

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 [2]: #if(!requireNamespace("BiocManager", quietly = TRUE))
#       install.packages("BiocManager")
```

```
In [3]: #BiocManager::install("Maaslin2")
#BiocManager::install('DESeq2')
#BiocManager::install('phyloseq')
#BiocManager::install('microbiome')
#BiocManager::install("DirichletMultinomial")
```

```
In [4]: #install.packages("remotes")
#remotes::install_github("mikemc/speedyseq")
```

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)
```

— Attaching packages — tidyverse 1.3.0 —

✓ ggplot2 3.3.2	✓ purrr 0.3.4
✓ tibble 3.0.4	✓ dplyr 1.0.2
✓ tidyr 1.1.2	✓ stringr 1.4.0
✓ readr 1.4.0	✓ forcats 0.5.0

```
— Conflicts — tidyverse_conflicts() —  
✖ dplyr::filter() masks stats::filter()  
✖ dplyr::lag() masks stats::lag()
```

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, colAvgPerRowSet, 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, rowAvgPerColSet,
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

```
In [6]: getwd()
        setwd('/home/jovyan/work/Jochum_3/jupyter_lab/GO_term_analysis/')
        '/home/jovyan/work/Jochum_3/jupyter_lab/GO_term_analysis/R_Maaslin2'
```

```
In [7]: 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 [8]: 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 [9]: 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", "Unclassified"),
    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 [10]: 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 [11]: 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 [12]: 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 phyloseq

```
In [13]: 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 [14]: 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 [15]: bio_bac_sam<-as.data.frame(read.table("Combined_BALF_GO_Terms_metadata.txt",header = T, sep = "\t",row.names = 1))
```

a little regex to fix the stupid filename

```
In [16]: rownames(bio_bac_sam)<-rownames(bio_bac_sam)%>%str_replace_all("NC1_SRR7796663", "NC1.SRR7796663")
bio_bac_sam$accession<-rownames(bio_bac_sam)
```

making physeq object

```
In [17]: 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 negative control and unknown samples

```
In [18]: 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:      [ 13846 taxa and 162 samples ]
sample_data() Sample Data:  [ 162 samples by 71 sample variables ]
tax_table()  Taxonomy Table: [ 13846 taxa by 3 taxonomic ranks ]
```

phyloseq-class experiment-level object

```
otu_table() OTU Table:      [ 13846 taxa and 141 samples ]
sample_data() Sample Data:  [ 141 samples by 71 sample variables ]
tax_table()  Taxonomy Table: [ 13846 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 [19]: #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 [20]: 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
```

13846

DESeq2 VST transformation

```
In [21]: 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

YAAAAAAAAAAAAAAAAASSSSSSSSSSSSSSS THANK YOU LIMMA

IT FIXED THE BATCH EFFECT!

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

```
In [22]: #vst_trans_count_tab2 <- limma::removeBatchEffect(vst_trans_count_tab, sample_info_tab$publication)
```

```
In [23]: 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 [24]: #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/Jochum_3/jupyter_lab/GO_term_analysis/R_Maaslin2'

```
In [25]: df_input_data2<-data.frame(t(otu_table(vst_physeq_comp)))
```



```
df_input_metadata2<-data.frame(sample_data(vst_physeq_comp))
```

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 maximum 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 mutiple test correction method to be done (BH=Benjamini-Hochberg)

```
In [26]: Maaslin2(
  input_data = df_input_data2,
  input_metadata = df_input_metadata2,
  output="./",
  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"),
  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] "Warning: Deleting existing log file: ../maaslin2.log"
2020-11-20 00:08:47 INFO::Writing function arguments to log file
2020-11-20 00:08:47 INFO::Verifying options selected are valid
2020-11-20 00:08:47 INFO::Determining format of input files
2020-11-20 00:08:47 INFO::Input format is data samples as rows and metadata samples as rows
2020-11-20 00:08:47 INFO::Formula for random effects: expr ~ (1 | sample_name) + (1 | publication)
2020-11-20 00:08:47 INFO::Formula for fixed effects: expr ~ case
```

2020-11-20 00:08:47 INFO::Filter data based on min abundance and min prevalence
2020-11-20 00:08:47 INFO::Total samples in data: 141
2020-11-20 00:08:47 INFO::Min samples required with min abundance for a feature not to be filtered: 1.410000
2020-11-20 00:08:47 INFO::Total filtered features: 13730
2020-11-20 00:08:47 INFO::Filtered feature names from abundance and prevalence filtering: GO.0045212.obsolete.neurotransmitter.receptor.biosynthetic.process, GO.0001319.obsolete.inheritance.of.oxidatively.modified.proteins.involved.in.replicative.cell.aging, GO.1900008.obsolete.negative.regulation.of.extrachromosomal.rDNA.circle.accumulation.involved.in.replicative.cell.aging, GO.0001302.obsolete.replicative.cell.aging, GO.0051704.multi.organism.process, GO.0051703.intraspecies.interaction.between.organisms, GO.0110148.biomineralization, GO.0043473.pigmentation, GO.0002376.immune.system.process, GO.0044419.interspecies.interaction.between.organisms, GO.0048511.rhythmic.process, GO.0023052.signaling, GO.0007610.behavior, GO.0015976.carbon.utilization, GO.0040011.locomotion, GO.0032502.developmental.process, GO.0032501.multicellular.organismal.process, GO.0040007.growth, GO.0009758.carbohydrate.utilization, GO.0022610.biological.adhesion, GO.0022414.reproductive.process, GO.0050896.response.to.stimulus, GO.0006791.sulfur.utilization, GO.0098754.detoxification, GO.0000003.reproduction, GO.0019740.nitrogen.utilization, GO.0008340.determination.of.adult.lifespan, GO.0019748.secondary.metabolic.process, GO.0060384.innervation, GO.0022413.reproductive.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.recognition, 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.organism.reproduction, 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.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.0002532.production.of.molecular.mediator.involved.in.inflammatory.response, GO.0010312.detoxification.of.zinc.ion, GO.0014854.response.to.inactivity, GO.0022611.dormancy.process, GO.0009653.anatomical.structure.morphogenesis, GO.0009607.response.to.biotic.stimulus, GO.0007566.embryo.implantation, GO.0009566.fertilization, GO.0051775.response.to.redox.state, GO.0036268.swimming, GO.0009847.spore.germination, GO.0016049.cell.growth, GO.0001775.cell.activation, GO.0033036.macromolecule.localization, GO.0045103.intermediate.filament.based.process, GO.0019674.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.maintenance, GO.0051301.cell.division, GO.0048532.anatomical.structure.arrangement, GO.0008283.cell.population.proliferation, GO.0042221.response.to.chemical, GO.0061919.process.utilizing.autophagic.mechanism, GO.0022412.cellular.process.involved.in.reproduction.in.multicellular.organism, GO.0007155.cell.adhesion, GO.0043934.sporulation, GO.0035640.exploration.behavior, GO.0051606.detection.of.stimulus, GO.0048646.anatomical.structure.formaton.involved.in.morphogenesis, GO.0007631.feeding.behavior, GO.0048589.developmental.growth, GO.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.methylation, 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.0035176.social.behavior, GO.0048869.cellular.developmental.process, GO.0007638.mechanosensory.behavior, GO.0032505.reproduction.of.a.single.celled.organism, GO.0050817.coagulation, GO.0051716.cellular.response.to.stimulus, GO.0030029.actin.filament.based.process, GO.0051641.cellular.localization, GO.0006276.plasmid.maintenance, GO.0006734.NADH.metabolic.process, GO.0014823.response.to.activity, GO.0032963.collagen.metabolic.process, GO.0009719.response.to.endogenous.stimulus, GO.0048856.anatomical.structure.development, GO.0019954.asexual.reproduction, GO.0002440.production.of.molecular.mediator.of.immune.response, GO.0009405.pathogenesis, GO.0007059.chromosome.segregation, GO.0007389.pattern.specification.process, GO.0065009.regulation.of.molecular.function, GO.0030431.sleep, GO.0097242.amyloid.beta.clearance, GO.0051189.prosthetic.group.metabolic.process, GO.0016203.muscle.attachment, GO.0065008.regulation.of.biological.quality, GO.0140029.exocytic.process, GO.0044703.multi.organism.reproductive.process, GO.0007272.en sheathment.of.neurons, GO.0090713.immunological.memory.process, GO.0035821.modulation.of.process.of.other.organism, GO.007098.demethylation, GO.0048771.tissue.remolding, GO.0046034.ATP.metabolic.process, GO.0007340.acrosome.reaction, GO.199074

8.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.0006928.movement.of.cell.or.subcellular.component, 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.0044403.symbiotic.process, GO.0010127.mycotoxin.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.0006950.response.to.stress, GO.0009838.abscission, GO.0007623.circadian.rhythm, GO.0009056.catabolic.process, GO.0009845.seed.germination, GO.0018933.nicotine.metabolic.process, GO.0006457.protein.folding, GO.0048870.cell.motility, 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.0044092.negative.regulation.of.molecular.function, 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.cuticle.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.0044282.small.molecule.catabolic.process, 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.0051649.establishment.of.localization.in.cell, GO.0048583.regulation.of.response.to.stimulus, 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.0006766.vitamin.metabolic.process, GO.0000320.re.entry.into.mitotic.cell.cycle, 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GO.0004984.olfactory.receptor.activity, GO.0034010.sulfolactate.sulfo.lyase.activity, GO.0046982.protein.heterodimerization.activity, GO.0008410.CoA.transferase.activity, GO.0098531.ligand.activated.transcription.factor.activity, GO.0009486.cytochrome.b03.ubiquinol.oxidase.activity, GO.0005501.retinoid.binding, GO.0016799.hydrolase.activity..hydrolyzing.N.glycosyl.compounds, GO.0047809.D.2.hydroxy.acid.dehydrogenase.activity, GO.0061798.GTP.3.8.cyclase.activity, GO.0052877.oxidoreductase.activity..acting.on.the.CH.NH2.group.of.donors..with.a.copper.protein.as.acceptor, GO.0051032.nucleic.acid.transmembrane.transporter.activity, GO.0008194.UDP.glycosyltransferase.activity, GO.0016250.N.sulfoglucosamine.sulfohydrolase.activity, GO.0051912.CoB..CoM.heterodisulfide.reductase.activity, GO.0048028.galacturonan.binding, GO.0051538.3.iron..4.sulfur.cluster.binding, GO.0019902.phosphatase.binding, GO.0034029.2.oxoglutarate.carboxylase.activity, GO.0030973.molybdate.ion.binding, GO.001685

