## rnaSeq.R

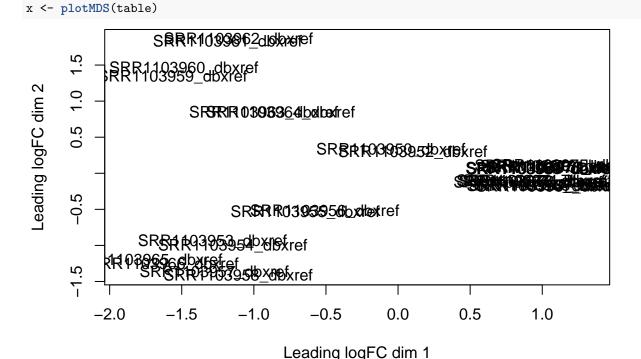
## phanthihonghuong 2019-04-27

```
library(edgeR)
## Loading required package: limma
library(ggplot2)
#declare your files so you can import them at once.
files = dir(pattern="*dbxref.count")
#import the reads into a DGE (differential gene expression) object for use in edgeR
table=readDGE(files)
## Meta tags detected: __no_feature, __ambiguous, __too_low_aQual, __not_aligned, __alignment_not_uniqu
dim(table)
## [1] 18754
               32
str(table)
## Formal class 'DGEList' [package "edgeR"] with 1 slot
    ..@ .Data:List of 2
    ....$:'data.frame': 32 obs. of 4 variables:
##
                        : chr [1:32] "SRR1103950 dbxref.count" "SRR1103952 dbxref.count" "SRR1103953
##
    .. .. ..$ files
    .. .. ..$ group
                         : Factor w/ 1 level "1": 1 1 1 1 1 1 1 1 1 ...
    .....$ lib.size : num [1:32] 20869121 69330557 28450156 64471339 14340640 ...
##
    ....$ norm.factors: num [1:32] 1 1 1 1 1 1 1 1 1 1 ...
##
    ....$: num [1:18754, 1:32] 1 368 193 5 36 108 186 355 4 5 ...
##
##
    ..... attr(*, "dimnames")=List of 2
                       : chr [1:18754] "VectorBase:AAEL000002-RA,GeneID:5563549,Genbank:XM_001647810.
##
    .. .. .. ..$ Tags
    ......$ Samples: chr [1:32] "SRR1103950_dbxref" "SRR1103952_dbxref" "SRR1103953_dbxref" "SRR1
#get rid of the meta tags (unfiltered reads, etc.) that are at the end of the table
MetaTags = grep("^ ", rownames(table))
table=table[-MetaTags,]
dim(table)
## [1] 18749
               32
#import the metadata downloaded from the SRA
#this can be obtained in the same place you originally downloaded the accession numbers
#in the NCBI SRA. Instead of downloading the accession list you ask for the run table,
#for all 32 libraries.
#note that as written, the command below will look for the file in your current #directory.
runtable = read.table(file="SraRunTable.txt", sep="\t", header=TRUE)
colnames(runtable[,c(12:14, 18:19, 20:22)])
## [1] "Run"
                            "SRA_Sample"
                                                  "Sample_Name"
## [4] "geographic_location" "isolate"
                                                  "label"
## [7] "phenotype"
                            "strain"
Groups = runtable[,c(12:14, 18:19, 20:22)]
```

```
#filter out low read samples. Since the smallest grouping (i.e., level of replication
#is 6, we are getting rid of genes that don't have at least 1 CPM in at least 6 #samples.
keep = rowSums(cpm(table)>1) >=6
table = table[keep,]
dim(table)

## [1] 10561 32
#apply TMM normalization
table = calcNormFactors(table)

#make a principal coordinates graph
```



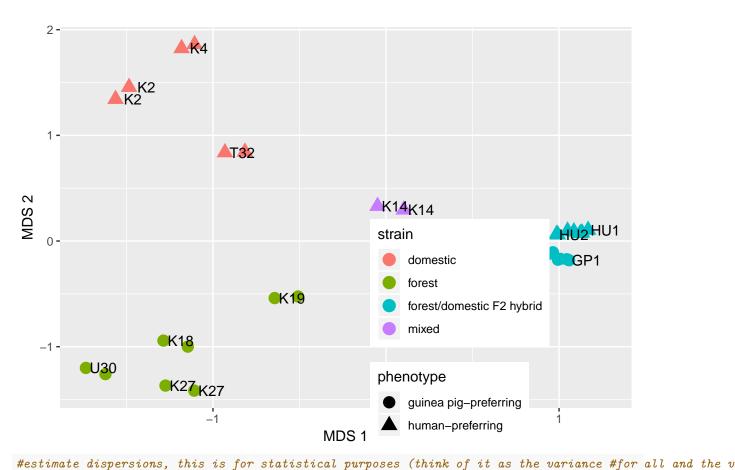
#to make this better and label things, it is best to bring out the data for use in #ggplot
#the data for the mds was saved within the DGE list and can be renamed as its own #object.
xy = x\$cmdscale.out

