class12

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Differential Expression Analysis

Loading required package: GenomicRanges

```
library(BiocManager)
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: 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"</pre>
countFile <- "GSE37704_featurecounts.csv"</pre>
# Import metadata and take a peak
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
# Import countdata
countData = read.csv(countFile, row.names=1)
head(countData)
                    length SRR493366 SRR493367 SRR493368 SRR493369 SRR493370
##
## ENSG0000186092
                       918
                                    0
                                              0
                                                         0
                                                                   0
                                                                              0
                                              0
                                                         0
                                                                   0
## ENSG00000279928
                       718
                                   0
                                                                              0
## ENSG00000279457
                      1982
                                   23
                                             28
                                                        29
                                                                   29
                                                                             28
## ENSG0000278566
                       939
                                    0
                                              0
                                                         0
                                                                   0
                                                                              0
## ENSG00000273547
                       939
                                    0
                                              0
                                                         0
                                                                   0
                                                                              0
## ENSG0000187634
                      3214
                                  124
                                            123
                                                       205
                                                                 207
                                                                            212
                    SRR493371
##
## ENSG0000186092
                            0
## ENSG00000279928
## ENSG00000279457
                           46
                            0
## ENSG0000278566
## ENSG00000273547
                            0
## ENSG0000187634
                          258
```

Q1. Complete the code below to remove the troublesome first column from countData

Note we need to remove the odd first \$length col

```
countData <- as.matrix(countData[,2:7])</pre>
head(countData)
                     SRR493366 SRR493367 SRR493368 SRR493369 SRR493370 SRR493371
##
## ENSG0000186092
                             0
                                         0
                                                    0
                                                               0
                                                                          0
## ENSG0000279928
                             0
                                         0
                                                    0
                                                               0
                                                                          0
                                                                                     0
## ENSG00000279457
                                                   29
                                                              29
                                                                         28
                            23
                                        28
                                                                                    46
                                                                                     0
## ENSG00000278566
                             0
                                         0
                                                    0
                                                               0
                                                                          0
## ENSG00000273547
                             0
                                         0
                                                    0
                                                               0
                                                                          0
                                                                                     0
## ENSG0000187634
                           124
                                       123
                                                 205
                                                             207
                                                                        212
                                                                                   258
```

Q2. 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?

rowSums(countData)