6.racemase.and.epimerase.activity..acting.on.hydroxy.acids.and.derivatives, GO.0003755.peptidyl.prolyl.cis.trans.isomerase.activity, GO.0009884.cytokinin.receptor.activity, GO.0008483.transaminase.activity, GO.0051907.S..hydroxymethyl.glutathione.synthase.activity, GO.0004019.adenylosuccinate.synthase.activity, GO.1990929.sulfoquinovosidase.activity, GO.0016900.oxidoreductase.activity..acting.on.the.CH.OH.group.of.donors..disulfide.as.acceptor, GO.0048018.receptor.ligand.activity, GO.0005338.nucleotide.sugar.transmembrane.transporter.activity, GO.0016861.intramolecular.oxidoreductase.activity..interconverting.aldoses.and.ketoses, GO.0097617.annealing.activity, GO.0018845.2.hydroxychromene.2.carboxylate.isomerase.activity, GO.0016405.CoA.ligase.activity, GO.0070679.inositol.1.4.5.trisphosphate.binding, GO.0001075.RNA.polymerase.II.general.transcription.initiation.factor.activity, GO.0030156.benzodiazepine.receptor.binding, GO.0001141.DNA.binding.transcription.repressor.activity, GO.0050499.oxidoreductase.activity..acting.on.phosphorus.or.arsenic.in.donors..with.NAD.P..as.acceptor, GO.0016723.oxidoreductase.activity..oxidizing.metal.ions..NAD.or.NADP.as.acceptor, GO.0045430.chalcone.isomerase.activity, GO.0016699.oxidoreductase.activity..acting.on.hydrogen.as.donor..iron.sulfur.protein.as.acceptor, GO.0042608.T.cell.receptor.binding, GO.0009000.selenocysteine.lyase.activity, GO.0033130.acetylcholine.receptor.binding, GO.0001540.amyloid.beta.binding, GO.0016668.oxidoreductase.activity..acting.on.a.sulfur.group.of.donors..NAD.P..as.acceptor, GO.0036122.BMP.binding, GO.0031593.polyubiquitin.modification.dependent.protein.binding, GO.0034038.deoxyhypusine.synthase.activity, GO.0050077.maleylpyruvate.isomerase.activity, GO.0032183.SUMO.binding, GO.0016814.hydrolase.activity..acting.on.carbon.nitrogen..but.not.peptide..bonds..in.cyclic.amidines, GO.0016634.oxidoreductase.activity..acting.on.the.CH.CH.group.of.donors..oxygen.as.acceptor, 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A. regulatory. subunit. binding, GO.0033720. .S. mandelate. dehydrogenase. activity, GO.0015094. lead. ion. transmembrane. transporter. activity, GO.0016664. oxidoreductase. activity. .acting. on. other. nitrogenous. compounds. as. donors. .iron. sulfur. protein. as. acceptor, GO.0017022. myosin. binding, GO.0036355. 2. iminoacetate. synthase. activity, GO.0042806. fucose. binding, GO.0019894. kinesin. binding, GO.0016780. phosphotransferase. activity. .for. other. substituted. phosphate. groups, GO.1901691. proton. binding, GO.0018740. 2. hydroxybiphenyl. 2. sulfinic. desulfinate. activity, GO.0000822. inositol. hexakisphosphate. binding, GO.0098623. selenite. reductase. activity, GO.0033792. bile. acid. 7alpha. dehydroxylase. activity, GO.0001140. DNA. binding. transcription. activation. activity, GO.0016790. thiolester. hydrolase. activity, GO.0008676. 3. deoxy. 8. phosphooctulonate. synthase. activity, GO.0005112. 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GO.0046912.transferase.activity..transferring.acyl.groups..acyl.groups.converted.into.alkyl.on.transfer, GO.0016867.intramolecular.transferase.activity..transferring.acyl.groups, GO.0070325.lipoprotein.particle.receptor.binding, GO.0016657.oxidoreductase.activity..acting.on.NAD.P.H..nitrogenous.group.as.acceptor, GO.0001875.lipopolysaccharide.immune.receptor.activity, GO.0052592.oxidoreductase.activity..acting.on.CH.or.CH2.groups..with.an.iron.sulfur.protein.as.acceptor, GO.0031177.phosphopantetheine.binding, GO.0044390.ubiquitin.like.protein.conjugating.enzyme.binding, GO.1903981.enterobactin.binding, GO.0016719.carotene.7.8.desaturase.activity, GO.0033727.aldehyde.dehydrogenase..FAD.independent..activity, GO.0004842.ubiquitin.protein.transferase.activity, GO.000609.alkylglycerone.phosphate.synthase.activity, GO.0042803.protein.homodimerization.activity, GO.0033748.hydrogenase..acceptor..activity, GO.0052856.NADHX.epimerase.activity, GO.0050461.L.mimosine.synthase.activity, 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0.0046974.histone.methyltransferase.activity..H3.K9.specific., GO.0052913.16S.rRNA..guanine.966..N.2...methyltransferase.activity, GO.0015093.ferrous.iron.transmembrane.transporter.activity, GO.0004535.poly.A..specific.ribonuclease.activity, GO.0052910.23S.rRNA..adenine.2085..N.6...dimethyltransferase.activity, GO.0008999.ribosomal.protein.alanine.N.acetyltransferase.activity, GO.0008512.sulfate.proton.symporter.activity, GO.0005248.voltage.gated.sodium.channel.activity, GO.0033677.DNA.RNA.helicase.activity, GO.0005432.calcium.sodium.antiporter.activity, GO.0000992.RNA.polymerase.III.cis.regulatory.region.sequence.specific.DNA.binding, GO.0015612.L.arabinose.importing.ATPase.activity, GO.0005351.carbohydrate.proton.symporter.activity, GO.0033680.DNA.RNA.helicase.activity, GO.0015091.ferric.iron.transmembrane.transporter.activity, GO.0015616.DNA.translocase.activity, GO.0042800.histone.methyltransferase.activity..H3.K4.specific., GO.0016463.zinc.transmembrane.transporter.activity..phosphorylative.mechanism, GO.0004008.copper.transmembrane.transporter.activity..phosphorylative.mechanism, GO.0015379.potassium.chloride.symporter.activity, GO.0008310.single.stranded.DNA.3.5.exodeoxyribonuclease.activity, GO.0005228.intracellular.sodium.activated.potassium.channel.activity, GO.0052912.23S.rRNA..guanine.748..N.1...methyltransferase.activity, GO.0015189.L.lysine.transmembrane.transporter.activity, GO.0015434.ATPase.coupled.cadmium.transmembrane.transporter.activity, GO.0033229.cysteine.transmembrane.transporter.activity, GO.0015333.peptide.proton.symporter.activity, GO.0005391.sodium.potassium.exchanging.ATPase.activity, GO.0015192.L.phenylalanine.transmembrane.transporter.activity, GO.0015183.L.aspartate.transmembrane.transporter.activity, GO.0046933.proton.transporting.ATP.synthase.activity..rotational.mechanism, GO.0005249.voltage.gated.potassium.channel.activity, GO.0046976.histone.methyltransferase.activity..H3.K27.specific., GO.0099604.ligand.gated.calcium.channel.activity, GO.0005302.L.tyrosine.transmembrane.transporter.activity, GO.0000978.RNA.polymerase.II.cis.regulatory.region.sequence.specific.DNA.binding, GO.0051908.double.stranded.DNA.5.3.exodeoxyribonuclease.activity, GO.0015321.sodium.phosphate.symporter.activity, GO.0015188.L.isoleucine.transmembrane.transporter.activity, GO.0052911.23S.rRNA..guanine.745..N.1...methyltransferase.activity, GO.0015568.L.idonate.transmembrane.transporter.activity, GO.0005294.neutral.L.amino.acid.secondary.active.transmembrane.transporter.activity, GO.0015184.L.cystine.transmembrane.transporter.activity, GO.0033170.protein.DNA.loading.ATPase.activity, GO.0004671.protein.C.terminal.S.isoprenylcysteine.carboxyl.O.methyltransferase.activity, GO.0015483.long.chain.fatty.acid.transporting.porin.activity, GO.0015134.hexuronate.transmembrane.transporter.activity, GO.0004004.RNA.helicase.activity, GO.0015279.store.operated.calcium.channel.activity, GO.0015280.ligand.gated.sodium.channel.activity, GO.0031151.histone.methyltransferase.activity..H3.K79.specific., GO.0015138.fumarate.transmembrane.transporter.activity, GO.0022883.zinc.efflux.transmembrane.transporter.activity, GO.0005313.L.glutamate.transmembrane.transporter.activity, GO.0022858.alanine.transmembrane.transporter.activity, GO.0004346.glucose.6.phosphatase.activity, GO.0009674.potassium.sodium.symporter.activity, GO.0044667.R..carnitine.4..trimethylammonio.butanoate.antiporter.activity, GO.0044020.histone.methyltransferase.activity..H4.R3.specific., GO.0000985.bacterial.type.RNA.polymerase.core.promoter.sequence.specific.DNA.binding, GO.0045145.single.stranded.DNA.5.3.exodeoxyribonuclease.activity, GO.0001150.bacterial.type.cis.regulatory.region.sequence.specific.DNA.binding, GO.0022841.potassium.ion.leak.channel.activity, GO.0005436.sodium.phosphate.symporter.activity, GO.0015140.malate.transmembrane.transporter.activity, GO.0015373.anion.sodium.symporter.activity, GO.0015196.L.tryptophan.transmembrane.transporter.activity, GO.0015195.L.threonine.transmembrane.transporter.activity, GO.0036327.VEGF.B.activated.receptor.activity, GO.0015445.silver.transmembrane.transporter.activity..phosphorylative.mechanism, GO.0005280.amino.acid.proton.symporter.activity, GO.0034639.L.amino.acid.efflux.transmembrane.transporter.activity, GO.0032143.single.thymine.insertion.binding, GO.0015269.calcium.activated.potassium.channel.activity, GO.0005451.monovalent.cation.proton.antiporter.activity, GO.0052907.23S.rRNA..adenine.1618..N.6...methyltransferase.activity, GO.0000979.RNA.polymerase.II.core.promoter.sequence.specific.DNA.binding, GO.0042970.homoserine.transmembrane.transporter.activity, GO.0043887.melibiose.sodium.symporter.activity, GO.0005247.voltage.gated.chloride.channel.activity, GO.0008553.proton.exporting.ATPase.activity..phosphorylative.mechanism, GO.0015181.arginine.transmembrane.transporter.activity, GO.0052914.16S.rRNA..guanine.1207..N.2...methyltransferase.activity, GO.0003678.DNA.helicase.activity, GO.0004439.phosphatidylinositol.4.5.bisphosphate.5.phosphatase.activity, GO.0036326.VEGF.A.activated.receptor.activity, GO.1901680.sulfur.containing.amino.acid.secondary.active.transmembrane.transporter.activity, GO.0051139.metal.ion.proton.antiporter.activity, GO.0000006.high.affinity.zinc.transmembrane.transporter.activity, GO.0005287.high.affinity.basic.amino.acid.transmembrane.transporter.activity, GO.0004402.histone.acetyltransferase.activity, GO.0005245.voltage.gated.calcium.channel.activity, GO.0005231.excitatory.extracellular.ligand.gated.ion.channel.activity, GO.000064.L.ornithine.transmembrane.transporter.activity, GO.0005290.L.histidine.transmembrane.transporter.activity, GO.1990939.ATP.dependent.microtubule.motor.activity, GO.0030698.5.10.methylenetetrahydrofolate.dependent.tRNA..m5U54..methyltransferase.activity, GO.0000986.bacterial.type.cis.regulatory.region.sequence.specific.DNA.binding, GO.0000062.fatty.acyl.Co

