

# Lab14

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## Data Import

```
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, saveRDS, setdiff,  
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

Loading in the files:

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

# Import metadata and take a peak
metadata <- read.csv(metaFile, row.names=1)
head(metadata)
```

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

```
# Import countdata
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					

Check correspondance of the columns of `counts` and the rows of `metadata`

```
colnames(countData)
```

```
[1] "length"      "SRR493366" "SRR493367" "SRR493368" "SRR493369" "SRR493370"
[7] "SRR493371"
```

```
metadata$id <- row.names(metadata)
metadata$id
```

```
[1] "SRR493366" "SRR493367" "SRR493368" "SRR493369" "SRR493370" "SRR493371"
```

Removing the first “length” column from the `counts`

```
# Note we need to remove the odd first $length col
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

Excluding genes with 0 read count:

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

	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

## DESeq Setup

```
dds = DESeqDataSetFromMatrix(countData=countData,  
                              colData=metadata,  
                              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

```
dds
```

```
class: DESeqDataSet
dim: 19808 6
metadata(1): version
assays(4): counts mu H cooks
rownames(19808): ENSG00000186092 ENSG00000279928 ... ENSG00000277475
               ENSG00000268674
rowData names(22): baseMean baseVar ... deviance maxCooks
colnames(6): SRR493366 SRR493367 ... SRR493370 SRR493371
colData names(3): condition id sizeFactor
```

```
##DESeq Analysis
```

Results for the HoxA1 knockdown versus control siRNA:

```
res = results(dds, contrast=c("condition", "hoxa1_kd", "control_siRNA"))
resultsNames(dds)
```

```
[1] "Intercept"                                "condition_hoxa1_kd_vs_control_siRNA"
```

Upregulated vs downregulated genes:

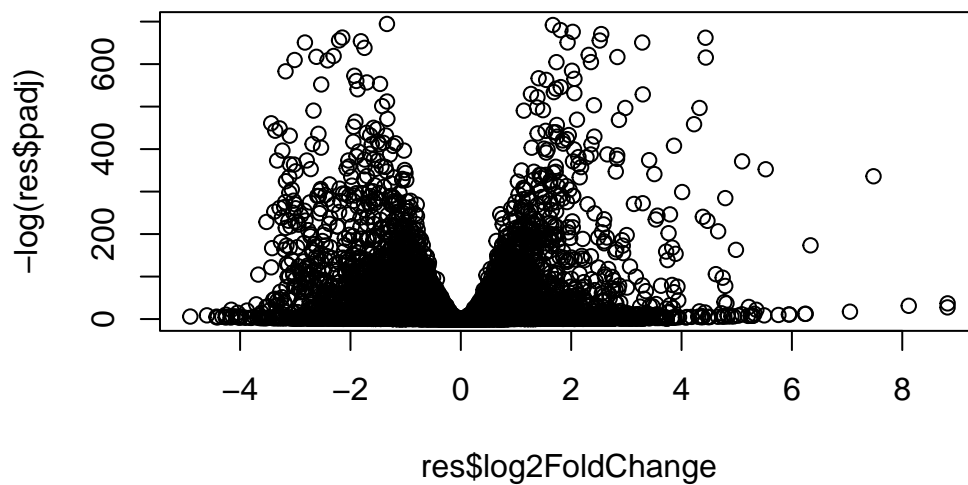
```
summary(res)
```

```
out of 15975 with nonzero total read count
adjusted p-value < 0.1
LFC > 0 (up)      : 4349, 27%
LFC < 0 (down)    : 4393, 27%
outliers [1]      : 0, 0%
low counts [2]    : 1221, 7.6%
(mean count < 0)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of ?results
```

## Result Visualization

Volcano plot of this data:

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



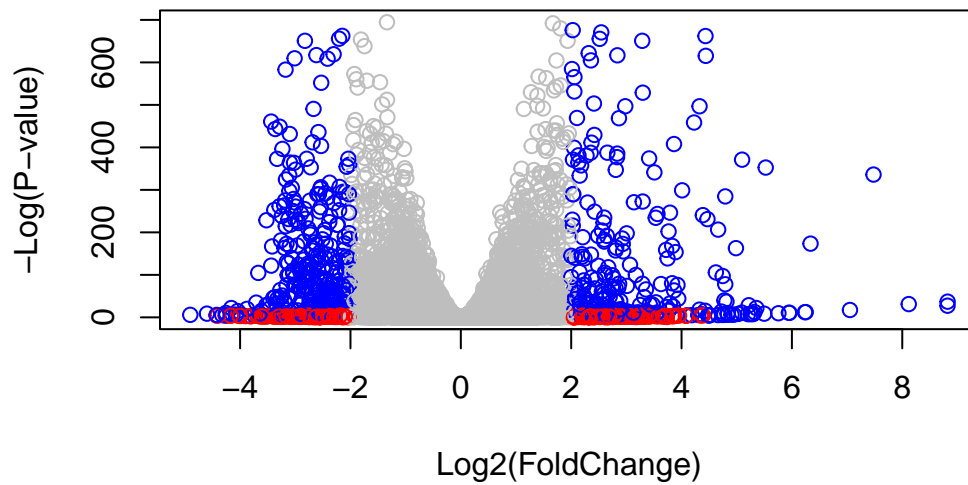
Fixing the color:

```
# Make a color vector for all genes
mycols <- rep("gray", nrow(res) )

# Color red the genes with absolute fold change above 2
mycols[ abs(res$log2FoldChange) > 2 ] <- "red"

# Color blue those with adjusted p-value less than 0.01
# and absolute fold change more than 2
inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "blue"

plot( res$log2FoldChange, -log(res$padj), col=mycols, xlab="Log2(FoldChange)", ylab="-Log(P-"
```



## Add Annotation

```
library("AnnotationDbi")
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"
```

```
res$symbol = mapIds(org.Hs.eg.db,
  keys=rownames(res),
  keytype="ENSEMBL",
```



```
column="SYMBOL",
multiVals="first")
```

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

```
res$entrez = mapIds(org.Hs.eg.db,
  keys=rownames(res),
  keytype="ENSEMBL",
  column="ENTREZID",
  multiVals="first")
```

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

```
res$name = mapIds(org.Hs.eg.db,
  keys=row.names(res),
  keytype="ENSEMBL",
  column="GENENAME",
  multiVals="first")
```

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

```
head(res, 10)
```

log2 fold change (MLE): condition hoxa1\_kd vs control\_sirna

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

DataFrame with 10 rows and 9 columns

	baseMean	log2FoldChange	lfcSE	stat	pvalue
	<numeric>	<numeric>	<numeric>	<numeric>	<numeric>
ENSG00000186092	0.0000	NA	NA	NA	NA
ENSG00000279928	0.0000	NA	NA	NA	NA
ENSG00000279457	29.9136	0.1792571	0.3248216	0.551863	5.81042e-01
ENSG00000278566	0.0000	NA	NA	NA	NA
ENSG00000273547	0.0000	NA	NA	NA	NA
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		name

	<numeric>	<character>	<character>	<character>
ENSG00000186092	NA	OR4F5	79501	olfactory receptor f..
ENSG00000279928	NA	NA	NA	NA
ENSG00000279457	6.87080e-01	NA	NA	NA
ENSG00000278566	NA	NA	NA	NA
ENSG00000273547	NA	NA	NA	NA
ENSG00000187634	5.16278e-03	SAMD11	148398	sterile alpha motif ..
ENSG00000188976	1.76741e-35	NOC2L	26155	NOC2 like nucleolar ..
ENSG00000187961	1.13536e-07	KLHL17	339451	kelch like family me..
ENSG00000187583	9.18988e-01	PLEKHN1	84069	pleckstrin homology ..
ENSG00000187642	4.03817e-01	PERM1	84808	PPARGC1 and ESRR ind..

```
res = res[order(res$pvalue),]
write.csv(res, file="deseq_results.csv")
```

## Pathway Analysis

Loading packages in:

```
library(pathview)
```

```
#####
Pathview is an open source software package distributed under GNU General
Public License version 3 (GPLv3). Details of GPLv3 is available at
http://www.gnu.org/licenses/gpl-3.0.html. Particullary, users are required to
formally cite the original Pathview paper (not just mention it) in publications
or products. For details, do citation("pathview") within R.
```

The pathview downloads and uses KEGG data. Non-academic uses may require a KEGG license agreement (details at <http://www.kegg.jp/kegg/legal.html>).

```
#####
```

Setting up KEGG datasets:

```
library(gage)
```

```
library(gageData)

data(kegg.sets.hs)
data(sigmet.idx.hs)

# Focus on signaling and metabolic pathways only
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]

# Examine the first 3 pathways
head(kegg.sets.hs, 3)
```

```
$`hsa00232 Caffeine metabolism`
```

```
[1] "10" "1544" "1548" "1549" "1553" "7498" "9"
```

```
$`hsa00983 Drug metabolism - other enzymes`
```

```
[1] "10" "1066" "10720" "10941" "151531" "1548" "1549" "1551"
[9] "1553" "1576" "1577" "1806" "1807" "1890" "221223" "2990"
[17] "3251" "3614" "3615" "3704" "51733" "54490" "54575" "54576"
[25] "54577" "54578" "54579" "54600" "54657" "54658" "54659" "54963"
[33] "574537" "64816" "7083" "7084" "7172" "7363" "7364" "7365"
[41] "7366" "7367" "7371" "7372" "7378" "7498" "79799" "83549"
[49] "8824" "8833" "9" "978"
```

```
$`hsa00230 Purine metabolism`
```

```
[1] "100" "10201" "10606" "10621" "10622" "10623" "107" "10714"
[9] "108" "10846" "109" "111" "11128" "11164" "112" "113"
[17] "114" "115" "122481" "122622" "124583" "132" "158" "159"
[25] "1633" "171568" "1716" "196883" "203" "204" "205" "221823"
[33] "2272" "22978" "23649" "246721" "25885" "2618" "26289" "270"
[41] "271" "27115" "272" "2766" "2977" "2982" "2983" "2984"
[49] "2986" "2987" "29922" "3000" "30833" "30834" "318" "3251"
[57] "353" "3614" "3615" "3704" "377841" "471" "4830" "4831"
[65] "4832" "4833" "4860" "4881" "4882" "4907" "50484" "50940"
[73] "51082" "51251" "51292" "5136" "5137" "5138" "5139" "5140"
[81] "5141" "5142" "5143" "5144" "5145" "5146" "5147" "5148"
[89] "5149" "5150" "5151" "5152" "5153" "5158" "5167" "5169"
[97] "51728" "5198" "5236" "5313" "5315" "53343" "54107" "5422"
[105] "5424" "5425" "5426" "5427" "5430" "5431" "5432" "5433"
[113] "5434" "5435" "5436" "5437" "5438" "5439" "5440" "5441"
[121] "5471" "548644" "55276" "5557" "5558" "55703" "55811" "55821"
[129] "5631" "5634" "56655" "56953" "56985" "57804" "58497" "6240"
[137] "6241" "64425" "646625" "654364" "661" "7498" "8382" "84172"
```

```
[145] "84265" "84284" "84618" "8622" "8654" "87178" "8833" "9060"
[153] "9061" "93034" "953" "9533" "954" "955" "956" "957"
[161] "9583" "9615"
```

```
foldchanges = res$log2FoldChange
names(foldchanges) = res$entrez
head(foldchanges)
```

```
      1266      54855      1465      51232      2034      2317
-2.422719  3.201955 -2.313738 -2.059631 -1.888019 -1.649792
```

Running gage:

```
# Get the results
keggres = gage(foldchanges, gsets=kegg.sets.hs)
```

```
attributes(keggres)
```

```
$names
[1] "greater" "less" "stats"
```

Looking at different pathways:

```
#Look at the first few down (less) pathways
head(keggres$less)
```

	p.geomean	stat.mean	p.val
hsa04110 Cell cycle	7.077982e-06	-4.432593	7.077982e-06
hsa03030 DNA replication	9.424076e-05	-3.951803	9.424076e-05
hsa03013 RNA transport	1.160132e-03	-3.080629	1.160132e-03
hsa04114 Oocyte meiosis	2.563806e-03	-2.827297	2.563806e-03
hsa03440 Homologous recombination	3.066756e-03	-2.852899	3.066756e-03
hsa00010 Glycolysis / Gluconeogenesis	4.360092e-03	-2.663825	4.360092e-03

	q.val	set.size	exp1
hsa04110 Cell cycle	0.001160789	124	7.077982e-06
hsa03030 DNA replication	0.007727742	36	9.424076e-05
hsa03013 RNA transport	0.063420543	149	1.160132e-03
hsa04114 Oocyte meiosis	0.100589607	112	2.563806e-03
hsa03440 Homologous recombination	0.100589607	28	3.066756e-03
hsa00010 Glycolysis / Gluconeogenesis	0.119175854	65	4.360092e-03

```
pathview(gene.data=foldchanges, pathway.id="hsa04110")
```

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

Info: Working in directory /Users/gretl\_mae/Desktop/Bioinformatics/Lab14

Info: Writing image file hsa04110.pathview.png

## Gene Ontology

Setting up gene ontology:

```
data(go.sets.hs)
data(go.subs.hs)

# Focus on Biological Process subset of GO
gobpsets = go.sets.hs[go.subs.hs$BP]

gobpres = gage(foldchanges, gsets=gobpsets, same.dir=TRUE)

lapply(gobpres, head)
```

\$greater

	p.geomean	stat.mean	p.val
GO:0007156 homophilic cell adhesion	1.734864e-05	4.210777	1.734864e-05
GO:0048729 tissue morphogenesis	5.407952e-05	3.888470	5.407952e-05
GO:0002009 morphogenesis of an epithelium	5.727599e-05	3.878706	5.727599e-05
GO:0030855 epithelial cell differentiation	2.053700e-04	3.554776	2.053700e-04
GO:0060562 epithelial tube morphogenesis	2.927804e-04	3.458463	2.927804e-04
GO:0048598 embryonic morphogenesis	2.959270e-04	3.446527	2.959270e-04

	q.val	set.size	expl
GO:0007156 homophilic cell adhesion	0.07584825	137	1.734864e-05
GO:0048729 tissue morphogenesis	0.08347021	483	5.407952e-05
GO:0002009 morphogenesis of an epithelium	0.08347021	382	5.727599e-05
GO:0030855 epithelial cell differentiation	0.16449701	299	2.053700e-04
GO:0060562 epithelial tube morphogenesis	0.16449701	289	2.927804e-04
GO:0048598 embryonic morphogenesis	0.16449701	498	2.959270e-04

\$less

p.geomean	stat.mean	p.val
-----------	-----------	-------

G0:0048285	organelle fission	6.626774e-16	-8.170439	6.626774e-16
G0:0000280	nuclear division	1.797050e-15	-8.051200	1.797050e-15
G0:0007067	mitosis	1.797050e-15	-8.051200	1.797050e-15
G0:0000087	M phase of mitotic cell cycle	4.757263e-15	-7.915080	4.757263e-15
G0:0007059	chromosome segregation	1.081862e-11	-6.974546	1.081862e-11
G0:0051301	cell division	8.718528e-11	-6.455491	8.718528e-11
		q.val	set.size	expl
G0:0048285	organelle fission	2.618901e-12	386	6.626774e-16
G0:0000280	nuclear division	2.618901e-12	362	1.797050e-15
G0:0007067	mitosis	2.618901e-12	362	1.797050e-15
G0:0000087	M phase of mitotic cell cycle	5.199689e-12	373	4.757263e-15
G0:0007059	chromosome segregation	9.459800e-09	146	1.081862e-11
G0:0051301	cell division	6.352901e-08	479	8.718528e-11

\$stats

	stat.mean	expl
G0:0007156 homophilic cell adhesion	4.210777	4.210777
G0:0048729 tissue morphogenesis	3.888470	3.888470
G0:0002009 morphogenesis of an epithelium	3.878706	3.878706
G0:0030855 epithelial cell differentiation	3.554776	3.554776
G0:0060562 epithelial tube morphogenesis	3.458463	3.458463
G0:0048598 embryonic morphogenesis	3.446527	3.446527

## Setup for REACTOME:

```
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]
print(paste("Total number of significant genes:", length(sig_genes)))
```

```
[1] "Total number of significant genes: 8146"
```

```
write.table(sig_genes, file="significant_genes.txt", row.names=FALSE, col.names=FALSE, quote=)
```

## Save Results