		ENSG00000279928	ENSG00000279457		ENSG00000273547
##	0 ENGG00000407604	U	183	0	U
##			ENSG00000187961		ENSG00000187642
##	1129	9907	1301	293	75
##			ENSG00000188157		ENSG00000131591
##	689	2144	56045	1	961
##		ENSG00000186891	ENSG00000186827		ENSG00000176022
##	0	4	5	28209	5572
##	ENSG00000184163		ENSG00000162572		ENSG00000169972
##	27	7559	198	6592	1379
##	ENSG00000127054		ENSG00000169962		ENSG00000162576
##	5574	1825	37	6063	33482
##		ENSG00000221978		ENSG00000235098	ENSG00000205116
##	6520	8382	4033	8	0
##		ENSG00000215915	ENSG00000160072		ENSG00000205090
##	660	3	2557	4280	245
##		ENSG00000279244		ENSG00000197530	ENSG00000189409
##	11346	0	1079	4602	15
##		ENSG00000189339		ENSG00000215790	ENSG00000008130
##	2901	10840	78	279	5827
##	ENSG00000078369	ENSG00000169885	ENSG00000178821	ENSG00000142609	ENSG00000187730
##	60694	9	16	0	0
##		ENSG00000162585		ENSG00000116151	ENSG00000157916
##	212	4005	10718	284	13099
##	ENSG00000157911	ENSG00000149527	ENSG00000157881		ENSG00000157873
##	1263	183	2648	0	151
##	ENSG00000157870		ENSG00000215912		ENSG00000142611
##	2325	2	15	0	24
##	ENSG00000130762	ENSG00000162591	ENSG00000158109	ENSG00000116213	ENSG00000078900
			11001	1000	2
##	5	741	11234	1203	6
## ##		741 ENSG00000235169	ENSG00000130764	ENSG00000116198	6 ENSG00000169598
## ## ##	5 ENSG00000162592 4	741 ENSG00000235169 49	ENSG00000130764 3910	ENSG00000116198 5316	598
## ## ## ##	5 ENSG00000162592 4 ENSG00000198912	741 ENSG00000235169 49 ENSG00000196581	ENSG00000130764 3910 ENSG00000131697	ENSG00000116198 5316 ENSG00000069424	6 ENSG00000169598 598 ENSG00000116254
## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669	741 ENSG00000235169 49 ENSG00000196581 16	ENSG00000130764 3910 ENSG00000131697 1398	ENSG00000116198 5316 ENSG00000069424 848	598 ENSG00000116254 30
## ## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673	598 ENSG00000116254 30 ENSG00000158292
## ## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673	598 ENSG00000116254 30 ENSG00000158292 1139
##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG00000097021	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG00000069812	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067 ENSG000001187017	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680
##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG00000097021 7661	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG00000069812 20	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067 ENSG000001187017	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657
## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG00000097021 7661 ENSG00000162408	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG00000069812 20 ENSG00000173662	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067 ENSG00000187017 4 ENSG00000204859	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331 ENSG00000162413	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657 ENSG00000116273
## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG00000097021 7661 ENSG00000162408 2185	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG00000069812 20 ENSG00000173662	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067 ENSG00000187017 4 ENSG00000204859 745	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331 ENSG00000162413 12770	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657 ENSG00000116273 2918
## ## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG00000097021 7661 ENSG00000162408 2185 ENSG00000041988	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG00000069812 20 ENSG00000173662 1 ENSG00000007923	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067 ENSG00000187017 4 ENSG000000204859 745 ENSG00000171735	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331 ENSG00000162413 12770 ENSG00000049245	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657 ENSG00000116273 2918 ENSG00000049246
## ## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG00000097021 7661 ENSG00000162408 2185 ENSG00000041988 772	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG00000069812 20 ENSG00000173662 1 ENSG0000007923 4034	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067 ENSG00000187017 4 ENSG000000204859 745 ENSG00000171735 1843	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331 ENSG00000162413 12770 ENSG00000049245 11842	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657 ENSG00000116273 2918 ENSG00000049246 892
## ## ## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG00000097021 7661 ENSG00000162408 2185 ENSG00000041988 772 ENSG00000049247	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG00000069812 20 ENSG00000173662 1 ENSG00000007923 4034 ENSG00000049249	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067 ENSG00000187017 4 ENSG000000204859 745 ENSG00000171735 1843 ENSG00000116288	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331 ENSG00000162413 12770 ENSG00000049245 11842 ENSG00000116285	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657 ENSG00000116273 2918 ENSG00000049246 892 ENSG00000162426
## ## ## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG00000097021 7661 ENSG00000162408 2185 ENSG00000041988 772 ENSG00000049247	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG00000069812 20 ENSG00000173662 1 ENSG000000173662 4034 ENSG00000049249 14	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067 ENSG00000187017 4 ENSG000000187017 51843 ENSG00000116288 13626	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331 ENSG00000162413 12770 ENSG00000049245 11842 ENSG00000116285 14356	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657 ENSG00000116273 2918 ENSG00000049246 892 ENSG00000162426 403
## ## ## ## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG0000097021 7661 ENSG00000162408 2185 ENSG00000041988 772 ENSG00000049247 2 ENSG00000142599	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG00000069812 20 ENSG00000173662 1 ENSG000000173662 4034 ENSG00000049249 14 ENSG00000074800	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067 ENSG00000187017 4 ENSG000000187017 45 ENSG00000171735 1843 ENSG00000116288 13626 ENSG00000131686	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331 ENSG00000162413 12770 ENSG00000049245 11842 ENSG00000116285 14356 ENSG00000197241	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657 ENSG00000116273 2918 ENSG00000049246 892 ENSG00000162426 403 ENSG00000142583
## ## ## ## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG0000097021 7661 ENSG00000162408 2185 ENSG00000041988 772 ENSG00000049247 2 ENSG00000142599 8669	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG0000069812 20 ENSG00000173662 1 ENSG000000173662 1 ENSG00000007923 4034 ENSG00000049249 14 ENSG00000074800 109127	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067 ENSG00000187017 4 ENSG000000187017 45 ENSG00000171735 1843 ENSG00000116288 13626 ENSG00000131686	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331 ENSG00000162413 12770 ENSG00000049245 11842 ENSG00000116285 14356 ENSG00000197241 0	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657 ENSG00000116273 2918 ENSG00000049246 892 ENSG00000162426 403 ENSG00000142583 5
## ## ## ## ## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG00000097021 7661 ENSG00000162408 2185 ENSG00000041988 772 ENSG00000049247 2 ENSG00000142599 8669 ENSG00000180758	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG00000069812 20 ENSG00000173662 1 ENSG00000007923 4034 ENSG00000049249 14 ENSG00000074800 109127 ENSG00000049239	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067 ENSG00000187017 4 ENSG00000187017 45 ENSG00000171735 1843 ENSG00000116288 13626 ENSG00000131686 1 ENSG00000171621	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331 ENSG00000162413 12770 ENSG00000049245 11842 ENSG00000116285 14356 ENSG00000197241 0 ENSG00000171612	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657 ENSG00000116273 2918 ENSG00000049246 892 ENSG00000162426 403 ENSG00000142583 5 ENSG00000188807
## ## ## ## ## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG00000097021 7661 ENSG00000162408 2185 ENSG00000014988 772 ENSG00000049247 2 ENSG000000142599 8669 ENSG00000180758 786	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG00000069812 20 ENSG00000173662 1 ENSG0000007923 4034 ENSG00000049249 14 ENSG00000074800 109127 ENSG00000049239 9650	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067 ENSG00000187017 4 ENSG000000204859 745 ENSG00000171735 1843 ENSG00000116288 13626 ENSG00000131686 1 ENSG00000171621 3728	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331 ENSG00000162413 12770 ENSG00000049245 11842 ENSG00000116285 14356 ENSG00000197241 0 ENSG00000171612 1041	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657 ENSG00000116273 2918 ENSG00000049246 892 ENSG00000162426 403 ENSG00000142583 5 ENSG00000188807 2088
## ## ## ## ## ## ## ## ## ## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG00000097021 7661 ENSG00000162408 2185 ENSG00000014288 772 ENSG00000049247 2 ENSG00000142599 8669 ENSG00000180758 786 ENSG00000171608	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG0000069812 20 ENSG00000173662 1 ENSG00000007923 4034 ENSG00000049249 14 ENSG00000074800 109127 ENSG00000049239 9650 ENSG00000171603	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067 ENSG00000187017 4 ENSG00000187017 45 ENSG00000171735 1843 ENSG00000116288 13626 ENSG00000131686 1 ENSG00000171621 3728 ENSG00000280113	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331 ENSG00000162413 12770 ENSG00000049245 11842 ENSG00000116285 ENSG00000171612 0 ENSG00000171612 1041 ENSG00000178585	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657 ENSG00000116273 2918 ENSG00000049246 892 ENSG00000162426 403 ENSG00000142583 5 ENSG00000188807 2088 ENSG00000162441
## ## ## ## ## ## ## ## ## ## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG00000097021 7661 ENSG00000162408 2185 ENSG00000041988 772 ENSG00000041988 772 ENSG00000142599 8669 ENSG00000180758 786 ENSG00000171608 3557	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG0000069812 20 ENSG00000173662 1 ENSG00000007923 4034 ENSG00000049249 14 ENSG00000074800 109127 ENSG00000049239 9650 ENSG00000171603 25190	ENSG00000130764	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331 ENSG00000162413 12770 ENSG00000049245 11842 ENSG00000116285 14356 ENSG00000177241 0 ENSG00000177612 1041 ENSG00000178585 2231	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657 ENSG00000116273 2918 ENSG00000049246 892 ENSG00000162426 403 ENSG00000142583 5 ENSG00000188807 2088 ENSG00000162441 1757
## ## ## ## ## ## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG0000097021 7661 ENSG00000162408 2185 ENSG00000041988 772 ENSG00000041988 275 ENSG00000142599 8669 ENSG00000180758 786 ENSG00000171608 3557 ENSG00000173614	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG0000069812 20 ENSG00000173662 1 ENSG00000007923 4034 ENSG00000049249 14 ENSG00000074800 109127 ENSG00000049239 9650 ENSG00000171603 25190 ENSG00000162444	ENSG00000130764 3910 ENSG00000131697 1398 ENSG00000116237 14067 ENSG00000187017 4 ENSG00000187017 45 ENSG00000171735 1843 ENSG00000116288 13626 ENSG00000131686 1 ENSG00000171621 3728 ENSG00000280113 0 ENSG00000130939	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331 ENSG00000162413 12770 ENSG00000049245 11842 ENSG00000116285 14356 ENSG00000177241 0 ENSG00000177241 1041 ENSG00000178585 2231 ENSG00000054523	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657 ENSG00000116273 2918 ENSG00000049246 892 ENSG00000162426 403 ENSG00000142583 5 ENSG00000188807 2088 ENSG00000162441 1757 ENSG00000142657
## ## ## ## ## ## ## ## ## ## ## ## ##	5 ENSG00000162592 4 ENSG00000198912 1669 ENSG00000116251 19132 ENSG0000097021 7661 ENSG00000162408 2185 ENSG00000041988 772 ENSG00000041988 275 ENSG00000142599 8669 ENSG00000180758 786 ENSG00000171608 3557 ENSG00000173614 1340	741 ENSG00000235169 49 ENSG00000196581 16 ENSG00000158286 101 ENSG0000069812 20 ENSG00000173662 1 ENSG0000007923 4034 ENSG00000049249 14 ENSG00000074800 109127 ENSG00000049239 9650 ENSG00000171603 25190 ENSG00000162444	ENSG00000130764	ENSG00000116198 5316 ENSG00000069424 848 ENSG00000173673 0 ENSG00000215788 331 ENSG00000162413 12770 ENSG00000049245 11842 ENSG00000116285 14356 ENSG00000171612 0 ENSG00000171612 1041 ENSG00000178585 2231 ENSG00000054523 10261	598 ENSG00000116254 30 ENSG00000158292 1139 ENSG00000171680 2657 ENSG00000116273 2918 ENSG00000049246 892 ENSG00000162426 403 ENSG00000142583 5 ENSG00000188807 2088 ENSG00000162441 1757 ENSG00000142657 24354
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                                                     3347
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## ENSG00000160999 ENSG00000260097 ENSG00000128563 ENSG00000160991 ENSG00000160993
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##
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                                                          Ω
##
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                             Ω
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## ENSG00000184905 ENSG00000204071 ENSG00000184515 ENSG00000269405 ENSG00000269437
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                               0
                                                         Ω
##
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## ENSG00000166352 ENSG00000148948 ENSG00000166181 ENSG00000052841 ENSG00000149084
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                                                         Ω
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                                                           3422
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                                                           Ω
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                   6
                                                            98
  ENSG00000100060 ENSG00000100065 ENSG00000128283 ENSG00000100079 ENSG00000100083
           2
                            2094
                                          9515
                                                            38
  ENSG00000100092 ENSG00000241360 ENSG00000100097 ENSG00000273899 ENSG00000100101
                            205
                                         112788
                                                          193
  ENSG00000100106 ENSG00000189060 ENSG00000100116 ENSG00000128310 ENSG00000100124
                                           1519
  ENSG00000100129 ENSG00000100139 ENSG00000128346 ENSG00000100142 ENSG00000100146
                            6654
                                            290
                                                             2
  ENSG00000100151 ENSG00000100156 ENSG00000128298 ENSG00000184381 ENSG00000185022
                            12
                                            74
                                                           205
  ENSG00000198792 ENSG00000213923 ENSG00000168135 ENSG00000100196 ENSG00000100201
            14204
                           22822
                                  9
                                                         12639
  ENSG00000100206 ENSG00000184949 ENSG00000100211 ENSG00000100216 ENSG00000100221
                             327
                                           1112
              17
  ENSG00000100226 ENSG00000100242 ENSG00000100246 ENSG00000221890 ENSG00000183741
##
             4494
                            7955
                                           1059
                                                          2032
  ENSG00000128383 ENSG00000179750 ENSG00000244509 ENSG00000243811 ENSG00000128394
              1
                                           7762
                            404
                                                            52
  ENSG00000239713 ENSG00000100298 ENSG00000100307 ENSG00000100311 ENSG00000100316
                                            263
             164
                             .3
                                                           240
  ENSG00000100321 ENSG00000100324 ENSG00000128268 ENSG00000100335 ENSG00000128272
                           2483
                                            236
##
                                                          6816
  ENSG00000187051 ENSG00000100346 ENSG00000176177 ENSG00000100351 ENSG00000133477
##
                                             0
                                                             8
                              1
  ENSG00000100354 ENSG00000239900 ENSG00000279624 ENSG00000100359 ENSG00000196588
                            5776
                                            0
                                                          4531
  ENSG00000128285 ENSG00000100372 ENSG00000100380 ENSG00000196236 ENSG00000172404
                                          28833
               36
                                                          1956
                            2111
  ENSG00000100387 ENSG00000100393 ENSG00000100395 ENSG00000100399 ENSG00000100401
                                           2571
##
             2682
                           10982
                                                            39
  ENSG00000100403 ENSG00000167074 ENSG00000183864 ENSG00000100410 ENSG00000100412
            33527
                            1907
                                           5000
                                                          1829
  ENSG00000100413 ENSG00000172346 ENSG00000100417 ENSG00000100418 ENSG00000196419
                            1625
                                          4337
                                                         7949
  ENSG00000100138 ENSG00000184208 ENSG00000167077 ENSG00000100147 ENSG00000198911
                  1268
                                            41
                                                          283
  ENSG00000234965 ENSG00000159958 ENSG00000100162 ENSG00000100167 ENSG00000183066
##
                               9
                                            333
```