A.binding, GO.0008311.double.stranded.DNA.3.5.exodeoxyribonuclease.activity, GO.0001165.RNA.polymerase.I.cis.regulatory.region.sequence.specific.DNA.binding, GO.0015128.gluconate.transmembrane.transporter.activity, GO.0015141.succinate.transmembrane.transporter.activity, GO.0005229.intracellular.calcium.activated.chloride.channel.activity, GO.0046975.histone.methyltransferase.activity..H3.K36.specific., GO.0015413.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.helicase.activity, GO.0052915.23S.rRNA..guanine.2445..N.2...methyltransferase.activity, GO.000980.RNA.polymerase.II.cis.regulatory.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.activity, GO.0043682.copper.transmembrane.transporter.activity..phosphorylative.mechanism, GO.0022824.transmitter.gated.ion.channel.activity, GO.0052929.ATP.3.cytidine.cytidine.tRNA.adenylyltransferase.activity, GO.0005343.organic.acid.sodium.symporter.activity, GO.0046961.proton.transporting.ATPase.activity..rotational.mechanism, GO.0061505.DNA.topoisomerase.type.II..double.strand.cut..ATP.hydrolyzing..activity, GO.0003918.DNA.topoisomerase.type.II..double.strand.cut..ATP.hydrolyzing..activity, GO.0008574.ATP.dependent.microtubule.motor.activity..plus.end.directed, GO.0086038.calcium.sodium.antiporter.activity.involved.in.regulation.of.cardiac.muscle.cell.membrane.potential, GO.0008332.low.voltage.gated.calcium.channel.activity, GO.0009675.high.affinity.sulfate.proton.symporter.activity, GO.0022851.GABA.gated.chloride.ion.channel.activity, GO.0033682.5.3.DNA.RNA.helicase.activity, GO.0061995.ATP.dependent.protein.DNA.complex.displacement.activity, GO.0004931.extracellularly.ATP.gated.cation.channel.activity, GO.0015518.arabinose.proton.symporter.activity, GO.0035497.cAMP.response.element.binding, GO.0061749.forked.DNA.dependent.helicase.activity, GO.0009679.hexose.proton.symporter.activity, GO.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.high.voltage.gated.calcium.channel.activity, GO.0015278.calcium.release.channel.activity, GO.0034335.DNA.negative.supercoiling.activity, GO.0043142.single.stranded.DNA.helicase.activity, GO.0042945.D.serine.transmembrane.transporter.activity, GO.0015271.outward.rectifier.potassium.channel.activity, GO.0004970.ionotropic.glutamate.receptor.activity, GO.0008507.sodium.iodide.symporter.activity, GO.1904315.transmitter.gated.ion.channel.activity.involved.in.regulation.of.postsynaptic.membrane.potential, GO.0043138.3.5.DNA.helicase.activity, GO.0015649.2.ke to.3.deoxygluconate.proton.symporter.activity, GO.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.specific.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.estrone.response.element.binding, GO.0017116.single.stranded.DNA.helicase.activity, GO.0015528.lactose.proton.symporter.activity, GO.0033230.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.helicase.activity, GO.0003689.DNA.clamp.loader.activity, GO.0015496.putrescine.ornithine.antiporter.activity, GO.0010485.H4.histone.acetyltransferase.activity, GO.0086008.voltage.gated.potassium.channel.activity.involved.in.cardiac.muscle.cell.action.potential.repolarization, GO.0043140.3.5.DNA.helicase.activity, GO.0043998.H2A.histone.acetyltransferase.activity, GO.0051978.lysophospholipid.sodium.symporter.activity, GO.0015386.potassium.proton.antiporter.activity, GO.0015369.calcium.proton.antiporter.activity, GO.0015341.zinc.efflux.active.transmembrane.transporter.activity, GO.0015565.threonine.efflux.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.ferric.iron.transmembrane.transporter.activity, GO.0005242.inward.rectifier.potassium.channel.activity, GO.0015366.malate.proton.symporter.activity, GO.0034459.3.5.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.activated.potassium.channel.activity, GO.0070888.E.box.binding, GO.0043858.arginine.ornithine.antiporter.activity, GO.0001003.RNA.polymerase.III.type.2.promoter.sequence.specific.DNA.binding, GO.0008551.cadmium.transmembrane.transporter.activity..phosphorylative.mechanism, GO.0009378.four.way.junction.helicase.activity, GO.0043995.histone.acetyltransferase.activity..H4.K5.specific., GO.0015655.alanine.sodium.symporter.activity, GO.0005298.proline.sodium.symporter.activity, GO.0008508.bile.acid.sodium.symporter.activity, GO.0043996.histone.acetyltransferase.activity..H4.K8.specific., GO.0015517.galactose.proton.symporter.activity, GO.0048763.ca

lcium.induced.calcium.release.activity, GO.0005219.ryanodine.sensitive.calcium.release.channel.activity, GO.0005295.neutral.amino.acid.sodium.symporter.activity, GO.0044668.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.activity, GO.0022848.acetylcholine.gated.cation.selective.channel.activity, GO.0015535.fucose.proton.symporter.activity, GO.0016934.extracellularly.glycine.gated.chloride.channel.activity

2020-11-20 00:08:48 INFO::Running selected normalization method: NONE

2020-11-20 00:08:48 INFO::Total filtered features with variance filtering: 0

2020-11-20 00:08:48 INFO::Filtered feature names from variance filtering:

2020-11-20 00:08:48 INFO::Applying z-score to standardize continuous metadata

2020-11-20 00:08:48 INFO::Running selected transform method: NONE

2020-11-20 00:08:48 INFO::Running selected analysis method: LM

2020-11-20 00:08:48 INFO::Creating cluster of 48 R processes

2020-11-20 00:09:50 INFO::Counting total values for each feature

2020-11-20 00:09:50 WARNING::Deleting existing residuals file: ../residuals.rds

2020-11-20 00:09:50 INFO::Writing residuals to file ../residuals.rds

2020-11-20 00:09:50 INFO::Writing all results to file (ordered by increasing q-values): ../all_results.tsv

2020-11-20 00:09:50 INFO::Writing the significant results (those which are less than or equal to the threshold of 0.250000) to file (ordered by increasing q-values): ../significant_results.tsv

2020-11-20 00:09:50 INFO::Writing heatmap of significant results to file: ../heatmap.pdf

2020-11-20 00:09:51 INFO::Writing association plots (one for each significant association) to output folder: ./

2020-11-20 00:09:51 INFO::Plotting associations from most to least significant, grouped by metadata

2020-11-20 00:09:51 INFO::Plotting data for metadata number 1, case

2020-11-20 00:09:51 INFO::Creating boxplot for categorical data, case vs GO.0034660.ncRNA.metabolic.process

2020-11-20 00:09:51 INFO::Creating boxplot for categorical data, case vs GO.0016740.transferase.activity

2020-11-20 00:09:51 INFO::Creating boxplot for categorical data, case vs GO.0140098.catalytic.activity..acting.on.RNA

2020-11-20 00:09:51 INFO::Creating boxplot for categorical data, case vs GO.0043170.macromolecule.metabolic.process

2020-11-20 00:09:51 INFO::Creating boxplot for categorical data, case vs GO.0016070.RNA.metabolic.process

2020-11-20 00:09:52 INFO::Creating boxplot for categorical data, case vs GO.0019538.protein.metabolic.process

2020-11-20 00:09:52 INFO::Creating boxplot for categorical data, case vs GO.0016740.transferase.activity

2020-11-20 00:09:52 INFO::Creating boxplot for categorical data, case vs GO.0043168.anion.binding

2020-11-20 00:09:52 INFO::Creating boxplot for categorical data, case vs GO.1901362.organic.cyclic.compound.biosynthetic.process

2020-11-20 00:09:52 INFO::Creating boxplot for categorical data, case vs GO.0044260.cellular.macromolecule.metabolic.process

2020-11-20 00:09:52 INFO::Creating boxplot for categorical data, case vs GO.0003676.nucleic.acid.binding

2020-11-20 00:09:52 INFO::Creating boxplot for categorical data, case vs GO.0065007.biological.regulation

2020-11-20 00:09:53 INFO::Creating boxplot for categorical data, case vs GO.0003677.DNA.binding

2020-11-20 00:09:53 INFO::Creating boxplot for categorical data, case vs GO.0016772.transferase.activity..transferring.phosphorus.containing.groups

2020-11-20 00:09:53 INFO::Creating boxplot for categorical data, case vs GO.0009987.cellular.process

2020-11-20 00:09:53 INFO::Creating boxplot for categorical data, case vs GO.0050794.regulation.of.cellular.process

2020-11-20 00:09:53 INFO::Creating boxplot for categorical data, case vs GO.0090304.nucleic.acid.metabolic.process

2020-11-20 00:09:53 INFO::Creating boxplot for categorical data, case vs GO.0050789.regulation.of.biological.process

2020-11-20 00:09:53 INFO::Creating boxplot for categorical data, case vs GO.0044260.cellular.macromolecule.metabolic.process

2020-11-20 00:09:54 INFO::Creating boxplot for categorical data, case vs GO.0016829.lyase.activity

2020-11-20 00:09:54 INFO::Creating boxplot for categorical data, case vs GO.0016779.nucleotidyltransferase.activity