#now change this xy object into a data.frame...it was a matrix by default
xy = as.data.frame(xy)

#this new table of coordinates can be modified so that the column names mean something
#and the labels are all part of the same table.
names(xy) = c("MDS\_1", "MDS\_2")

#before merging you need to make sure your df's are in the same order
#they are not, so sort Groups by Run
Groups2 = Groups[order(Groups\$Run),]
xy = cbind(xy, Groups2)

mdsplot = ggplot(data=xy, aes(x=MDS\_1, y=MDS\_2, color=strain, label=label, pch=phenotype))+geom\_point(smdsplot)



```
table=estimateCommonDisp(table)
table=estimateTagwiseDisp(table)
table$samples$group <- Groups2$phenotype</pre>
#do a pairwise test
de = exactTest(table, pair=c("guinea pig-preferring", "human-preferring"))
#summarize the results...this just shows how many genes are up or down regulated
#by default this corrects for false discovery (FDR)
summary(decideTestsDGE(de))
##
          human-preferring-guinea pig-preferring
## Down
                                             618
## NotSig
                                            8999
## Up
                                             944
#what the above shows is that there are 618 genes up-regulated in human-preferring and
#944 in guinea pig preferring.
#But we are using the whole dataset. Let's subset it and then repeat.
#here we are subsetting to only include the first 16 libraries, i.e., no F2s
#note that the normalizations and calculated dispersions stay with this object and #don't need to be re
table2=table[,1:16]
de2 = exactTest(table2, pair=c("guinea pig-preferring", "human-preferring"))
summary(decideTestsDGE(de2))
```