```
## ENSG00000198951 ENSG00000177096 ENSG00000183172 ENSG00000184983 ENSG00000100197
##
            3279
                          1414
                                         1241
                                                       3933
  ENSG00000100207 ENSG00000235568 ENSG00000189306 ENSG00000183569 ENSG00000100227
                             4
                                         3462
                                                        109
  ENSG00000100243 ENSG00000249222 ENSG00000128274 ENSG00000242247 ENSG00000100266
                             2
                                         1982
                                                       7362
  ENSG00000100271 ENSG00000100290 ENSG00000100294 ENSG00000100300 ENSG00000100304
                            8
                                         974
                                                       10676
  ENSG00000159307 ENSG00000186732 ENSG00000186976 ENSG00000130540 ENSG00000100341
                            0
               Ω
                                          92
                                                        220
  ENSG00000100344 ENSG00000100347 ENSG00000188677 ENSG00000138964 ENSG00000138944
             911
                         4539
                                        2092
                                                         0
  ENSG00000188636 ENSG00000186654 ENSG00000248405 ENSG00000241484 ENSG00000056487
                          1022 0 0
  ENSG00000093000 ENSG00000100364 ENSG00000100373 ENSG00000100376 ENSG00000077935
                          12061
                                0
                                                       1734
  ENSG00000128408 ENSG00000077942 ENSG00000130638 ENSG00000188064 ENSG00000182257
               6
                         10424
                                       12812
                                                        179
  ENSG00000186951 ENSG00000205643 ENSG00000130943 ENSG00000075234 ENSG00000075218
                           552
                                          15
                                                       5102
  ENSG00000100416 ENSG00000075275 ENSG00000075240 ENSG00000100422 ENSG00000054611
                           810
                                        1377
  ENSG00000280080 ENSG00000278881 ENSG00000219438 ENSG00000100425 ENSG00000100426
               0
                             2
                                           8
                                                       3411
  ENSG00000182858 ENSG00000184164 ENSG00000198355 ENSG00000188263 ENSG00000138892
            1241
                         4561
                                        3291
                                                         1
  ENSG00000100427 ENSG00000073146 ENSG00000073150 ENSG00000170638 ENSG00000073169
                           106
                                          579
                                                       2603
  ENSG00000128159 ENSG00000100429 ENSG00000188130 ENSG00000185386 ENSG00000196576
                            65
                                         3966
                                                       1639
  ENSG00000205593 ENSG00000100239 ENSG00000100241 ENSG00000128165 ENSG00000100253
##
             297
                          8359
                                        14649
                                                       1264
  ENSG00000100258 ENSG00000025770 ENSG00000130489 ENSG00000025708 ENSG00000177989
                          2305
                                          860
            9217
                                                        140
  ENSG00000130487 ENSG00000217442 ENSG00000205560 ENSG00000254413 ENSG00000100288
                            1
               4
                                          .3
                                                          2
  ENSG00000008735 ENSG00000100299 ENSG00000100312 ENSG00000079974 ENSG00000279493
                          3233
##
              58
                                           1
                                                       1087
  ENSG00000277117 ENSG00000280071 ENSG00000276612 ENSG00000275464 ENSG00000280433
##
                           299
                                           0
              77
                                                        582
  ENSG00000275993 ENSG00000274276 ENSG00000275895 ENSG00000276076 ENSG00000273590
               7
                            8
                                         45
                                                          0
  ENSG00000276289 ENSG00000274391 ENSG00000280108 ENSG00000166351 ENSG00000188992
                      0
                                   0 0
               3
  ENSG00000185272 ENSG00000155304 ENSG00000155307 ENSG00000243440 ENSG00000180530
                                1 0
                          8863
              1
  ENSG00000155313 ENSG00000154639 ENSG00000154640 ENSG00000154642 ENSG00000154645
                           656
                                         3232
                                                       2243
  ENSG00000154646 ENSG00000154654 ENSG00000154719 ENSG00000154721 ENSG00000154723
              0
                           764
                                1509
                                                       1047
  ENSG00000154727 ENSG00000142192 ENSG00000166265 ENSG00000154734 ENSG00000154736
            4135 94799
                                 0 59299
  ENSG00000156239 ENSG00000198862 ENSG00000156253 ENSG00000156256 ENSG00000156261
                          4382
                                         1946
                                                       4560
##
```

