Differential Gene Expression Analysis

Chuck E. Denniston
Dr.Biologie

Differential Gene Expression Analysis using DeSeq2

This document is a tutorial for differential gene expression analysis using DeSeq2 package. I assume a basic understanding of the development of the software. For more information about the DeSeq2 package, please consult the Bioconductor website and the DeSeq2 Bioconductor Package Page of Google.

Set current working directory where everything will be saved and closed

```
setwd("~/Desktop/CSCC-RNA-Seq/Bioinformatics_and_Genomics_Lecture/Week_10/RNA-Sequencing/Deseq2")
```

Load in a package which will be needed

```
library(DESeq2)
```

1. Load in the count data

```
cscc_count_data <- read.table("GSE124634_CSCC_Illumina_Human_Gene_Expression_Profiling_RNA-Seq_RNA-Seq_Sequencing_Raw_Counts_Human_Skin_Control_patient_all.txt", sep="\t", header = TRUE)
```

The file can now be viewed as a dataframe in the R environment

```
head(cscc_count_data, n=3L)
```

```
  ..... Control
ID ..... GSM3543470
1 ..... 0
2 ..... 0
3 ..... 0
  ..... GSM3543471
1 ..... 0
2 ..... 0
3 ..... 0
  ..... GSM3543472
1 ..... 0
2 ..... 0
3 ..... 0
  .....
```

Transform your factor normalised read counts to log2 scale using a pseudocount of 1 :

```
log2.norm.counts <- log2(norm.counts + 1)
```

can also use the command :

```
log2.norm.counts <- cpm(norm.counts)
```

Control vs A549

```
> head(log2.norm.counts, n = 5)
> head(log2.norm.counts, n = 5)
Transforming raw counts into log2 scale using a pseudocount of 1 :
Log-cpm counts dim: 5 (genes)x24 (samples)
```

Control vs A549

```
> head(log2.norm.counts, n = 5)
> head(log2.norm.counts, n = 5)
Transforming raw counts into log2 scale using a pseudocount of 1 :
Log-cpm counts dim: 5 (genes)x24 (samples)
```

Plotting the normalised counts

```
par(mfrow=c(1,2))
par(mfrow=c(1,2))
boxplot(log2.norm.counts, ylab = "Log2 of cpm and the following two values: pseudocount and cpmnorm", cex.axis=0.7, las=2)
title(main="Log2 transformed read counts")
abline(h=median(log2.norm.counts),col="blue")
text(x=1, y=median(log2.norm.counts), labels="median", pos=3, cex=0.7, col="blue")
boxplot(log2.norm.counts, ylab = "Log2 of cpm and the following two values: pseudocount and cpmnorm", cex.axis=0.7, las=2)
title(main="Log2 transformed read counts")
abline(h=median(log2.norm.counts),col="blue")
text(x=1, y=median(log2.norm.counts), labels="median", pos=3, cex=0.7, col="blue")
```

7. Visualise the normalised data

Plot mean-SD in ggplot2 style :

```
plotSD(log.norm.counts, ylab = "sd", xlab = "mean", main="Normalised read counts")
```

