.protein.deacetylase.activity, GO.0045545.syndecan.binding, GO.0061650.ubiquitin.like.protein.conjugating.enzyme.activity, GO.0046997.oxidoreductase.activity..acting.on.the.CH.NH.group.of.donors..flavin.as.acceptor, GO.0010521.telomerase.inhibitor.activity, GO.0008201.heparin.binding, GO.0016643.oxidoreductase.activity..acting.on.the.CH.NH2.group.of.donors..iron.sulfur.protein.as.acceptor, GO.0016823.hydrolase.activity..acting.on.acid.carbon.carbon.bonds..in.ketonic.substances, GO.0055131.C3HC4.type.RING.finger.domain.binding, GO.2001065.mannan.binding, GO.0070205.2.succinyl.6.hydroxy.2.4.cyclohexadiene.1.carboxylate.synthase.activity, GO.0052381.tRNA.dimethylallyltransferase.activity, GO.0061799.cyclic.pyranopterin.monophosphate.synthase.activity, GO.0018849.muconate.cycloisomerase.activity, GO.0016812.hydrolase.activity..acting.on.carbon.nitrogen..but.not.peptide..bonds..in.cyclic.amides, GO.0030975.thiamine.binding, 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199.cholest.4.en.3.one.26.monooxygenase.activity, GO.0018753.cyanuric.acid.amidohydrolase.activity, GO.0008892.guanine.de
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GO.0004644.phosphoribosylglycinamide.formyltransferase.activity, GO.0050067.lysine.2.monooxygenase.activity, GO.0008792.arginine.decarboxylase.activity, GO.0047475.phenylacetate.CoA.ligase.activity, GO.0050661.NADP.binding, GO.0033705.GDP.4.dehydro.6.deoxy.D.mannose.reductase.activity, GO.0008763.UDP.N.acetylmuramate.L.alanine.ligase.activity, GO.0035888.isoguanine.deaminase.activity, GO.0051766.inositol.trisphosphate.kinase.activity, GO.0019205.nucleobase.containing.compound.kinase.activity, GO.0043866.adenylyl.sulfate.reductase..thioredoxin..activity, GO.0016692.NADH.peroxidase.activity, GO.0035064.methylated.histone.binding, GO.0050046.lathosterol.oxidase.activity, GO.0010285.L.L.diaminopimelate.aminotransferase.activity, GO.0030368.interleukin.17.receptor.activity, GO.0060698.endoribonuclease.inhibitor.activity, GO.0042379.chemokine.receptor.binding, GO.0018626.2.chlorobenzoate.1.2.dioxygenase.activity, GO.0051073.adenosylcobinamide.GDP.ribazoletransferase.activity, 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activity, GO.0008419.RNA.lariat.debranching.enzyme.activity, GO.0005003.ephrin.receptor.activity, GO.0033862.UMP.kinase.a
ctivity, GO.0015125.bile.acid.transmembrane.transporter.activity, GO.0004533.exoribonuclease.H.activity, GO.0072320.volum
e.sensitive.chloride.channel.activity, GO.0015099.nickel.cation.transmembrane.transporter.activity, GO.0046353.aminoglyco
side.3.N.acetyltransferase.activity, GO.0050309.sugar.terminal.phosphatase.activity, GO.0005089.Rho.guanyl.nucleotide.exc
hange.factor.activity, GO.0004137.deoxycytidine.kinase.activity, GO.0031459.ATPase.coupled.glycine.betaine.transporter.ac
tivity, GO.0001054.RNA.polymerase.I.activity, GO.0070042.rRNA..uridine.N3...methyltransferase.activity, GO.0008420.RNA.po
lymerase.II.CTD.heptapeptide.repeat.phosphatase.activity, GO.0036402.proteasome.activating.ATPase.activity, GO.0009383.rR
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vity, GO.0030552.cAMP.binding, GO.0004706.JUN.kinase.kinase.kinase.activity, GO.0015486.glycoside.pentoside.hexuronide.ca
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ty, GO.0030743.rRNA..adenosine.2.O...methyltransferase.activity, GO.0004445.inositol.polyphosphate.5.phosphatase.activit
y, GO.0015133.uronic.acid.transmembrane.transporter.activity, GO.0001147.transcription.termination.site.sequence.specifi
c.DNA.binding, GO.0061778.intracellular.chloride.channel.activity, GO.0016150.translation.release.factor.activity..codon.
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vity, GO.0004938.alpha2.adrenergic.receptor.activity, GO.0015411.ATPase.coupled.taurine.transporter.activity, GO.0015080.
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on.specific, GO.0035241.protein.arginine.omega.N.monomethyltransferase.activity, GO.0015087.cobalt.ion.transmembrane.tran
sporter.activity, GO.0052832.inositol.monophosphate.3.phosphatase.activity, GO.0015412.ATPase.coupled.molybdate.transmemb
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endent.tRNA..m5U54..methyltransferase.activity, GO.0052916.23S.rRNA..guanine.1835..N.2...methyltransferase.activity, GO.0
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pecific.ribonuclease.activity, GO.0052910.23S.rRNA..adenine.2085..N.6...dimethyltransferase.activity, GO.0008999.ribosoma
l.protein.alanine.N.acetyltransferase.activity, GO.0008512.sulfate.proton.symporter.activity, GO.0005248.voltage.gated.so
dium.channel.activity, GO.0033677.DNA.RNA.helicase.activity, GO.0005432.calcium.sodium.antiporter.activity, GO.0000992.RN
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ane.transporter.activity, GO.0015616.DNA.translocase.activity, GO.0042800.histone.methyltransferase.activity..H3.K4.speci
fic., GO.0016463.zinc.transmembrane.transporter.activity..phosphorylative.mechanism, GO.0004008.copper.transmembrane.tran
sporter.activity..phosphorylative.mechanism, GO.0015379.potassium.chloride.symporter.activity, GO.0008310.single.strande
d.DNA.3.5.exodeoxyribonuclease.activity, GO.0005228.intracellular.sodium.activated.potassium.channel.activity, GO.005291
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O.0015333.peptide.proton.symporter.activity, GO.0005391.sodium.potassium.exchanging.ATPase.activity, GO.0015192.L.phenyla
lanine.transmembrane.transporter.activity, GO.0015183.L.aspartate.transmembrane.transporter.activity, GO.0046933.proton.t
ransporting.ATP.synthase.activity..rotational.mechanism, GO.0046976.histone.methyltransferase.activity..H3.K27.specific.,
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orter.activity..phosphorylative.mechanism, GO.0005280.amino.acid.proton.symporter.activity, GO.0034639.L.amino.acid.efflu
x.transmembrane.transporter.activity, GO.0032143.single.thymine.insertion.binding, GO.0015269.calcium.activated.potassiu
m.channel.activity, GO.0005451.monovalent.cation.proton.antiporter.activity, GO.0052907.23S.rRNA..adenine.1618..N.6...met
hyltransferase.activity, GO.0000979.RNA.polymerase.II.core.promoter.sequence.specific.DNA.binding, GO.0042970.homoserine.
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ino.acid.secondary.active.transmembrane.transporter.activity, GO.0051139.metal.ion.proton.antiporter.activity, GO.000000
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 GO.0005290.L.histidine.transmembrane.transporter.activity, GO.1990939.ATP.dependent.microtubule.motor.activity, GO.00306
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nel.activity, GO.0052929.ATP.3.cytidine.cytidine.tRNA.adenylyltransferase.activity, GO.0005343.organic.acid.sodium.sympor
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r.activity.involved.in.regulation.of.cardiac.muscle.cell.membrane.potential, GO.0008332.low.voltage.gated.calcium.channe
l.activity, GO.0009675.high.affinity.sulfate.proton.symporter.activity, GO.0022851.GABA.gated.chloride.ion.channel.activi
ty, GO.0033682.5.3.DNA.RNA.helicase.activity, GO.0061995.ATP.dependent.protein.DNA.complex.displacement.activity, GO.0004
931.extracellularly.ATP.gated.cation.channel.activity, GO.0015518.arabinose.proton.symporter.activity, GO.0035497.cAMP.re
sponse.element.binding, GO.0061749.forked.DNA.dependent.helicase.activity, GO.0009679.hexose.proton.symporter.activity, G
O.0016933.extracellularly.glycine.gated.ion.channel.activity, GO.0015191.L.methionine.transmembrane.transporter.activity,
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ng.activity, GO.0043142.single.stranded.DNA.helicase.activity, GO.0042945.D.serine.transmembrane.transporter.activity, G
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ion.potential.repolarization, GO.0043140.3.5.DNA.helicase.activity, GO.0043998.H2A.histone.acetyltransferase.activity, G
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GO.0046972.histone.acetyltransferase.activity..H4.K16.specific., GO.0008511.sodium.potassium.chloride.symporter.activity,
GO.0015501.glutamate.sodium.symporter.activity, GO.0000102.L.methionine.secondary.active.transmembrane.transporter.activi
ty, GO.0022848.acetylcholine.gated.cation.selective.channel.activity, GO.0015535.fucose.proton.symporter.activity, GO.001
6934.extracellularly.glycine.gated.chloride.channel.activity
2020-11-23 19:01:05 INFO::Running selected normalization method: NONE
2020-11-23 19:01:05 INFO::Total filtered features with variance filtering: 0
2020-11-23 19:01:05 INFO::Filtered feature names from variance filtering:
2020-11-23 19:01:05 INFO::Applying z-score to standardize continuous metadata
2020-11-23 19:01:05 INFO::Running selected transform method: NONE
2020-11-23 19:01:05 INFO::Running selected analysis method: LM
2020-11-23 19:01:05 INFO::Creating cluster of 48 R processes
2020-11-23 19:02:13 INFO::Counting total values for each feature
2020-11-23 19:02:13 INFO::Writing residuals to file ./outcome/residuals.rds
2020-11-23 19:02:13 INFO::Writing all results to file (ordered by increasing q-values): ./outcome/all_results.tsv
2020-11-23 19:02:13 INFO::Writing the significant results (those which are less than or equal to the threshold of 0.77000
0 ) to file (ordered by increasing q-values): ./outcome/significant_results.tsv
2020-11-23 19:02:13 INFO::Writing heatmap of significant results to file: ./outcome/heatmap.pdf
[1] "There is not enough metadata in the associations to create a heatmap plot. Please review the associations in text ou
tput file."
2020-11-23 19:02:13 INFO::Writing association plots (one for each significant association) to output folder: ./outcome
2020-11-23 19:02:13 INFO::Plotting associations from most to least significant, grouped by metadata
2020-11-23 19:02:13 INFO::Plotting data for metadata number 1, outcome
```

```
2020-11-23 19:02:13 INFO::Creating boxplot for categorical data, outcome vs GO.0032502.developmental.process
2020-11-23 19:02:13 INFO::Creating boxplot for categorical data, outcome vs GO.0022904.respiratory.electron.transport.cha
in
2020-11-23 19:02:14 INFO::Creating boxplot for categorical data, outcome vs GO.0043177.organic.acid.binding
2020-11-23 19:02:14 INFO::Creating boxplot for categorical data, outcome vs GO.0031406.carboxylic.acid.binding
2020-11-23 19:02:14 INFO::Creating boxplot for categorical data, outcome vs GO.0016763.transferase.activity..transferrin
g.pentosyl.groups
2020-11-23 19:02:14 INFO::Creating boxplot for categorical data, outcome vs GO.0030554.adenyl.nucleotide.binding
2020-11-23 19:02:14 INFO::Creating boxplot for categorical data, outcome vs GO.0032559.adenyl.ribonucleotide.binding
2020-11-23 19:02:14 INFO::Creating boxplot for categorical data, outcome vs GO.0005524.ATP.binding
2020-11-23 19:02:15 INFO::Creating boxplot for categorical data, outcome vs GO.0033293.monocarboxylic.acid.binding
2020-11-23 19:02:15 INFO::Creating boxplot for categorical data, outcome vs GO.0004721.phosphoprotein.phosphatase.activit
y
2020-11-23 19:02:15 INFO::Creating boxplot for categorical data, outcome vs GO.0022900.electron.transport.chain
2020-11-23 19:02:15 INFO::Creating boxplot for categorical data, outcome vs GO.0015291.secondary.active.transmembrane.tra
nsporter.activity
2020-11-23 19:02:15 INFO::Creating boxplot for categorical data, outcome vs GO.0004252.serine.type.endopeptidase.activity
2020-11-23 19:02:15 INFO::Creating boxplot for categorical data, outcome vs GO.0009055.electron.transfer.activity
2020-11-23 19:02:15 INFO::Creating boxplot for categorical data, outcome vs GO.0006996.organelle.organization
2020-11-23 19:02:16 INFO::Creating boxplot for categorical data, outcome vs GO.0016817.hydrolase.activity..acting.on.aci
d.anhydrides
2020-11-23 19:02:16 INFO::Creating boxplot for categorical data, outcome vs GO.0016818.hydrolase.activity..acting.on.aci
d.anhydrides..in.phosphorus.containing.anhydrides
```

In [36]:

```
#I need to figure out how to change how to pivot wider the case colum for an age analysis
df_input_metadata2$age<-as.numeric(df_input_metadata2$age)

age<-Maaslin2(
  input_data = df_input_data2,
  input_metadata = df_input_metadata2,
  output="./age",
  min_abundance = 0.001,
  min_prevalence = 0.001,
  normalization = "NONE",
  transform = "NONE",
  analysis_method = "LM",
  max_significance = 0.25,
  #random_effects = c("sample_name","publication","collection_location","sequence_type"),
  random_effects = c("sample_name","publication"),
  fixed_effects = c("case","age"),
  correction="BH",
  standardize = TRUE,
  cores = 48,
  plot_heatmap = TRUE,
  plot_scatter = TRUE,
  heatmap_first_n = 100,
  reference=c("case,COVID19"))
```

```
[1] "Warning: Deleting existing log file: ./age/maaslin2.log"
2020-11-23 20:03:14 INFO::Writing function arguments to log file
2020-11-23 20:03:14 INFO::Verifying options selected are valid
2020-11-23 20:03:14 INFO::Determining format of input files
2020-11-23 20:03:14 INFO::Input format is data samples as rows and metadata samples as rows
2020-11-23 20:03:15 INFO::Formula for random effects: expr ~ (1 | sample_name) + (1 | publication)
2020-11-23 20:03:15 INFO::Formula for fixed effects: expr ~  case + age
2020-11-23 20:03:15 INFO::Filter data based on min abundance and min prevalence
2020-11-23 20:03:15 INFO::Total samples in data: 141
2020-11-23 20:03:15 INFO::Min samples required with min abundance for a feature not to be filtered: 0.141000
2020-11-23 20:03:15 INFO::Total filtered features: 13051
2020-11-23 20:03:15 INFO::Filtered feature names from abundance and prevalence filtering: GO.0045212.obsolete.neurotransm
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d.DNA.3.5.exodeoxyribonuclease.activity, GO.0005228.intracellular.sodium.activated.potassium.channel.activity, GO.005291
2.23S.rRNA..guanine.748..N.1...methyltransferase.activity, GO.0015189.L.lysine.transmembrane.transporter.activity, GO.001
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GO.0099604.ligand.gated.calcium.channel.activity, GO.0005302.L.tyrosine.transmembrane.transporter.activity, GO.0000978.RN
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98.5.10.methylenetetrahydrofolate.dependent.tRNA..m5U54..methyltransferase.activity, GO.0000986.bacterial.type.cis.regula
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nel.activity, GO.0052929.ATP.3.cytidine.cytidine.tRNA.adenylyltransferase.activity, GO.0005343.organic.acid.sodium.sympor
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ty, GO.0033682.5.3.DNA.RNA.helicase.activity, GO.0061995.ATP.dependent.protein.DNA.complex.displacement.activity, GO.0004
931.extracellularly.ATP.gated.cation.channel.activity, GO.0015518.arabinose.proton.symporter.activity, GO.0035497.cAMP.re
sponse.element.binding, GO.0061749.forked.DNA.dependent.helicase.activity, GO.0009679.hexose.proton.symporter.activity, G
O.0016933.extracellularly.glycine.gated.ion.channel.activity, GO.0015191.L.methionine.transmembrane.transporter.activity,
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GO.0005222.intracellular.cAMP.activated.cation.channel.activity, GO.0001006.RNA.polymerase.III.type.3.promoter.sequence.s

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GO.0046972.histone.acetyltransferase.activity..H4.K16.specific., GO.0008511.sodium.potassium.chloride.symporter.activity,
GO.0015501.glutamate.sodium.symporter.activity, GO.0000102.L.methionine.secondary.active.transmembrane.transporter.activi
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6934.extracellularly.glycine.gated.chloride.channel.activity
2020-11-23 20:03:15 INFO::Running selected normalization method: NONE
2020-11-23 20:03:15 INFO::Total filtered features with variance filtering: 0
2020-11-23 20:03:15 INFO::Filtered feature names from variance filtering:
2020-11-23 20:03:15 INFO::Applying z-score to standardize continuous metadata
2020-11-23 20:03:15 INFO::Running selected transform method: NONE
2020-11-23 20:03:15 INFO::Running selected analysis method: LM
2020-11-23 20:03:15 INFO::Creating cluster of 48 R processes
2020-11-23 20:04:26 INFO::Counting total values for each feature
2020-11-23 20:04:26 WARNING::Deleting existing residuals file: ./age/residuals.rds
2020-11-23 20:04:26 INFO::Writing residuals to file ./age/residuals.rds
2020-11-23 20:04:26 INFO::Writing all results to file (ordered by increasing q-values): ./age/all_results.tsv
2020-11-23 20:04:26 INFO::Writing the significant results (those which are less than or equal to the threshold of 0.25000
0 ) to file (ordered by increasing q-values): ./age/significant_results.tsv
2020-11-23 20:04:26 INFO::Writing heatmap of significant results to file: ./age/heatmap.pdf
2020-11-23 20:04:26 INFO::Writing association plots (one for each significant association) to output folder: ./age
2020-11-23 20:04:26 INFO::Plotting associations from most to least significant, grouped by metadata
2020-11-23 20:04:26 INFO::Plotting data for metadata number 1, case
2020-11-23 20:04:26 INFO::Creating boxplot for categorical data, case vs GO.0003676.nucleic.acid.binding
2020-11-23 20:04:26 INFO::Creating boxplot for categorical data, case vs GO.0044271.cellular.nitrogen.compound.biosynthet
ic.process
2020-11-23 20:04:27 INFO::Creating boxplot for categorical data, case vs GO.0051020.GTPase.binding
2020-11-23 20:04:27 INFO::Creating boxplot for categorical data, case vs GO.0005085.guanyl.nucleotide.exchange.factor.act
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2020-11-23 20:04:27 INFO::Creating boxplot for categorical data, case vs GO.0003677.DNA.binding
2020-11-23 20:04:27 INFO::Creating boxplot for categorical data, case vs GO.0018130.heterocycle.biosynthetic.process
2020-11-23 20:04:27 INFO::Creating boxplot for categorical data, case vs GO.0007264.small.GTPase.mediated.signal.transduc
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2020-11-23 20:04:28 INFO::Creating boxplot for categorical data, case vs GO.0016740.transferase.activity
2020-11-23 20:04:28 INFO::Creating boxplot for categorical data, case vs GO.0005102.signaling.receptor.binding
2020-11-23 20:04:28 INFO::Creating boxplot for categorical data, case vs GO.0097435.supramolecular.fiber.organization
2020-11-23 20:04:28 INFO::Creating boxplot for categorical data, case vs GO.0007015.actin.filament.organization
2020-11-23 20:04:28 INFO::Creating boxplot for categorical data, case vs GO.0015980.energy.derivation.by.oxidation.of.org
anic.compounds
2020-11-23 20:04:29 INFO::Creating boxplot for categorical data, case vs GO.0035556.intracellular.signal.transduction
2020-11-23 20:04:29 INFO::Creating boxplot for categorical data, case vs GO.0050839.cell.adhesion.molecule.binding
2020-11-23 20:04:29 INFO::Creating boxplot for categorical data, case vs GO.0016477.cell.migration
2020-11-23 20:04:29 INFO::Creating boxplot for categorical data, case vs GO.0033627.cell.adhesion.mediated.by.integrin
2020-11-23 20:04:29 INFO::Creating boxplot for categorical data, case vs GO.0007160.cell.matrix.adhesion
2020-11-23 20:04:29 INFO::Creating boxplot for categorical data, case vs GO.0005178.integrin.binding
2020-11-23 20:04:30 INFO::Creating boxplot for categorical data, case vs GO.0050660.flavin.adenine.dinucleotide.binding
2020-11-23 20:04:30 INFO::Creating boxplot for categorical data, case vs GO.0040011.locomotion
2020-11-23 20:04:30 INFO::Creating boxplot for categorical data, case vs GO.0031589.cell.substrate.adhesion
2020-11-23 20:04:30 INFO::Creating boxplot for categorical data, case vs GO.0016051.carbohydrate.biosynthetic.process
2020-11-23 20:04:30 INFO::Creating boxplot for categorical data, case vs GO.0005976.polysaccharide.metabolic.process
2020-11-23 20:04:30 INFO::Creating boxplot for categorical data, case vs GO.0044264.cellular.polysaccharide.metabolic.pro
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2020-11-23 20:04:31 INFO::Creating boxplot for categorical data, case vs GO.0034637.cellular.carbohydrate.biosynthetic.pr
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2020-11-23 20:04:31 INFO::Creating boxplot for categorical data, case vs GO.0033692.cellular.polysaccharide.biosynthetic.
process
2020-11-23 20:04:31 INFO::Creating boxplot for categorical data, case vs GO.0007229.integrin.mediated.signaling.pathway
2020-11-23 20:04:31 INFO::Creating boxplot for categorical data, case vs GO.0046379.extracellular.polysaccharide.metaboli
c.process
2020-11-23 20:04:31 INFO::Creating boxplot for categorical data, case vs GO.0045226.extracellular.polysaccharide.biosynth
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2020-11-23 20:04:32 INFO::Creating boxplot for categorical data, case vs GO.0019899.enzyme.binding
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2020-11-23 20:04:33 INFO::Creating boxplot for categorical data, case vs GO.0022610.biological.adhesion
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2020-11-23 20:04:33 INFO::Creating boxplot for categorical data, case vs GO.0016043.cellular.component.organization
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2020-11-23 20:04:35 INFO::Creating boxplot for categorical data, case vs GO.0005102.signaling.receptor.binding
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2020-11-23 20:04:35 INFO::Creating boxplot for categorical data, case vs GO.0010646.regulation.of.cell.communication
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2020-11-23 20:04:36 INFO::Creating boxplot for categorical data, case vs GO.0022613.ribonucleoprotein.complex.biogenesis
2020-11-23 20:04:36 INFO::Creating boxplot for categorical data, case vs GO.0072330.monocarboxylic.acid.biosynthetic.proc
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2020-11-23 20:04:36 INFO::Creating boxplot for categorical data, case vs GO.0034470.ncRNA.processing
2020-11-23 20:04:36 INFO::Creating boxplot for categorical data, case vs GO.0005543.phospholipid.binding
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metabolic.process
2020-11-23 20:04:37 INFO::Creating boxplot for categorical data, case vs GO.0008289.lipid.binding
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2020-11-23 20:04:37 INFO::Creating boxplot for categorical data, case vs GO.0140110.transcription.regulator.activity
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2020-11-23 20:04:38 INFO::Creating boxplot for categorical data, case vs GO.0006364.rRNA.processing
2020-11-23 20:04:38 INFO::Creating boxplot for categorical data, case vs GO.0098772.molecular.function.regulator
2020-11-23 20:04:38 INFO::Creating boxplot for categorical data, case vs GO.0000451.rRNA.2.O.methylation
2020-11-23 20:04:38 INFO::Creating boxplot for categorical data, case vs GO.0062105.RNA.2.O.methyltransferase.activity
2020-11-23 20:04:38 INFO::Creating boxplot for categorical data, case vs GO.0016477.cell.migration
2020-11-23 20:04:38 INFO::Creating boxplot for categorical data, case vs GO.0019438.aromatic.compound.biosynthetic.proces
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2020-11-23 20:04:39 INFO::Creating boxplot for categorical data, case vs GO.0036211.protein.modification.process
2020-11-23 20:04:39 INFO::Creating boxplot for categorical data, case vs GO.0006464.cellular.protein.modification.process
2020-11-23 20:04:39 INFO::Creating boxplot for categorical data, case vs GO.0000470.maturation.of.LSU.rRNA
2020-11-23 20:04:39 INFO::Creating boxplot for categorical data, case vs GO.0000460.maturation.of.5.8S.rRNA
2020-11-23 20:04:39 INFO::Creating boxplot for categorical data, case vs GO.0000466.maturation.of.5.8S.rRNA.from.tricistr
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2020-11-23 20:04:41 INFO::Creating boxplot for categorical data, case vs GO.0023051.regulation.of.signaling
2020-11-23 20:04:41 INFO::Creating boxplot for categorical data, case vs GO.0010646.regulation.of.cell.communication
2020-11-23 20:04:41 INFO::Creating boxplot for categorical data, case vs GO.0052689.carboxylic.ester.hydrolase.activity
2020-11-23 20:04:42 INFO::Creating boxplot for categorical data, case vs GO.0005975.carbohydrate.metabolic.process
2020-11-23 20:04:42 INFO::Creating boxplot for categorical data, case vs GO.0009055.electron.transfer.activity
2020-11-23 20:04:42 INFO::Creating boxplot for categorical data, case vs GO.0016301.kinase.activity
2020-11-23 20:04:42 INFO::Creating boxplot for categorical data, case vs GO.0034660.ncRNA.metabolic.process
2020-11-23 20:04:42 INFO::Creating boxplot for categorical data, case vs GO.0016477.cell.migration
2020-11-23 20:04:42 INFO::Creating boxplot for categorical data, case vs GO.0007165.signal.transduction
2020-11-23 20:04:43 INFO::Creating boxplot for categorical data, case vs GO.0071704.organic.substance.metabolic.process
2020-11-23 20:04:43 INFO::Creating boxplot for categorical data, case vs GO.0005544.calcium.dependent.phospholipid.bindin
g
2020-11-23 20:04:43 INFO::Creating boxplot for categorical data, case vs GO.0003676.nucleic.acid.binding
2020-11-23 20:04:43 INFO::Creating boxplot for categorical data, case vs GO.0004557.alpha.galactosidase.activity
2020-11-23 20:04:43 INFO::Creating boxplot for categorical data, case vs GO.0061024.membrane.organization
2020-11-23 20:04:43 INFO::Creating boxplot for categorical data, case vs GO.0022900.electron.transport.chain
2020-11-23 20:04:44 INFO::Creating boxplot for categorical data, case vs GO.0016742.hydroxymethyl...formyl..and.related.t
ransferase.activity
2020-11-23 20:04:44 INFO::Creating boxplot for categorical data, case vs GO.0042398.cellular.modified.amino.acid.biosynth
etic.process
2020-11-23 20:04:44 INFO::Creating boxplot for categorical data, case vs GO.0022904.respiratory.electron.transport.chain
2020-11-23 20:04:44 INFO::Creating boxplot for categorical data, case vs GO.0006428.isoleucyl.tRNA.aminoacylation
2020-11-23 20:04:44 INFO::Creating boxplot for categorical data, case vs GO.0004822.isoleucine.tRNA.ligase.activity
2020-11-23 20:04:44 INFO::Creating boxplot for categorical data, case vs GO.0071496.cellular.response.to.external.stimulu
s
2020-11-23 20:04:45 INFO::Creating boxplot for categorical data, case vs GO.0031668.cellular.response.to.extracellular.st
imulus
2020-11-23 20:04:45 INFO::Creating boxplot for categorical data, case vs GO.0044260.cellular.macromolecule.metabolic.proc
ess
2020-11-23 20:04:45 INFO::Creating boxplot for categorical data, case vs GO.0009991.response.to.extracellular.stimulus
2020-11-23 20:04:45 INFO::Creating boxplot for categorical data, case vs GO.0007154.cell.communication
2020-11-23 20:04:45 INFO::Creating boxplot for categorical data, case vs GO.0045333.cellular.respiration
2020-11-23 20:04:45 INFO::Creating boxplot for categorical data, case vs GO.0015711.organic.anion.transport
2020-11-23 20:04:46 INFO::Creating boxplot for categorical data, case vs GO.0006304.DNA.modification
2020-11-23 20:04:46 INFO::Creating boxplot for categorical data, case vs GO.0007166.cell.surface.receptor.signaling.pathw
ay
2020-11-23 20:04:46 INFO::Creating boxplot for categorical data, case vs GO.0003723.RNA.binding
2020-11-23 20:04:46 INFO::Creating boxplot for categorical data, case vs GO.0043565.sequence.specific.DNA.binding
2020-11-23 20:04:46 INFO::Creating boxplot for categorical data, case vs GO.0006793.phosphorus.metabolic.process
2020-11-23 20:04:46 INFO::Creating boxplot for categorical data, case vs GO.0022607.cellular.component.assembly
```

