class 14

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Today we will complete an RNASeq analysis from counts to pathways.

We will work with data on differential analysis of lung fibroblasts in response to loss of developmental transciption factor HOXA1.

Data Import

```
library(DESeq2)
```

```
Loading required package: S4Vectors

Loading required package: stats4

Loading required package: BiocGenerics


Attaching package: 'BiocGenerics'

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

    IQR, mad, sd, var, xtabs

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

    anyDuplicated, aperm, append, as.data.frame, basename, cbind,
    colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
    get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
    match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
    Position, rank, rbind, Reduce, rownames, sapply, saveRDS, setdiff,
    table, tapply, union, unique, unsplit, which.max, which.min
```

```
Attaching package: 'S4Vectors'

The following object is masked from 'package:utils':

    findMatches

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

    expand.grid, I, unname

Loading required package: IRanges

Loading required package: GenomicRanges

Loading required package: GenomeInfoDb

Loading required package: SummarizedExperiment

Loading required package: MatrixGenerics

Loading required package: matrixStats


Attaching package: 'MatrixGenerics'

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

    colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
    colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
    colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
    colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
    colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
    colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
    colWeightedMeans, colWeightedMedians, colWeightedSds,
    colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
    rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
    rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
    rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
```

```
    rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
    rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
    rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
    rowWeightedSds, rowWeightedVars


Loading required package: Biobase


Welcome to Bioconductor

    Vignettes contain introductory material; view with
    'browseVignettes()'. To cite Bioconductor, see
    'citation("Biobase")', and for packages 'citation("pkgname")'.



Attaching package: 'Biobase'


The following object is masked from 'package:MatrixGenerics':

    rowMedians


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

    anyMissing, rowMedians
```

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

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

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

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

```
                length SRR493366 SRR493367 SRR493368 SRR493369 SRR493370
ENSG00000186092    918         0         0         0         0         0
ENSG00000279928    718         0         0         0         0         0
ENSG00000279457   1982        23        28        29        29        28
ENSG00000278566    939         0         0         0         0         0
ENSG00000273547    939         0         0         0         0         0
ENSG00000187634   3214       124       123       205       207       212
                SRR493371
ENSG00000186092         0
ENSG00000279928         0
ENSG00000279457        46
ENSG00000278566         0
ENSG00000273547         0
ENSG00000187634       258
```

Check correspondence of the columns of 'counts' and rows of 'metadata'

```
colnames(countData)
```

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

```
metadata$id
```

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

We need to remove the first length column from our counts

```
counts <- countData[,-1]
colnames(counts) == metadata$id
```

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

Q. How many genes do we have?

```
nrow(counts)
```

```
[1] 19808
```

There are tones of zero count genes that we can get rid of.

```
to.rm <- rowSums(counts) == 0
#counts[!to.rm,]
```

```
to.keep <- rowSums(counts) == !0
countData[to.keep,]
```

	length	SRR493366	SRR493367	SRR493368	SRR493369	SRR493370
ENSG00000237330	1902	0	0	0	0	1
ENSG00000173662	2892	0	0	1	0	0
ENSG00000131686	2662	0	0	1	0	0
ENSG00000277726	2205	0	0	1	0	0
ENSG00000120952	1642	0	0	0	0	0
ENSG00000277058	1148	0	0	0	0	1
ENSG00000275774	1148	0	0	1	0	0
ENSG00000162438	3486	0	0	0	0	1
ENSG00000186510	3476	0	0	0	0	0
ENSG00000187144	4506	0	0	0	1	0
ENSG00000158748	1984	0	1	0	0	0
ENSG00000162543	5571	0	0	0	0	0
ENSG00000158816	6377	0	0	0	1	0
ENSG00000070886	5358	0	0	0	0	0
ENSG00000188822	5254	0	0	0	1	0
ENSG00000131914	4008	0	0	0	0	1
ENSG00000182866	3339	0	0	1	0	0
ENSG00000188910	2387	0	0	0	0	1
ENSG00000187513	1717	0	0	0	0	0
ENSG00000271741	3099	0	0	0	0	0
ENSG00000274944	3251	0	0	0	1	0
ENSG00000204060	2398	0	0	0	0	0
ENSG00000127129	1639	0	0	0	0	1
ENSG00000177181	8981	0	0	0	0	0
ENSG00000253313	1516	1	0	0	0	0
ENSG00000142700	3225	0	0	0	0	0
ENSG00000134709	6758	0	0	0	1	0
ENSG00000177414	1838	0	1	0	0	0

