

# Class 14: RNASeq Mini-Project

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Here we run through a complete RNAseq analysis from counts to pathways to biological insight.

## Data Import

```
# Import metadata
colData = read.csv("GSE37704_metadata.csv", row.names=1)

colData

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

```
# Import countdata
countData = read.csv("GSE37704_featurecounts.csv", row.names =1)

# remove first $length column
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

```
# filter count data where you have 0 read count across all samples
non_zero <- rowSums(countData) != 0
filtered_countData <- countData[non_zero,]

head(filtered_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

## Setup for 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, 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, 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

## Running DESeq

```
dds = DESeqDataSetFromMatrix(countData = filtered_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

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

## Add gene annotation data (gene names etc.)

```
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),
                     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(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

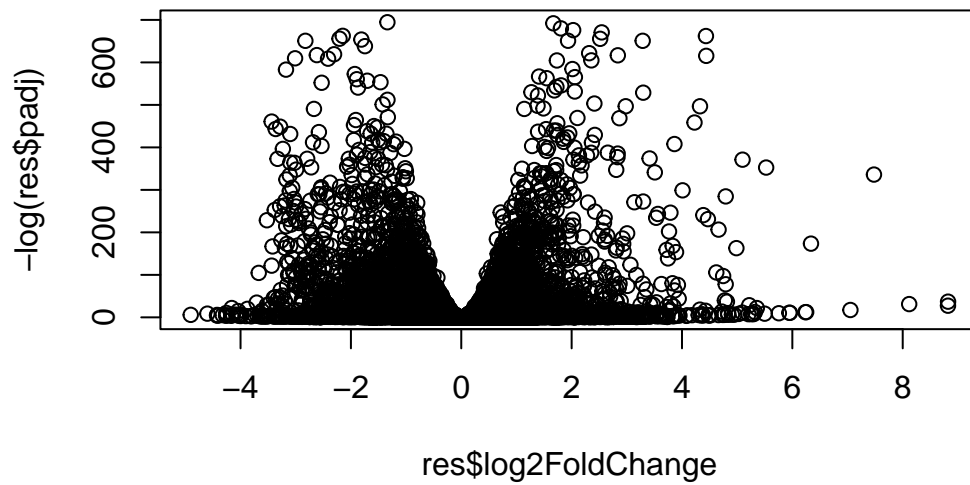
Wald test p-value: condition hoxa1 kd vs control sirna

DataFrame with 10 rows and 9 columns

	baseMean	log2FoldChange	lfcSE	stat	pvalue
	<numeric>	<numeric>	<numeric>	<numeric>	<numeric>
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	name	
	<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	AGR1	375790	agrin	
ENSG00000237330	NA	RNF223	401934	ring finger protein ..	

## Results visualization

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



Make it colorful :)

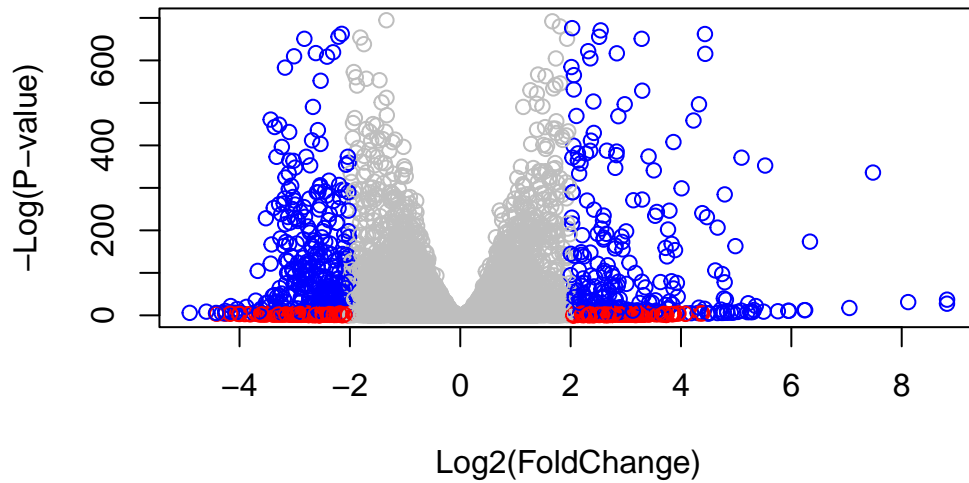
```
# Make a color vector for all genes
mycols <- rep("gray", nrow(res) )

# 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(
```





## Save our results

```
res = res[order(res$pvalue),]
write.csv(res, file = "deseq_results_lab14.csv")
```

## Pathway analysis (KEGG, GO, Reactome)

### Kegg analysis:

```
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>).

