

Class14_RNAseq mini project

Table of contents

```
library(DESeq2)
```

Loading required package: S4Vectors

Loading required package: stats4

Loading required package: BiocGenerics

Attaching package: 'BiocGenerics'

The following objects are masked from 'package:stats':

IQR, mad, sd, var, xtabs

The following objects are masked from 'package:base':

anyDuplicated, aperm, 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 object is masked from 'package:utils':

findMatches

The following objects are masked from 'package:base':

expand.grid, I, unname

Loading required package: IRanges

Loading required package: GenomicRanges

Loading required package: GenomeInfoDb

Loading required package: SummarizedExperiment

Loading required package: MatrixGenerics

Loading required package: matrixStats

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

```
metaFile <- "GSE37704_metadata.csv"
countFile <- "GSE37704_featurecounts.csv"
```

```
colData = read.csv(metaFile, row.names=1)
head(colData)
```

```
          condition
SRR493366 control_sirna
SRR493367 control_sirna
SRR493368 control_sirna
SRR493369      hoxa1_kd
SRR493370      hoxa1_kd
SRR493371      hoxa1_kd
```

```
countData = read.csv(countFile, row.names=1)
head(countData)
```

	length	SRR493366	SRR493367	SRR493368	SRR493369	SRR493370
ENSG00000186092	918	0	0	0	0	0
ENSG00000279928	718	0	0	0	0	0
ENSG00000279457	1982	23	28	29	29	28
ENSG00000278566	939	0	0	0	0	0
ENSG00000273547	939	0	0	0	0	0
ENSG00000187634	3214	124	123	205	207	212

	SRR493371
ENSG00000186092	0
ENSG00000279928	0
ENSG00000279457	46
ENSG00000278566	0
ENSG00000273547	0
ENSG00000187634	258

```
countData <- as.matrix(countData[,-1])
head(countData)
```

	SRR493366	SRR493367	SRR493368	SRR493369	SRR493370	SRR493371
ENSG00000186092	0	0	0	0	0	0
ENSG00000279928	0	0	0	0	0	0
ENSG00000279457	23	28	29	29	28	46
ENSG00000278566	0	0	0	0	0	0
ENSG00000273547	0	0	0	0	0	0
ENSG00000187634	124	123	205	207	212	258

```
nrow(countData)
```

```
[1] 19808
```

- Find the rowSums() this will be zero for any genes with no count data
- Find the zero sum genes
- Remove them before doing our DESeq

```
!c(T,F,T)
```

```
[1] FALSE TRUE FALSE
```

```
to.rm.ind <- rowSums(countData) == 0
countData <- countData[!to.rm.ind,]
head(countData)
```

	SRR493366	SRR493367	SRR493368	SRR493369	SRR493370	SRR493371
ENSG00000279457	23	28	29	29	28	46
ENSG00000187634	124	123	205	207	212	258
ENSG00000188976	1637	1831	2383	1226	1326	1504
ENSG00000187961	120	153	180	236	255	357
ENSG00000187583	24	48	65	44	48	64
ENSG00000187642	4	9	16	14	16	16

```
dds = DESeqDataSetFromMatrix(countData=countData,
                              colData=colData,
                              design=~condition)
```

Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in design formula are characters, converting to factors

```
dds = DESeq(dds)
```

estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing

Now run our main DESeq analysis:

```
dds <- DESeq(dds)
res <- results(dds)
head(res)
```

log2 fold change (MLE): condition hoxa1 kd vs control sirna

Wald test p-value: condition hoxa1 kd vs control sirna

DataFrame with 6 rows and 6 columns

	baseMean	log2FoldChange	lfcSE	stat	pvalue
	<numeric>	<numeric>	<numeric>	<numeric>	<numeric>
ENSG00000279457	29.9136	0.1792571	0.3248216	0.551863	5.81042e-01
ENSG00000187634	183.2296	0.4264571	0.1402658	3.040350	2.36304e-03
ENSG00000188976	1651.1881	-0.6927205	0.0548465	-12.630158	1.43990e-36
ENSG00000187961	209.6379	0.7297556	0.1318599	5.534326	3.12428e-08
ENSG00000187583	47.2551	0.0405765	0.2718928	0.149237	8.81366e-01
ENSG00000187642	11.9798	0.5428105	0.5215598	1.040744	2.97994e-01
	padj				
	<numeric>				
ENSG00000279457	6.86555e-01				
ENSG00000187634	5.15718e-03				
ENSG00000188976	1.76549e-35				
ENSG00000187961	1.13413e-07				
ENSG00000187583	9.19031e-01				
ENSG00000187642	4.03379e-01				

```
summary(res)
```