2020-11-20 00:09:54 INFO::Creating boxplot for categorical data, case vs GO.0034645.cellular.macromolecule.biosynthetic.p

rocess

2020-11-20 00:09:54 INFO::Creating boxplot for categorical data, case vs GO.0044281.small.molecule.metabolic.process
 2020-11-20 00:09:54 INFO::Creating boxplot for categorical data, case vs GO.0044283.small.molecule.biosynthetic.process
 2020-11-20 00:09:54 INFO::Creating boxplot for categorical data, case vs GO.0009987.cellular.process
 2020-11-20 00:09:54 INFO::Creating boxplot for categorical data, case vs GO.0009059.macromolecule.biosynthetic.process
 2020-11-20 00:09:55 INFO::Creating boxplot for categorical data, case vs GO.0019438.aromatic.compound.biosynthetic.proces
 s
 2020-11-20 00:09:55 INFO::Creating boxplot for categorical data, case vs GO.0044262.cellular.carbohydrate.metabolic.proce
 ss
 2020-11-20 00:09:55 INFO::Creating boxplot for categorical data, case vs GO.0019438.aromatic.compound.biosynthetic.proces
 s
 2020-11-20 00:09:55 INFO::Creating boxplot for categorical data, case vs GO.1901566.organonitrogen.compound.biosynthetic.
 process
 2020-11-20 00:09:55 INFO::Creating boxplot for categorical data, case vs GO.0006139.nucleobase.containing.compound.metabo
 lic.process
 2020-11-20 00:09:55 INFO::Creating boxplot for categorical data, case vs GO.0043168.anion.binding
 2020-11-20 00:09:56 INFO::Creating boxplot for categorical data, case vs GO.0034641.cellular.nitrogen.compound.metabolic.
 process
 2020-11-20 00:09:56 INFO::Creating boxplot for categorical data, case vs GO.0006793.phosphorus.metabolic.process
 2020-11-20 00:09:56 INFO::Creating boxplot for categorical data, case vs GO.0006796.phosphate.containing.compound.metabol
 ic.process
 2020-11-20 00:09:56 INFO::Creating boxplot for categorical data, case vs GO.1901265.nucleoside.phosphate.binding
 2020-11-20 00:09:56 INFO::Creating boxplot for categorical data, case vs GO.0000166.nucleotide.binding
 2020-11-20 00:09:56 INFO::Creating boxplot for categorical data, case vs GO.0036094.small.molecule.binding
 2020-11-20 00:09:56 INFO::Creating boxplot for categorical data, case vs GO.0006082.organic.acid.metabolic.process
 2020-11-20 00:09:56 INFO::Creating boxplot for categorical data, case vs GO.0044249.cellular.biosynthetic.process
 2020-11-20 00:09:57 INFO::Creating boxplot for categorical data, case vs GO.0044281.small.molecule.metabolic.process
 2020-11-20 00:09:57 INFO::Creating boxplot for categorical data, case vs GO.0043170.macromolecule.metabolic.process
 2020-11-20 00:09:57 INFO::Creating boxplot for categorical data, case vs GO.1901576.organic.substance.biosynthetic.proces
 s
 2020-11-20 00:09:57 INFO::Creating boxplot for categorical data, case vs GO.0043436.oxoacid.metabolic.process
 2020-11-20 00:09:57 INFO::Creating boxplot for categorical data, case vs GO.0019752.carboxylic.acid.metabolic.process
 2020-11-20 00:09:57 INFO::Creating boxplot for categorical data, case vs GO.1901265.nucleoside.phosphate.binding
 2020-11-20 00:09:57 INFO::Creating boxplot for categorical data, case vs GO.0000166.nucleotide.binding
 2020-11-20 00:09:58 INFO::Creating boxplot for categorical data, case vs GO.0034660.ncRNA.metabolic.process
 2020-11-20 00:09:58 INFO::Creating boxplot for categorical data, case vs GO.0044238.primary.metabolic.process
 2020-11-20 00:09:58 INFO::Creating boxplot for categorical data, case vs GO.0005975.carbohydrate.metabolic.process
 2020-11-20 00:09:58 INFO::Creating boxplot for categorical data, case vs GO.0019538.protein.metabolic.process
 2020-11-20 00:09:58 INFO::Creating boxplot for categorical data, case vs GO.0036094.small.molecule.binding
 2020-11-20 00:09:58 INFO::Creating boxplot for categorical data, case vs GO.0008652.cellular.amino.acid.biosynthetic.proc
 ess
 2020-11-20 00:09:58 INFO::Creating boxplot for categorical data, case vs GO.0044877.protein.containing.complex.binding
 2020-11-20 00:09:58 INFO::Creating boxplot for categorical data, case vs GO.0016835.carbon.oxygen.lyase.activity
 2020-11-20 00:09:59 INFO::Creating boxplot for categorical data, case vs GO.0008652.cellular.amino.acid.biosynthetic.proc
 ess
 2020-11-20 00:09:59 INFO::Creating boxplot for categorical data, case vs GO.0046483.heterocycle.metabolic.process
 2020-11-20 00:09:59 INFO::Creating boxplot for categorical data, case vs GO.0016836.hydro.lyase.activity
 2020-11-20 00:09:59 INFO::Creating boxplot for categorical data, case vs GO.0044283.small.molecule.biosynthetic.process
 2020-11-20 00:09:59 INFO::Creating boxplot for categorical data, case vs GO.0016053.organic.acid.biosynthetic.process

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2020-11-20 00:09:59 INFO::Creating boxplot for categorical data, case vs GO.0046394.carboxylic.acid.biosynthetic.process
2020-11-20 00:09:59 INFO::Creating boxplot for categorical data, case vs GO.0006520.cellular.amino.acid.metabolic.process
2020-11-20 00:10:00 INFO::Creating boxplot for categorical data, case vs GO.0016835.carbon.oxygen.lyase.activity
2020-11-20 00:10:00 INFO::Creating boxplot for categorical data, case vs GO.0009072.aromatic.amino.acid.family.metabolic.
process
2020-11-20 00:10:00 INFO::Creating boxplot for categorical data, case vs GO.0016836.hydro.lyase.activity
2020-11-20 00:10:00 INFO::Creating boxplot for categorical data, case vs GO.0046417.chorismate.metabolic.process
2020-11-20 00:10:00 INFO::Creating boxplot for categorical data, case vs GO.0044877.protein.containing.complex.binding
2020-11-20 00:10:00 INFO::Creating boxplot for categorical data, case vs GO.0009423.chorismate.biosynthetic.process
2020-11-20 00:10:01 INFO::Creating boxplot for categorical data, case vs GO.0009073.aromatic.amino.acid.family.biosynthet
ic.process
2020-11-20 00:10:01 INFO::Creating boxplot for categorical data, case vs GO.0009423.chorismate.biosynthetic.process
2020-11-20 00:10:01 INFO::Creating boxplot for categorical data, case vs GO.0003677.DNA.binding
2020-11-20 00:10:01 INFO::Creating boxplot for categorical data, case vs GO.0006082.organic.acid.metabolic.process
2020-11-20 00:10:01 INFO::Creating boxplot for categorical data, case vs GO.0043436.oxoacid.metabolic.process
2020-11-20 00:10:01 INFO::Creating boxplot for categorical data, case vs GO.0043650.dicarboxylic.acid.biosynthetic.proces
s
2020-11-20 00:10:01 INFO::Creating boxplot for categorical data, case vs GO.0043650.dicarboxylic.acid.biosynthetic.proces
s
2020-11-20 00:10:02 INFO::Creating boxplot for categorical data, case vs GO.0003855.3.dehydroquinate.dehydratase.activity
2020-11-20 00:10:02 INFO::Creating boxplot for categorical data, case vs GO.0019752.carboxylic.acid.metabolic.process
2020-11-20 00:10:02 INFO::Creating boxplot for categorical data, case vs GO.0003855.3.dehydroquinate.dehydratase.activity
2020-11-20 00:10:02 INFO::Creating boxplot for categorical data, case vs GO.0097367.carbohydrate.derivative.binding
2020-11-20 00:10:02 INFO::Creating boxplot for categorical data, case vs GO.0097367.carbohydrate.derivative.binding
2020-11-20 00:10:02 INFO::Creating boxplot for categorical data, case vs GO.0046417.chorismate.metabolic.process
2020-11-20 00:10:02 INFO::Creating boxplot for categorical data, case vs GO.0032553.ribonucleotide.binding
2020-11-20 00:10:02 INFO::Creating boxplot for categorical data, case vs GO.0016053.organic.acid.biosynthetic.process
2020-11-20 00:10:03 INFO::Creating boxplot for categorical data, case vs GO.0046394.carboxylic.acid.biosynthetic.process
2020-11-20 00:10:03 INFO::Creating boxplot for categorical data, case vs GO.0043648.dicarboxylic.acid.metabolic.process
2020-11-20 00:10:03 INFO::Creating boxplot for categorical data, case vs GO.0032553.ribonucleotide.binding
2020-11-20 00:10:03 INFO::Creating boxplot for categorical data, case vs GO.0016070.RNA.metabolic.process
2020-11-20 00:10:03 INFO::Creating boxplot for categorical data, case vs GO.1901566.organonitrogen.compound.biosynthetic.
process
2020-11-20 00:10:03 INFO::Creating boxplot for categorical data, case vs GO.0006807.nitrogen.compound.metabolic.process
2020-11-20 00:10:03 INFO::Creating boxplot for categorical data, case vs GO.0003674.molecular_function
2020-11-20 00:10:04 INFO::Creating boxplot for categorical data, case vs GO.0032555.purine.ribonucleotide.binding
2020-11-20 00:10:04 INFO::Creating boxplot for categorical data, case vs GO.0017076.purine.nucleotide.binding
2020-11-20 00:10:04 INFO::Creating boxplot for categorical data, case vs GO.0051540.metal.cluster.binding
2020-11-20 00:10:04 INFO::Creating boxplot for categorical data, case vs GO.0032555.purine.ribonucleotide.binding
2020-11-20 00:10:04 INFO::Creating boxplot for categorical data, case vs GO.0017076.purine.nucleotide.binding
2020-11-20 00:10:04 INFO::Creating boxplot for categorical data, case vs GO.0004518.nuclease.activity
2020-11-20 00:10:04 INFO::Creating boxplot for categorical data, case vs GO.0051536.iron.sulfur.cluster.binding
2020-11-20 00:10:04 INFO::Creating boxplot for categorical data, case vs GO.0035639.purine.ribonucleoside.triphosphate.bi
nding
2020-11-20 00:10:05 INFO::Creating boxplot for categorical data, case vs GO.0140098.catalytic.activity..acting.on.RNA
2020-11-20 00:10:05 INFO::Creating boxplot for categorical data, case vs GO.0035639.purine.ribonucleoside.triphosphate.bi
nding
2020-11-20 00:10:05 INFO::Creating boxplot for categorical data, case vs GO.0009058.biosynthetic.process
2020-11-20 00:10:05 INFO::Creating boxplot for categorical data, case vs GO.0051539.4.iron..4.sulfur.cluster.binding

```