Check for heteroscedasticity (less statistical bias) and variance across that data is homoscedastic, or flat all variables have similar variance. Moreover, data with large differences among the rows of the technical observations often shows heteroskedastic behavior. One way to visually check for heteroskedasticity is to plot the standard deviation against the mean.

```
> library("vsn")
> library("ggplot2")
> meanSdPlot(log.norm.counts,ylab="sd",
                 main="Sequencing depth normalised log2 (read counts )")
                 ylim = c(0,5)
> meanSdPlot(log.norm.counts,ylab="sd",
                 main="Sequencing depth normalised log2 (read counts )")
                 ylim = c(0,5)
```

```
>H1      -1.5    -1.5    -1.5    -1.5    -1.5    -1.5    -1.5    -1.5
>H2      -1.5    -1.5    -1.5    -1.5    -1.5    -1.5    -1.5    -1.5
>H3 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333
>H4 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333
>H5 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333
>H6 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333
>H7 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333 -0.333333
```

```
Seurat.obj.DE <- FindMarkers(Seurat.obj, ident.1 = "Treated")
head(Seurat.obj.DE, n = 3)
```

```
                   p_val avg_log2FC pct.1 pct.2    p_val_adj
Ccl2       0.000000e+00   7.000000 0.994 0.000 0.000000e+00
Ccl7       0.000000e+00   6.000000 0.994 0.000 0.000000e+00
Cxcl10     0.000000e+00   5.000000 0.994 0.000 0.000000e+00
Ccl12      0.000000e+00   4.000000 0.994 0.000 0.000000e+00
Ccl8       0.000000e+00   3.000000 0.994 0.000 0.000000e+00
Ccl6       0.000000e+00   2.000000 0.994 0.000 0.000000e+00
Ccl9       0.000000e+00   1.000000 0.994 0.000 0.000000e+00
Ccl3       0.000000e+00   0.000000 0.994 0.000 0.000000e+00
Ccl4       0.000000e+00  -1.000000 0.000 0.994 0.000000e+00
Ccl5       0.000000e+00  -2.000000 0.000 0.994 0.000000e+00
Cxcl1      0.000000e+00  -3.000000 0.000 0.994 0.000000e+00
Cxcl2      0.000000e+00  -4.000000 0.000 0.994 0.000000e+00
Cxcl3      0.000000e+00  -5.000000 0.000 0.994 0.000000e+00
Cxcl5      0.000000e+00  -6.000000 0.000 0.994 0.000000e+00
Cxcl9      0.000000e+00  -7.000000 0.000 0.994 0.000000e+00
Ccl11      0.000000e+00  -8.000000 0.000 0.994 0.000000e+00
Ccl17      0.000000e+00  -9.000000 0.000 0.994 0.000000e+00
Ccl22      0.000000e+00 -10.000000 0.000 0.994 0.000000e+00
Cxcl13     0.000000e+00 -11.000000 0.000 0.994 0.000000e+00
Ccl24      0.000000e+00 -12.000000 0.000 0.994 0.000000e+00
Cxcl16     0.000000e+00 -13.000000 0.000 0.994 0.000000e+00
Ccl20      0.000000e+00 -14.000000 0.000 0.994 0.000000e+00
Cxcl12     0.000000e+00 -15.000000 0.000 0.994 0.000000e+00
Ccl19      0.000000e+00 -16.000000 0.000 0.994 0.000000e+00
Ccl21      0.000000e+00 -17.000000 0.000 0.994 0.000000e+00
Cxcl14     0.000000e+00 -18.000000 0.000 0.994 0.000000e+00
Ccl27      0.000000e+00 -19.000000 0.000 0.994 0.000000e+00
Cxcl15     0.000000e+00 -20.000000 0.000 0.994 0.000000e+00
```

To Differential expression results for markers in a cluster

```
table(Seurat.obj.DE$avg_log2FC > 0.25)
```

```
FALSE  TRUE
   20    20
```

```
table(Seurat.obj.DE$avg_log2FC > 0.25 & Seurat.obj.DE$p_val_adj<0.05)
```

```
FALSE  TRUE
   20    20
```

Combine the deseq2 results with gc values

```
deseq2.positive <- deseq2.results.ko[deseq2.results.ko$log2FC > 0.25,]
deseq2.positive <- deseq2.positive[deseq2.positive$pvalue < 0.05,]
deseq2.positive <- deseq2.positive[deseq2.positive$baseMean > 1,]
deseq2.positive <- deseq2.positive[deseq2.positive$gene.chr!="chrM",]
```

Sort results based on FC value

```
deseq2.positive <- deseq2.positive[order(deseq2.positive$log2FC),]
deseq2.positive$gene.chr <- factor(deseq2.positive$gene.chr, levels=c("chr1","chr2","chr3","chr4","chr5","chr6","chr7","chr8","chr9","chr10","chr11","chr12","chr13","chr14","chr15","chr16","chr17","chr18","chr19","chrX","chrY"))
deseq2.positive <- deseq2.positive[order(deseq2.positive$gene.chr),]
deseq2.positive$gene.name <- factor(deseq2.positive$gene.name, levels=deseq2.positive$gene.name)
```