```
2020-11-23 20:04:46 INFO::Creating boxplot for categorical data, case vs GO.0015980.energy.derivation.by.oxidation.of.org
anic.compounds
2020-11-23 20:04:47 INFO::Creating boxplot for categorical data, case vs GO.0006450.regulation.of.translational.fidelity
2020-11-23 20:04:47 INFO::Creating boxplot for categorical data, case vs GO.0006796.phosphate.containing.compound.metabol
ic.process
2020-11-23 20:04:47 INFO::Creating boxplot for categorical data, case vs GO.0002161.aminoacyl.tRNA.editing.activity
2020-11-23 20:04:47 INFO::Creating boxplot for categorical data, case vs GO.0106074.aminoacyl.tRNA.metabolism.involved.i
n.translational.fidelity
2020-11-23 20:04:47 INFO::Creating boxplot for categorical data, case vs GO.0031167.rRNA.methylation
2020-11-23 20:04:47 INFO::Creating boxplot for categorical data, case vs GO.0004672.protein.kinase.activity
2020-11-23 20:04:48 INFO::Creating boxplot for categorical data, case vs GO.0000154.rRNA.modification
2020-11-23 20:04:48 INFO::Creating boxplot for categorical data, case vs GO.0097435.supramolecular.fiber.organization
2020-11-23 20:04:48 INFO::Creating boxplot for categorical data, case vs GO.0007015.actin.filament.organization
2020-11-23 20:04:48 INFO::Creating boxplot for categorical data, case vs GO.0001510.RNA.methylation
2020-11-23 20:04:48 INFO::Creating boxplot for categorical data, case vs GO.0015849.organic.acid.transport
2020-11-23 20:04:48 INFO::Creating boxplot for categorical data, case vs GO.0033036.macromolecule.localization
2020-11-23 20:04:49 INFO::Creating boxplot for categorical data, case vs GO.0008104.protein.localization
2020-11-23 20:04:49 INFO::Creating boxplot for categorical data, case vs GO.0140102.catalytic.activity..acting.on.a.rRNA
2020-11-23 20:04:49 INFO::Creating boxplot for categorical data, case vs GO.0008649.rRNA.methyltransferase.activity
2020-11-23 20:04:49 INFO::Creating boxplot for categorical data, case vs GO.0042578.phosphoric.ester.hydrolase.activity
2020-11-23 20:04:49 INFO::Creating boxplot for categorical data, case vs GO.0016773.phosphotransferase.activity..alcohol.
group.as.acceptor
2020-11-23 20:04:49 INFO::Creating boxplot for categorical data, case vs GO.0003677.DNA.binding
2020-11-23 20:04:50 INFO::Creating boxplot for categorical data, case vs GO.0046942.carboxylic.acid.transport
2020-11-23 20:04:50 INFO::Creating boxplot for categorical data, case vs GO.0045184.establishment.of.protein.localization
2020-11-23 20:04:50 INFO::Creating boxplot for categorical data, case vs GO.0008173.RNA.methyltransferase.activity
2020-11-23 20:04:50 INFO::Creating boxplot for categorical data, case vs GO.0033627.cell.adhesion.mediated.by.integrin
2020-11-23 20:04:50 INFO::Creating boxplot for categorical data, case vs GO.0007160.cell.matrix.adhesion
2020-11-23 20:04:50 INFO::Creating boxplot for categorical data, case vs GO.0005178.integrin.binding
2020-11-23 20:04:51 INFO::Creating boxplot for categorical data, case vs GO.0006468.protein.phosphorylation
2020-11-23 20:04:51 INFO::Creating boxplot for categorical data, case vs GO.0065008.regulation.of.biological.quality
2020-11-23 20:04:51 INFO::Creating boxplot for categorical data, case vs GO.0051020.GTPase.binding
2020-11-23 20:04:51 INFO::Creating boxplot for categorical data, case vs GO.0005085.guanyl.nucleotide.exchange.factor.act
ivity
2020-11-23 20:04:51 INFO::Creating boxplot for categorical data, case vs GO.0007155.cell.adhesion
2020-11-23 20:04:51 INFO::Creating boxplot for categorical data, case vs GO.0006082.organic.acid.metabolic.process
2020-11-23 20:04:52 INFO::Creating boxplot for categorical data, case vs GO.0019752.carboxylic.acid.metabolic.process
2020-11-23 20:04:52 INFO::Creating boxplot for categorical data, case vs GO.0008652.cellular.amino.acid.biosynthetic.proc
ess
2020-11-23 20:04:52 INFO::Creating boxplot for categorical data, case vs GO.0043436.oxoacid.metabolic.process
2020-11-23 20:04:52 INFO::Creating boxplot for categorical data, case vs GO.0050839.cell.adhesion.molecule.binding
2020-11-23 20:04:52 INFO::Creating boxplot for categorical data, case vs GO.0031589.cell.substrate.adhesion
2020-11-23 20:04:52 INFO::Creating boxplot for categorical data, case vs GO.0015940.pantothenate.biosynthetic.process
2020-11-23 20:04:53 INFO::Creating boxplot for categorical data, case vs GO.0008863.formate.dehydrogenase..NAD...activity
2020-11-23 20:04:53 INFO::Creating boxplot for categorical data, case vs GO.0022610.biological.adhesion
2020-11-23 20:04:53 INFO::Creating boxplot for categorical data, case vs GO.0007264.small.GTPase.mediated.signal.transduc
tion
2020-11-23 20:04:53 INFO::Creating boxplot for categorical data, case vs GO.0043412.macromolecule.modification
2020-11-23 20:04:53 INFO::Creating boxplot for categorical data, case vs GO.0016053.organic.acid.biosynthetic.process
```