```
## ENSG00000156265 ENSG00000156273 ENSG00000171189 ENSG00000156282 ENSG00000156284
##
             2005
                           12899
                                              50
                                                              0
  ENSG00000188694 ENSG00000232263 ENSG00000197683 ENSG00000206107 ENSG00000182816
                               0
                                              Ω
                                                              Ω
  ENSG00000186980 ENSG00000198390 ENSG00000240432 ENSG00000186971 ENSG00000186970
                Ω
                               Ω
                                              0
                                                              Ω
   ENSG00000184351 ENSG00000244025 ENSG00000186965 ENSG00000186967 ENSG00000186977
                0
                               0
                                              0
                                                              0
  ENSG00000186925 ENSG00000244362 ENSG00000206106 ENSG00000212938 ENSG00000186930
                0
                               0
                                              0
                                                              Ω
   ENSG00000186924 ENSG00000184724 ENSG00000244624 ENSG00000206105 ENSG00000184032
                0
                               0
                                              0
                                                              0
   ENSG00000206104 ENSG00000231068 ENSG00000187026 ENSG00000187005 ENSG00000183640
                0
                               Ω
                                              0
                                                              Ω
   ENSG00000274749 ENSG00000182591 ENSG00000206102 ENSG00000156299 ENSG00000142168
                0
                   0
                                  0
                                                           1249
##
   ENSG00000156304 ENSG00000142149 ENSG00000159055 ENSG00000170262 ENSG00000142207
                             402
                                             703
                                                              0
  ENSG00000166979 ENSG00000242220 ENSG00000265590 ENSG00000159079 ENSG00000159082
                              8
                                  0
                                                            727
  ENSG00000159086 ENSG00000205929 ENSG00000205927 ENSG00000184221 ENSG00000159110
                                             0
                              1
  ENSG00000249624 ENSG00000243646 ENSG00000142166 ENSG00000159128 ENSG00000142188
##
                0
                            1532
                                            7276
                                                           4623
   ENSG00000177692 ENSG00000159131 ENSG00000159140 ENSG00000159147 ENSG00000249209
               70
                            6095
                                          28501
                                                           1012
  ENSG00000205758 ENSG00000205726 ENSG00000241837 ENSG00000243927 ENSG00000198743
             1286
                            6093
                                            2277
                                                           2379
   ENSG00000159197 ENSG00000205670 ENSG00000222018 ENSG00000243627 ENSG00000180509
                               0
                                              0
                                                              0
               1
   ENSG00000159200 ENSG00000159212 ENSG00000159216 ENSG00000280170 ENSG00000185917
##
            11290
                              10
                                           10631
                                                              2
   ENSG00000159228 ENSG00000159231 ENSG00000142197 ENSG00000159256 ENSG00000159259
                             234
                                            3237
                                                           3022
   ENSG00000159261 ENSG00000159263 ENSG00000159267 ENSG00000183145 ENSG00000185808
                             863
##
                9
                                            2543
                                                              7
   ENSG00000182670 ENSG00000157538 ENSG00000157540 ENSG00000157542 ENSG00000184029
            39228
                            3977
                                            4876
##
                                                            118
   ENSG00000198054 ENSG00000157551 ENSG00000157554 ENSG00000157557 ENSG00000183527
##
                Ω
                             162
                                                          13431
                                               1
   ENSG00000185658 ENSG00000205581 ENSG00000182093 ENSG00000157578 ENSG00000185437
                           15402
                                           3350
                                                             52
   ENSG00000183778 ENSG00000183067 ENSG00000183036 ENSG00000171587 ENSG00000182240
               10
                               2
                                              Ω
                                                             15
   ENSG00000183844 ENSG00000183486 ENSG00000157601 ENSG00000184012 ENSG00000183421
                0
                                                              3
##
                              43
                                             89
   ENSG00000141956 ENSG00000157617 ENSG00000173276 ENSG00000177398 ENSG00000160179
                            7568
                                            4620
                                                            12
  ENSG00000160180 ENSG00000160181 ENSG00000160182 ENSG00000160183 ENSG00000160185
               0
                              0
                                             0
                                                             0
  ENSG00000160188 ENSG00000160190 ENSG00000160191 ENSG00000160193 ENSG00000160194
##
                3
                             171
                                            319 1465
  ENSG00000160199 ENSG00000160200 ENSG00000160201 ENSG00000160202 ENSG00000142178
##
             3641
                             440
                                              96
```