Add the deseq2 results to the gc values

```
rbind.deseq2.gene.values <- rbind(deseq2.positive, deseq2.negative)
rbind.deseq2.gene.values$gene.name <- factor(rbind.deseq2.gene.values$gene.name, levels=rbind.deseq2.gene.values$gene.name)
rbind.deseq2.gene.values$gene.chr <- factor(rbind.deseq2.gene.values$gene.chr, levels=c("chr1","chr2","chr3","chr4","chr5","chr6","chr7","chr8","chr9","chr10","chr11","chr12","chr13","chr14","chr15","chr16","chr17","chr18","chr19","chrX","chrY"))
rbind.deseq2.gene.values$gene.name[1:5]
```

```
[1] Ccl2  Ccl7  Ccl8  Ccl9  Ccl6
20 Levels: Ccl2 Ccl7 Ccl8 Ccl9 Ccl6 Ccl3 Ccl4 Ccl5 Cxcl1 Cxcl2 Cxcl3 Cxcl5 Cxcl9 Ccl11 Ccl17 Ccl22 Cxcl13 Ccl24 Cxcl16 Ccl20
```

Take the top 20 genes according to FC values to create their GO and pathway

```
rbind.deseq2.gene.values[rbind.deseq2.gene.values$log2FC > 0.25,]
rbind.deseq2.gene.values[rbind.deseq2.gene.values$log2FC > 0.25,c(1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100,101,102,103,104,105,106,107,108,109,110,111,112,113,114,115,116,117,118,119,120,121,122,123,124,125,126,127,128,129,130,131,132,133,134,135,136,137,138,139,140,141,142,143,144,145,146,147,148,149,150,151,152,153,154,155,156,157,158,159,160,161,162,163,164,165,166,167,168,169,170,171,172,173,174,175,176,177,178,179,180,181,182,183,184,185,186,187,188,189,190,191,192,193,194,195,196,197,198,199,200,201,202,203,204,205,206,207,208,209,210,211,212,213,214,215,216,217,218,219,220,221,222,223,224,225,226,227,228,229,230,231,232,233,234,235,236,237,238,239,240,241,242,243,244,245,246,247,248,249,250,251,252,253,254,255,256,257,258,259,260,261,262,263,264,265,266,267,268,269,270,271,272,273,274,275,276,277,278,279,280,281,282,283,284,285,286,287,288,289,290,291,292,293,294,295,296,297,298,299,300,301,302,303,304,305,306,307,308,309,310,311,312,313,314,315,316,317,318,319,320,321,322,323,324,325,326,327,328,329,330,331,332,333,334,335,336,337,338,339,340,341,342,343,344,345,346,347,348,349,350,351,352,353,354,355,356,357,358,359,360,361,362,363,364,365,366,367,368,369,370,371,372,373,374,375,376,377,378,379,380,381,382,383,384,385,386,387,388,389,390,391,392,393,394,395,396,397,398,399,400,401,402,403,404,405,406,407,408,409,410,411,412,413,414,415,416,417,418,419,420,421,422,423,424,425,426,427,428,429,430,431,432,433,434,435,436,437,438,439,440,441,442,443,444,445,446,447,448,449,450,451,452,453,454,455,456,457,458,459,460,461,462,463,464,465,466,467,468,469,470,471,472,473,474,475,476,477,478,479,480,481,482,483,484,485,486,487,488,489,490,491,492,493,494,495,496,497,498,499,500,501,502,503,504,505,506,507,508,509,510,511,512,513,514,515,516,517,518,519,520,521,522,523,524,525,526,527,528,529,530,531,532,533,534,535,536,537,538,539,540,541,542,543,544,545,546,547,548,549,550,551,552,553,554,555,556,557,558,559,560,561,562,563,564,565,566,567,568,569,570,571,572,573,574,575,576,577,578,579,580,581,582,583,584,585,586,587,588,589,590,591,592,593,594,595,596,597,598,599,600,601,602,603,604,605,606,607,608,609,610,611,612,613,614,615,616,617,618,619,620,621,622,623,624,625,626,627,628,629,630,631,632,633,634,635,636,637,638,639,640,641,642,643,644,645,646,647,648,649,650,651,652,653,654,655,656,657,658,659,660,661,662,663,664,665,666,667,668,669,670,671,672,673,674,675,676,677,678,679,680,681,682,683,684,685,686,687,688,689,690,691,692,693,694,695,696,697,698,699,700,701,702,703,704,705,706,707,708,709,710,711,712,713,714,715,716,717,718,719,720,721,722,723,724,725,726,727,728,729,730,731,732,733,734,735,736,737,738,739,740,741,742,743,744,745,746,747,748,749,750,751,752,753,754,755,756,757,758,759,760,761,762,763,764,765,766,767,768,769,770,771,772,773,774,775