```
2020-11-23 20:04:53 INFO::Creating boxplot for categorical data, case vs GO.0046394.carboxylic.acid.biosynthetic.process
2020-11-23 20:04:54 INFO::Creating boxplot for categorical data, case vs GO.0007229.integrin.mediated.signaling.pathway
2020-11-23 20:04:54 INFO::Creating boxplot for categorical data, case vs GO.0006399.tRNA.metabolic.process
2020-11-23 20:04:54 INFO::Creating boxplot for categorical data, case vs GO.0043170.macromolecule.metabolic.process
2020-11-23 20:04:54 INFO::Creating boxplot for categorical data, case vs GO.0004725.protein.tyrosine.phosphatase.activity
2020-11-23 20:04:54 INFO::Creating boxplot for categorical data, case vs GO.0016651.oxidoreductase.activity..acting.on.NA
D.P.H
2020-11-23 20:04:54 INFO::Creating boxplot for categorical data, case vs GO.0006865.amino.acid.transport
2020-11-23 20:04:55 INFO::Creating boxplot for categorical data, case vs GO.0016627.oxidoreductase.activity..acting.on.th
e.CH.CH.group.of.donors
2020-11-23 20:04:55 INFO::Creating boxplot for categorical data, case vs GO.0015939.pantothenate.metabolic.process
2020-11-23 20:04:55 INFO::Creating boxplot for categorical data, case vs GO.0006305.DNA.alkylation
2020-11-23 20:04:55 INFO::Creating boxplot for categorical data, case vs GO.0044728.DNA.methylation.or.demethylation
2020-11-23 20:04:55 INFO::Creating boxplot for categorical data, case vs GO.0006306.DNA.methylation
2020-11-23 20:04:55 INFO::Creating boxplot for categorical data, case vs GO.0016655.oxidoreductase.activity..acting.on.NA
D.P.H..quinone.or.similar.compound.as.acceptor
2020-11-23 20:04:56 INFO::Creating boxplot for categorical data, case vs GO.0071709.membrane.assembly
2020-11-23 20:04:56 INFO::Creating boxplot for categorical data, case vs GO.0016624.oxidoreductase.activity..acting.on.th
e.aldehyde.or.oxo.group.of.donors..disulfide.as.acceptor
2020-11-23 20:04:56 INFO::Creating boxplot for categorical data, case vs GO.0009008.DNA.methyltransferase.activity
2020-11-23 20:04:56 INFO::Creating boxplot for categorical data, case vs GO.0000287.magnesium.ion.binding
2020-11-23 20:04:56 INFO::Creating boxplot for categorical data, case vs GO.0015980.energy.derivation.by.oxidation.of.org
anic.compounds
2020-11-23 20:04:56 INFO::Creating boxplot for categorical data, case vs GO.0099177.regulation.of.trans.synaptic.signalin
g
2020-11-23 20:04:56 INFO::Creating boxplot for categorical data, case vs GO.0050804.modulation.of.chemical.synaptic.trans
mission
2020-11-23 20:04:57 INFO::Creating boxplot for categorical data, case vs GO.0007187.G.protein.coupled.receptor.signaling.
pathway..coupled.to.cyclic.nucleotide.second.messenger
2020-11-23 20:04:57 INFO::Creating boxplot for categorical data, case vs GO.0051966.regulation.of.synaptic.transmission..
glutamatergic
2020-11-23 20:04:57 INFO::Creating boxplot for categorical data, case vs GO.0007188.adenylate.cyclase.modulating.G.protei
n.coupled.receptor.signaling.pathway
2020-11-23 20:04:57 INFO::Creating boxplot for categorical data, case vs GO.1902600.proton.transmembrane.transport
2020-11-23 20:04:57 INFO::Creating boxplot for categorical data, case vs GO.0007193.adenylate.cyclase.inhibiting.G.protei
n.coupled.receptor.signaling.pathway
2020-11-23 20:04:57 INFO::Creating boxplot for categorical data, case vs GO.0007196.adenylate.cyclase.inhibiting.G.protei
n.coupled.glutamate.receptor.signaling.pathway
2020-11-23 20:04:58 INFO::Creating boxplot for categorical data, case vs GO.0098988.G.protein.coupled.glutamate.receptor.
activity
2020-11-23 20:04:58 INFO::Creating boxplot for categorical data, case vs GO.0001640.adenylate.cyclase.inhibiting.G.protei
n.coupled.glutamate.receptor.activity
2020-11-23 20:04:58 INFO::Creating boxplot for categorical data, case vs GO.0001642.group.III.metabotropic.glutamate.rece
ptor.activity
2020-11-23 20:04:58 INFO::Creating boxplot for categorical data, case vs GO.0016311.dephosphorylation
2020-11-23 20:04:58 INFO::Creating boxplot for categorical data, case vs GO.0044271.cellular.nitrogen.compound.biosynthet
ic.process
2020-11-23 20:04:58 INFO::Creating boxplot for categorical data, case vs GO.0044255.cellular.lipid.metabolic.process
2020-11-23 20:04:59 INFO::Creating boxplot for categorical data, case vs GO.0019438.aromatic.compound.biosynthetic.proces
```

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s
2020-11-23 20:04:59 INFO::Creating boxplot for categorical data, case vs GO.0009987.cellular.process
2020-11-23 20:04:59 INFO::Creating boxplot for categorical data, case vs GO.0018130.heterocycle.biosynthetic.process
2020-11-23 20:04:59 INFO::Creating boxplot for categorical data, case vs GO.0006520.cellular.amino.acid.metabolic.process
2020-11-23 20:04:59 INFO::Creating boxplot for categorical data, case vs GO.0030420.establishment.of.competence.for.trans
formation
2020-11-23 20:04:59 INFO::Creating boxplot for categorical data, case vs GO.0003676.nucleic.acid.binding
2020-11-23 20:05:00 INFO::Creating boxplot for categorical data, case vs GO.0046835.carbohydrate.phosphorylation
2020-11-23 20:05:00 INFO::Creating boxplot for categorical data, case vs GO.1901681.sulfur.compound.binding
2020-11-23 20:05:00 INFO::Creating boxplot for categorical data, case vs GO.0004747.ribokinase.activity
2020-11-23 20:05:00 INFO::Creating boxplot for categorical data, case vs GO.0016791.phosphatase.activity
2020-11-23 20:05:00 INFO::Creating boxplot for categorical data, case vs GO.0033218.amide.binding
2020-11-23 20:05:00 INFO::Creating boxplot for categorical data, case vs GO.0006766.vitamin.metabolic.process
2020-11-23 20:05:01 INFO::Creating boxplot for categorical data, case vs GO.0006767.water.soluble.vitamin.metabolic.proce
ss
2020-11-23 20:05:01 INFO::Creating boxplot for categorical data, case vs GO.0009064.glutamine.family.amino.acid.metaboli
c.process
2020-11-23 20:05:01 INFO::Creating boxplot for categorical data, case vs GO.0009110.vitamin.biosynthetic.process
2020-11-23 20:05:01 INFO::Creating boxplot for categorical data, case vs GO.0042364.water.soluble.vitamin.biosynthetic.pr
ocess
2020-11-23 20:05:01 INFO::Creating boxplot for categorical data, case vs GO.0016645.oxidoreductase.activity..acting.on.th
e.CH.NH.group.of.donors
2020-11-23 20:05:01 INFO::Creating boxplot for categorical data, case vs GO.0016310.phosphorylation
2020-11-23 20:05:02 INFO::Creating boxplot for categorical data, case vs GO.0038023.signaling.receptor.activity
2020-11-23 20:05:02 INFO::Creating boxplot for categorical data, case vs GO.0016070.RNA.metabolic.process
2020-11-23 20:05:02 INFO::Creating boxplot for categorical data, case vs GO.0008238.exopeptidase.activity
2020-11-23 20:05:02 INFO::Creating boxplot for categorical data, case vs GO.0016741.transferase.activity..transferring.on
e.carbon.groups
2020-11-23 20:05:02 INFO::Creating boxplot for categorical data, case vs GO.0046417.chorismate.metabolic.process
2020-11-23 20:05:02 INFO::Creating boxplot for categorical data, case vs GO.0009423.chorismate.biosynthetic.process
2020-11-23 20:05:03 INFO::Creating boxplot for categorical data, case vs GO.0008757.S.adenosylmethionine.dependent.methyl
transferase.activity
2020-11-23 20:05:03 INFO::Creating boxplot for categorical data, case vs GO.0032775.DNA.methylation.on.adenine
2020-11-23 20:05:03 INFO::Creating boxplot for categorical data, case vs GO.0004721.phosphoprotein.phosphatase.activity
2020-11-23 20:05:03 INFO::Creating boxplot for categorical data, case vs GO.0009007.site.specific.DNA.methyltransferase..
adenine.specific..activity
2020-11-23 20:05:03 INFO::Creating boxplot for categorical data, case vs GO.0060089.molecular.transducer.activity
2020-11-23 20:05:04 INFO::Creating boxplot for categorical data, case vs GO.0003855.3.dehydroquinate.dehydratase.activity
2020-11-23 20:05:04 INFO::Creating boxplot for categorical data, case vs GO.0004673.protein.histidine.kinase.activity
2020-11-23 20:05:04 INFO::Creating boxplot for categorical data, case vs GO.0043414.macromolecule.methylation
2020-11-23 20:05:04 INFO::Creating boxplot for categorical data, case vs GO.0043650.dicarboxylic.acid.biosynthetic.proces
s
2020-11-23 20:05:04 INFO::Creating boxplot for categorical data, case vs GO.0009073.aromatic.amino.acid.family.biosynthet
ic.process
2020-11-23 20:05:04 INFO::Creating boxplot for categorical data, case vs GO.0060089.molecular.transducer.activity
2020-11-23 20:05:05 INFO::Creating boxplot for categorical data, case vs GO.0016775.phosphotransferase.activity..nitrogen
ous.group.as.acceptor
2020-11-23 20:05:05 INFO::Creating boxplot for categorical data, case vs GO.0046873.metal.ion.transmembrane.transporter.a
ctivity
```

```
2020-11-23 20:05:05 INFO::Creating boxplot for categorical data, case vs GO.0003678.DNA.helicase.activity
2020-11-23 20:05:05 INFO::Creating boxplot for categorical data, case vs GO.0032259.methylation
2020-11-23 20:05:05 INFO::Creating boxplot for categorical data, case vs GO.0022613.ribonucleoprotein.complex.biogenesis
2020-11-23 20:05:05 INFO::Creating boxplot for categorical data, case vs GO.0016874.ligase.activity
2020-11-23 20:05:06 INFO::Creating boxplot for categorical data, case vs GO.0038023.signaling.receptor.activity
2020-11-23 20:05:06 INFO::Creating boxplot for categorical data, case vs GO.0008237.metallopeptidase.activity
2020-11-23 20:05:06 INFO::Creating boxplot for categorical data, case vs GO.0051641.cellular.localization
2020-11-23 20:05:08 INFO::Plotting data for metadata number 2, age
2020-11-23 20:05:08 INFO::Creating scatter plot for continuous data, age vs GO.0016407.acetyltransferase.activity
`geom_smooth()` using formula 'y ~ x'

Warning message:
“Removed 29 rows containing missing values (geom_point).”
2020-11-23 20:05:08 INFO::Creating scatter plot for continuous data, age vs GO.0044036.cell.wall.macromolecule.metabolic.
process
`geom_smooth()` using formula 'y ~ x'

Warning message:
“Removed 28 rows containing missing values (geom_point).”
2020-11-23 20:05:08 INFO::Creating scatter plot for continuous data, age vs GO.0016667.oxidoreductase.activity..acting.o
n.a.sulfur.group.of.donors
`geom_smooth()` using formula 'y ~ x'

Warning message:
“Removed 28 rows containing missing values (geom_point).”
2020-11-23 20:05:08 INFO::Creating scatter plot for continuous data, age vs GO.0016831.carboxy.lyase.activity
`geom_smooth()` using formula 'y ~ x'

Warning message:
“Removed 29 rows containing missing values (geom_point).”
`geom_smooth()` using formula 'y ~ x'

Warning message:
“Removed 29 rows containing missing values (geom_point).”
`geom_smooth()` using formula 'y ~ x'

Warning message:
“Removed 28 rows containing missing values (geom_point).”
`geom_smooth()` using formula 'y ~ x'

Warning message:
“Removed 28 rows containing missing values (geom_point).”
`geom_smooth()` using formula 'y ~ x'

Warning message:
“Removed 29 rows containing missing values (geom_point).”
```

MaAsLin2 outcome analyisis (pruned_samples)

In [167…

```
bac_pseq_outcome<-subset_samples(physeq = bac_pseq_no_neg,outcome!="NA")
#bac_pseq_outcome<-subset_samples(physeq = bac_pseq_no_neg, case=="COVID19")
bac_pseq_outcome
sample_info_tab<-sample_data(bac_pseq_outcome)
sample_info_tab_phy <- sample_data(sample_info_tab)
deseq_counts<-phyloseq_to_deseq2(physeq = bac_pseq_outcome,design = ~ 1)
deseq_counts_vst <- estimateSizeFactors(deseq_counts, type = "poscounts")
vst_trans_count_tab <- assay(deseq_counts_vst)
vst_count_phy <- otu_table(vst_trans_count_tab, taxa_are_rows=T)
vst_tax_phy <- tax_table(bac_pseq_no_neg)
vst_physeq <- phyloseq(vst_count_phy, vst_tax_phy,sample_data(bac_pseq_outcome))
vst_physeq_comp<-microbiome::transform(x = vst_physeq,transform = "compositional")
df_input_data2<-data.frame(t(otu_table(vst_physeq_comp)))
df_input_metadata2<-data.frame(sample_data(vst_physeq_comp))
```

```
phyloseq-class experiment-level object
```

```
otu_table()   OTU Table:         [ 13844 taxa and 39 samples ]
sample_data() Sample Data:       [ 39 samples by 71 sample variables ]
tax_table()   Taxonomy Table:    [ 13844 taxa by 3 taxonomic ranks ]
converting counts to integer mode
```

ok thats interesting it looks like it just through everything else into NA regardless of case... I guess lets trying it again with only covid cases and also look at age

In [39]:

```
df_input_metadata2$age<-as.numeric(df_input_metadata2$age)
subset_outcome<-Maaslin2(
  input_data = df_input_data2,
  input_metadata = df_input_metadata2,
  output="./subset_outcome",
  min_abundance = 0.001,
  min_prevalence = 0.001,
  normalization = "NONE",
  transform = "NONE",
  analysis_method = "LM",
  max_significance = 0.25,
  random_effects = c("sample_name","publication"),
  fixed_effects = c("age","outcome"),
  correction="BH",
  standardize = TRUE,
  cores = 48,
  plot_heatmap = TRUE,
  plot_scatter = TRUE,
  heatmap_first_n = 100,
  reference="outcome,Deceased")
```

```
[1] "Warning: Deleting existing log file: ./subset_outcome/maaslin2.log"
2020-11-23 20:34:52 INFO::Writing function arguments to log file
2020-11-23 20:34:52 INFO::Verifying options selected are valid
2020-11-23 20:34:52 INFO::Determining format of input files
2020-11-23 20:34:52 INFO::Input format is data samples as rows and metadata samples as rows
2020-11-23 20:34:52 INFO::Formula for random effects: expr ~ (1 | sample_name) + (1 | publication)
2020-11-23 20:34:52 INFO::Formula for fixed effects: expr ~  age + outcome
2020-11-23 20:34:52 INFO::Filter data based on min abundance and min prevalence
2020-11-23 20:34:52 INFO::Total samples in data: 141
2020-11-23 20:34:52 INFO::Min samples required with min abundance for a feature not to be filtered: 0.141000
2020-11-23 20:34:52 INFO::Total filtered features: 13051
2020-11-23 20:34:52 INFO::Filtered feature names from abundance and prevalence filtering: GO.0045212.obsolete.neurotransm
itter.receptor.biosynthetic.process, GO.0001319.obsolete.inheritance.of.oxidatively.modified.proteins.involved.in.replica
tive.cell.aging, GO.1900008.obsolete.negative.regulation.of.extrachromosomal.rDNA.circle.accumulation.involved.in.replica
tive.cell.aging, GO.0001302.obsolete.replicative.cell.aging, GO.0051704.multi.organism.process, GO.0051703.intraspecies.i
nteraction.between.organisms, GO.0110148.biomineralization, GO.0043473.pigmentation, GO.0002376.immune.system.process, G
O.0048511.rhythmic.process, GO.0023052.signaling, GO.0007610.behavior, GO.0015976.carbon.utilization, GO.0040007.growth,
```