```
## ENSG00000160207 ENSG00000234289 ENSG00000160208 ENSG00000160209 ENSG00000160213
##
                                10
                                              6635
                59
                                                             17419
   ENSG00000160214 ENSG00000160216 ENSG00000160218 ENSG00000241945 ENSG00000160221
                             14261
                                              3555
   ENSG00000160223 ENSG00000142182 ENSG00000160224 ENSG00000141959 ENSG00000160226
                 8
                                 2
                                                 9
                                                             12350
   ENSG00000142185 ENSG00000160233 ENSG00000175894 ENSG00000215455 ENSG00000205445
##
                 3
                               321
                                                 8
                                                                 0
   ENSG00000212935 ENSG00000215454 ENSG00000241123 ENSG00000188155 ENSG00000272804
                 0
                                 0
                                                 0
                                                                 Ω
   ENSG00000187766 ENSG00000221837 ENSG00000221859 ENSG00000243489 ENSG00000205439
                 0
##
                                 0
                                                 0
                                                                 0
   ENSG00000212933 ENSG00000221864 ENSG00000187175 ENSG00000189169 ENSG00000184787
                                 0
                                                                 0
                 0
                                                 0
   ENSG00000184900 ENSG00000183255 ENSG00000160255 ENSG00000160256 ENSG00000197381
##
                             36882
                                                 8
                                                              1104
   ENSG00000186866 ENSG00000182871 ENSG00000173638 ENSG00000183570 ENSG00000142156
                            43293
                                              1829
                                                               482
  ENSG00000142173 ENSG00000160282 ENSG00000160284 ENSG00000160285 ENSG00000160294
            310920
                               34
                                             1046
                                                              9362
   ENSG00000182362 ENSG00000160298 ENSG00000160299 ENSG00000160305 ENSG00000160307
                                              4358
   ENSG00000160310 ENSG00000198888 ENSG00000198763 ENSG00000198804 ENSG00000198712
                            148984
                                            139615
                                                            907247
##
   ENSG00000228253 ENSG00000198899 ENSG00000198938 ENSG00000198840 ENSG00000212907
            51515
                            312082
                                            400678
                                                             33202
   ENSG00000198886 ENSG00000198786 ENSG00000198695 ENSG00000198727 ENSG00000274175
##
            610441
                            293037
                                             93767
                                                            208079
   ENSG00000275869 ENSG00000273554 ENSG00000278782 ENSG00000277761 ENSG00000277836
                 0
                                 6
                                                 0
   ENSG00000277666 ENSG00000276760 ENSG00000275249 ENSG00000274792 ENSG00000278704
##
                 0
                                 0
                                                 0
                                                                 0
   ENSG00000277400 ENSG00000274847 ENSG00000273496 ENSG00000276256 ENSG00000278198
                                 Ω
                                                 Ω
                                                               567
                 1
   ENSG00000273748 ENSG00000276017 ENSG00000278817 ENSG00000277196 ENSG00000277630
                                0
                                               15
                                                                 0
              216
  ENSG00000278384 ENSG00000278633 ENSG00000276345 ENSG00000277856 ENSG00000275063
                                 0
                                               445
## ENSG00000271254 ENSG00000277475 ENSG00000268674
              1080
                                 0
```

Filter count data where you have 0 read count across all samples.
countData = countData[rowSums(countData) != 0,]
head(countData)

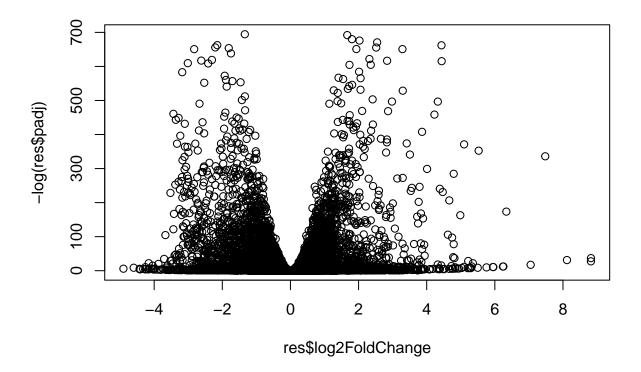
##	SRR493366	SRR493367	SRR493368	SRR493369	SRR493370	SRR493371
## ENSG00000279457	23	28	29	29	28	46
## ENSG00000187634	124	123	205	207	212	258
## ENSG00000188976	1637	1831	2383	1226	1326	1504
## ENSG00000187961	120	153	180	236	255	357
## ENSG00000187583	24	48	65	44	48	64
## ENSG00000187642	4	9	16	14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

Running DESeq2