ENSG00000116783	4719	1	0	0	0	0
ENSG00000137976	1476	0	0	1	0	0
ENSG00000213512	2454	0	0	0	1	0
ENSG00000134184	2512	0	0	1	0	0
ENSG00000134256	3828	0	0	0	0	1
ENSG00000271567	785	1	0	0	0	0
ENSG00000265107	3292	1	0	0	0	0
ENSG00000263464	600	0	1	0	0	0
ENSG00000203852	1656	0	1	0	0	0
ENSG00000143452	2405	0	0	0	1	0
ENSG00000159409	4439	0	0	0	1	0
ENSG00000143520	9124	0	1	0	0	0
ENSG00000159455	612	0	1	0	0	0
ENSG00000203786	2492	0	0	0	0	0
ENSG00000240386	357	0	0	0	0	0
ENSG00000169418	5003	0	0	1	0	0
ENSG00000143595	2292	0	0	0	0	1
ENSG00000163239	2706	0	0	0	1	0
ENSG00000173080	1240	0	0	0	0	1
ENSG00000132698	1699	0	0	0	0	0
ENSG00000260238	1007	0	0	0	0	0
ENSG00000242252	1190	0	1	0	0	0
ENSG00000189030	676	0	1	0	0	0
ENSG00000125462	12314	0	0	0	0	0
ENSG00000198400	4356	0	0	0	0	0
ENSG00000253831	1976	0	1	0	0	0
ENSG00000163568	2009	0	0	1	0	0
ENSG00000085552	6628	0	0	1	0	0
ENSG00000177807	5293	0	0	0	0	0
ENSG00000143194	3148	0	0	0	1	0
ENSG00000198771	5718	0	0	0	0	1
ENSG00000198734	7158	0	0	0	0	0
ENSG00000180999	1725	0	1	0	0	0
ENSG00000203737	1088	0	0	0	1	0
ENSG00000198216	17771	0	0	0	1	0
ENSG00000157060	2982	0	0	1	0	0
ENSG00000173627	2653	0	1	0	0	0
ENSG00000159166	3482	0	1	0	0	0
ENSG00000163435	7191	0	0	0	0	0
ENSG00000143851	5906	0	0	1	0	0
ENSG00000133055	1805	0	0	1	0	0
ENSG00000162896	4376	0	0	0	0	0
ENSG00000123843	3219	0	0	1	0	0

ENSG00000197721	2768	1	0	0	0	0
ENSG00000198570	4266	1	0	0	0	0
ENSG00000196660	5770	0	0	0	1	0
ENSG00000116141	5417	0	0	0	0	1
ENSG00000197520	2603	0	0	0	0	0
ENSG00000186197	3726	0	1	0	0	0
ENSG00000198626	17777	0	0	0	0	1
ENSG00000180287	9545	0	0	0	0	1
ENSG00000135747	3197	0	0	0	0	0
ENSG00000169224	4680	0	0	0	0	1
ENSG00000177462	939	1	0	0	0	0
ENSG00000084674	14828	0	0	0	0	1
ENSG00000280034	375	0	0	0	1	0
ENSG00000189350	19816	1	0	0	0	0
ENSG00000205221	3710	0	0	1	0	0
ENSG00000170577	2205	0	0	0	0	0
ENSG00000178021	3089	1	0	0	0	0
ENSG00000163217	1584	0	0	0	1	0
ENSG00000169605	1149	1	0	0	0	0
ENSG00000135638	5516	0	1	0	0	0
ENSG00000144035	947	0	0	0	0	0
ENSG00000274049	2040	0	0	1	0	0
ENSG00000214336	2804	0	0	0	0	0
ENSG00000188886	1654	0	0	0	0	0
ENSG00000279515	690	0	0	0	0	0
ENSG00000256671	2553	0	0	0	0	1
ENSG00000144130	4225	0	0	0	0	1
ENSG00000136688	1380	0	0	0	1	0
ENSG00000163064	2886	0	0	0	1	0
ENSG00000144119	2043	0	0	0	0	0
ENSG00000163075	9655	0	1	0	0	0
ENSG00000281676	42	0	0	0	0	1
ENSG00000054219	8798	0	0	0	0	0
ENSG00000115290	2958	0	0	0	0	1
ENSG00000204334	601	0	0	1	0	0
ENSG00000175879	2629	1	0	0	0	0
ENSG00000281192	72	0	0	0	0	1
ENSG00000152430	4004	0	1	0	0	0
ENSG00000144410	1209	0	0	0	1	0
ENSG00000127831	6920	0	0	0	0	0
ENSG00000135925	3412	0	0	0	0	1
ENSG00000135903	4633	0	1	0	0	0
ENSG00000144460	4828	0	0	0	0	1

ENSG00000185038	5730	0	0	0	0	0
ENSG00000178602	645	0	1	0	0	0
ENSG00000172478	2772	0	0	0	0	0
ENSG00000144550	2335	0	0	0	0	0
ENSG00000183960	6148	0	0	0	0	0
ENSG00000206557	8685	0	0	0	1	0
ENSG00000183813	3095	0	0	0	0	0
ENSG00000168329	3655	0	0	1	0	0
ENSG00000157119	2517	0	1	0	0	0
ENSG00000180432	4441	0	0	1	0	0
ENSG00000163810	5443	0	0	0	0	0
ENSG00000173578	5403	0	0	0	1	0
ENSG00000012223	4247	0	0	1	0	0
ENSG00000163825	1321	1	0	0	0	0
ENSG00000241186	5013	0	0	0	1	0
ENSG00000187492	3163	0	0	1	0	0
ENSG00000179564	1494	0	0	0	0	1
ENSG00000272104	408	0	1	0	0	0
ENSG00000239732	3870	0	0	0	0	0
ENSG00000010319	5011	0	1	0	0	0
ENSG00000055955	5462	0	1	0	0	0
ENSG00000186451	2413	0	0	0	1	0
ENSG00000064835	1569	0	0	0	0	1
ENSG00000235156	668	1	0	0	0	0
ENSG00000163530	1383	1	0	0	0	0
ENSG00000144837	2202	0	0	0	1	0
ENSG00000145103	2953	0	0	1	0	0
ENSG00000189366	843	0	1	0	0	0
ENSG00000180767	1789	0	0	1	0	0
ENSG00000163914	2592	0	0	0	0	0
ENSG00000170893	1978	0	0	0	0	1
ENSG00000169313	1859	0	1	0	0	0
ENSG00000188306	1827	1	0	0	0	0
ENSG00000197584	3542	1	0	0	0	0
ENSG00000181449	2513	0	0	0	0	0
ENSG00000172578	10546	0	0	0	0	1
ENSG00000145192	2353	0	1	0	0	0
ENSG00000181092	4613	0	0	0	1	0
ENSG00000145107	1899	0	0	0	0	0
ENSG00000109684	6206	1	0	0	0	0
ENSG00000137440	1359	0	0	0	1	0
ENSG00000137441	1250	0	0	1	0	0
ENSG00000177752	1813	0	0	0	0	0