GO.0009758.carbohydrate.utilization, GO.0022414.reproductive.process, GO.0006791.sulfur.utilization, GO.0098754.detoxific
ation, GO.0000003.reproduction, GO.0019740.nitrogen.utilization, GO.0008340.determination.of.adult.lifespan, GO.0019748.s
econdary.metabolic.process, GO.0060384.innervation, GO.0022413.reproductive.process.in.single.celled.organism, GO.000962
8.response.to.abiotic.stimulus, GO.0009791.post.embryonic.development, GO.0007585.respiratory.gaseous.exchange.by.respira
tory.system, GO.0008037.cell.recognition, GO.0009846.pollen.germination, GO.0043335.protein.unfolding, GO.0060033.anatomi
cal.structure.regression, GO.0071722.detoxification.of.arsenic.containing.substance, GO.0032504.multicellular.organism.re
production, GO.0000920.septum.digestion.after.cytokinesis, GO.0003006.developmental.process.involved.in.reproduction, GO.
0045494.photoreceptor.cell.maintenance, GO.0001503.ossification, GO.0007568.aging, GO.0030537.larval.behavior, GO.009719
4.execution.phase.of.apoptosis, GO.0001906.cell.killing, GO.0007017.microtubule.based.process, GO.0051702.interaction.wit
h.symbiont, GO.0098727.maintenance.of.cell.number, GO.0002532.production.of.molecular.mediator.involved.in.inflammatory.r
esponse, GO.0010312.detoxification.of.zinc.ion, GO.0014854.response.to.inactivity, GO.0022611.dormancy.process, GO.000756
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ino.acid.secondary.active.transmembrane.transporter.activity, GO.0051139.metal.ion.proton.antiporter.activity, GO.000000
6.high.affinity.zinc.transmembrane.transporter.activity, GO.0005287.high.affinity.basic.amino.acid.transmembrane.transpor
ter.activity, GO.0004402.histone.acetyltransferase.activity, GO.0005245.voltage.gated.calcium.channel.activity, GO.000523

1.excitatory.extracellular.ligand.gated.ion.channel.activity, GO.0000064.L.ornithine.transmembrane.transporter.activity,
GO.0005290.L.histidine.transmembrane.transporter.activity, GO.1990939.ATP.dependent.microtubule.motor.activity, GO.00306
98.5.10.methylenetetrahydrofolate.dependent.tRNA..m5U54..methyltransferase.activity, GO.0000986.bacterial.type.cis.regula
tory.region.sequence.specific.DNA.binding, GO.0000062.fatty.acyl.CoA.binding, GO.0008311.double.stranded.DNA.3.5.exodeoxy
ribonuclease.activity, GO.0001165.RNA.polymerase.I.cis.regulatory.region.sequence.specific.DNA.binding, GO.0015128.glucon
ate.transmembrane.transporter.activity, GO.0015141.succinate.transmembrane.transporter.activity, GO.0005229.intracellula
r.calcium.activated.chloride.channel.activity, GO.0046975.histone.methyltransferase.activity..H3.K36.specific., GO.001541
3.ATPase.coupled.nickel.transmembrane.transporter.activity, GO.0043865.methionine.transmembrane.transporter.activity, GO.
0015506.nucleoside.proton.symporter.activity, GO.1990189.peptide.serine.N.acetyltransferase.activity, GO.0004003.DNA.heli
case.activity, GO.0052915.23S.rRNA..guanine.2445..N.2...methyltransferase.activity, GO.0000980.RNA.polymerase.II.cis.regu
latory.region.sequence.specific.DNA.binding, GO.0061994.ATP.dependent.protein.nucleic.acid.complex.displacement.activity,
GO.0005304.L.valine.transmembrane.transporter.activity, GO.0015425.ATPase.coupled.nonpolar.amino.acid.transporter.activit
y, GO.0043682.copper.transmembrane.transporter.activity..phosphorylative.mechanism, GO.0022824.transmitter.gated.ion.chan
nel.activity, GO.0052929.ATP.3.cytidine.cytidine.tRNA.adenylyltransferase.activity, GO.0005343.organic.acid.sodium.sympor
ter.activity, GO.0046961.proton.transporting.ATPase.activity..rotational.mechanism, GO.0061505.DNA.topoisomerase.type.I
I..double.strand.cut..ATP.hydrolyzing..activity, GO.0003918.DNA.topoisomerase.type.II..double.strand.cut..ATP.hydrolyzin
g..activity, GO.0008574.ATP.dependent.microtubule.motor.activity..plus.end.directed, GO.0086038.calcium.sodium.antiporte
r.activity.involved.in.regulation.of.cardiac.muscle.cell.membrane.potential, GO.0008332.low.voltage.gated.calcium.channe
l.activity, GO.0009675.high.affinity.sulfate.proton.symporter.activity, GO.0022851.GABA.gated.chloride.ion.channel.activi
ty, GO.0033682.5.3.DNA.RNA.helicase.activity, GO.0061995.ATP.dependent.protein.DNA.complex.displacement.activity, GO.0004
931.extracellularly.ATP.gated.cation.channel.activity, GO.0015518.arabinose.proton.symporter.activity, GO.0035497.cAMP.re
sponse.element.binding, GO.0061749.forked.DNA.dependent.helicase.activity, GO.0009679.hexose.proton.symporter.activity, G
O.0016933.extracellularly.glycine.gated.ion.channel.activity, GO.0015191.L.methionine.transmembrane.transporter.activity,
GO.0008273.calcium..potassium.sodium.antiporter.activity, GO.0005291.high.affinity.L.histidine.transmembrane.transporter.
activity, GO.0005283.amino.acid.sodium.symporter.activity, GO.0015378.sodium.chloride.symporter.activity, GO.0008331.hig
h.voltage.gated.calcium.channel.activity, GO.0015278.calcium.release.channel.activity, GO.0034335.DNA.negative.supercoili
ng.activity, GO.0043142.single.stranded.DNA.helicase.activity, GO.0042945.D.serine.transmembrane.transporter.activity, G
O.0015271.outward.rectifier.potassium.channel.activity, GO.0004970.ionotropic.glutamate.receptor.activity, GO.0008507.sod
ium.iodide.symporter.activity, GO.1904315.transmitter.gated.ion.channel.activity.involved.in.regulation.of.postsynaptic.m
embrane.potential, GO.0043138.3.5.DNA.helicase.activity, GO.0015649.2.keto.3.deoxygluconate.proton.symporter.activity, G
O.0033676.double.stranded.DNA.helicase.activity, GO.0008569.ATP.dependent.microtubule.motor.activity..minus.end.directed,
GO.0005222.intracellular.cAMP.activated.cation.channel.activity, GO.0001006.RNA.polymerase.III.type.3.promoter.sequence.s
pecific.DNA.binding, GO.0140161.monocarboxylate.sodium.symporter.activity, GO.0001002.RNA.polymerase.III.type.1.promoter.
sequence.specific.DNA.binding, GO.0048474.D.methionine.transmembrane.transporter.activity, GO.0034056.estrogen.response.e
lement.binding, GO.0017116.single.stranded.DNA.helicase.activity, GO.0015528.lactose.proton.symporter.activity, GO.003323
0.ATPase.coupled.cysteine.transmembrane.transporter.activity, GO.0015385.sodium.proton.antiporter.activity, GO.0061459.L.
arginine.transmembrane.transporter.activity, GO.0017153.sodium.dicarboxylate.symporter.activity, GO.0043141.5.3.DNA.helic
ase.activity, GO.0003689.DNA.clamp.loader.activity, GO.0015496.putrescine.ornithine.antiporter.activity, GO.0010485.H4.hi
stone.acetyltransferase.activity, GO.0086008.voltage.gated.potassium.channel.activity.involved.in.cardiac.muscle.cell.act
ion.potential.repolarization, GO.0043140.3.5.DNA.helicase.activity, GO.0043998.H2A.histone.acetyltransferase.activity, G
O.0051978.lysophospholipid.sodium.symporter.activity, GO.0015386.potassium.proton.antiporter.activity, GO.0015369.calciu
m.proton.antiporter.activity, GO.0015341.zinc.efflux.active.transmembrane.transporter.activity, GO.0015565.threonine.effl
ux.transmembrane.transporter.activity, GO.0036042.long.chain.fatty.acyl.CoA.binding, GO.0005300.high.affinity.tryptophan.
transmembrane.transporter.activity, GO.0008900.potassium.proton.exchanging.ATPase.activity, GO.0015408.ATPase.coupled.fer
ric.iron.transmembrane.transporter.activity, GO.0005242.inward.rectifier.potassium.channel.activity, GO.0015366.malate.pr
oton.symporter.activity, GO.0034459.3.5.RNA.helicase.activity, GO.0015661.L.lysine.efflux.transmembrane.transporter.activ
ity, GO.0034458.3.5.RNA.helicase.activity, GO.0005223.intracellular.cGMP.activated.cation.channel.activity, GO.0016286.sm
all.conductance.calcium.activated.potassium.channel.activity, GO.0070888.E.box.binding, GO.0043858.arginine.ornithine.ant
iporter.activity, GO.0001003.RNA.polymerase.III.type.2.promoter.sequence.specific.DNA.binding, GO.0008551.cadmium.transme

```
mbrane.transporter.activity..phosphorylative.mechanism, GO.0009378.four.way.junction.helicase.activity, GO.0043995.histon
e.acetyltransferase.activity..H4.K5.specific., GO.0015655.alanine.sodium.symporter.activity, GO.0005298.proline.sodium.sy
mporter.activity, GO.0008508.bile.acid.sodium.symporter.activity, GO.0043996.histone.acetyltransferase.activity..H4.K8.sp
ecific., GO.0015517.galactose.proton.symporter.activity, GO.0048763.calcium.induced.calcium.release.activity, GO.0005219.
ryanodine.sensitive.calcium.release.channel.activity, GO.0005295.neutral.amino.acid.sodium.symporter.activity, GO.004466
8.sodium.malonate.symporter.activity, GO.0005220.inositol.1.4.5.trisphosphate.sensitive.calcium.release.channel.activity,
GO.0046972.histone.acetyltransferase.activity..H4.K16.specific., GO.0008511.sodium.potassium.chloride.symporter.activity,
GO.0015501.glutamate.sodium.symporter.activity, GO.0000102.L.methionine.secondary.active.transmembrane.transporter.activi
ty, GO.0022848.acetylcholine.gated.cation.selective.channel.activity, GO.0015535.fucose.proton.symporter.activity, GO.001
6934.extracellularly.glycine.gated.chloride.channel.activity
2020-11-23 20:34:52 INFO::Running selected normalization method: NONE
2020-11-23 20:34:52 INFO::Total filtered features with variance filtering: 0
2020-11-23 20:34:52 INFO::Filtered feature names from variance filtering:
2020-11-23 20:34:52 INFO::Applying z-score to standardize continuous metadata
2020-11-23 20:34:52 INFO::Running selected transform method: NONE
2020-11-23 20:34:52 INFO::Running selected analysis method: LM
2020-11-23 20:34:52 INFO::Creating cluster of 48 R processes
2020-11-23 20:36:05 INFO::Counting total values for each feature
2020-11-23 20:36:05 WARNING::Deleting existing residuals file: ./subset_outcome/residuals.rds
2020-11-23 20:36:05 INFO::Writing residuals to file ./subset_outcome/residuals.rds
2020-11-23 20:36:05 INFO::Writing all results to file (ordered by increasing q-values): ./subset_outcome/all_results.tsv
2020-11-23 20:36:05 INFO::Writing the significant results (those which are less than or equal to the threshold of 0.25000
0 ) to file (ordered by increasing q-values): ./subset_outcome/significant_results.tsv
2020-11-23 20:36:05 INFO::Writing heatmap of significant results to file: ./subset_outcome/heatmap.pdf
[1] "There is not enough metadata in the associations to create a heatmap plot. Please review the associations in text ou
tput file."
2020-11-23 20:36:05 INFO::Writing association plots (one for each significant association) to output folder: ./subset_out
come
2020-11-23 20:36:05 INFO::Plotting associations from most to least significant, grouped by metadata
2020-11-23 20:36:05 INFO::Plotting data for metadata number 1, outcome
2020-11-23 20:36:05 INFO::Creating boxplot for categorical data, outcome vs GO.0032502.developmental.process
2020-11-23 20:36:06 INFO::Creating boxplot for categorical data, outcome vs GO.0043177.organic.acid.binding
2020-11-23 20:36:06 INFO::Creating boxplot for categorical data, outcome vs GO.0031406.carboxylic.acid.binding
2020-11-23 20:36:06 INFO::Creating boxplot for categorical data, outcome vs GO.0035639.purine.ribonucleoside.triphosphat
e.binding
2020-11-23 20:36:06 INFO::Creating boxplot for categorical data, outcome vs GO.0017076.purine.nucleotide.binding
2020-11-23 20:36:06 INFO::Creating boxplot for categorical data, outcome vs GO.0032555.purine.ribonucleotide.binding
2020-11-23 20:36:07 INFO::Creating boxplot for categorical data, outcome vs GO.0030554.adenyl.nucleotide.binding
2020-11-23 20:36:07 INFO::Creating boxplot for categorical data, outcome vs GO.0032559.adenyl.ribonucleotide.binding
2020-11-23 20:36:07 INFO::Creating boxplot for categorical data, outcome vs GO.0005524.ATP.binding
```