```

2020-11-20 00:10:05 INFO::Creating boxplot for categorical data, case vs GO.0043648.dicarboxylic.acid.metabolic.process
2020-11-20 00:10:05 INFO::Creating boxplot for categorical data, case vs GO.0034641.cellular.nitrogen.compound.metabolic.
process
2020-11-20 00:10:05 INFO::Creating boxplot for categorical data, case vs GO.0009072.aromatic.amino.acid.family.metabolic.
process
2020-11-20 00:10:06 INFO::Creating boxplot for categorical data, case vs GO.0071941.nitrogen.cycle.metabolic.process
2020-11-20 00:10:06 INFO::Creating boxplot for categorical data, case vs GO.0009399.nitrogen.fixation
2020-11-20 00:10:06 INFO::Creating boxplot for categorical data, case vs GO.0009073.aromatic.amino.acid.family.biosynthet
ic.process
2020-11-20 00:10:06 INFO::Creating boxplot for categorical data, case vs GO.0003824.catalytic.activity
2020-11-20 00:10:06 INFO::Creating boxplot for categorical data, case vs GO.0065007.biological.regulation
2020-11-20 00:10:06 INFO::Creating boxplot for categorical data, case vs GO.0050794.regulation.of.cellular.process
2020-11-20 00:10:06 INFO::Creating boxplot for categorical data, case vs GO.0003676.nucleic.acid.binding
2020-11-20 00:10:06 INFO::Creating boxplot for categorical data, case vs GO.0003824.catalytic.activity
2020-11-20 00:10:07 INFO::Creating boxplot for categorical data, case vs GO.0006520.cellular.amino.acid.metabolic.process
2020-11-20 00:10:07 INFO::Creating boxplot for categorical data, case vs GO.0046483.heterocycle.metabolic.process
2020-11-20 00:10:07 INFO::Creating boxplot for categorical data, case vs GO.0050789.regulation.of.biological.process
2020-11-20 00:10:07 INFO::Creating boxplot for categorical data, case vs GO.0016874.ligase.activity
2020-11-20 00:10:07 INFO::Creating boxplot for categorical data, case vs GO.0051540.metal.cluster.binding
2020-11-20 00:10:07 INFO::Creating boxplot for categorical data, case vs GO.0016772.transferase.activity..transferring.ph
osphorus.containing.groups
2020-11-20 00:10:07 INFO::Creating boxplot for categorical data, case vs GO.0051536.iron.sulfur.cluster.binding
2020-11-20 00:10:08 INFO::Creating boxplot for categorical data, case vs GO.0051539.4.iron..4.sulfur.cluster.binding
2020-11-20 00:10:08 INFO::Creating boxplot for categorical data, case vs GO.0008150.biological_process
2020-11-20 00:10:08 INFO::Creating boxplot for categorical data, case vs GO.0071941.nitrogen.cycle.metabolic.process
2020-11-20 00:10:08 INFO::Creating boxplot for categorical data, case vs GO.0009399.nitrogen.fixation
2020-11-20 00:10:08 INFO::Creating boxplot for categorical data, case vs GO.0044271.cellular.nitrogen.compound.biosynthet
ic.process
2020-11-20 00:10:08 INFO::Creating boxplot for categorical data, case vs GO.1901362.organic.cyclic.compound.biosynthetic.
process
2020-11-20 00:10:08 INFO::Creating boxplot for categorical data, case vs GO.0044249.cellular.biosynthetic.process
2020-11-20 00:10:08 INFO::Creating boxplot for categorical data, case vs GO.1901576.organic.substance.biosynthetic.proces
s
2020-11-20 00:10:09 INFO::Creating boxplot for categorical data, case vs GO.0034645.cellular.macromolecule.biosynthetic.p
rocess
2020-11-20 00:10:09 INFO::Creating boxplot for categorical data, case vs GO.0009059.macromolecule.biosynthetic.process
2020-11-20 00:10:09 INFO::Creating boxplot for categorical data, case vs GO.0055086.nucleobase.containing.small.molecule.
metabolic.process
2020-11-20 00:10:09 INFO::Creating boxplot for categorical data, case vs GO.0006725.cellular.aromatic.compound.metabolic.
process
2020-11-20 00:10:09 INFO::Creating boxplot for categorical data, case vs GO.1901564.organonitrogen.compound.metabolic.pro
cess
2020-11-20 00:10:09 INFO::Creating boxplot for categorical data, case vs GO.0019637.organophosphate.metabolic.process
2020-11-20 00:10:09 INFO::Creating boxplot for categorical data, case vs GO.0016788.hydrolase.activity..acting.on.ester.b
onds
2020-11-20 00:10:10 INFO::Creating boxplot for categorical data, case vs GO.0016817.hydrolase.activity..acting.on.acid.an
hydrides
2020-11-20 00:10:10 INFO::Creating boxplot for categorical data, case vs GO.0016818.hydrolase.activity..acting.on.acid.an
hydrides..in.phosphorus.containing.anhydrides

```