,776,777,778,779,780,781,782,783,784,785,786,787,788,789,790,791,792,793,794,795,796,797,798,799,800,801,802,803,804,805,806,807,808,809,810,811,812,813,814,815,816,817,818,819,820,821,822,823,824,825,826,827,828,829,830,831,832,833,834,835,836,837,838,839,840,841,842,843,844,845,846,847,848,849,850,851,852,853,854,855,856,857,858,859,860,861,862,863,864,865,866,867,868,869,870,871,872,873,874,875,876,877,878,879,880,881,882,883,884,885,886,887,888,889,890,891,892,893,894,895,896,897,898,899,900,901,902,903,904,905,906,907,908,909,910,911,912,913,914,915,916,917,918,919,920,921,922,923,924,925,926,927,928,929,930,931,932,933,934,935,936,937,938,939,940,941,942,943,944,945,946,947,948,949,950,951,952,953,954,955,956,957,958,959,960,961,962,963,964,965,966,967,968,969,970,971,972,973,974,975,976,977,978,979,980,981,982,983,984,985,986,987,988,989,990,991,992,993,994,995,996,997,998,999,1000,1001,1002,1003,1004,1005,1006,1007,1008,1009,1010,1011,1012,1013,1014,1015,1016,1017,1018,1019,1020,1021,1022,1023,1024,1025,1026,1027,1028,1029,1030,1031,1032,1033,1034,1035,1036,1037,1038,1039,1040,1041,1042,1043,1044,1045,1046,1047,1048,1049,1050,1051,1052,1053,1054,1055,1056,1057,1058,1059,1060,1061,1062,1063,1064,1065,1066,1067,1068,1069,1070,1071,1072,1073,1074,1075,1076,1077,1078,1079,1080,1081,1082,1083,1084,1085,1086,1087,1088,1089,1090,1091,1092,1093,1094,1095,1096,1097,1098,1099,1100,1101,1102,1103,1104,1105,1106,1107,1108,1109,1110,1111,1112,1113,1114,1115,1116,1117,1118,1119,1120,1121,1122,1123,1124,1125,1126,1127,1128,1129,1130,1131,1132,1133,1134,1135,1136,1137,1138,1139,1140,1141,1142,1143,1144,1145,1146,1147,1148,1149,1150,1151,1152,1153,1154,1155,1156,1157,1158,1159,1160,1161,1162,1163,1164,1165,1166,1167,1168,1169,1170,1171,1172,1173,1174,1175,1176,1177,1178,1179,1180,1181,1182,1183,1184,1185,1186,1187,1188,1189,1190,1191,1192,1193,1194,1195,1196,1197,1198,1199,1200,1201,1202,1203,1204,1205,1206,1207,1208,1209,1210,1211,1212,1213,1214,1215,1216,1217,1218,1219,1220,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[4] "Loading required package: ggplot2"
[5] "Loading required package: ggsankey"
[6] "Loading required package: ggbump"
[7] "Loading required package: ggbreak"
[8] "Loading required package: scales"
[9] "Loading required package: patchwork"
[10] "Loading required package: ggsankeyfier"
[11] "Loading required package: ggforce"
[12] "Loading required package: ggrepel"
[13] "Loading required package: ggsankey"
[14] "Loading required package: ggbump"
[15] "Loading required package: ggbreak"
[16] "Loading required package: scales"
[17] "Loading required package: patchwork"
[18] "Loading required package: ggsankeyfier"
[19] "Loading required package: ggforce"
[20] "Loading required package: ggrepel"
[21] "Loading required package: ggsankeyfier"
[22] "Loading required package: ggforce"
[23] "Loading required package: ggrepel"
[24] "Loading required package: ggsankeyfier"
[25] "Loading required package: ggforce"
[26] "Loading required package: ggrepel"
```

```
                    col="transparent",
                    fill = c(alpha("#7FF75BA",0.5), alpha('#ACE7FF',0.5)), cex=2,
                    # font inside circles
                    fontface = "plain",
                    fontfamily = "sans",
                    # category names
                    cat.cex=2, cat.fontface="bold", cat.default.pos="outer",
                    cat.pos=c(-10,10), cat.dist=c(0.055,0.055), cat.fontfamily="sans",
                    filename=NULL,alpha=0.7, scaled=TRUE)

# have a look at the default plot
grid.newpage()
grid.draw(v)
```

We now know that 314 genes are overlapping and significant in both KOs.