#####

```
library(gage)
```

```
library(gageData)
```

```
data(kegg.sets.hs)
```

```
data(sigmet.idx.hs)
```

```
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]
```

```
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"

```

Make named vector of fold changes

```

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

```

Gage pathway analysis:

```

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

Make a pathway plot:

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

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

Info: Working in directory /Users/bernicelozada/Desktop/BIMM 143/Class14

Info: Writing image file hsa04110.pathview.png

Focus on top 5 upregulated pathways and make pathway plot:

```
keggrespathways_up <- rownames(keggres$greater)[1:5]

# Extract IDs (8 characters long)
keggresids_up = substr(keggrespathways_up, start = 1, stop = 8)
keggresids_up
```

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

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

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

Info: Working in directory /Users/bernicelozada/Desktop/BIMM 143/Class14

Info: Writing image file hsa04640.pathview.png

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

Info: Working in directory /Users/bernicelozada/Desktop/BIMM 143/Class14

Info: Writing image file hsa04630.pathview.png

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

Info: Working in directory /Users/bernicelozada/Desktop/BIMM 143/Class14

Info: Writing image file hsa00140.pathview.png

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

Info: Working in directory /Users/bernicelozada/Desktop/BIMM 143/Class14

Info: Writing image file hsa04142.pathview.png

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

Info: Working in directory /Users/bernicelozada/Desktop/BIMM 143/Class14

Info: Writing image file hsa04330.pathview.png

Making pathway plot for top 5 downregulated pathways:

```
keggrespathways_down <- rownames(keggres$less)[1:5]
```

```
# Extract IDs (8 characters long)
```

```
keggresids_down = substr(keggrespathways_down, start = 1, stop = 8)
```

```
keggresids_down
```

```
[1] "hsa04110" "hsa03030" "hsa03013" "hsa03440" "hsa04114"
```

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

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

Info: Working in directory /Users/bernicelozada/Desktop/BIMM 143/Class14

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory /Users/bernicelozada/Desktop/BIMM 143/Class14

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory /Users/bernicelozada/Desktop/BIMM 143/Class14

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory /Users/bernicelozada/Desktop/BIMM 143/Class14

Info: Writing image file hsa03440.pathview.png

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

Info: Working in directory /Users/bernicelozada/Desktop/BIMM 143/Class14

Info: Writing image file hsa04114.pathview.png

## GO Analysis

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

# Focusing 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	1.925222e-04	3.565432	1.925222e-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	0.1952430	113	8.519724e-05
G0:0002009	morphogenesis of an epithelium	0.1952430	339	1.396681e-04
G0:0048729	tissue morphogenesis	0.1952430	424	1.432451e-04
G0:0007610	behavior	0.1968058	426	1.925222e-04
G0:0060562	epithelial tube morphogenesis	0.3566193	257	5.932837e-04
G0:0035295	tube development	0.3566193	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	5.843127e-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

\$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	3.565432	3.565432
G0:0060562	epithelial tube morphogenesis	3.261376	3.261376
G0:0035295	tube development	3.253665	3.253665

## Reactome Analysis

Get list of significant genes and output as a plain text file:

```
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]  
head(sig_genes)
```

```
ENSG00000117519 ENSG00000183508 ENSG00000159176 ENSG00000150938 ENSG00000116016  
      "CNN3"      "TENT5C"      "CSRP1"      "CRIM1"      "EPAS1"  
ENSG00000136068  
      "FLNB"
```

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

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 cell cycle pathway has the most significant “entities p-value”. The most significant pathways in the Reactome analysis have to do with the cell cycle, which is the same for the KEGG pathways. However, the KEGG pathway analysis also implicated glycolysis as a significantly changed pathway, while it was not in the top 20 for Reactome. This is likely because the Reactome has more detailed, overlapping reactions within one pathway, while KEGG just reveals the overall pathway.

## GO Online

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 negative regulation of glycogen biosynthetic process has the most significant entities p-value. Though the order of significant pathways is different between the KEGG and GO analysis, the content is the same. This may be because GO will annotate and organize by specific reactions, while KEGG will annotate general pathways by simply detecting genes that are all implicated in a general pathway.