```

2020-11-20 00:10:10 INFO::Creating boxplot for categorical data, case vs GO.0006725.cellular.aromatic.compound.metabolic.
process
2020-11-20 00:10:10 INFO::Creating boxplot for categorical data, case vs GO.0006793.phosphorus.metabolic.process
2020-11-20 00:10:10 INFO::Creating boxplot for categorical data, case vs GO.0006796.phosphate.containing.compound.metabol
ic.process
2020-11-20 00:10:10 INFO::Creating boxplot for categorical data, case vs GO.0016462.pyrophosphatase.activity
2020-11-20 00:10:10 INFO::Creating boxplot for categorical data, case vs GO.0016787.hydrolase.activity
2020-11-20 00:10:11 INFO::Creating boxplot for categorical data, case vs GO.0008150.biological_process
2020-11-20 00:10:11 INFO::Creating boxplot for categorical data, case vs GO.0008233.peptidase.activity
2020-11-20 00:10:11 INFO::Creating boxplot for categorical data, case vs GO.0006810.transport
2020-11-20 00:10:11 INFO::Creating boxplot for categorical data, case vs GO.0006753.nucleoside.phosphate.metabolic.proces
s
2020-11-20 00:10:11 INFO::Creating boxplot for categorical data, case vs GO.0009117.nucleotide.metabolic.process
2020-11-20 00:10:11 INFO::Creating boxplot for categorical data, case vs GO.0003674.molecular_function
2020-11-20 00:10:11 INFO::Creating boxplot for categorical data, case vs GO.0006810.transport
2020-11-20 00:10:12 INFO::Creating boxplot for categorical data, case vs GO.0016787.hydrolase.activity
2020-11-20 00:10:12 INFO::Creating boxplot for categorical data, case vs GO.0051234.establishment.of.localization
2020-11-20 00:10:12 INFO::Creating boxplot for categorical data, case vs GO.0017111.nucleoside.triphosphatase.activity
2020-11-20 00:10:12 INFO::Creating boxplot for categorical data, case vs GO.0051179.localization
2020-11-20 00:10:12 INFO::Creating boxplot for categorical data, case vs GO.0043167.ion.binding
2020-11-20 00:10:12 INFO::Creating boxplot for categorical data, case vs GO.0140096.catalytic.activity..acting.on.a.prote
in
2020-11-20 00:10:12 INFO::Creating boxplot for categorical data, case vs GO.0016779.nucleotidyltransferase.activity
2020-11-20 00:10:12 INFO::Creating boxplot for categorical data, case vs GO.0072521.purine.containing.compound.metabolic.
process
2020-11-20 00:10:13 INFO::Creating boxplot for categorical data, case vs GO.0016829.lyase.activity

```

\$results

A data.frame: 232 × 10

	feature	metadata	value	coef
	<chr>	<chr>	<chr>	<dbl>
	GO.0034660.ncRNA.metabolic.process	case	Control_Healthy	0.003981729 0.00
	GO.0016740.transferase.activity	case	Control_Sick	0.009075342 0.00
	GO.0140098.catalytic.activity..acting.on.RNA	case	Control_Healthy	0.002885018 0.00
	GO.0043170.macromolecule.metabolic.process	case	Control_Healthy	0.005589451 0.00
	GO.0016070.RNA.metabolic.process	case	Control_Healthy	0.004123315 0.00
	GO.0019538.protein.metabolic.process	case	Control_Healthy	0.001399883 0.00

	feature	metadata	value	coef
	<chr>	<chr>	<chr>	<dbl>
GO.0016740.transferase.activity		case	Control_Healthy	0.007359575 0.00
GO.0043168.anion.binding		case	Control_Healthy	0.001858915 0.00
GO.1901362.organic.cyclic.compound.biosynthetic.process		case	Control_Healthy	-0.002910851 0.00
GO.0044260.cellular.macromolecule.metabolic.process		case	Control_Healthy	0.002850432 0.00
GO.0003676.nucleic.acid.binding		case	Control_Sick	-0.002974457 0.00
GO.0065007.biological.regulation		case	Control_Healthy	0.002747947 0.00
GO.0003677.DNA.binding		case	Control_Sick	-0.002225029 0.00
GO.0016772.transferase.activity..transferring.phosphorus.containing.groups		case	Control_Sick	0.005475526 0.00
GO.0009987.cellular.process		case	Control_Healthy	0.006062118 0.00
GO.0050794.regulation.of.cellular.process		case	Control_Healthy	0.002335097 0.00
GO.0090304.nucleic.acid.metabolic.process		case	Control_Healthy	0.003677832 0.00
GO.0050789.regulation.of.biological.process		case	Control_Healthy	0.002322823 0.00
GO.0044260.cellular.macromolecule.metabolic.process		case	Control_Sick	0.002371719 0.00
GO.0016829.lyase.activity		case	Control_Healthy	-0.004643479 0.00
GO.0016779.nucleotidyltransferase.activity		case	Control_Sick	0.003828213 0.00

	feature	metadata	value	coef
	<chr>	<chr>	<chr>	<dbl>
GO.0034645.cellular.macromolecule.biosynthetic.process		case	Control_Sick	0.001793419 0.00
GO.0044281.small.molecule.metabolic.process		case	Control_Healthy	-0.004324793 0.00
GO.0044283.small.molecule.biosynthetic.process		case	Control_Healthy	-0.004540546 0.00
GO.0009987.cellular.process		case	Control_Sick	0.004905174 0.00
GO.0009059.macromolecule.biosynthetic.process		case	Control_Sick	0.001745069 0.00
GO.0019438.aromatic.compound.biosynthetic.process		case	Control_Healthy	-0.002051294 0.00
GO.0044262.cellular.carbohydrate.metabolic.process		case	Control_Sick	0.002421115 0.00
GO.0019438.aromatic.compound.biosynthetic.process		case	Control_Sick	-0.001938171 0.00
GO.1901566.organonitrogen.compound.biosynthetic.process		case	Control_Healthy	-0.002981672 0.00
:	:	:	:	:
GO.0001882.nucleoside.binding		case	Control_Healthy	5.863199e-05 1.07
GO.0032549.ribonucleoside.binding		case	Control_Healthy	5.872475e-05 1.07
GO.0019001.guanyl.nucleotide.binding		case	Control_Sick	5.523391e-05 1.02
GO.0032561.guanyl.ribonucleotide.binding		case	Control_Sick	5.493212e-05 1.02
GO.0043169.cation.binding		case	Control_Sick	4.762072e-04 9.03
GO.0071704.organic.substance.metabolic.process		case	Control_Healthy	5.885809e-04 1.13
GO.0005215.transporter.activity		case	Control_Sick	-2.138713e-04 4.30
GO.0001883.purine.nucleoside.binding		case	Control_Sick	4.841929e-05 1.01

	feature	metadata	value	coef
	<chr>	<chr>	<chr>	<dbl>
GO.0046872.metal.ion.binding		case	Control_Sick	4.318028e-04 9.02
GO.0032550.purine.ribonucleoside.binding		case	Control_Sick	4.848646e-05 1.01
GO.0019001.guanyl.nucleotide.binding		case	Control_Healthy	5.140863e-05 1.06
GO.0032561.guanyl.ribonucleotide.binding		case	Control_Healthy	5.147866e-05 1.06
GO.0005525.GTP.binding		case	Control_Sick	4.844792e-05 1.01
GO.0003924.GTPase.activity		case	Control_Sick	4.632208e-05 1.00
GO.0001883.purine.nucleoside.binding		case	Control_Healthy	4.579257e-05 1.06
GO.0032550.purine.ribonucleoside.binding		case	Control_Healthy	4.596168e-05 1.06
GO.0005525.GTP.binding		case	Control_Healthy	4.587574e-05 1.06
GO.0003924.GTPase.activity		case	Control_Healthy	4.409975e-05 1.05
GO.0006163.purine.nucleotide.metabolic.process		case	Control_Sick	-8.193882e-05 2.90
GO.0072521.purine.containing.compound.metabolic.process		case	Control_Sick	-7.894296e-05 2.85
GO.0016788.hydrolase.activity..acting.on.ester.bonds		case	Control_Healthy	-1.431759e-04 5.41
GO.0006508.proteolysis		case	Control_Sick	3.226418e-05 1.48
GO.0009058.biosynthetic.process		case	Control_Sick	-2.985806e-04 1.47
GO.0043022.ribosome.binding		case	Control_Sick	1.573443e-05 9.75
GO.0043021.ribonucleoprotein.complex.binding		case	Control_Sick	1.426256e-05 9.76
GO.1901360.organic.cyclic.compound.metabolic.process		case	Control_Sick	-9.724336e-05 6.95
GO.0043021.ribonucleoprotein.complex.binding		case	Control_Healthy	-9.348902e-06 1.01
GO.0016491.oxidoreductase.activity		case	Control_Healthy	-6.169740e-05 7.80

\$residuals

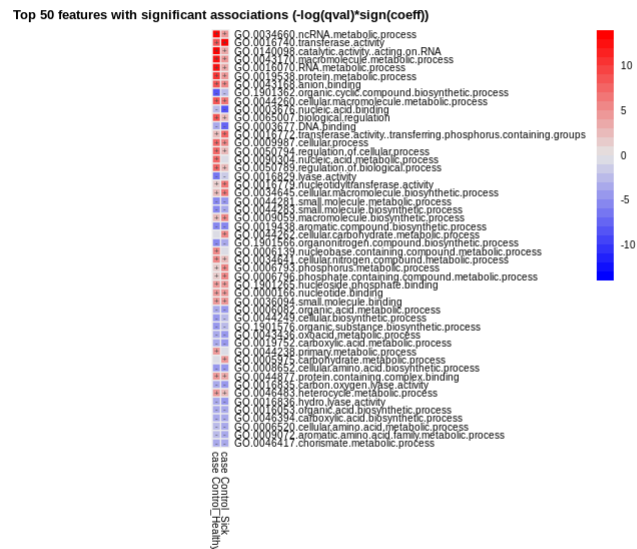
feature		metadata	value	coef
<chr>		<chr>	<chr>	<dbl>
GO.0043022.ribosome.binding		case	Control_Healthy	-6.899274e-06 1.01
GO.0044238.primary.metabolic.process		case	Control_Sick	-1.558900e-05 6.95
1				
GO.0008150.biological_process				1.050308e-03 0.002671
GO.0008152.metabolic.process				1.967926e-03 0.002084
GO.0051179.localization				-1.096149e-03 -0.000227
GO.0065007.biological.regulation				-1.735470e-04 -0.000647
GO.0009987.cellular.process				4.027215e-03 0.003487
GO.0044237.cellular.metabolic.process				3.258478e-03 0.002986
GO.0050789.regulation.of.biological.process				-4.025308e-04 -0.000741
GO.0044238.primary.metabolic.process				-8.640054e-05 -0.001704
GO.0071704.organic.substance.metabolic.process				2.111104e-03 0.000530
GO.0006807.nitrogen.compound.metabolic.process				5.247134e-04 -0.002390
GO.0051234.establishment.of.localization				-1.095399e-03 -0.000227
GO.0009058.biosynthetic.process				2.696740e-03 0.003096

GO.0044281.small.molecule.metabolic.process	-4.001232e-04	-0.001376
GO.0044283.small.molecule.biosynthetic.process	3.578024e-04	-0.000598
GO.1901360.organic.cyclic.compound.metabolic.process	3.092047e-03	0.000303
GO.0034641.cellular.nitrogen.compound.metabolic.process	3.431093e-03	0.001800
GO.0046483.heterocycle.metabolic.process	3.578329e-03	0.001777
GO.0006082.organic.acid.metabolic.process	-3.351721e-03	-0.004956
GO.0043170.macromolecule.metabolic.process	2.782860e-03	0.000701
GO.0006725.cellular.aromatic.compound.metabolic.process	-7.259887e-04	-0.003856
GO.0006810.transport	-1.130341e-03	-0.000262
GO.0071941.nitrogen.cycle.metabolic.process	-1.256314e-03	-0.001511
GO.0044249.cellular.biosynthetic.process	9.054021e-04	0.000920
GO.0050794.regulation.of.cellular.process	-3.717222e-04	-0.000776
GO.0005975.carbohydrate.metabolic.process	1.194613e-03	0.002485
GO.1901564.organonitrogen.compound.metabolic.process	9.787501e-04	0.000407
GO.0006793.phosphorus.metabolic.process	1.465548e-04	0.001891
GO.1901576.organic.substance.biosynthetic.process	9.139901e-04	0.000952

		1
GO.1901362.organic.cyclic.compound.biosynthetic.process	1.503030e-03	0.001804
GO.1901566.organonitrogen.compound.biosynthetic.process	1.100834e-03	0.000877
:	:	
GO.0001882.nucleoside.binding	-3.003590e-07	1.041706
GO.0016788.hydrolase.activity..acting.on.ester.bonds	5.390783e-04	-2.862556
GO.0043021.ribonucleoprotein.complex.binding	5.093344e-08	4.446446
GO.1901265.nucleoside.phosphate.binding	-2.767435e-04	-2.390776
GO.0003676.nucleic.acid.binding	-1.417264e-03	-1.839516
GO.0051536.iron.sulfur.cluster.binding	-2.660612e-03	-3.132336
GO.0043169.cation.binding	2.233725e-03	2.501046
GO.0004518.nuclease.activity	4.558782e-04	-2.475266
GO.0016779.nucleotidyltransferase.activity	2.006040e-04	2.479736
GO.0001883.purine.nucleoside.binding	-3.579259e-07	-1.449427
GO.0046872.metal.ion.binding	2.219931e-03	2.434186
GO.0003677.DNA.binding	-5.606521e-04	-1.005526
GO.0016836.hydro.lyase.activity	-4.785183e-04	-8.838047

		1
	GO.0043022.ribosome.binding	2.553842e-09 4.35893e
GO.0016818.hydrolase.activity..acting.on.acid.anhydrides..in.phosphorus.containing.anhydrides		-1.012493e-04 -6.87043e
	GO.0035639.purine.ribonucleoside.triphosphate.binding	-8.854684e-05 -6.67497e
	GO.0051539.4.iron..4.sulfur.cluster.binding	-1.307937e-03 -1.56568e
	GO.0000166.nucleotide.binding	-2.767435e-04 -2.39077e
	GO.0032549.ribonucleoside.binding	-2.981624e-07 1.06389e
	GO.0016462.pyrophosphatase.activity	-1.055595e-04 -6.68668e
	GO.0032550.purine.ribonucleoside.binding	-3.557352e-07 -1.42927e
	GO.0003855.3.dehydroquinase.dehydratase.activity	-5.109609e-04 -9.64509e
	GO.0032553.ribonucleotide.binding	-7.401135e-05 -3.10159e
	GO.0017076.purine.nucleotide.binding	-8.525448e-05 -5.48154e
	GO.0032555.purine.ribonucleotide.binding	-8.417166e-05 -5.64370e
	GO.0019001.guanyl.nucleotide.binding	-3.225791e-07 -6.09219e
	GO.0017111.nucleoside.triphosphatase.activity	-1.094893e-04 -7.33570e
	GO.0032561.guanyl.ribonucleotide.binding	-3.297811e-07 -7.31027e
	GO.0003924.GTPase.activity	-3.044794e-07 -2.25532e

GO.0005525.GTP.binding -3.547081e-07 -1.432634



In []:

OK OK OK OK NEXT I HAVE TO DO THIS ON THE COVID19 CASES ONLY (OR MAYBE ALL THE CASES AND JUST DELINEATE COVID19 BY OUTCOME) AND DO IT BY OUTCOME

DMM modeling using the MaAslin2 derived terms

Ok, lets import the MaAsLin2 derived significant terms