ENSG00000109255	975	0	0	0	1	0
ENSG00000087128	2138	0	0	0	0	1
ENSG00000156096	2489	0	0	0	0	0
ENSG00000171209	836	1	0	0	0	0
ENSG00000171195	2798	0	0	0	0	1
ENSG00000056291	2359	0	0	0	0	0
ENSG00000163737	604	0	0	0	0	0
ENSG00000152208	7508	0	0	0	1	0
ENSG00000198099	2453	0	0	0	0	1
ENSG00000248144	1860	1	0	0	0	0
ENSG00000168743	6117	0	0	0	0	1
ENSG00000109471	1119	0	0	0	0	0
ENSG00000151475	1841	1	0	0	0	0
ENSG00000145423	2032	0	0	0	1	0
ENSG00000121207	7562	0	0	1	0	0
ENSG00000256394	1692	0	1	0	0	0
ENSG00000168843	5355	0	0	0	1	0
ENSG00000185758	663	1	0	0	0	0
ENSG00000088926	3364	0	0	0	1	0
ENSG00000205097	2103	0	1	0	0	0
ENSG00000185028	1573	0	0	0	0	0
ENSG00000281325	1802	0	0	0	1	0
ENSG00000113100	4203	0	0	1	0	0
ENSG00000273294	3424	0	1	0	0	0
ENSG00000082074	6100	0	0	0	0	0
ENSG00000016082	2882	0	1	0	0	0
ENSG00000164287	4496	1	0	0	0	0
ENSG00000280447	375	0	0	0	1	0
ENSG00000134061	5668	1	0	0	0	0
ENSG00000122012	12028	0	0	0	0	0
ENSG00000145692	3519	1	0	0	0	0
ENSG00000145721	4115	0	0	0	0	1
ENSG00000145794	9007	0	0	0	0	1
ENSG00000186367	2354	0	0	0	0	1
ENSG00000239642	1671	0	0	1	0	0
ENSG00000113520	818	0	1	0	0	0
ENSG00000205089	3162	0	0	1	0	0
ENSG00000198944	3211	1	0	0	0	0
ENSG00000145826	2175	1	0	0	0	0
ENSG00000061492	2089	0	0	0	0	0
ENSG00000281227	48	0	0	0	0	0
ENSG00000204965	7114	0	0	0	0	0
ENSG00000251664	5454	0	0	0	0	0

ENSG00000120329	1417	1	0	0	0	0
ENSG00000275740	3311	0	0	0	0	1
ENSG00000091010	1182	1	0	0	0	0
ENSG00000132915	7415	0	0	0	0	0
ENSG00000279109	69	1	0	0	0	0
ENSG00000186335	4263	0	0	0	0	0
ENSG00000145888	2434	0	0	0	1	0
ENSG00000113302	2761	0	0	1	0	0
ENSG00000182132	3126	0	0	0	1	0
ENSG00000113749	2565	0	0	0	0	0
ENSG00000170074	8528	0	0	0	0	1
ENSG00000204677	4285	0	0	0	0	1
ENSG00000164304	3512	0	0	1	0	0
ENSG00000259040	3302	0	0	0	1	0
ENSG00000181355	4807	0	0	0	1	0
ENSG00000153157	3962	1	0	0	0	0
ENSG00000278705	438	0	0	1	0	0
ENSG00000124557	2895	1	0	0	0	0
ENSG00000274997	387	0	1	0	0	0
ENSG00000158553	3354	1	0	0	0	0
ENSG00000273703	381	1	0	0	0	0
ENSG00000275126	312	0	0	0	0	1
ENSG00000204657	1584	0	0	1	0	0
ENSG00000204539	2552	0	0	1	0	0
ENSG00000226979	1737	0	0	0	0	0
ENSG00000232810	1676	0	0	0	1	0
ENSG00000227507	1535	0	0	0	1	0
ENSG00000204475	1212	0	0	1	0	0
ENSG00000204287	1280	0	0	0	0	0
ENSG00000250264	2705	0	0	0	1	0
ENSG00000231389	5748	0	0	0	0	0
ENSG00000270800	876	0	0	0	0	0
ENSG00000197753	2961	0	0	0	0	0
ENSG00000180316	2567	0	1	0	0	0
ENSG00000124721	15055	0	1	0	0	0
ENSG00000164626	3756	0	0	1	0	0
ENSG00000124701	1151	0	0	0	0	0
ENSG00000171611	1168	0	0	0	1	0
ENSG00000146215	2453	1	0	0	0	0
ENSG00000181577	3917	0	0	0	0	0
ENSG00000137225	3549	0	0	0	0	0
ENSG00000112782	8997	0	0	0	0	1
ENSG00000112796	4264	0	1	0	0	0