out of 15975 with nonzero total read count

adjusted p-value < 0.1

LFC > 0 (up) : 4349, 27%

LFC < 0 (down) : 4396, 28%

outliers [1] : 0, 0%

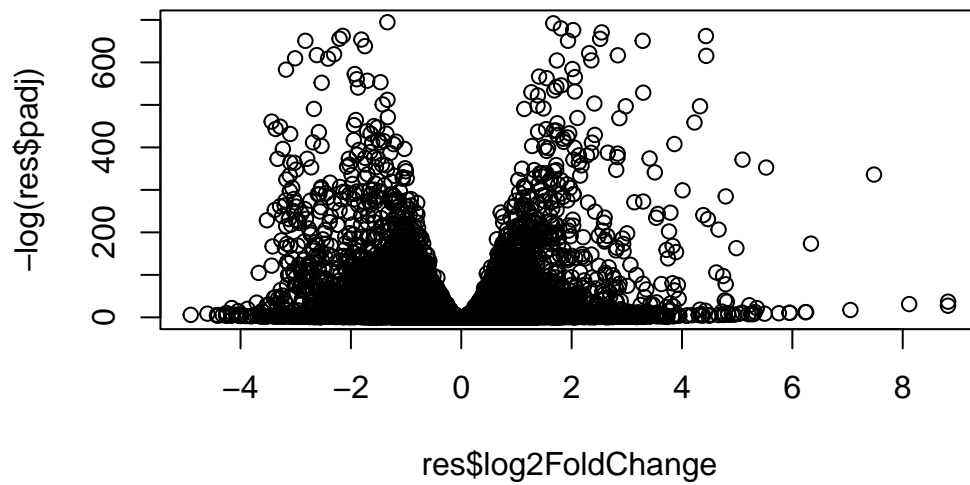
low counts [2] : 1237, 7.7%

(mean count < 0)

[1] see 'cooksCutoff' argument of ?results

[2] see 'independentFiltering' argument of ?results

```
plot( res$log2FoldChange, -log(res$padj) )
```



Let's add some color and annotation data to this plot.

```
library(AnnotationDbi)
```

Warning: package 'AnnotationDbi' was built under R version 4.3.2

```
library(org.Hs.eg.db)
```

```
columns(org.Hs.eg.db)
```

[1] "ACCNUM"	"ALIAS"	"ENSEMBL"	"ENSEMBLPROT"	"ENSEMBLTRANS"
[6] "ENTREZID"	"ENZYME"	"EVIDENCE"	"EVIDENCEALL"	"GENENAME"
[11] "GENETYPE"	"GO"	"GOALL"	"IPI"	"MAP"
[16] "OMIM"	"ONTOLOGY"	"ONTOLOGYALL"	"PATH"	"PFAM"
[21] "PMID"	"PROSITE"	"REFSEQ"	"SYMBOL"	"UCSCKG"
[26] "UNIPROT"				

```
head(row.names(countData))
```

```
[1] "ENSG00000279457" "ENSG00000187634" "ENSG00000188976" "ENSG00000187961"
[5] "ENSG00000187583" "ENSG00000187642"
```

```
res$symbol <- mapIds(org.Hs.eg.db,
  keys=row.names(countData),
  keytype="ENSEMBL",
  column="SYMBOL",
  multiVals="first")
```

'select()' returned 1:many mapping between keys and columns

```
res$entrez <- mapIds(org.Hs.eg.db,
  keys=row.names(countData),
  keytype="ENSEMBL",
  column="ENTREZID",
  multiVals="first")
```

'select()' returned 1:many mapping between keys and columns

```
head(res)
```

log2 fold change (MLE): condition hoxa1 kd vs control sirna

Wald test p-value: condition hoxa1 kd vs control sirna

DataFrame with 6 rows and 8 columns

	baseMean	log2FoldChange	lfcSE	stat	pvalue
	<numeric>	<numeric>	<numeric>	<numeric>	<numeric>
ENSG00000279457	29.9136	0.1792571	0.3248216	0.551863	5.81042e-01
ENSG00000187634	183.2296	0.4264571	0.1402658	3.040350	2.36304e-03
ENSG00000188976	1651.1881	-0.6927205	0.0548465	-12.630158	1.43990e-36
ENSG00000187961	209.6379	0.7297556	0.1318599	5.534326	3.12428e-08
ENSG00000187583	47.2551	0.0405765	0.2718928	0.149237	8.81366e-01
ENSG00000187642	11.9798	0.5428105	0.5215598	1.040744	2.97994e-01
	padj	symbol	entrez		
	<numeric>	<character>	<character>		
ENSG00000279457	6.86555e-01	NA	NA		

ENSG00000187634	5.15718e-03	SAMD11	148398
ENSG00000188976	1.76549e-35	NOC2L	26155
ENSG00000187961	1.13413e-07	KLHL17	339451
ENSG00000187583	9.19031e-01	PLEKHN1	84069
ENSG00000187642	4.03379e-01	PERM1	84808

```
library(gage)
library(gageData)
library(pathview)
```

The `gage()` function wants a “vector of importance” in our case here it will be fold-change values with associated entrez gene names.

```
foldchange <- res$log2FoldChange
names(foldchange) <- res$entrez

data("kegg.sets.hs")
keggres = gage(foldchange, gsets=kegg.sets.hs)

head(keggres$less)
```

	p.geomean	stat.mean
hsa04110 Cell cycle	8.995727e-06	-4.378644
hsa03030 DNA replication	9.424076e-05	-3.951803
hsa05130 Pathogenic Escherichia coli infection	1.405864e-04	-3.765330
hsa03013 RNA transport	1.246882e-03	-3.059466
hsa03440 Homologous recombination	3.066756e-03	-2.852899
hsa04114 Oocyte meiosis	3.784520e-03	-2.698128

	p.val	q.val
hsa04110 Cell cycle	8.995727e-06	0.001889103
hsa03030 DNA replication	9.424076e-05	0.009841047
hsa05130 Pathogenic Escherichia coli infection	1.405864e-04	0.009841047
hsa03013 RNA transport	1.246882e-03	0.065461279
hsa03440 Homologous recombination	3.066756e-03	0.128803765
hsa04114 Oocyte meiosis	3.784520e-03	0.132458191

	set.size	exp1
hsa04110 Cell cycle	121	8.995727e-06
hsa03030 DNA replication	36	9.424076e-05
hsa05130 Pathogenic Escherichia coli infection	53	1.405864e-04
hsa03013 RNA transport	144	1.246882e-03

hsa03440 Homologous recombination	28 3.066756e-03
hsa04114 Oocyte meiosis	102 3.784520e-03

```
pathview(gene.data=foldchange, pathway.id="hsa00230")
```

'select()' returned 1:1 mapping between keys and columns

Info: Working in directory /Users/billywegeng/Desktop/BGGN213/Class 14

Info: Writing image file hsa00230.pathview.png

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of vector length (arg 2)

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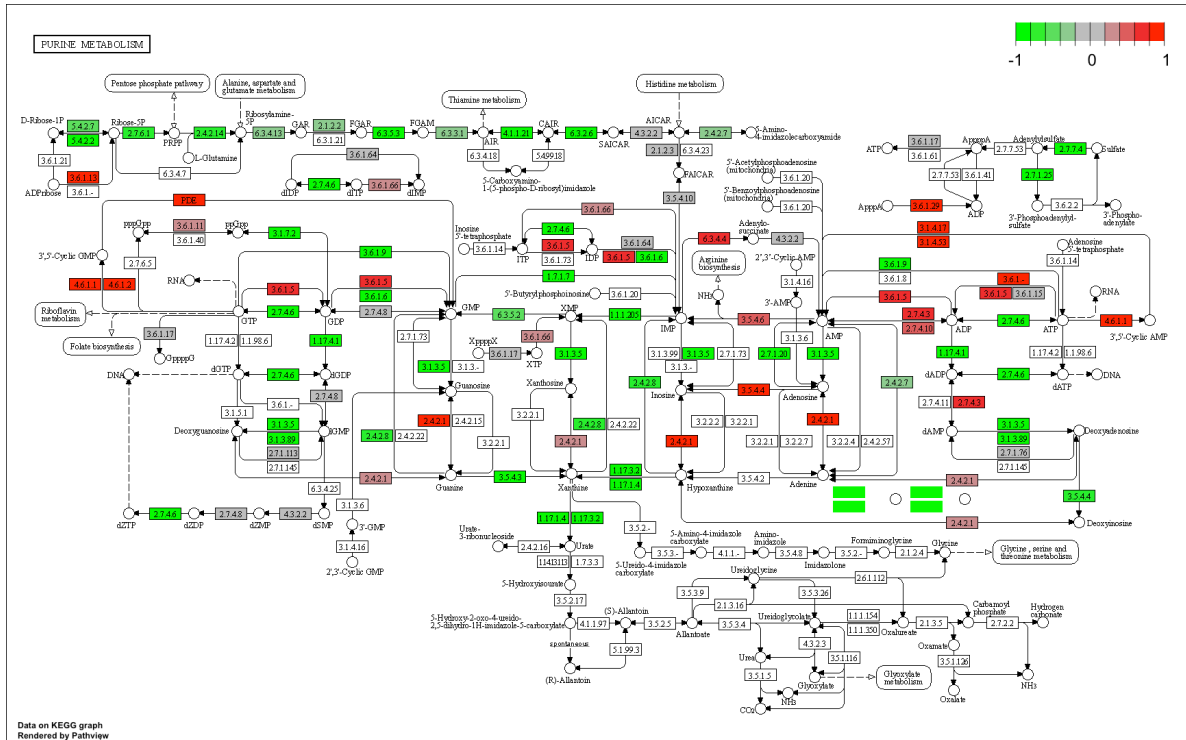


Figure 1: Purine Metabolism hsa00230

```
data(go.sets.hs)
data(go.subs.hs)
gobpsets = go.sets.hs[go.subs.hs$BP]

gobpres = gage(foldchange, gsets=gobpsets,)

head(gobpres$less)
```

	p.geomean	stat.mean	p.val
G0:0048285 organelle fission	1.536227e-15	-8.063910	1.536227e-15

G0:0000280	nuclear division	4.286961e-15	-7.939217	4.286961e-15
G0:0007067	mitosis	4.286961e-15	-7.939217	4.286961e-15
G0:0000087	M phase of mitotic cell cycle	1.169934e-14	-7.797496	1.169934e-14
G0:0007059	chromosome segregation	2.028624e-11	-6.878340	2.028624e-11
G0:0000236	mitotic prometaphase	1.729553e-10	-6.695966	1.729553e-10
		q.val	set.size	exp1
G0:0048285	organelle fission	5.843127e-12	376	1.536227e-15
G0:0000280	nuclear division	5.843127e-12	352	4.286961e-15
G0:0007067	mitosis	5.843127e-12	352	4.286961e-15
G0:0000087	M phase of mitotic cell cycle	1.195965e-11	362	1.169934e-14
G0:0007059	chromosome segregation	1.659009e-08	142	2.028624e-11
G0:0000236	mitotic prometaphase	1.178690e-07	84	1.729553e-10

We will use the online version of Reactome. It wants a list of your genes. We will write this out from R here:

```
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]
write.table(sig_genes, file="significant_genes.txt", row.names=FALSE, col.names=FALSE, quo
```

<https://reactome.org/PathwayBrowser/#TOOL=AT>