```
In [27]: sig<-read.table("Significant_Go_terms_for_Beth_Maaslin2.txt",header = T,sep = "\t")
head(sig)
```

A data.frame: 6 × 12

	GO_Tag	name	metadata	value.as.compared.to.COVID19.	coef	stderr	N	N.not.0	pval	qval	
	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>	<int>	<int>	<dbl>	<dbl>	
1	GO:0034660	ncRNA metabolic process	case	Control_Healthy	0.003981729	0.000636300	141	133	6.14e- 09	9.21e- 07	GO.
2	GO:0016740	transferase activity	case	Control_Sick	0.009075342	0.001469230	141	135	1.00e- 08	9.21e- 07	
3	GO:0140098	catalytic activity acting on RNA	case	Control_Healthy	0.002885018	0.000469004	141	134	1.19e- 08	9.21e- 07	GO.01400
4	GO:0043170	macromolecule metabolic process	case	Control_Healthy	0.005589451	0.000911267	141	136	5.98e- 08	3.47e- 06	GO.0043170
5	GO:0016070	RNA metabolic process	case	Control_Healthy	0.004123315	0.000725339	141	135	9.00e- 08	4.17e- 06	G
6	GO:0019538	protein metabolic process	case	Control_Healthy	0.001399883	0.000264455	141	134	6.08e- 07	2.35e- 05	GO.(

DMM Preprocessing /filtering

Ok lets filter out the GO_tag matches from our phyloseq object

```
In [28]: Terms<-sig$GO_Tag
```

```
In [29]: 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:      [ 13846 taxa and 141 samples ]
sample_data() Sample Data:  [ 141 samples by 71 sample variables ]
tax_table()  Taxonomy Table: [ 13846 taxa by 3 taxonomic ranks ]
```

```
In [30]: bac_pseq_prune<-prune_taxa(x = bac_pseq_no_neg, taxa = Terms)
bac_pseq_prune #[ 92 taxa and 141 samples ]
```

```

phyloseq-class experiment-level object
otu_table() OTU Table:      [ 92 taxa and 141 samples ]
sample_data() Sample Data:  [ 141 samples by 71 sample variables ]
tax_table()  Taxonomy Table: [ 92 taxa by 3 taxonomic ranks ]

```

ok lets rename our GO_terms again

```

In [31]: tax<-data.frame(tax_table(bac_pseq_prune))
names<-paste(rownames(tax),tax$name,sep="-")
length(names)
taxa_names(bac_pseq_prune)<-names

```

92

filter out the depth 0 mol fxn and bio proc, empty GO Terms, and empty samples

```

In [32]: bac_pseq_prune
filtme<-c("GO:0003674-molecular_function")
bac_pseq_prune <- prune_taxa(taxa=taxa_names(bac_pseq_prune)!=filtme, bac_pseq_prune)
bac_pseq_prune
filtme<-c("GO:0008150-biological_process")
bac_pseq_prune <- prune_taxa(taxa=taxa_names(bac_pseq_prune)!=filtme, bac_pseq_prune)
bac_pseq_prune
bac_pseq_prune <- prune_samples(sample_sums(bac_pseq_prune) > 1, bac_pseq_prune)
#bac_pseq_prune#[ 92 taxa and 141 samples ]
bac_pseq_prune <- prune_taxa(taxa_sums(bac_pseq_prune) > 1, bac_pseq_prune)
bac_pseq_prune #[ 92 taxa and 141 samples ]

```

```

phyloseq-class experiment-level object
otu_table() OTU Table:      [ 92 taxa and 141 samples ]
sample_data() Sample Data:  [ 141 samples by 71 sample variables ]
tax_table()  Taxonomy Table: [ 92 taxa by 3 taxonomic ranks ]
phyloseq-class experiment-level object
otu_table() OTU Table:      [ 91 taxa and 141 samples ]
sample_data() Sample Data:  [ 141 samples by 71 sample variables ]
tax_table()  Taxonomy Table: [ 91 taxa by 3 taxonomic ranks ]
phyloseq-class experiment-level object
otu_table() OTU Table:      [ 90 taxa and 141 samples ]
sample_data() Sample Data:  [ 141 samples by 71 sample variables ]
tax_table()  Taxonomy Table: [ 90 taxa by 3 taxonomic ranks ]
phyloseq-class experiment-level object
otu_table() OTU Table:      [ 90 taxa and 141 samples ]
sample_data() Sample Data:  [ 141 samples by 71 sample variables ]
tax_table()  Taxonomy Table: [ 90 taxa by 3 taxonomic ranks ]

```

DMM modeling time

convert counts to a matrix

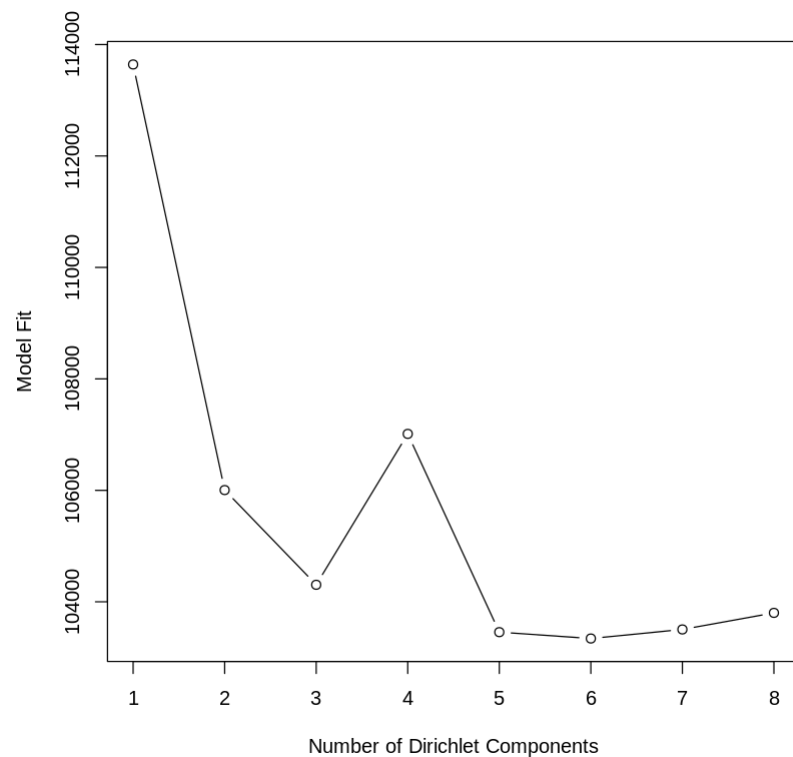
```
In [33]: dat <- abundances(bac_pseq_prune)
count <- as.matrix(t(dat))
```

Fit the dmm model

```
In [34]: fit <- mclapply(1:8, dmn, count = count, verbose=TRUE)
```

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

```
In [35]: 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 [36]: #identify the number of clusters that best fits the model
```

```
In [37]: best <- fit[[which.min(lplc)]]
```



```
best <- fit[[3]]
best
```

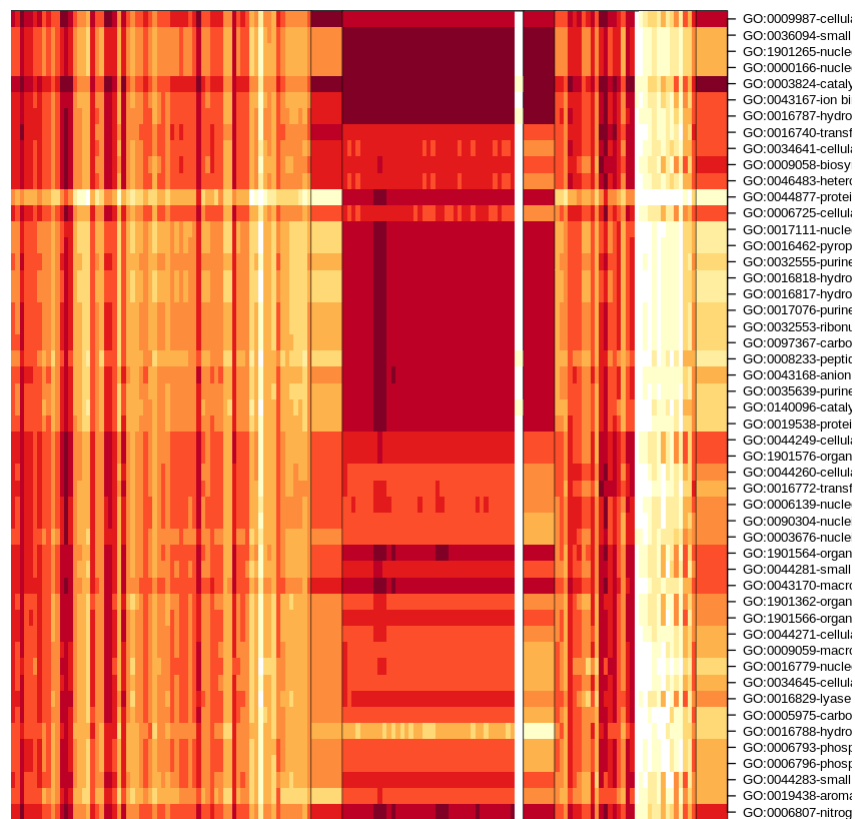
```
class: DMN
k: 3
samples x taxa: 141 x 90
Laplace: 104305.2 BIC: 104203.3 AIC: 103802.2
```