ENSG00000170950	1254	0	0	1	0	0
ENSG00000168143	3167	0	1	0	0	0
ENSG00000243501	1636	0	1	0	0	0
ENSG00000256980	953	0	0	0	1	0
ENSG00000272514	3613	1	0	0	0	0
ENSG00000118432	6357	0	0	0	0	0
ENSG00000186231	4124	0	1	0	0	0
ENSG00000152034	2392	0	0	0	0	1
ENSG00000112246	9274	0	0	0	0	0
ENSG00000187772	5505	0	0	0	1	0
ENSG00000004809	3907	0	0	0	0	1
ENSG00000182747	2359	0	0	0	1	0
ENSG00000257065	762	0	0	0	1	0
ENSG00000074771	2042	0	1	0	0	0
ENSG00000112530	2612	1	0	0	0	0
ENSG00000112539	2240	0	0	1	0	0
ENSG00000187546	3587	0	0	0	0	0
ENSG00000106511	2500	0	0	0	0	1
ENSG00000171243	2473	0	1	0	0	0
ENSG00000229937	1090	0	0	0	0	0
ENSG00000281889	123	0	0	0	1	0
ENSG00000196335	6183	0	0	0	1	0
ENSG00000106069	10660	0	0	0	0	0
ENSG00000122574	6065	0	0	0	0	1
ENSG00000106631	1458	0	0	1	0	0
ENSG00000164746	2275	0	0	0	0	1
ENSG00000158517	3462	1	0	0	0	0
ENSG00000243566	2660	0	0	0	0	1
ENSG00000185040	3611	0	1	0	0	0
ENSG00000160868	3141	0	0	0	0	1
ENSG00000021461	2637	0	0	0	0	1
ENSG00000077080	2389	1	0	0	0	0
ENSG00000106436	1009	0	0	0	0	0
ENSG00000187416	1900	0	1	0	0	0
ENSG00000172209	3683	1	0	0	0	0
ENSG00000281656	48	0	0	0	1	0
ENSG00000106328	3214	1	0	0	0	0
ENSG00000227471	3325	0	0	0	0	0
ENSG00000214102	3061	1	0	0	0	0
ENSG00000197993	3923	0	1	0	0	0
ENSG00000106526	6460	0	0	0	1	0
ENSG00000171115	4184	0	0	0	0	1
ENSG00000185960	4384	0	0	0	0	0

ENSG00000198223	3830	0	0	0	1	0
ENSG00000196433	1846	1	0	0	0	0
ENSG00000130234	3926	0	0	1	0	0
ENSG00000169906	455	0	0	0	0	0
ENSG00000147036	10298	0	0	1	0	0
ENSG00000279849	375	0	1	0	0	0
ENSG00000147113	6720	1	0	0	0	0
ENSG00000130988	2811	1	0	0	0	0
ENSG00000165583	1668	0	1	0	0	0
ENSG00000158578	2134	0	0	0	0	1
ENSG00000238269	507	0	0	0	1	0
ENSG00000050030	11757	0	0	0	0	0
ENSG00000078596	2051	0	0	0	1	0
ENSG00000184905	1666	0	0	1	0	0
ENSG00000204065	1168	0	0	0	1	0
ENSG00000188828	2722	0	1	0	0	0
ENSG00000123576	1495	1	0	0	0	0
ENSG00000147223	1190	0	0	0	1	0
ENSG00000198088	1867	0	0	0	0	0
ENSG00000101890	3714	0	0	0	0	0
ENSG00000183918	2450	0	0	0	0	1
ENSG00000147255	6818	0	0	0	0	0
ENSG00000147257	2711	0	0	0	0	0
ENSG00000227234	469	0	0	0	0	0
ENSG00000267978	1921	0	0	1	0	0
ENSG00000171116	1963	0	0	0	1	0
ENSG00000123584	1814	0	0	0	1	0
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ENSG00000183837	3748	0	0	0	0	1
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ENSG00000143595	0
ENSG00000163239	0
ENSG00000173080	0
ENSG00000132698	1
ENSG00000260238	1
ENSG00000242252	0
ENSG00000189030	0
ENSG00000125462	1
ENSG00000198400	1
ENSG00000253831	0
ENSG00000163568	0
ENSG00000085552	0
ENSG00000177807	1
ENSG00000143194	0
ENSG00000198771	0
ENSG00000198734	1
ENSG00000180999	0
ENSG00000203737	0
ENSG00000198216	0

ENSG00000157060	0
ENSG00000173627	0
ENSG00000159166	0
ENSG00000163435	1
ENSG00000143851	0
ENSG00000133055	0
ENSG00000162896	1
ENSG00000123843	0
ENSG00000197721	0
ENSG00000198570	0
ENSG00000196660	0
ENSG00000116141	0
ENSG00000197520	1
ENSG00000186197	0
ENSG00000198626	0
ENSG00000180287	0
ENSG00000135747	1
ENSG00000169224	0
ENSG00000177462	0
ENSG00000084674	0
ENSG00000280034	0
ENSG00000189350	0
ENSG00000205221	0
ENSG00000170577	1
ENSG00000178021	0
ENSG00000163217	0
ENSG00000169605	0
ENSG00000135638	0
ENSG00000144035	1
ENSG00000274049	0
ENSG00000214336	1
ENSG00000188886	1
ENSG00000279515	1
ENSG00000256671	0
ENSG00000144130	0
ENSG00000136688	0
ENSG00000163064	0
ENSG00000144119	1
ENSG00000163075	0
ENSG00000281676	0
ENSG00000054219	1
ENSG00000115290	0
ENSG00000204334	0

ENSG00000175879	0
ENSG00000281192	0
ENSG00000152430	0
ENSG00000144410	0
ENSG00000127831	1
ENSG00000135925	0
ENSG00000135903	0
ENSG00000144460	0
ENSG00000185038	1
ENSG00000178602	0
ENSG00000172478	1
ENSG00000144550	1
ENSG00000183960	1
ENSG00000206557	0
ENSG00000183813	1
ENSG00000168329	0
ENSG00000157119	0
ENSG00000180432	0
ENSG00000163810	1
ENSG00000173578	0
ENSG00000012223	0
ENSG00000163825	0
ENSG00000241186	0
ENSG00000187492	0
ENSG00000179564	0
ENSG00000272104	0
ENSG00000239732	1
ENSG00000010319	0
ENSG00000055955	0
ENSG00000186451	0
ENSG00000064835	0
ENSG00000235156	0
ENSG00000163530	0
ENSG00000144837	0
ENSG00000145103	0
ENSG00000189366	0
ENSG00000180767	0
ENSG00000163914	1
ENSG00000170893	0
ENSG00000169313	0
ENSG00000188306	0
ENSG00000197584	0
ENSG00000181449	1