DMM modeling using the MaAslin2 derived terms

Ok, lets import the MaAsLin2 derived significant terms

In [240…

```
res<-case$results%>%filter(pval<=0.05)
```

DMM Preprocessing /filtering

Ok lets filter out the GO_tag mataches from our phyloseq object

In [241…
```
Terms<-gsub("GO.","GO:",res$feature)
Terms<-gsub("[.]"," ",Terms)
Terms<-sub(" ","-",Terms)
Terms<-as_tibble(Terms)
Terms<-separate(data = Terms,col = value,sep = "-",into =  c("Term", "name"))
```

In [242…
```
bac_pseq_no_neg<-subset_samples(bac_pseq, sample_type!="neg_control")
bac_pseq_no_neg<-subset_samples(bac_pseq_no_neg, sample_type!="Unknown")
bac_pseq_no_neg #[ 13846 taxa and 141 samples ]:
```

```
phyloseq-class experiment-level object
otu_table()   OTU Table:         [ 13844 taxa and 141 samples ]
sample_data() Sample Data:       [ 141 samples by 71 sample variables ]
tax_table()   Taxonomy Table:    [ 13844 taxa by 3 taxonomic ranks ]
```

'GO:0045212' · 'GO:0001319' · 'GO:1900008' · 'GO:0001302' · 'GO:0051704' · 'GO:0008152'

In [243…
```
bac_pseq_prune <- prune_taxa(taxa = Terms$Term,x =  bac_pseq_no_neg)
bac_pseq_prune #[85 taxa and 141 samples ]
```

```
phyloseq-class experiment-level object
otu_table()   OTU Table:         [ 85 taxa and 141 samples ]
sample_data() Sample Data:       [ 141 samples by 71 sample variables ]
tax_table()   Taxonomy Table:    [ 85 taxa by 3 taxonomic ranks ]
```

Ok lets fix our names again

In [245…
```
tax<-data.frame(tax_table(bac_pseq_prune))
names<-paste(rownames(tax),tax$name,sep="-")
taxa_names(bac_pseq_prune)<-names
```

DMM modeling time

convert counts to a matrix

In [247…
```
dat <- abundances(bac_pseq_prune)
count <- as.matrix(t(dat))
```

Fit the dmm model

In [248…
```
fit <- mclapply(1:8, dmn, count = count, verbose=TRUE)
```

Check the model fit with different number of mixture componenets using standard information criteria

In [249…

```
lplc <- sapply(fit, laplace) # AIC / BIC / Laplace
aic  <- sapply(fit, AIC) # AIC / BIC / Laplace
bic  <- sapply(fit, BIC) # AIC / BIC / Laplace
plot(lplc, type="b", xlab="Number of Dirichlet Components", ylab="Model Fit")
```

In [250…

```
#identify the number of clusters that best fits the model
```

In [284…

```
best <- fit[[which.min(lplc)]]
best <-fit[[4]]
best
```

```
class: DMN
k: 4
samples x taxa: 141 x 85
Laplace: 98563.38 BIC: 98388.17 AIC: 97882.46
```

In [285…

```
#save.image(file = "go_terms_dmm.rdata")
```

make a heatmap visualization of the cluster

log 2 Heatmap

In [309…

```
options(repr.plot.width=15, repr.plot.height=15)
heatmapdmn(count, fit[[1]], best,ntaxa = 50,
           transform =log2, lblwidth = 0.2 * nrow(count))
```

square root version

In [310…

```
options(repr.plot.width=15, repr.plot.height=15)
heatmapdmn(count, fit[[1]], best,ntaxa = 50,
           transform =sqrt, lblwidth = 0.2 * nrow(count))
```

print out the theta values

In [311…

```
mixturewt(best)
```

A data.frame: 4 × 2

pi	theta
<dbl>	<dbl>

pi	theta
<dbl>	<dbl>
0.3319495	718.30307
0.2786808	9132.92640
0.2229376	1060.40832
0.1664321	67.82893

save the datasheet that show which GO terms contributed to each dmm group

In [312…

```
write.table(fitted(best),"GO_TERMS_DMM_contributions.tsv", sep="\t")
```

save a datasheet that identifies which sample belongs to which dmm group

In [313…

```
ass <- apply(mixture(best), 1, which.max)
write.table(ass,"GO_TERMS_DMM_groups.tsv",sep="")
```

In [314…

```
#add the dmm group to the metadata
sample_data(bac_pseq_prune)$dmn<-ass
bac_pseq_prune_comp<-microbiome::transform(bac_pseq_prune,"compositional")
#melt the phyloseq object into tidy form
tmp<-psmelt(bac_pseq_prune_comp)
tmp<-as_tibble(tmp)
```

In [318…

```
options(repr.plot.width=5, repr.plot.height=5)
gghistogram(tmp,x = "Abundance",y = "..count..")+scale_x_log10() #move each dmm group into a colum of its own
```

```
Warning message:
“Using `bins = 30` by default. Pick better value with the argument `bins`.”
Warning message:
“Transformation introduced infinite values in continuous x-axis”
Warning message:
“Removed 790 rows containing non-finite values (stat_bin).”
```

In [319…
```
#tmp$log2Abundance<-log2(tmp$Abundance)
```

In [320…
```
#subset the dataset to only include the case, Go_term, count, and dmm group.
#obtain the avergage count for each Go term
#order the go terms from hight to lowest count
```

I added these filtering commands to pull out the counts with less than 1% relabund or greater tahn 22% (ie:molecular function)

In [321…
```
d2<-tmp %>%
  select(case,OTU,Abundance, dmn)%>%
  group_by(OTU,case, dmn) %>%
  summarise(avg = mean(Abundance)) %>%
  arrange(desc(avg))
```

```
`summarise()` regrouping output by 'OTU', 'case' (override with `.groups` argument)
```

In [322…
```
#d2$avg<-sqrt(d2$avg)
```

In [323…
```
#get the total count of the go terms and oder from greates to lowest
```

In [332…
```
d3<-tidyr::spread(d2,dmn,avg)
d3$tot<-rowSums(d3[3:6], na.rm = T)
d3<-d3%>%arrange(desc(tot))
```

```
d3$tot<-NULL
head(d3)
```

A grouped_df: 6 × 6

OTU	case	1	2	3	4
<chr>	<chr>	<dbl>	<dbl>	<dbl>	<dbl>
GO:0003824-catalytic activity-catalytic activity	COVID19	0.19709584	NA	0.10062902	0.10722726
GO:0003824-catalytic activity-catalytic activity	Control_Healthy	0.10882747	0.08690861	0.09912470	0.08661512
GO:0003824-catalytic activity-catalytic activity	Community_acquired_pneumonia	0.10989682	NA	0.09972762	0.09453713
GO:0009987-cellular process-cellular process	Control_Healthy	0.06354380	0.02466242	0.07351454	0.07790732
GO:0009987-cellular process-cellular process	Community_acquired_pneumonia	0.06165796	NA	0.07537258	0.05883757
GO:0071704-organic substance metabolic process-organic substance metabolic process	Control_Healthy	0.04647188	0.04741581	0.04935608	0.05252678

In [333…

```
d3<-d3%>%gather(data = d3,avg,3:6)
colnames(d3)<-c("name","case", "dmn","avg")
```

make the balloon plot

In [336…

```
d4<-d3%>%filter(avg>0.01)%>%arrange(name,case,dmn)
d4<-d4[1:108,]
d4
```

A grouped_df: 108 × 4

name	case	dmn	avg
<chr>	<chr>	<chr>	<dbl>
GO:0000166-nucleotide binding-nucleotide binding	Community_acquired_pneumonia	3	0.01465015
GO:0000166-nucleotide binding-nucleotide binding	Community_acquired_pneumonia	4	0.01871800
GO:0000166-nucleotide binding-nucleotide binding	Control_Healthy	2	0.06631550
GO:0000166-nucleotide binding-nucleotide binding	Control_Healthy	3	0.01261766
GO:0000166-nucleotide binding-nucleotide binding	Control_Healthy	4	0.01247959
GO:0000166-nucleotide binding-nucleotide binding	Control_Sick	2	0.06611459
GO:0000166-nucleotide binding-nucleotide binding	COVID19	3	0.01867085

name	case	dmn	avg
<chr>	<chr>	<chr>	<dbl>
GO:0003676-nucleic acid binding-nucleic acid binding	Community_acquired_pneumonia	3	0.01082450
GO:0003676-nucleic acid binding-nucleic acid binding	Control_Healthy	3	0.01093655
GO:0003676-nucleic acid binding-nucleic acid binding	Control_Healthy	4	0.01184144
GO:0003676-nucleic acid binding-nucleic acid binding	COVID19	1	0.01078878
GO:0003676-nucleic acid binding-nucleic acid binding	COVID19	3	0.01459786
GO:0003676-nucleic acid binding-nucleic acid binding	COVID19	4	0.01690512
GO:0003677-DNA binding-DNA binding	COVID19	4	0.01027344
GO:0003824-catalytic activity-catalytic activity	Community_acquired_pneumonia	1	0.10989682
GO:0003824-catalytic activity-catalytic activity	Community_acquired_pneumonia	3	0.09972762
GO:0003824-catalytic activity-catalytic activity	Community_acquired_pneumonia	4	0.09453713
GO:0003824-catalytic activity-catalytic activity	Control_Healthy	1	0.10882747
GO:0003824-catalytic activity-catalytic activity	Control_Healthy	2	0.08690861
GO:0003824-catalytic activity-catalytic activity	Control_Healthy	3	0.09912470
GO:0003824-catalytic activity-catalytic activity	Control_Healthy	4	0.08661512
GO:0003824-catalytic activity-catalytic activity	Control_Sick	2	0.08759088
GO:0003824-catalytic activity-catalytic activity	COVID19	1	0.19709584
GO:0003824-catalytic activity-catalytic activity	COVID19	3	0.10062902
GO:0003824-catalytic activity-catalytic activity	COVID19	4	0.10722726
GO:0005975-carbohydrate metabolic process-carbohydrate metabolic process	Community_acquired_pneumonia	1	0.01415885
GO:0005975-carbohydrate metabolic process-carbohydrate metabolic process	Community_acquired_pneumonia	4	0.01001309
GO:0005975-carbohydrate metabolic process-carbohydrate metabolic process	Control_Healthy	1	0.01472692
GO:0006082-organic acid metabolic process-organic acid metabolic process	Control_Healthy	4	0.01318295
GO:0006082-organic acid metabolic process-organic acid metabolic process	COVID19	3	0.01079695
⋮	⋮	⋮	⋮

name	case	dmn	avg
<chr>	<chr>	<chr>	<dbl>
GO:0009987-cellular process-cellular process	Control_Sick	2	0.02392001
GO:0009987-cellular process-cellular process	COVID19	1	0.05546210
GO:0009987-cellular process-cellular process	COVID19	3	0.06773506
GO:0009987-cellular process-cellular process	COVID19	4	0.05238402
GO:0016053-organic acid biosynthetic process-organic acid biosynthetic process	COVID19	4	0.01625205
GO:0016070-RNA metabolic process-RNA metabolic process	Community_acquired_pneumonia	3	0.01368030
GO:0016070-RNA metabolic process-RNA metabolic process	Community_acquired_pneumonia	4	0.01045458
GO:0016070-RNA metabolic process-RNA metabolic process	Control_Healthy	3	0.01844819
GO:0016070-RNA metabolic process-RNA metabolic process	Control_Healthy	4	0.02430556
GO:0016070-RNA metabolic process-RNA metabolic process	COVID19	3	0.01016752
GO:0016310-phosphorylation-phosphorylation	Control_Healthy	1	0.01009932
GO:0016491-oxidoreductase activity-oxidoreductase activity	Community_acquired_pneumonia	3	0.01144033
GO:0016491-oxidoreductase activity-oxidoreductase activity	Community_acquired_pneumonia	4	0.01496509
GO:0016491-oxidoreductase activity-oxidoreductase activity	Control_Healthy	3	0.01102109
GO:0016491-oxidoreductase activity-oxidoreductase activity	Control_Healthy	4	0.01159283
GO:0016491-oxidoreductase activity-oxidoreductase activity	COVID19	1	0.01028801
GO:0016491-oxidoreductase activity-oxidoreductase activity	COVID19	3	0.01474157
GO:0016740-transferase activity-transferase activity	Community_acquired_pneumonia	1	0.04442776
GO:0016740-transferase activity-transferase activity	Community_acquired_pneumonia	3	0.03760361
GO:0016740-transferase activity-transferase activity	Community_acquired_pneumonia	4	0.03276395
GO:0016740-transferase activity-transferase activity	Control_Healthy	1	0.04848999
GO:0016740-transferase activity-transferase activity	Control_Healthy	3	0.03752979
GO:0016740-transferase activity-transferase activity	Control_Healthy	4	0.03055851
GO:0016740-transferase activity-transferase activity	COVID19	1	0.03046940

name	case	dmn	avg
<chr>	<chr>	<chr>	<dbl>
GO:0016740-transferase activity-transferase activity	COVID19	3	0.03182015
GO:0016740-transferase activity-transferase activity	COVID19	4	0.01267244
GO:0016772-transferase activity, transferring phosphorus-containing groups-transferase activity, transferring phosphorus-containing groups	Community_acquired_pneumonia	1	0.02812732
GO:0016772-transferase activity, transferring phosphorus-containing groups-transferase activity, transferring phosphorus-containing groups	Community_acquired_pneumonia	3	0.01478601
GO:0016772-transferase activity, transferring phosphorus-containing groups-transferase activity, transferring phosphorus-containing groups	Community_acquired_pneumonia	4	0.01942445
GO:0016772-transferase activity, transferring phosphorus-containing groups-transferase activity, transferring phosphorus-containing groups	Control_Healthy	1	0.02884304

In [335…

```
my_pal<-viridis(n = 256, alpha = 1, begin = 0, end = 1, direction = 1)
```

In [338…

```
options(repr.plot.width=20, repr.plot.height=20)
a<-ggplot(data = d4,mapping = aes(x = factor(dmn),y =reorder(name,avg),size=avg,color=avg))+
geom_point()+
#theme(text=element_text(size=20))+
scale_colour_gradientn(colours = my_pal,trans="log2")+
facet_grid(facets = ~ case)+
theme_minimal(base_size = 16)

a
```

In [340…

```
ggballoonplot(d4, y ="name",x = "dmn", size = "avg", facet.by = "case",fill = "avg",ggtheme = theme_minimal())+
     guides(size = FALSE)+
    font("y.text", size = 12)+scale_fill_viridis_c()
```

Community_acquired_pneumonia Control_Healthy

GO:0016772-transferase activity, transferring phosphorus-containing groups-transferase activity, transferring phosphorus-containing groups
GO:0016740-transferase activity-transferase activity
GO:0016491-oxidoreductase activity-oxidoreductase activity
GO:0016310-phosphorylation-phosphorylation
GO:0016070-RNA metabolic process-RNA metabolic process
GO:0016053-organic acid biosynthetic process-organic acid biosynthetic process
GO:0009987-cellular process-cellular process
GO:0009059-macromolecule biosynthetic process-macromolecule biosynthetic process
GO:0009058-biosynthetic process-biosynthetic process
GO:0008652-cellular amino acid biosynthetic process-cellular amino acid biosynthetic process
GO:0006807-nitrogen compound metabolic process-nitrogen compound metabolic process
GO:0006796-phosphate-containing compound metabolic process-phosphate-containing compound metabolic process
GO:0006793-phosphorus metabolic process-phosphorus metabolic process
GO:0006520-cellular amino acid metabolic process-cellular amino acid metabolic process
GO:0006139-nucleobase-containing compound metabolic process-nucleobase-containing compound metabolic process
GO:0006082-organic acid metabolic process-organic acid metabolic process
GO:0005975-carbohydrate metabolic process-carbohydrate metabolic process
GO:0003824-catalytic activity-catalytic activity
GO:0003677-DNA binding-DNA binding
GO:0003676-nucleic acid binding-nucleic acid binding
GO:0000166-nucleotide binding-nucleotide binding
Control_Sick
COVID19
avg
0.15
0.10
0.05
GO:0016772-transferase activity, transferring phosphorus-containing groups-transferase activity, transferring phosphorus-containing groups
GO:0016740-transferase activity-transferase activity
GO:0016491-oxidoreductase activity-oxidoreductase activity
GO:0016310-phosphorylation-phosphorylation
GO:0016070-RNA metabolic process-RNA metabolic process
GO:0016053-organic acid biosynthetic process-organic acid biosynthetic process
GO:0009987-cellular process-cellular process
GO:0009059-macromolecule biosynthetic process-macromolecule biosynthetic process
GO:0009058-biosynthetic process-biosynthetic process
GO:0008652-cellular amino acid biosynthetic process-cellular amino acid biosynthetic process
GO:0006807-nitrogen compound metabolic process-nitrogen compound metabolic process
GO:0006796-phosphate-containing compound metabolic process-phosphate-containing compound metabolic process
GO:0006793-phosphorus metabolic process-phosphorus metabolic process
GO:0006520-cellular amino acid metabolic process-cellular amino acid metabolic process
GO:0006139-nucleobase-containing compound metabolic process-nucleobase-containing compound metabolic process
GO:0006082-organic acid metabolic process-organic acid metabolic process

In [341…

```
save.image("GO_TERM_Maaslin2_23_NOV_2020.rda")
#load.Rdata("GO_TERM_Maaslin2_19_NOV_2020.rda")
```

DATA VISUALIZATION TIME and outcome comparison time

In [395…

```
sam<-as_tibble(sample_data(bac_pseq_prune))
tbl<-tally(x = case~dmn,sam, format = "count")
```

```
Warning message in class(x) <- c(setdiff(subclass, tibble_class), tibble_class):
“Setting class(x) to multiple strings ("tbl_df", "tbl", ...); result will no longer be an S4 object”
```

In [456…

```
case<-as.vector(tally(~case,sam))
case
dmn<-as.vector(tally(~dmn,sam))
dmn
cor.test(case,dmn)
cor(case,dmn)
cov(case,dmn)
```

$25 \cdot 32 \cdot 36 \cdot 48$

$48 \cdot 39 \cdot 31 \cdot 23$

```
        Pearson's product-moment correlation

data:  x and y
t = -6.206, df = 2, p-value = 0.025
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.9994979 -0.2211418
sample estimates:
       cor
-0.9750048
```

-0.975004755823038

-100.75

ok lets do a binomial test to see if we can use this

In [454…

```
out_tbl<-tally(x = case~dmn,sam)
result2 <-chisq.test(table(out_tbl))
out_tbl
result2
chisq(result2)
```

```
Warning message in chisq.test(table(out_tbl)):
“Chi-squared approximation may be incorrect”
                              dmn
case                            1  2  3  4
  Community_acquired_pneumonia 14  0  7  4
  Control_Healthy              13  3 13  3
  Control_Sick                  0 36  0  0
  COVID19                      21  0 11 16
        Chi-squared test for given probabilities

data:  table(out_tbl)
X-squared = 9, df = 9, p-value = 0.4373
```

X.squared: 9

YAAAASSSSSS CASE ~OUTCOME Chi-squared TEST P=0.0517 LESGOOOO

In [ ]:

HEATMAP TIME

In [457…

```
##########################################
###Dont forget to save you shit HERE#######
##########################################
#save.image(file = "bac_go_terms_dmm.rdata")
#
```

In [493…

```
count<-abundances(bac_pseq_prune)
select <- order(rowMeans(count),decreasing=TRUE)
select3<-order(rowSdDiffs(count),decreasing=T)
select2<-log1p((count)[select3,])
tmp<-rownames(select2)
dim(select2)
```

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In [494…

```
#select2$mol<-tmp
select_tibb<-as_tibble(select2)
```

```
rownames(select_tibb)<-rownames(select2)
write.table(select2,file = "GO_Terms_results")
```

```
Warning message:
“Setting row names on a tibble is deprecated.”
```

In [495…

```
#library(matrixTests)
#library(genefilter)
#select3<-as_tibble(select2)%>%summarise(std=rowFtests(select2))%>% arrange(desc(std))
#select2$mol<-tmp
select2<-as_tibble(select2)
#select2$mean<-rowMeans(select2)
```

In [497…

```
rownames(select2)<-tmp
rownames(select2)
sam<-data.frame(sample_data(bac_pseq_prune))
df<-as.data.frame(sample_data(bac_pseq_prune))
df<-as_tibble(df)
df<-df%>%select(dmn, publication, sample_type,case)#,dmn,body_site)
df<-as.data.frame(df)
```

```
Warning message:
“Setting row names on a tibble is deprecated.”
```

'GO:0003824-catalytic activity-catalytic activity' · 'GO:0009987-cellular process-cellular process' · 'GO:0071704-organic substance metabolic process-organic substance metabolic process' · 'GO:0044238-primary metabolic process-primary metabolic process' · 'GO:0006807-nitrogen compound metabolic process-nitrogen compound metabolic process' · 'GO:0043170-macromolecule metabolic process-macromolecule metabolic process' · 'GO:0034641-cellular nitrogen compound metabolic process-cellular nitrogen compound metabolic process' · 'GO:0016740-transferase activity-transferase activity' · 'GO:0046483-heterocycle metabolic process-heterocycle metabolic process' · 'GO:0006139-nucleobase-containing compound metabolic process-nucleobase-containing compound metabolic process' · 'GO:0090304-nucleic acid metabolic process-nucleic acid metabolic process' · 'GO:0009058-biosynthetic process-biosynthetic process' · 'GO:0044260-cellular macromolecule metabolic process-cellular macromolecule metabolic process' · 'GO:0016070-RNA metabolic process-RNA metabolic process' · 'GO:1901576-organic substance biosynthetic process-organic substance biosynthetic process' · 'GO:0044249-cellular biosynthetic process-cellular biosynthetic process' · 'GO:0034660-ncRNA metabolic process-ncRNA metabolic process' · 'GO:0016772-transferase activity, transferring phosphorus-containing groups-transferase activity, transferring phosphorus-containing groups' ·

'GO:0065007-biological regulation-biological regulation' · 'GO:0003676-nucleic acid binding-nucleic acid binding' ·
'GO:0036094-small molecule binding-small molecule binding' ·
'GO:0140098-catalytic activity, acting on RNA-catalytic activity, acting on RNA' ·
'GO:0044281-small molecule metabolic process-small molecule metabolic process' ·
'GO:1901265-nucleoside phosphate binding-nucleoside phosphate binding' · 'GO:0000166-nucleotide binding-nucleotide binding' ·
'GO:0016829-lyase activity-lyase activity' · 'GO:0016491-oxidoreductase activity-oxidoreductase activity' ·
'GO:0050789-regulation of biological process-regulation of biological process' ·
'GO:0050794-regulation of cellular process-regulation of cellular process' ·
'GO:1901566-organonitrogen compound biosynthetic process-organonitrogen compound biosynthetic process' ·
'GO:0044271-cellular nitrogen compound biosynthetic process-cellular nitrogen compound biosynthetic process' ·
'GO:0004518-nuclease activity-nuclease activity' · 'GO:0016779-nucleotidyltransferase activity-nucleotidyltransferase activity' ·
'GO:0043169-cation binding-cation binding' · 'GO:0046872-metal ion binding-metal ion binding' ·
'GO:0009059-macromolecule biosynthetic process-macromolecule biosynthetic process' · 'GO:0016874-ligase activity-ligase activity' ·
'GO:0034645-cellular macromolecule biosynthetic process-cellular macromolecule biosynthetic process' ·
'GO:0006793-phosphorus metabolic process-phosphorus metabolic process' ·
'GO:1901362-organic cyclic compound biosynthetic process-organic cyclic compound biosynthetic process' ·
'GO:0006796-phosphate-containing compound metabolic process-phosphate-containing compound metabolic process' ·
'GO:0018130-heterocycle biosynthetic process-heterocycle biosynthetic process' · 'GO:0043168-anion binding-anion binding' ·
'GO:0044283-small molecule biosynthetic process-small molecule biosynthetic process' ·
'GO:0005975-carbohydrate metabolic process-carbohydrate metabolic process' ·
'GO:0019538-protein metabolic process-protein metabolic process' · 'GO:0003677-DNA binding-DNA binding' ·
'GO:0006082-organic acid metabolic process-organic acid metabolic process' ·
'GO:0043436-oxoacid metabolic process-oxoacid metabolic process' ·
'GO:0019752-carboxylic acid metabolic process-carboxylic acid metabolic process' ·
'GO:0044262-cellular carbohydrate metabolic process-cellular carbohydrate metabolic process' ·
'GO:0016301-kinase activity-kinase activity' · 'GO:0016310-phosphorylation-phosphorylation' ·
'GO:0019438-aromatic compound biosynthetic process-aromatic compound biosynthetic process' ·
'GO:0006520-cellular amino acid metabolic process-cellular amino acid metabolic process' ·
'GO:0097367-carbohydrate derivative binding-carbohydrate derivative binding' ·
'GO:0032553-ribonucleotide binding-ribonucleotide binding' · 'GO:0017076-purine nucleotide binding-purine nucleotide binding' ·
'GO:0032555-purine ribonucleotide binding-purine ribonucleotide binding' ·
'GO:0035639-purine ribonucleoside triphosphate binding-purine ribonucleoside triphosphate binding' ·
'GO:0016051-carbohydrate biosynthetic process-carbohydrate biosynthetic process' ·