human-preferring-guinea pig-preferring

##

```
## Down
                                                771
## NotSig
                                               8718
## Up
                                               1072
table3=table[,17:32]
de3 = exactTest(table3, pair=c("guinea pig-preferring", "human-preferring"))
summary(decideTestsDGE(de3))
##
          human-preferring-guinea pig-preferring
## Down
## NotSig
                                              10561
## Up
#for simplicity we will ask for a report of the top 100
topTags(de2, n=100)
   Comparison of groups: human-preferring-guinea pig-preferring
##
                                                                                                     logFC
## VectorBase: AAEL006425-RA, GeneID: 5567964, Genbank: XM 001657736.1, VectorBase: AAEL006425
                                                                                                -7.2984658
## VectorBase: AAEL007320-RA, GeneID: 5569019, Genbank: XM_001658225.1, VectorBase: AAEL007320
                                                                                                -7.4582095
## VectorBase: AAEL015450-RA, GeneID: 5579425, Genbank: XM 001647675.1, VectorBase: AAEL015450
                                                                                                -7.2345252
## VectorBase: AAEL005480-RA, GeneID: 5566574, Genbank: XM_001650898.1, VectorBase: AAEL005480
                                                                                                -5.3291735
## VectorBase: AAEL001291-RA, GeneID: 5569809, Genbank: XM_001652976.1, VectorBase: AAEL001291
                                                                                                 3.3332324
## VectorBase: AAEL013566-RB, GeneID: 5578228, Genbank: XM_001656819.2, VectorBase: AAEL013566
                                                                                                -3.5120309
## VectorBase: AAEL007489-RA, GeneID: 5569242, Genbank: XM 001652755.1, VectorBase: AAEL007489
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## VectorBase: AAEL013841-RA, GeneID: 5578731, Genbank: XM 001663983.1, VectorBase: AAEL013841
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## VectorBase: AAEL005753-RA, GeneID: 5567003, Genbank: XM_001651367.1, VectorBase: AAEL005753
                                                                                                 3.5825014
## VectorBase: AAEL006207-RA, GeneID: 5567606, Genbank: XM_001657533.1, VectorBase: AAEL006207
                                                                                                 2.2956864
## VectorBase: AAEL000028-RA, GeneID: 5563616, Genbank: XM_001647815.2, VectorBase: AAEL000028
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                                                                                                 1.3080217
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```

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##
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##
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   VectorBase: AAEL007320-RA, GeneID: 5569019, Genbank: XM_001658225.1, VectorBase: AAEL007320
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                                                                                                 6.585824380
   VectorBase: AAEL017246-RA, GeneID: 23687666, Genbank: XM 011495454.1, VectorBase: AAEL017246
                                                                                                 1.292750967
   VectorBase: AAEL012311-RA, GeneID: 5576085, Genbank: XM 001662363.1, VectorBase: AAEL012311
                                                                                                 8.285778588
   VectorBase: AAEL014311-RA, GeneID: 5564094, Genbank: XM_001648370.1, VectorBase: AAEL014311
                                                                                                 2.398833038
   VectorBase: AAEL013839-RA, GeneID: 5578730, Genbank: XM_001663980.1, VectorBase: AAEL013839
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                                                                                                 4.920514937
   VectorBase: AAEL003431-RA, GeneID: 5578121, Genbank: XM_001656769.1, VectorBase: AAEL003431
                                                                                                 5.372648288
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                                                                                                 7.106185064
   VectorBase: AAEL002668-RA, GeneID: 5575578, Genbank: XM_001662012.1, VectorBase: AAEL002668
                                                                                                 3.838364614
   VectorBase: AAEL007984-RA, GeneID: 5569909, Genbank: XM_001658756.1, VectorBase: AAEL007984
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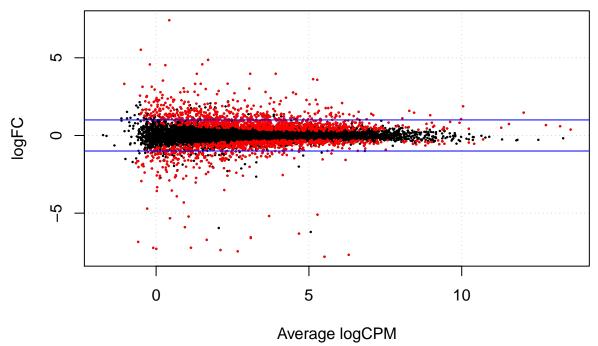
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##
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                                                                                               9.104288e-05
  VectorBase: AAEL007830-RA, GeneID: 5569684, Genbank: XM_001658623.2, VectorBase: AAEL007830
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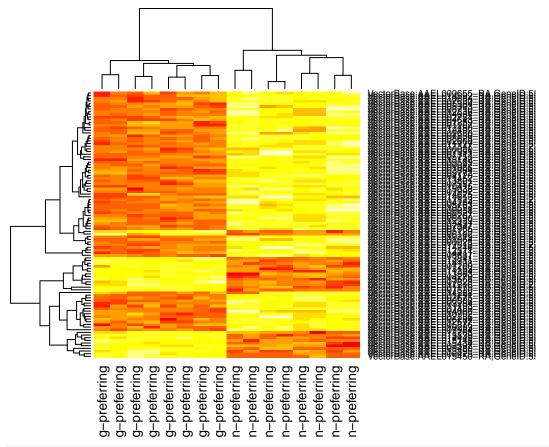
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                                                                                               2.926003e-04
## VectorBase: AAEL007260-RA, GeneID: 5568952, Genbank: XM_001652572.1, VectorBase: AAEL007260
                                                                                               2.961362e-04
topTags = topTags(de2, n=10561)
write.table(topTags, file="topTags.txt")
#another useful visualization for these data is a smear plot. This plots the
#level of expression against the fold change difference of your treatments.
#typically, highly expressed genes tend not to be too different between treatments
#so the differentially expressed, very different, gene expression patterns
#are of lesser expressed genes. This then creates a pattern of decreasing
#variance in fold change with increasing expression level.
de2_decide = decideTestsDGE(de2)
#differentiates the differentially expressed genes
```

```
detags = rownames(table)[as.logical(de2_decide)]
#converts those diff expressed genes with 1 or 0
plotSmear(de2, de.tags=detags)
#the plotting function, the de.tags are those that are diff expressed, by default red
abline(h=c(-1, 1), col="blue")
```



#adds lines at +/-1 (i.e., 2 fold difference in expression since it is log2)

#lastly, if you want to do a heatmap, which can look pretty cool, here are some basics
topde2 = topTags(de2, n=100)
detags2 = rownames(topde2)
cpm\_de2 = cpm(table2, log=TRUE)[detags2,]
heatmap(cpm\_de2, labRow=table2\$group, labCol=Groups2\$phenotype)



#this can be further modified as wanted