```
In [38]: #save.image(file = "go_terms_dmm.rdata")
```

make a heatmap visualization of the cluster

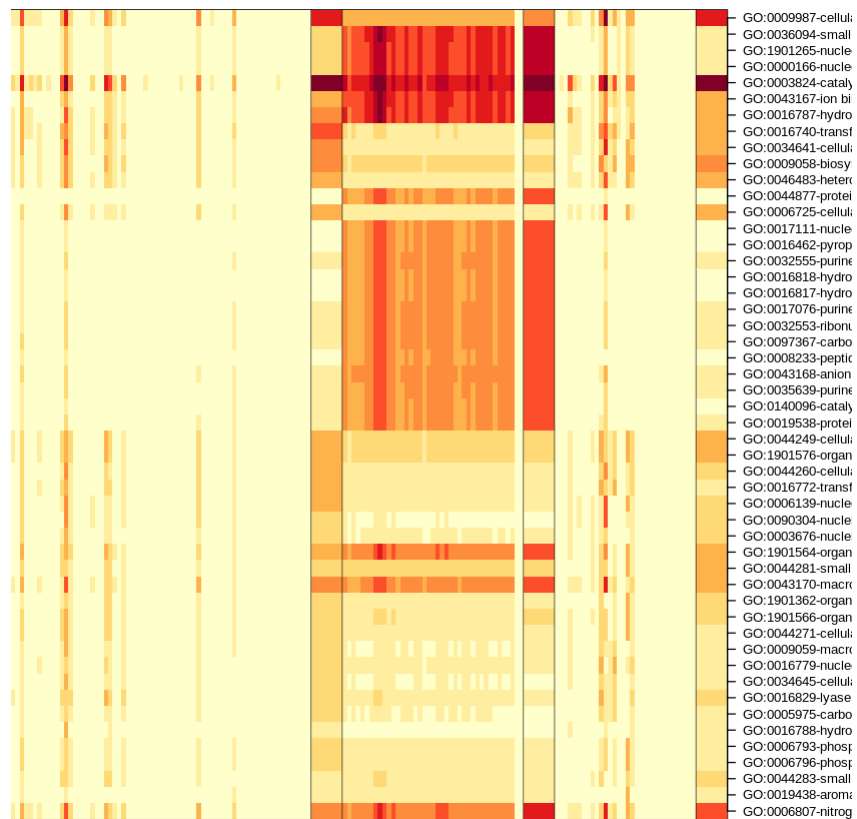
log 2 Heatmap

```
In [39]: heatmapdmn(count, fit[[1]], best, ntaxa = 50,
  transform = log2, lblwidth = 0.2 * nrow(count))
```



square root version

```
In [40]: heatmapdmn(count, fit[[1]], best, ntaxa = 50,
               transform =sqrt, lblwidth = 0.2 * nrow(count))
```



print out the theta values

```
In [41]: mixturewt(best)
```

A data.frame: 3 × 2

pi	theta
<dbl>	<dbl>
0.4823277	708.70467
0.2906241	10503.53447
0.2270482	79.21304

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

```
In [42]: write.table(fitted(best),"GO_TERMS_DMM_contributions.tsv", sep="\t")
```

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

```
In [43]: ass <- apply(mixture(best), 1, which.max)
write.table(ass,"GO_TERMS_DMM_groups.tsv",sep="")
```

```
In [44]: #add the dmm group to the metadata
sample_data(bac_pseq_prune)$dmm<-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 [45]: gghistogram(tmp,x = "Abundance",y = "..count..")+scale_x_log10() #move each dmm group into a colum of its own
```

Warning message:

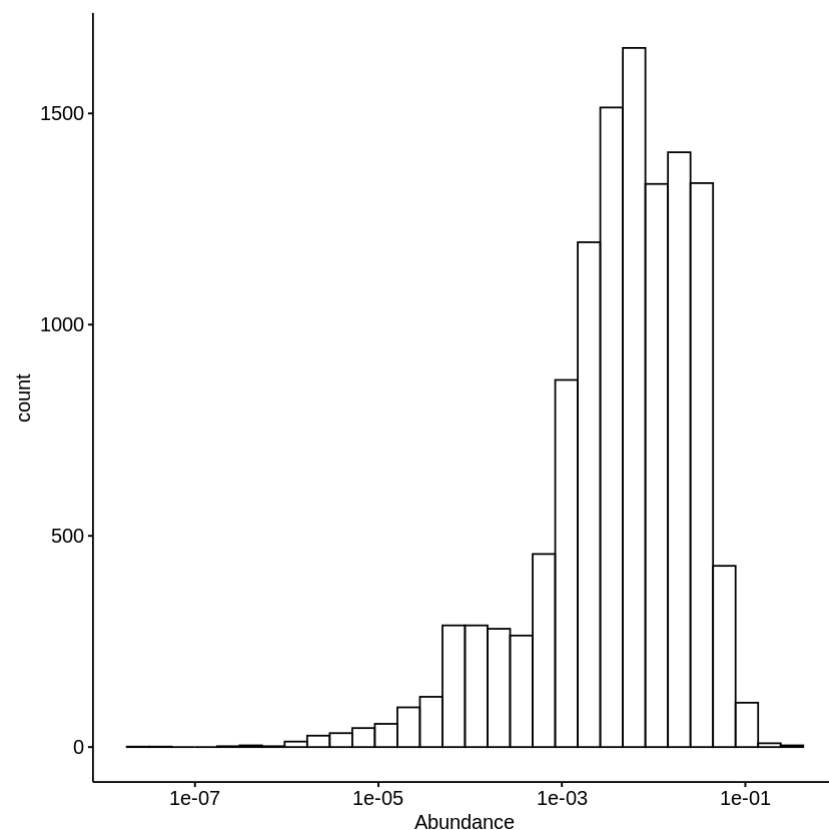
"Using `bins = 30` by default. Pick better value with the argument `bins`."

Warning message:

"Transformation introduced infinite values in continuous x-axis"

Warning message:

"Removed 861 rows containing non-finite values (stat_bin)."



```
In [46]: #tmp$Log2Abundance<-Log2(tmp$Abundance)
```

```
In [47]: #subset the dataset to only include the case, Go_term, count, and dmm group.
#obtain the average 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 [48]: 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 [49]: #d2$avg<-sqrt(d2$avg)
```

```
In [50]: d3<-tidyr::spread(d2,dmn, avg)
```

```
In [51]: #get the total count of the go terms and oder from greates to lowest
```

```
In [52]: d3<-tidyr::spread(d2,dmn,avg)
d3$tot<-rowSums(d3[,3:5], na.rm = T)
d3<-d3%>%arrange(desc(tot))
d3$tot<-NULL
head(d3)
```

A grouped_df: 6 × 5

	OTU	case	1	2	3
	<chr>	<chr>	<dbl>	<dbl>	<dbl>
	GO:0003824-catalytic activity	COVID19	0.109352879	0.29285714	0.097175968
	GO:0016787-hydrolase activity	COVID19	0.028312042	0.29285714	0.028805605
	GO:0003824-catalytic activity	Control_Sick	0.108973363	0.06954130	0.113480076
	GO:0003824-catalytic activity	Control_Healthy	0.107499082	0.06891539	0.096723461
	GO:0140096-catalytic activity, acting on a protein	COVID19	0.004725500	0.20714286	0.005114505
	GO:0008233-peptidase activity	COVID19	0.003253628	0.20714286	0.004649460

```
In [53]: d3<-d3%>%gather(data = d3,avg,3:5)
colnames(d3)<-c("name", "case", "dmn", "avg")
```

make the balloon plot

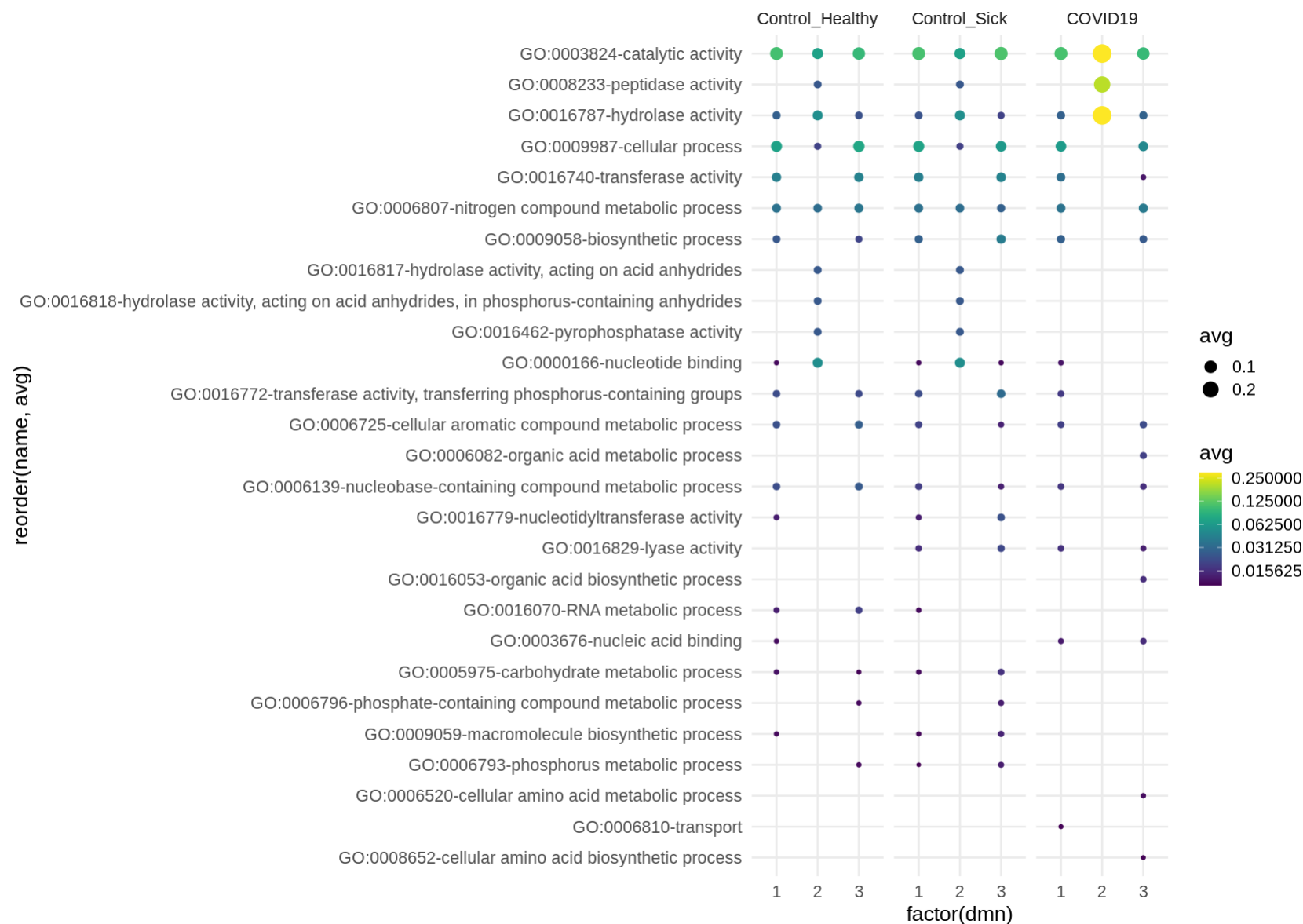
```
In [54]: d4<-d3%>%filter(avg>0.01)%>%arrange(name,case,dmn)
d4<-d4[,1:108,]
```

```
In [93]: my_pal<-viridis(n = 256, alpha = 1, begin = 0, end = 1, direction = 1)
```

```
In [116... options(repr.plot.width=14, repr.plot.height=10)
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 [ ]: # 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()
```

In []: