

Class 13: RNASeq Mini Project

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The data for hands-on session comes from GEO entry: GSE37704, which is associated with the following publication: Trapnell C, Hendrickson DG, Sauvageau M, Goff L et al. "Differential analysis of gene regulation at transcript resolution with RNA-seq". Nat Biotechnol 2013 Jan;31(1):46-53. PMID: 23222703

The authors report on differential analysis of lung fibroblasts in response to loss of the developmental transcription factor HOXA1.

##RNASeq Analysis

Need 2 things: countData and colData

```
colData <- read.csv("GSE37704_metadata.csv", 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("GSE37704_featurecounts.csv", 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					

There is an unwanted first column “length” in the countData. I will need to remove this first before going on to further analysis:

```
counts <- countData[,-1]
head(counts)
```

	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

##Removing Zero count genes

Q. Complete the code below to filter countData to exclude genes (i.e. rows) where we have 0 read count across all samples (i.e. columns).

Tip: What will rowSums() of countData return and how could you use it in this context?

```
head(counts)
```

	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

```
to.keep <- rowSums(counts) >0
counts <- counts[to.keep,]
head(counts)
```

	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

How many genes do we have left?

```
nrow(counts)
```

```
[1] 15975
```

```
colnames(counts)==rownames(colData)
```

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

Time to use DESeq

```
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, 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: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, 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, rowAveragesPerColSet,  
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
```

1st step required by DESeq

```
dds = DESeqDataSetFromMatrix(countData=counts,  
                              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

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

Call the `summary()` function on your results to get a sense of how many genes are up or down-regulated at the default 0.1 p-value cutoff.

```
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%
```

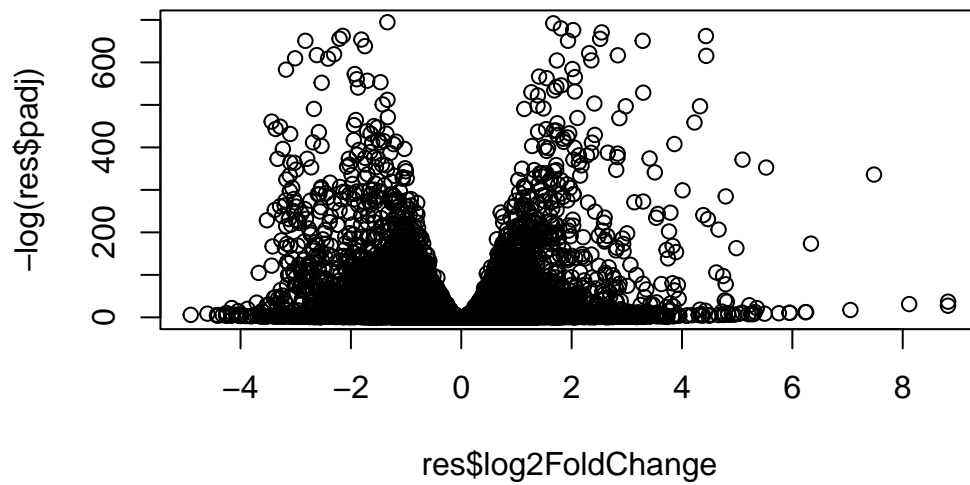
```
(mean count < 0)
```

```
[1] see 'cooksCutoff' argument of ?results
```

```
[2] see 'independentFiltering' argument of ?results
```

```
##Volcano Plot
```

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



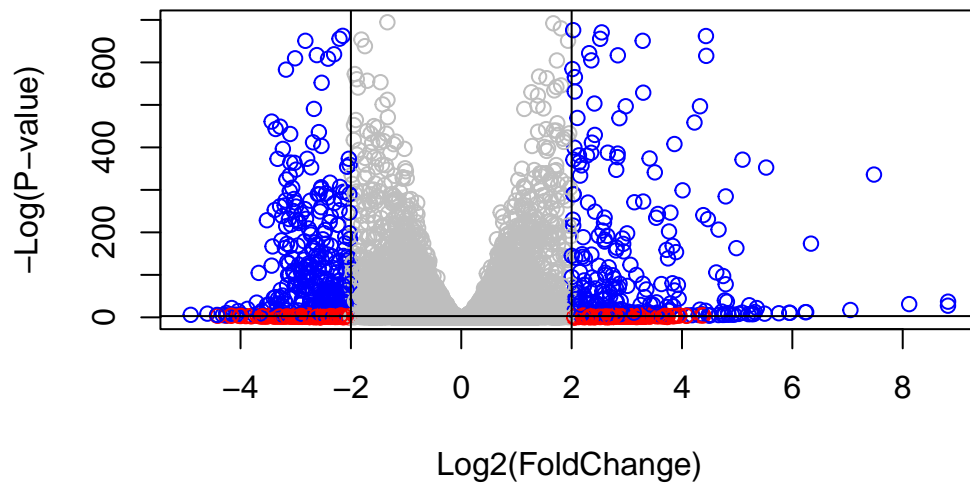
Adding Color

```
# 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(
abline(v=c(-2,+2))
abline(h=-log(0.05))
```



Adding Gene 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=row.names(res), # Our genenames
```



```

keytype="ENSEMBL",      # The format of our genenames
column="SYMBOL",        # The new format we want to add
multiVals="first")

```

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

```

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

```

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

```

res$genename <- mapIds(org.Hs.eg.db,
  keys=row.names(res),
  column="GENENAME",
  keytype="ENSEMBL",
  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>
ENSG00000279457	29.913579	0.1792571	0.3248216	0.551863	5.81042e-01
ENSG00000187634	183.229650	0.4264571	0.1402658	3.040350	2.36304e-03
ENSG00000188976	1651.188076	-0.6927205	0.0548465	-12.630158	1.43990e-36
ENSG00000187961	209.637938	0.7297556	0.1318599	5.534326	3.12428e-08
ENSG00000187583	47.255123	0.0405765	0.2718928	0.149237	8.81366e-01
ENSG00000187642	11.979750	0.5428105	0.5215598	1.040744	2.97994e-01
ENSG00000188290	108.922128	2.0570638	0.1969053	10.446970	1.51282e-25
ENSG00000187608	350.716868	0.2573837	0.1027266	2.505522	1.22271e-02

ENSG00000188157	9128.439422	0.3899088	0.0467163	8.346304	7.04321e-17
ENSG00000237330	0.158192	0.7859552	4.0804729	0.192614	8.47261e-01
	padj	symbol	entrez		genename
	<numeric>	<character>	<character>		<character>
ENSG00000279457	6.86555e-01	NA	NA		NA
ENSG00000187634	5.15718e-03	SAMD11	148398	sterile alpha motif ..	
ENSG00000188976	1.76549e-35	NOC2L	26155	NOC2 like nucleolar ..	
ENSG00000187961	1.13413e-07	KLHL17	339451	kelch like family me..	
ENSG00000187583	9.19031e-01	PLEKHN1	84069	pleckstrin homology ..	
ENSG00000187642	4.03379e-01	PERM1	84808	PPARGC1 and ESRR ind..	
ENSG00000188290	1.30538e-24	HES4	57801	hes family bHLH tran..	
ENSG00000187608	2.37452e-02	ISG15	9636	ISG15 ubiquitin like..	
ENSG00000188157	4.21963e-16	AGRN	375790		agrin
ENSG00000237330	NA	RNF223	401934	ring finger protein ..	

Save my results so far to a CSV file >Q. Finally for this section let's reorder these results by adjusted p-value and save them to a CSV file in your current project directory.

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

##Pathway Analysis

Again we will use the 'gage()' package and functino with a focus first on KEGG and sigment

```
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"
```

Recall that 'gage()' wants only the vector

```
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
```

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

```
attributes(keggres)
```

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

```
head(keggres$less)
```

	p.geomean	stat.mean	p.val
hsa04110 Cell cycle	8.995727e-06	-4.378644	8.995727e-06
hsa03030 DNA replication	9.424076e-05	-3.951803	9.424076e-05
hsa03013 RNA transport	1.375901e-03	-3.028500	1.375901e-03
hsa03440 Homologous recombination	3.066756e-03	-2.852899	3.066756e-03
hsa04114 Oocyte meiosis	3.784520e-03	-2.698128	3.784520e-03
hsa00010 Glycolysis / Gluconeogenesis	8.961413e-03	-2.405398	8.961413e-03

	q.val	set.size	exp1
hsa04110 Cell cycle	0.001448312	121	8.995727e-06
hsa03030 DNA replication	0.007586381	36	9.424076e-05
hsa03013 RNA transport	0.073840037	144	1.375901e-03
hsa03440 Homologous recombination	0.121861535	28	3.066756e-03
hsa04114 Oocyte meiosis	0.121861535	102	3.784520e-03
hsa00010 Glycolysis / Gluconeogenesis	0.212222694	53	8.961413e-03

Generating a colored pathway figure for hsa04110 cell cycle

```
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.
```

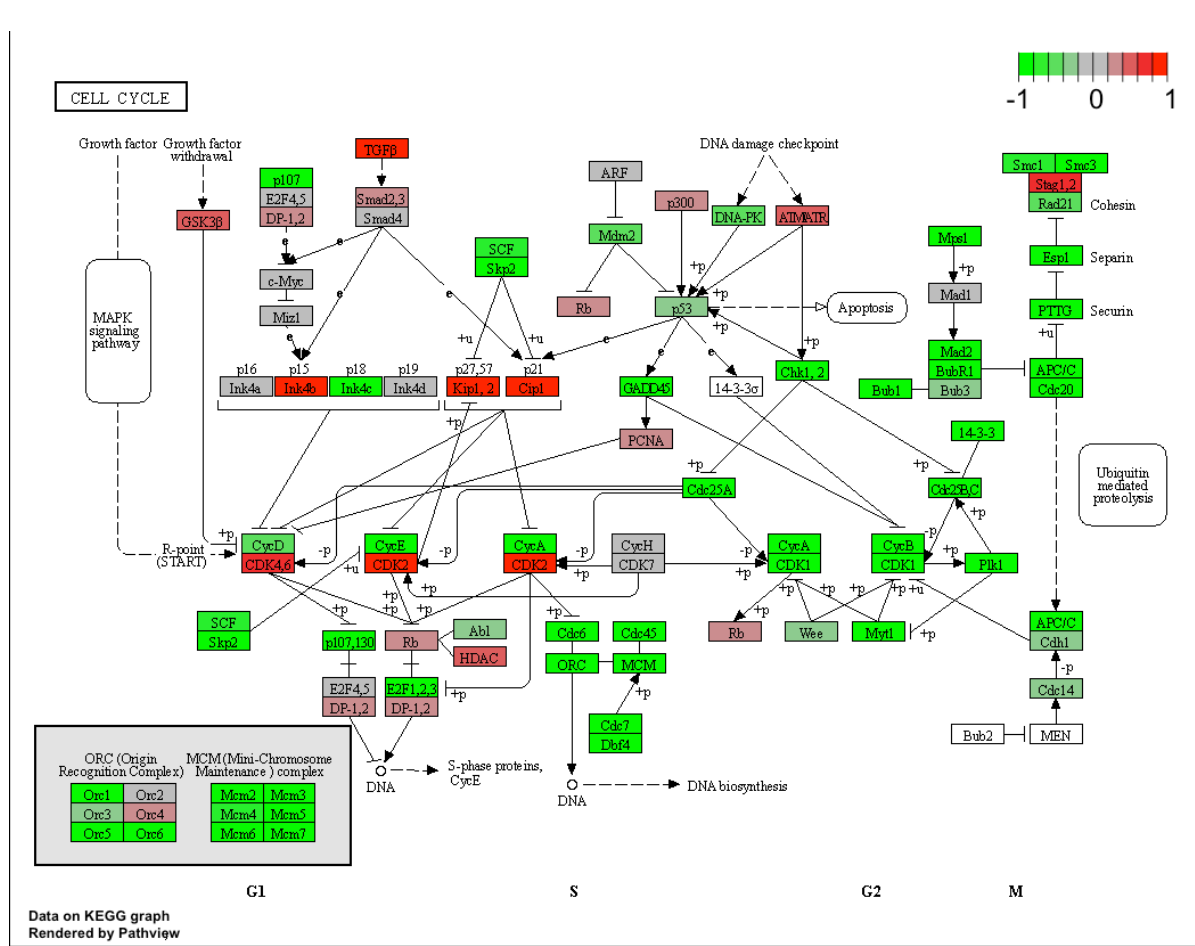
#####

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

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

Info: Working in directory /Users/marcuslau/Desktop/BIMM143/class13

Info: Writing image file hsa04110.pathview.png



```
## Focus on top 5 upregulated pathways here for demo purposes only  
keggrespathways <- rownames(keggres$greater)[1:5]
```

```
# Extract the 8 character long IDs part of each string  
keggresids = substr(keggrespathways, start=1, stop=8)  
keggresids
```

```
[1] "hsa04640" "hsa04630" "hsa00140" "hsa04142" "hsa04330"
```

```
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
```

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

```
Info: Working in directory /Users/marcuslau/Desktop/BIMM143/class13
```

```
Info: Writing image file hsa04640.pathview.png
```

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

```
Info: Working in directory /Users/marcuslau/Desktop/BIMM143/class13
```

```
Info: Writing image file hsa04630.pathview.png
```

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

```
Info: Working in directory /Users/marcuslau/Desktop/BIMM143/class13
```

```
Info: Writing image file hsa00140.pathview.png
```

```
Info: Downloading xml files for hsa04142, 1/1 pathways..
```

```
Info: Downloading png files for hsa04142, 1/1 pathways..
```

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

```
Info: Working in directory /Users/marcuslau/Desktop/BIMM143/class13
```

Info: Writing image file hsa04142.pathview.png

Info: some node width is different from others, and hence adjusted!

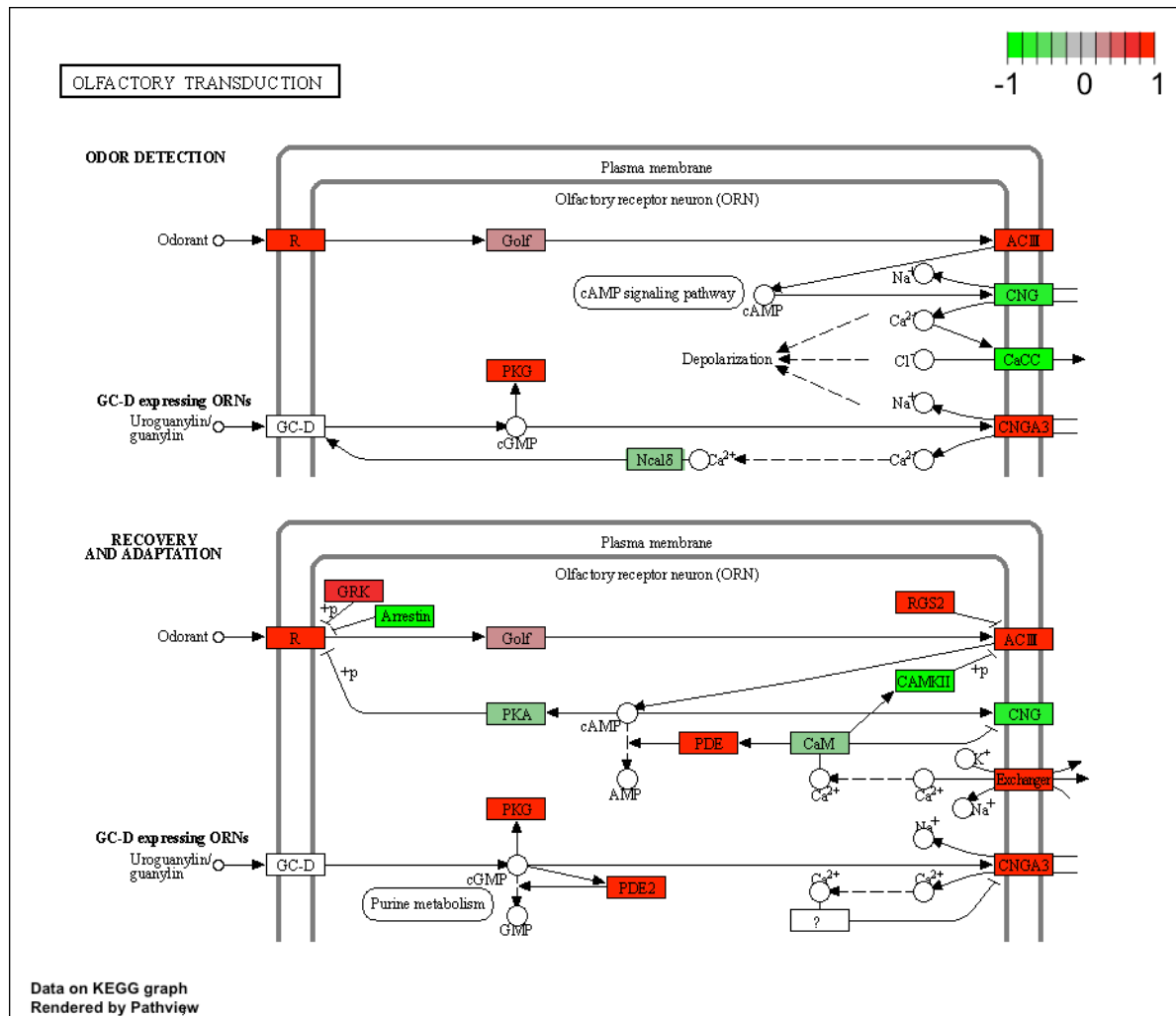
Info: Downloading xml files for hsa04330, 1/1 pathways..

Info: Downloading png files for hsa04330, 1/1 pathways..

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

Info: Working in directory /Users/marcuslau/Desktop/BIMM143/class13

Info: Writing image file hsa04330.pathview.png




```
# 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
G0:0007156 homophilic cell adhesion	8.519724e-05	3.824205	8.519724e-05
G0:0002009 morphogenesis of an epithelium	1.396681e-04	3.653886	1.396681e-04
G0:0048729 tissue morphogenesis	1.432451e-04	3.643242	1.432451e-04
G0:0007610 behavior	2.195494e-04	3.530241	2.195494e-04
G0:0060562 epithelial tube morphogenesis	5.932837e-04	3.261376	5.932837e-04
G0:0035295 tube development	5.953254e-04	3.253665	5.953254e-04
	q.val	set.size	exp1
G0:0007156 homophilic cell adhesion	0.1951953	113	8.519724e-05
G0:0002009 morphogenesis of an epithelium	0.1951953	339	1.396681e-04
G0:0048729 tissue morphogenesis	0.1951953	424	1.432451e-04
G0:0007610 behavior	0.2243795	427	2.195494e-04
G0:0060562 epithelial tube morphogenesis	0.3711390	257	5.932837e-04
G0:0035295 tube development	0.3711390	391	5.953254e-04

\$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	5.841698e-12	376	1.536227e-15
G0:0000280 nuclear division	5.841698e-12	352	4.286961e-15
G0:0007067 mitosis	5.841698e-12	352	4.286961e-15
G0:0000087 M phase of mitotic cell cycle	1.195672e-11	362	1.169934e-14
G0:0007059 chromosome segregation	1.658603e-08	142	2.028624e-11
G0:0000236 mitotic prometaphase	1.178402e-07	84	1.729553e-10

\$stats

stat.mean	exp1
-----------	------

G0:0007156	homophilic cell adhesion	3.824205	3.824205
G0:0002009	morphogenesis of an epithelium	3.653886	3.653886
G0:0048729	tissue morphogenesis	3.643242	3.643242
G0:0007610	behavior	3.530241	3.530241
G0:0060562	epithelial tube morphogenesis	3.261376	3.261376
G0:0035295	tube development	3.253665	3.253665

##Reactome Analysis

```
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: 8147"
```

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

Q: What pathway has the most significant “Entities p-value”? Do the most significant pathways listed match your previous KEGG results? What factors could cause differences between the two methods?

The pathway with the most significant p-value is Endosomal/Vacuolar pathway. P value was 1.67E-4. Some of the most significant pathways do match our previous KEGG results. Factors that caused these differences include different data bases.