'GO:0005976-polysaccharide metabolic process-polysaccharide metabolic process' · 'GO:0000271-polysaccharide biosynthetic process-polysaccharide biosynthetic process' · 'GO:0044264-cellular polysaccharide metabolic process-cellular polysaccharide metabolic process' · 'GO:0034637-cellular carbohydrate biosynthetic process-cellular carbohydrate biosynthetic process' · 'GO:0033692-cellular polysaccharide biosynthetic process-cellular polysaccharide biosynthetic process' · 'GO:0046379-extracellular polysaccharide metabolic process-extracellular polysaccharide metabolic process' · 'GO:0045226-extracellular polysaccharide biosynthetic process-extracellular polysaccharide biosynthetic process' · 'GO:0008879-glucose-1-phosphate thymidylyltransferase activity-glucose-1-phosphate thymidylyltransferase activity' · 'GO:0006811-ion transport-ion transport' · 'GO:0016053-organic acid biosynthetic process-organic acid biosynthetic process' · 'GO:0046394-carboxylic acid biosynthetic process-carboxylic acid biosynthetic process' · 'GO:0008652-cellular amino acid biosynthetic process-cellular amino acid biosynthetic process' · 'GO:0044877-protein-containing complex binding-protein-containing complex binding' · 'GO:0051540-metal cluster binding-metal cluster binding' · 'GO:0051536-iron-sulfur cluster binding-iron-sulfur cluster binding' · 'GO:0009072-aromatic amino acid family metabolic process-aromatic amino acid family metabolic process' · 'GO:0009073-aromatic amino acid family biosynthetic process-aromatic amino acid family biosynthetic process' · 'GO:0016835-carbon-oxygen lyase activity-carbon-oxygen lyase activity' · 'GO:0043648-dicarboxylic acid metabolic process-dicarboxylic acid metabolic process' · 'GO:0016836-hydro-lyase activity-hydro-lyase activity' · 'GO:0046417-chorismate metabolic process-chorismate metabolic process' · 'GO:0043650-dicarboxylic acid biosynthetic process-dicarboxylic acid biosynthetic process' · 'GO:0009423-chorismate biosynthetic process-chorismate biosynthetic process' · 'GO:0003855-3-dehydroquinate dehydratase activity-3-dehydroquinate dehydratase activity'

```
Warning message in class(x) <- c(setdiff(subclass, tibble_class), tibble_class):
“Setting class(x) to multiple strings ("tbl_df", "tbl", ...); result will no longer be an S4 object”
```

In [498…

```
#select2<-sqrt((count)[select,])
dim(select2)
colnames(select2) <- colnames(otu_table(bac_pseq_prune))
length(rownames(select2))
length(row.names(df))
row.names(df) <- colnames(select2)
```

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85

141

In [499…

```
library(ggsci)
```

In [501…

```
mypal <- pal_aaas("default", alpha = 1)(10)
mypal
library("scales")
library(RColorBrewer)
library(viridis)
library(pheatmap)
df_row<-as.data.frame(fitted(best))
#colnames(df_row)<-c("1","2","3","4","5","6")
df<-data.frame(df)
colnames(df)<-c("dmm_cluster", "Publication","Sample_Type","Case")
```

'#3B4992FF' · '#EE0000FF' · '#008B45FF' · '#631879FF' · '#008280FF' · '#BB0021FF' · '#5F559BFF' · '#A20056FF' · '#808180FF' · '#1B1919FF'

In [502…

```
# Specify colors
```

In [521…

```
ann_colors = list(
  dmm_cluster=c("1"="#3B4992FF","2"="#EE0000FF","3"="#008B45FF","4"="#631879FF"),#,"5"="#008280FF"),
  Publication=c("Xiong"="#008280FF", "Shen"="#631879FF","Michalovich"="#BB0021FF","Chen"="#3B4992FF","Wu"="#EE0000FF","Zh
  Sample_Type =c("COVID_19"="firebrick",
                 "Healthy"="forestgreen",
                 "Community_acquired_pneumonia"="darkorange1",
                 "Obese_Asthma"="goldenrod3",
                 "Obese_Smoker"="goldenrod4",
                 "Obese"="goldenrod1",
                 "Asthma"= "dodgerblue2",
                 "Asthma_Smoker"="dodgerblue4",
                 "Asthma_Ex_smoker"="dodgerblue3",
                 "Smoker"="gray27",
                 "Obese_Asthma_Smoker"="black"),
  Case=c("COVID19"="firebrick","Community_acquired_pneumonia"="darkorange1", "Control_Healthy"="forestgreen","Control_Sic
```

In [522…

```
select3<-select2%>%filter(rowMeans(select2)>8)
dim(select2)
dim(select3)
```

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45 · 141

In [523…

```
options(repr.plot.width=24, repr.plot.height=15)
xx <- pheatmap(mat = select3,
               color = colorRampPalette(rev(brewer.pal(n = 7, name ="RdYlBu")))(1000),
               annotation_col=df,
```

```
                 annotation_colors = ann_colors,
                 clustering_distance_rows = "euclidean",
                 clustering_distance_cols = "euclidean",
                annotation_row = df_row)
```

In [524…

```
options(repr.plot.width=24, repr.plot.height=20)
xx <- pheatmap(mat = select2,
                 color = colorRampPalette(rev(brewer.pal(n = 7, name ="RdYlBu")))(10),
                 annotation_col=df,
                 annotation_colors = ann_colors,
                 clustering_distance_rows = "euclidean",
```

```
                 clustering_distance_cols = "euclidean",
                 annotation_row = df_row,
                 cluster_row = T,
                 cluster_col=T)
```

Case
Sample_Type
Publication
dmm_cluster
GO:0003855-3-dehydroquinate dehydratase activity-3-dehydroquinate dehydratase activity
GO:0046417-chorismate metabolic process-chorismate metabolic process
GO:0009423-chorismate biosynthetic process-chorismate biosynthetic process
GO:0003824-catalytic activity-catalytic activity
GO:0009987-cellular process-cellular process
GO:0006807-nitrogen compound metabolic process-nitrogen compound metabolic process
GO:0071704-organic substance metabolic process-organic substance metabolic process
GO:0044238-primary metabolic process-primary metabolic process
GO:0006520-cellular amino acid metabolic process-cellular amino acid metabolic process
GO:0006082-organic acid metabolic process-organic acid metabolic process
GO:0043436-oxoacid metabolic process-oxoacid metabolic process
GO:0019752-carboxylic acid metabolic process-carboxylic acid metabolic process
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GO:0046483-heterocycle metabolic process-heterocycle metabolic process
GO:0006139-nucleobase-containing compound metabolic process-nucleobase-containing compound metabolic process
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GO:0050789-regulation of biological process-regulation of biological process
GO:0050794-regulation of cellular process-regulation of cellular process
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GO:0043168-anion binding-anion binding
GO:0019538-protein metabolic process-protein metabolic process
GO:0017076-purine nucleotide binding-purine nucleotide binding
GO:0032555-purine ribonucleotide binding-purine ribonucleotide binding
GO:0035639-purine ribonucleoside triphosphate binding-purine ribonucleoside triphosphate binding
GO:0097367-carbohydrate derivative binding-carbohydrate derivative binding
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GO:0043170-macromolecule metabolic process-macromolecule metabolic process
GO:0016740-transferase activity-transferase activity
GO:0043169-cation binding-cation binding
GO:0046872-metal ion binding-metal ion binding
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GO:0016836-hydro-lyase activity-hydro-lyase activity
GO:0009072-aromatic amino acid family metabolic process-aromatic amino acid family metabolic process
GO:0009073-aromatic amino acid family biosynthetic process-aromatic amino acid family biosynthetic process
GO:0043648-dicarboxylic acid metabolic process-dicarboxylic acid metabolic process
GO:0051540-metal cluster binding-metal cluster binding
GO:0051536-iron-sulfur cluster binding-iron-sulfur cluster binding
V4
V3
V2
V1
15
10
5
0
Case
COVID19
Community_acquired_pneumonia
Control_Healthy
Control_Sick
Sample_Type
COVID_19
Healthy
Community_acquired_pneumonia
Obese_Asthma
Obese_Smoker
Obese
Asthma
Asthma_Smoker
Asthma_Ex_smoker
Smoker
Obese_Asthma_Smoker
Publication
Xiong
Shen
Michalovich
Chen
Wu
Zhou
Ren
dmm_cluster
4
1
V4
6
1
V3
100
20
V2
600
200
V1
60
20

OK GREAT JOB

NEXT STEPS:

REMOVE THE MIKALOVICH SAMPLES

ADD A COLUMN FOR OUTCOME ON THE HEATMAP

In [ ]:

In [ ]:

```
##################ANYTHING BELOW THIS LINE IS TRASH FOR NOW###########
#####################################################
# Correlation between dmm groups and cases
##################################################




library(matrixTests)
p<-psmelt(bac_pseq_prune)
dmn_sum<-p%>%
  select(Sample,OTU,dmn,Abundance)%>%
  group_by(OTU,dmn)%>%
  pivot_wider(id_cols = c(Sample,dmn), names_from = OTU,values_from = Abundance)
write.table(dmn_sum, "dmn_sum.tsv",sep="\t")
# an example with offsets from Venables & Ripley (2002, p.189)
dmn_case<-pairwise.wilcox.test(p$Abundance, p$case,p.adjust.method = "BH")
dmn_wilcox<-pairwise.wilcox.test(p$Abundance, p$dmn,p.adjust.method = "BH")


p$lg<-log1p(p$Abundance)
p$dmn<-as_factor(p$dmn)
p$case<-factor(x = p$case, levels = c("Control_Healthy","Control_Sick","COVID19"))


library(lmerTest)
library(lmer)
case_dmn_glm <- lmer(lg ~ dmn +(dmn|case),data = p)
f<-ls_means(case_dmn_glm)

# b<-glm( lg ~ dmn+case, data = p, family = gaussian)
library(mosaic)
msummary(b)
```

```
b$coefficients
c<-anova(b)
d<-aov(b)
e<-TukeyHSD(d)
library(lmerTest)
f<-ls_means(case_dmn_glm)

msummary(e$case)
e$case
mat<-dmn_sum[,3:length(dmn_sum)]
krus_dmn<-col_kruskalwallis(x =mat,g = dmn_sum$dmn)
welch_dmn<-col_oneway_welch(x=mat, g=dmn_sum$dmn)

krus_dmn_sig<-krus_dmn%>%filter(pvalue<0.01)%>%arrange(pvalue)
welch_dmn_sig<-welch_dmn%>%filter(pvalue<0.01)%>%arrange(pvalue)

TukeyHSD(welch_dmn_sig)
library(mosaic)
msummary(welch_dmn_sig)
dim(krus_dmn_sig)
dim(welch_dmn_sig)
krus_sig
write.table(krus,"krus_dmn.tsv",sep = "\t")

colnames(krus_sig)
colnames(krus_sig)
krus_sig_names<-intersect(rownames(krus_case_sig), rownames(krus_dmn_sig))
welch_sig_names<-intersect(rownames(welch_case_sig), rownames(welch_dmn_sig))
sig_names<-intersect(krus_sig_names,welch_sig_names)
```