```
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
dds
## class: DESeqDataSet
## dim: 15975 6
## metadata(1): version
## assays(4): counts mu H cooks
## rownames(15975): ENSG00000279457 ENSG00000187634 ... ENSG00000276345
    ENSG00000271254
## rowData names(22): baseMean baseVar ... deviance maxCooks
## colnames(6): SRR493366 SRR493367 ... SRR493370 SRR493371
## colData names(2): condition sizeFactor
Results for HoxA1 knockdown vs. control siRNA
res = results(dds, contrast=c("condition", "hoxa1_kd", "control_sirna"))
    Q3. 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)</pre>
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
```

Volcano Plot

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



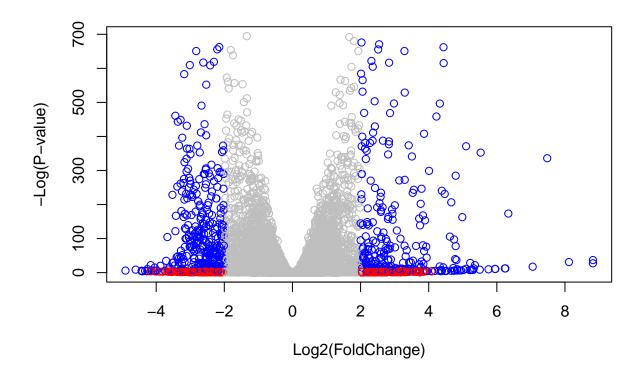
Q4. Improve this plot by completing the below code, which adds color and axis labels