ENSG00000172578	0
ENSG00000145192	0
ENSG00000181092	0
ENSG00000145107	1
ENSG00000109684	0
ENSG00000137440	0
ENSG00000137441	0
ENSG00000177752	1
ENSG00000109255	0
ENSG00000087128	0
ENSG00000156096	1
ENSG00000171209	0
ENSG00000171195	0
ENSG00000056291	1
ENSG00000163737	1
ENSG00000152208	0
ENSG00000198099	0
ENSG00000248144	0
ENSG00000168743	0
ENSG00000109471	1
ENSG00000151475	0
ENSG00000145423	0
ENSG00000121207	0
ENSG00000256394	0
ENSG00000168843	0
ENSG00000185758	0
ENSG00000088926	0
ENSG00000205097	0
ENSG00000185028	1
ENSG00000281325	0
ENSG00000113100	0
ENSG00000273294	0
ENSG00000082074	1
ENSG00000016082	0
ENSG00000164287	0
ENSG00000280447	0
ENSG00000134061	0
ENSG00000122012	1
ENSG00000145692	0
ENSG00000145721	0
ENSG00000145794	0
ENSG00000186367	0
ENSG00000239642	0

ENSG00000113520	0
ENSG00000205089	0
ENSG00000198944	0
ENSG00000145826	0
ENSG00000061492	1
ENSG00000281227	1
ENSG00000204965	1
ENSG00000251664	1
ENSG00000120329	0
ENSG00000275740	0
ENSG00000091010	0
ENSG00000132915	1
ENSG00000279109	0
ENSG00000186335	1
ENSG00000145888	0
ENSG00000113302	0
ENSG00000182132	0
ENSG00000113749	1
ENSG00000170074	0
ENSG00000204677	0
ENSG00000164304	0
ENSG00000259040	0
ENSG00000181355	0
ENSG00000153157	0
ENSG00000278705	0
ENSG00000124557	0
ENSG00000274997	0
ENSG00000158553	0
ENSG00000273703	0
ENSG00000275126	0
ENSG00000204657	0
ENSG00000204539	0
ENSG00000226979	1
ENSG00000232810	0
ENSG00000227507	0
ENSG00000204475	0
ENSG00000204287	1
ENSG00000250264	0
ENSG00000231389	1
ENSG00000270800	1
ENSG00000197753	1
ENSG00000180316	0
ENSG00000124721	0

ENSG00000164626	0
ENSG00000124701	1
ENSG00000171611	0
ENSG00000146215	0
ENSG00000181577	1
ENSG00000137225	1
ENSG00000112782	0
ENSG00000112796	0
ENSG00000170950	0
ENSG00000168143	0
ENSG00000243501	0
ENSG00000256980	0
ENSG00000272514	0
ENSG00000118432	1
ENSG00000186231	0
ENSG00000152034	0
ENSG00000112246	1
ENSG00000187772	0
ENSG00000004809	0
ENSG00000182747	0
ENSG00000257065	0
ENSG00000074771	0
ENSG00000112530	0
ENSG00000112539	0
ENSG00000187546	1
ENSG00000106511	0
ENSG00000171243	0
ENSG00000229937	1
ENSG00000281889	0
ENSG00000196335	0
ENSG00000106069	1
ENSG00000122574	0
ENSG00000106631	0
ENSG00000164746	0
ENSG00000158517	0
ENSG00000243566	0
ENSG00000185040	0
ENSG00000160868	0
ENSG00000021461	0
ENSG00000077080	0
ENSG00000106436	1
ENSG00000187416	0
ENSG00000172209	0

ENSG00000281656	0
ENSG00000106328	0
ENSG00000227471	1
ENSG00000214102	0
ENSG00000197993	0
ENSG00000106526	0
ENSG00000171115	0
ENSG00000185960	1
ENSG00000198223	0
ENSG00000196433	0
ENSG00000130234	0
ENSG00000169906	1
ENSG00000147036	0
ENSG00000279849	0
ENSG00000147113	0
ENSG00000130988	0
ENSG00000165583	0
ENSG00000158578	0
ENSG00000238269	0
ENSG00000050030	1
ENSG00000078596	0
ENSG00000184905	0
ENSG00000204065	0
ENSG00000188828	0
ENSG00000123576	0
ENSG00000147223	0
ENSG00000198088	1
ENSG00000101890	1
ENSG00000183918	0
ENSG00000147255	1
ENSG00000147257	1
ENSG00000227234	1
ENSG00000267978	0
ENSG00000171116	0
ENSG00000123584	0
ENSG00000156009	0
ENSG00000183837	0
ENSG00000130829	1
ENSG00000203870	0
ENSG00000223443	0
ENSG00000168546	0
ENSG00000248235	1
ENSG00000015592	0

ENSG00000189233	0
ENSG00000214050	0
ENSG00000188778	0
ENSG00000196166	0
ENSG00000104371	1
ENSG00000104237	0
ENSG00000172817	0
ENSG00000261787	0
ENSG00000076554	0
ENSG00000147606	0
ENSG00000174226	1
ENSG00000147647	1
ENSG00000212993	0
ENSG00000254402	0
ENSG00000183784	0
ENSG00000205549	0
ENSG00000107105	1
ENSG00000165269	1
ENSG00000256966	0
ENSG00000070601	0
ENSG00000183148	0
ENSG00000273514	0
ENSG00000107242	0
ENSG00000119125	0
ENSG00000165023	0
ENSG00000148123	1
ENSG00000188386	0
ENSG00000229314	0
ENSG00000228278	0
ENSG00000165702	0
ENSG00000107147	0
ENSG00000180549	0
ENSG00000184709	0
ENSG00000197768	0
ENSG00000278919	0
ENSG00000180176	0
ENSG00000184698	0
ENSG00000180919	0
ENSG00000265264	0
ENSG00000170790	0
ENSG00000170782	1
ENSG00000170683	0
ENSG00000166405	0

ENSG00000166407	0
ENSG00000133800	0
ENSG00000006611	0
ENSG00000179817	0
ENSG00000173432	1
ENSG00000110786	0
ENSG00000255359	0
ENSG00000148942	0
ENSG00000052850	0
ENSG00000066336	0
ENSG00000196666	0
ENSG00000134817	0
ENSG00000254462	0
ENSG00000186509	1
ENSG00000214787	1
ENSG00000156738	0
ENSG00000110448	0
ENSG00000133328	0
ENSG00000219435	0
ENSG00000273003	0
ENSG00000204710	1
ENSG00000173237	0
ENSG00000256514	0
ENSG00000204571	0
ENSG00000182103	0
ENSG00000077498	0
ENSG00000255501	0
ENSG00000170290	0
ENSG00000110777	0
ENSG00000255292	0
ENSG00000149295	0
ENSG00000255663	1
ENSG00000137634	0
ENSG00000110324	1
ENSG00000176302	0
ENSG00000137709	0
ENSG00000149418	0
ENSG00000166105	0
ENSG00000282246	0
ENSG00000148482	0
ENSG00000078114	0
ENSG00000077327	1
ENSG00000263639	0

ENSG00000204175	0
ENSG00000243251	0
ENSG00000165443	1
ENSG00000227877	0
ENSG00000107736	0
ENSG00000138308	0
ENSG00000185303	0
ENSG00000133661	0
ENSG00000185737	1
ENSG00000204022	0
ENSG00000138136	0
ENSG00000107831	1
ENSG00000148795	1
ENSG00000185933	0
ENSG00000254536	0
ENSG00000111704	0
ENSG00000150045	0
ENSG00000213809	0
ENSG00000212128	1
ENSG00000256188	0
ENSG00000121335	1
ENSG00000273079	0
ENSG00000151490	0
ENSG00000111404	0
ENSG00000177359	0
ENSG00000185610	0
ENSG00000139223	0
ENSG00000197376	0
ENSG00000139549	0
ENSG00000135406	0
ENSG00000086159	0
ENSG00000123388	0
ENSG00000139572	0
ENSG00000172551	0
ENSG00000257390	0
ENSG00000139540	0
ENSG00000175336	0
ENSG00000166856	1
ENSG00000166866	0
ENSG00000166148	0
ENSG00000185306	0
ENSG00000156076	0
ENSG00000090382	0

ENSG00000180318	0
ENSG00000182050	0
ENSG00000197651	1
ENSG00000083782	0
ENSG00000183395	0
ENSG00000111783	0
ENSG00000089116	0
ENSG00000089250	1
ENSG00000170890	0
ENSG00000120669	1
ENSG00000250709	0
ENSG00000080618	0
ENSG00000152207	0
ENSG00000152192	1
ENSG00000134873	1
ENSG00000182968	0
ENSG00000176198	0
ENSG00000165799	0
ENSG00000255804	0
ENSG00000259522	1
ENSG00000129467	0
ENSG00000092009	0
ENSG00000100453	0
ENSG00000165496	0
ENSG00000258644	0
ENSG00000182732	0
ENSG00000279461	0
ENSG00000165548	0
ENSG00000280308	1
ENSG00000197734	0
ENSG00000165409	0
ENSG00000170099	0
ENSG00000133937	0
ENSG00000140107	0
ENSG00000156414	0
ENSG00000213145	0
ENSG00000261739	0
ENSG00000261247	0
ENSG00000134160	0
ENSG00000249931	0
ENSG00000184507	0
ENSG00000255192	0
ENSG00000175779	1

ENSG00000137843	0
ENSG00000233041	1
ENSG00000159337	0
ENSG00000223572	0
ENSG00000074803	1
ENSG00000170236	0
ENSG00000103569	0
ENSG00000171989	1
ENSG00000205502	1
ENSG00000259316	0
ENSG00000183324	0
ENSG00000080644	0
ENSG00000260836	0
ENSG00000250021	0
ENSG00000140557	0
ENSG00000183571	1
ENSG00000127588	0
ENSG00000167945	0
ENSG00000103355	0
ENSG00000213937	0
ENSG00000130182	0
ENSG00000103313	0
ENSG00000224712	0
ENSG00000134398	1
ENSG00000233232	0
ENSG00000052344	1
ENSG00000140798	0
ENSG00000102924	0
ENSG00000102970	0
ENSG00000070729	0
ENSG00000159723	0
ENSG00000260914	0
ENSG00000157335	0
ENSG00000280252	0
ENSG00000260537	0
ENSG00000140835	0
ENSG00000280264	0
ENSG00000103089	1
ENSG00000135702	0
ENSG00000168589	0
ENSG00000168418	1
ENSG00000129993	0
ENSG00000126856	0

ENSG00000180090	0
ENSG00000108381	0
ENSG00000262304	0
ENSG00000108405	0
ENSG00000167741	0
ENSG00000182896	1
ENSG00000281523	1
ENSG00000179593	0
ENSG00000125414	0
ENSG00000189375	0
ENSG00000171931	0
ENSG00000154016	1
ENSG00000142494	0
ENSG00000108255	0
ENSG00000181374	0
ENSG00000270806	0
ENSG00000270379	0
ENSG00000277363	0
ENSG00000275489	1
ENSG00000204952	0
ENSG00000161405	0
ENSG00000171431	0
ENSG00000006059	0
ENSG00000108417	0
ENSG00000108759	0
ENSG00000186847	0
ENSG00000184502	0
ENSG00000161610	0
ENSG00000161649	0
ENSG00000179673	1
ENSG00000170703	0
ENSG00000108813	1
ENSG00000049283	0
ENSG00000153930	0
ENSG00000121053	1
ENSG00000005381	0
ENSG00000007312	1
ENSG00000007314	0
ENSG00000091583	0
ENSG00000204347	0
ENSG00000257008	0
ENSG00000167850	0
ENSG00000186407	0

ENSG00000182040	0
ENSG00000259120	0
ENSG00000257949	0
ENSG00000167880	0
ENSG00000185332	0
ENSG00000182459	0
ENSG00000141579	0
ENSG00000079101	0
ENSG00000185231	1
ENSG00000134755	0
ENSG00000101746	0
ENSG00000101489	0
ENSG00000206181	0
ENSG00000267699	0
ENSG00000141655	0
ENSG00000166573	1
ENSG00000256463	0
ENSG00000198326	0
ENSG00000088827	1
ENSG00000172264	1
ENSG00000101441	0
ENSG00000149435	0
ENSG00000077984	0
ENSG00000260903	0
ENSG00000254806	0
ENSG00000182931	0
ENSG00000168634	0
ENSG00000101180	1
ENSG00000101197	0
ENSG00000185198	0
ENSG00000077009	0
ENSG00000105251	0
ENSG00000105428	0
ENSG00000267157	0
ENSG00000171124	0
ENSG00000267314	0
ENSG00000267952	0
ENSG00000188000	1
ENSG00000267303	0
ENSG00000076662	0
ENSG00000130167	1
ENSG00000196361	0
ENSG00000213297	0

ENSG00000188032	0
ENSG00000105143	0
ENSG00000186529	0
ENSG00000186115	0
ENSG00000171903	0
ENSG00000196684	0
ENSG00000268790	0
ENSG00000105641	0
ENSG00000268173	0
ENSG00000269067	0
ENSG00000021488	0
ENSG00000089356	1
ENSG00000266964	0
ENSG00000105679	0
ENSG00000188223	1
ENSG00000105261	0
ENSG00000171777	1
ENSG00000182472	0
ENSG00000171747	0
ENSG00000262484	1
ENSG00000188505	0
ENSG00000198077	0
ENSG00000221826	1
ENSG00000124467	0
ENSG00000237452	0
ENSG00000268434	0
ENSG00000104941	0
ENSG00000170608	0
ENSG00000008438	0
ENSG00000204866	0
ENSG00000118156	0
ENSG00000177202	1
ENSG00000189052	0
ENSG00000261949	0
ENSG00000126467	0
ENSG00000269404	0
ENSG00000086967	0
ENSG00000167755	0
ENSG00000167759	0
ENSG00000129437	0
ENSG00000269403	0
ENSG00000198633	0
ENSG00000268964	0

```
ENSG00000129991        0
ENSG00000180043        0
ENSG00000185792        0
ENSG00000223638        0
ENSG00000160505        0
ENSG00000197213        1
ENSG00000092377        0
ENSG00000154620        0
ENSG00000279115        0
ENSG00000099960        0
ENSG00000128322        0
ENSG00000128218        0
ENSG00000100122        0
ENSG00000196431        0
ENSG00000099985        1
ENSG00000128253        0
ENSG00000128310        0
ENSG00000128383        0
ENSG00000100346        1
ENSG00000188263        0
ENSG00000217442        0
ENSG00000100312        0
ENSG00000185272        0
ENSG00000155307        1
ENSG00000205929        0
ENSG00000159197        0
ENSG00000157554        0
ENSG00000183421        1
ENSG00000278704        0
ENSG00000277400        0
```

Q. How many genes do we have left?

```
nrow(counts)
```

```
[1] 19808
```

DESeq setup

```
library(DESeq2)
```

DESeq Analysis

```
dds <- DESeqDataSetFromMatrix(countData = counts,
                              colData = metadata,
                              design = ~condition)
```

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

```
dds
```

```
class: DESeqDataSet
dim: 19808 6
metadata(1): version
assays(1): counts
rownames(19808): ENSG00000186092 ENSG00000279928 ... ENSG00000277475
  ENSG00000268674
rowData names(0):
colnames(6): SRR493366 SRR493367 ... SRR493370 SRR493371
colData names(2): condition id
```

```
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)
```

```
head(res)
```

```
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 6 rows and 6 columns
                  baseMean log2FoldChange      lfcSE      stat      pvalue
                 <numeric>      <numeric> <numeric> <numeric>   <numeric>
ENSG00000186092    0.0000             NA        NA        NA          NA
ENSG00000279928    0.0000             NA        NA        NA          NA
ENSG00000279457   29.9136       0.179257  0.324822  0.551863  0.58104205
ENSG00000278566    0.0000             NA        NA        NA          NA
ENSG00000273547    0.0000             NA        NA        NA          NA
ENSG00000187634  183.2296       0.426457  0.140266  3.040350  0.00236304
                       padj
                  <numeric>
ENSG00000186092          NA
ENSG00000279928          NA
ENSG00000279457  0.68707978
ENSG00000278566          NA
ENSG00000273547          NA
ENSG00000187634  0.00516278
```
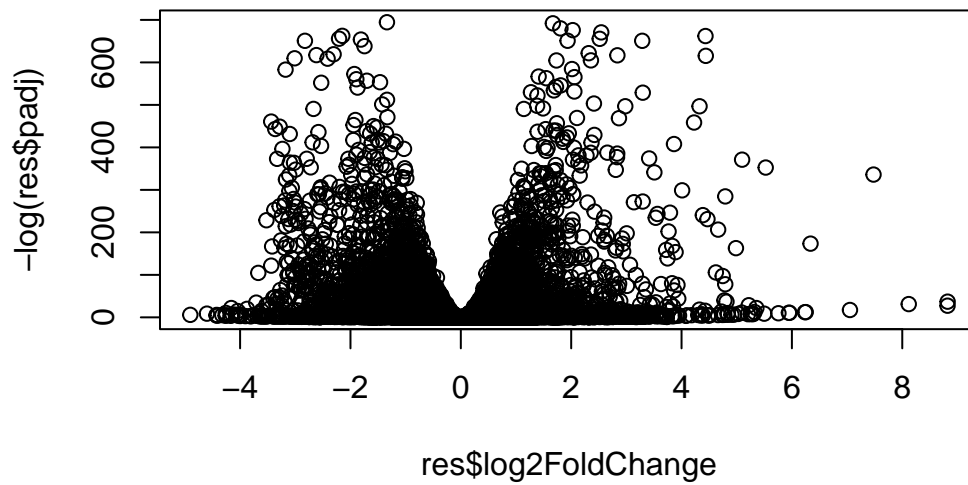
Result Visualization

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

Add Annotataion

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

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

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

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

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

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

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

```
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 8 columns
                  baseMean log2FoldChange      lfcSE        stat      pvalue
                 <numeric>      <numeric>  <numeric>   <numeric>   <numeric>
ENSG00000186092    0.0000             NA         NA          NA          NA
ENSG00000279928    0.0000             NA         NA          NA          NA
ENSG00000279457   29.9136      0.1792571  0.3248216    0.551863 5.81042e-01
ENSG00000278566    0.0000             NA         NA          NA          NA
ENSG00000273547    0.0000             NA         NA          NA          NA
ENSG00000187634  183.2296      0.4264571  0.1402658    3.040350 2.36304e-03
ENSG00000188976 1651.1881     -0.6927205  0.0548465  -12.630158 1.43990e-36
ENSG00000187961  209.6379      0.7297556  0.1318599    5.534326 3.12428e-08
ENSG00000187583   47.2551      0.0405765  0.2718928    0.149237 8.81366e-01
ENSG00000187642   11.9798      0.5428105  0.5215598    1.040744 2.97994e-01
                      padj      symbol      entrez
                 <numeric> <character> <character>
ENSG00000186092         NA       OR4F5       79501
ENSG00000279928         NA          NA          NA
ENSG00000279457 6.87080e-01         NA          NA
ENSG00000278566         NA          NA          NA
ENSG00000273547         NA          NA          NA
ENSG00000187634 5.16278e-03      SAMD11      148398
ENSG00000188976 1.76741e-35       NOC2L       26155
ENSG00000187961 1.13536e-07      KLHL17      339451
ENSG00000187583 9.18988e-01     PLEKHN1       84069
ENSG00000187642 4.03817e-01       PERM1       84808
```

Pathway Analysis

```
library(gage)
```

```
library(gageData)
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).
################################################################################
```

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

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

```
    79501       <NA>       <NA>       <NA>       <NA>     148398
       NA         NA 0.1792571         NA         NA 0.4264571
```

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

```
head(keggres$less, 3)
```

```
                                      p.geomean stat.mean
hsa04110 Cell cycle              7.077982e-06 -4.432593
hsa03030 DNA replication         9.424076e-05 -3.951803
```

```
hsa05130 Pathogenic Escherichia coli infection 1.076420e-04 -3.835716
                                                    p.val       q.val
hsa04110 Cell cycle                            7.077982e-06 0.001507610
hsa03030 DNA replication                       9.424076e-05 0.007642585
hsa05130 Pathogenic Escherichia coli infection 1.076420e-04 0.007642585
                                                 set.size       exp1
hsa04110 Cell cycle                                   124 7.077982e-06
hsa03030 DNA replication                               36 9.424076e-05
hsa05130 Pathogenic Escherichia coli infection         55 1.076420e-04
```

```
head(keggres$greater, 3)
```

```
                                                 p.geomean stat.mean
hsa04740 Olfactory transduction                6.512007e-08  5.345472
hsa04060 Cytokine-cytokine receptor interaction 8.703597e-08  5.313429
hsa05323 Rheumatoid arthritis                  4.392802e-05  4.030693
                                                    p.val       q.val
hsa04740 Olfactory transduction                6.512007e-08 9.269331e-06
hsa04060 Cytokine-cytokine receptor interaction 8.703597e-08 9.269331e-06
hsa05323 Rheumatoid arthritis                  4.392802e-05 3.118889e-03
                                                 set.size       exp1
hsa04740 Olfactory transduction                     354 6.512007e-08
hsa04060 Cytokine-cytokine receptor interaction     263 8.703597e-08
hsa05323 Rheumatoid arthritis                        87 4.392802e-05
```

```
nrow(countData)
```

```
[1] 19808
```

```
nrow(res)
```

```
[1] 19808
```

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

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

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

Save Results

```r
write.csv(res, file="myresults.csv")
```