```
# 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$pvalue < 0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "blue"

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



Adding Gene Annotation

library("AnnotationDbi")

[26] "UNIPROT"

Q5. Use the mapIDs() function multiple times to add SYMBOL, ENTREZID and GENENAME annotation to our results by completing the code below.

```
library("org.Hs.eg.db")
##
columns(org.Hs.eg.db)
                         "ALIAS"
                                         "ENSEMBL"
                                                          "ENSEMBLPROT"
                                                                          "ENSEMBLTRANS"
    [1] "ACCNUM"
        "ENTREZID"
                         "ENZYME"
                                         "EVIDENCE"
                                                          "EVIDENCEALL"
                                                                          "GENENAME"
##
                                                                          "MAP"
   [11]
        "GENETYPE"
                         "GO"
                                         "GOALL"
                                                          "IPI"
        "OMIM"
                         "ONTOLOGY"
                                         "ONTOLOGYALL"
                                                          "PATH"
                                                                          "PFAM"
##
   [16]
        "PMID"
                         "PROSITE"
                                         "REFSEQ"
                                                          "SYMBOL"
                                                                          "UCSCKG"
```

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

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

'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
## ENSG0000187634
                   183.229650
                                    0.4264571 0.1402658
                                                           3.040350 2.36304e-03
## ENSG00000188976 1651.188076
                                    -0.6927205 0.0548465 -12.630158 1.43990e-36
## ENSG0000187961
                   209.637938
                                    0.7297556 0.1318599
                                                           5.534326 3.12428e-08
## ENSG0000187583
                     47.255123
                                    0.0405765 0.2718928
                                                           0.149237 8.81366e-01
## ENSG0000187642
                     11.979750
                                                           1.040744 2.97994e-01
                                    0.5428105 0.5215598
## ENSG0000188290
                    108.922128
                                    2.0570638 0.1969053
                                                          10.446970 1.51282e-25
                                    0.2573837 0.1027266
                                                           2.505522 1.22271e-02
## ENSG0000187608
                    350.716868
## 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
                                    WASH9P
                                              102723897 WAS protein family h..
## ENSG00000187634 5.15718e-03
                                                 148398 sterile alpha motif ...
                                     SAMD11
                                                  26155 NOC2 like nucleolar ...
## ENSG00000188976 1.76549e-35
                                      NOC2L
## 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
                                                   9636 ISG15 ubiquitin like..
                                      ISG15
## ENSG00000188157 4.21963e-16
                                       AGRN
                                                                          agrin
## ENSG00000237330
                            NΔ
                                    RNF223
                                                 401934 ring finger protein ..
```

Q6. 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, "deseq_results.csv")
```

Pathway Analysis

KEGG Pathways

```
library(pathview)
```

##

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

\$'hsa00230 Purine metabolism'

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

Used mapIDs() to get Entrez gene IDs and fold change results from DESeq2 analysis.

```
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 and look at the object returned from gage().

```
keggres = gage(foldchanges, gsets=kegg.sets.hs)
attributes(keggres)
```

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

```
# Look at the first few down (less) pathways
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
                                         0.073840037
## hsa03013 RNA transport
                                                         144 1.375901e-03
## hsa03440 Homologous recombination
                                       0.121861535
                                                          28 3.066756e-03
## hsa04114 Oocyte meiosis
                                                          102 3.784520e-03
                                         0.121861535
## hsa00010 Glycolysis / Gluconeogenesis 0.212222694
                                                           53 8.961413e-03
Make a pathway plot with RNA-Seq expression results with pathview().
pathview(gene.data=foldchanges, pathway.id="hsa04110")
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/admin/Documents/college/class12
## Info: Writing image file hsa04110.pathview.png
PDF graph
pathview(gene.data=foldchanges, pathway.id="hsa04110", kegg.native=FALSE)
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/admin/Documents/college/class12
## Info: Writing image file hsa04110.pathview.pdf
## Focus on top 5 upregulated pathways here for demo purposes only
keggrespathways <- rownames(keggres$greater)[1:5]</pre>
# Extract the 8 character long IDs part of each string
keggresids = substr(keggrespathways, start=1, stop=8)
keggresids
## [1] "hsa04640" "hsa04630" "hsa00140" "hsa04142" "hsa04330"
Draw plots for all top 5 pathways
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/admin/Documents/college/class12
## Info: Writing image file hsa04640.pathview.png
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/admin/Documents/college/class12
```

```
## Info: Writing image file hsa04630.pathview.png
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/admin/Documents/college/class12
## Info: Writing image file hsa00140.pathview.png
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/admin/Documents/college/class12
## Info: Writing image file hsa04142.pathview.png
## Info: some node width is different from others, and hence adjusted!
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/admin/Documents/college/class12
## Info: Writing image file hsa04330.pathview.png
    Q7. Can you do the same procedure as above to plot the pathview figures for the top 5 down-
    reguled pathways?
Yes
## Focus on top 5 downregulated pathways
keggresless <- rownames(keggres$less)[1:5]</pre>
# Extract the 8 character long IDs part of each string
keggresidsLess = substr(keggresless, start=1, stop=8)
keggresidsLess
## [1] "hsa04110" "hsa03030" "hsa03013" "hsa03440" "hsa04114"
pathview(gene.data=foldchanges, pathway.id=keggresidsLess, species="hsa")
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/admin/Documents/college/class12
## Info: Writing image file hsa04110.pathview.png
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/admin/Documents/college/class12
```

```
## Info: Writing image file hsa03030.pathview.png
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/admin/Documents/college/class12
## Info: Writing image file hsa03013.pathview.png
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/admin/Documents/college/class12
## Info: Writing image file hsa03440.pathview.png
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/admin/Documents/college/class12
## Info: Working in directory /Users/admin/Documents/college/class12
## Info: Writing image file hsa04114.pathview.png
```

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

```
##
                                               p.geomean stat.mean
                                                                           p.val
## GO:0048285 organelle fission
                                            1.536227e-15 -8.063910 1.536227e-15
                                            4.286961e-15 -7.939217 4.286961e-15
## GO:0000280 nuclear division
## GO: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
## GO:0007059 chromosome segregation
                                            2.028624e-11 -6.878340 2.028624e-11
## GO:0000236 mitotic prometaphase
                                            1.729553e-10 -6.695966 1.729553e-10
                                                   q.val set.size
## GO:0048285 organelle fission
                                            5.841698e-12
                                                              376 1.536227e-15
## GO:0000280 nuclear division
                                            5.841698e-12
                                                              352 4.286961e-15
## GO:0007067 mitosis
                                            5.841698e-12
                                                              352 4.286961e-15
## GO:0000087 M phase of mitotic cell cycle 1.195672e-11
                                                              362 1.169934e-14
## GO:0007059 chromosome segregation
                                            1.658603e-08
                                                              142 2.028624e-11
## GO:0000236 mitotic prometaphase
                                            1.178402e-07
                                                               84 1.729553e-10
##
## $stats
##
                                             stat.mean
                                                            exp1
## GO:0007156 homophilic cell adhesion
                                              3.824205 3.824205
## GD:0002009 morphogenesis of an epithelium 3.653886 3.653886
## GO:0048729 tissue morphogenesis
                                              3.643242 3.643242
## GO:0007610 behavior
                                              3.530241 3.530241
## GO:0060562 epithelial tube morphogenesis
                                              3.261376 3.261376
## GO: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, quote=FALSE)</pre>
```

Q8. 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?

Endosomal/Vacuolor Pathway has the most significant "Entities p-value" being the lowest at 2.02E-4. It does not match the previous KEGG results since te KEGG results showed Cell Cycle being the most significant p-value. Cell cycle is second in the reactome analysis. The reason there are differences is because we focused on signaling and metabolic pathways only for KEGG.

GO Online

Q9. 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?

"detection of chemical stimulus involved in sensory perception" with a p-value of 6.17E-64. This does not match the KEGG that had cell cycle with the lowest p-value. I'm not sure what factors cause differences.