3. Extract the list of genes in each group

```
# have a look at the names in the plot object v
# We are interested in the labels
lapply(v, function(i) i$label)
```

```
## [[1]]
## NULL
##
## [[2]]
## NULL
##
## [[3]]
## NULL
##
## [[4]]
## NULL
##
## [[5]]
## [1] "379"
##
## [[6]]
## [1] "350"
##
## [[7]]
## [1] "314"
##
## [[8]]
## [1] "WTvsKOA"
##
## [[9]]
## [1] "WTvsKOB"
```

```
# Over-write labels (5 to 7 chosen by manual check of labels)
# in KOA only
v[[5]]$label <- paste(setdiff(x$WTvsKOA, x$WTvsKOB), collapse="\n")
only_in_KOA  <- data.frame(paste(setdiff(x$WTvsKOA, x$WTvsKOB)))

# in KOB only
v[[6]]$label <- paste(setdiff(x$WTvsKOB, x$WTvsKOA)  , collapse="\n")
only_in_KOB  <- data.frame(paste(setdiff(x$WTvsKOB, x$WTvsKOA)))

# intersection i.e the genes that are significant in both analyses
v[[7]]$label <- paste(intersect(x$WTvsKOA, x$WTvsKOB), collapse="\n")
KOA_KOB  <- data.frame(paste(intersect(x$WTvsKOA, x$WTvsKOB)))

## Obtain gene names and their values for each list
# overlapping list
KOA_KOBSGeneid <- KOA_KOBSpaste.intersect.x.WTvsKOA..x.WTvsKOB..
KOA_KOB_values <- merge(KOA_KOB, merge_KOA_KOB, by="Geneid")
sum(abs(KOA_KOB_values$padj.WTvsKOB)<0.05)
```

```
## [1] 0
```

```
# Only significant DEG in KOA
only_in_KOASGeneid <- only_in_KOASpaste.setdiff.x.WTvsKOA..x.WTvsKOB..
only_in_KOA_values <- merge(only_in_KOA, merge_KOA_KOB, by="Geneid")

# Only significant DEG in KOB
only_in_KOBSGeneid <- only_in_KOBSpaste.setdiff.x.WTvsKOB..x.WTvsKOA..
only_in_KOB_values <- merge(only_in_KOB, merge_KOA_KOB, by="Geneid")
```

4. Create a column with the mean PC values

```
# Create a column of mean PC values
KOA_KOB_values$meanPC <- (KOA_KOB_values$PC1.WTvsKOA + KOA_KOB_values$PC1.WTvsKOB)/2
```

5. Merge with wt vs all KO file to see if they come up in main analysis

```
KOA_KOB_values_combined <- merge(KOA_KOB_values, merged_pcresults, by="Geneid")
sum(KOA_KOB_values_combined$padj<0.05, na.rm=T)
```

```
## [1] 4
```

6. Sort by mean PC value

```
KOA_KOB_values_combined_sortedPC <- KOA_KOB_values_combined[order(-KOA_KOB_values_combined$meanPC), ]
```

7. Obtain a list of Upregulated and Downregulated genes

```
#### Upregulated genes
upreg_KOA_KOB_overlap <- KOA_KOB_values_combined_sortedPC[KOA_KOB_values_combined_sortedPC$log2FoldChange>0,]
#write.table(upreg_KOA_KOB_overlap, file='../Overlapping_genes_KOA_KOB_analysis_Upregulated.txt', sep='\t', row.n
ames=F, quote=F)

#### Downregulated genes
downreg_KOA_KOB_overlap <- KOA_KOB_values_combined_sortedPC[KOA_KOB_values_combined_sortedPC$log2FoldChange<0,]
#write.table(downreg_KOA_KOB_overlap, file='../Overlapping_genes_KOA_KOB_analysis_Downregulated.txt', sep='\t', r
ow.names=F, quote=F)
```

8. Plot all of the overlapping genes on a heat map

```
row.names(KOA_KOB_values_combined_sortedPC) <- KOA_KOB_values_combined_sortedPC$Geneid
overlapping_genes <- rownames(KOA_KOB_values_combined_sortedPC)
library(plyr)
library(RColorBrewer)
mat <- rlDMatrix[overlapping_genes,]
mat <- mat - rowMeans(mat)
mat2 <- data.frame(mat)
mat2$Geneid <- row.names(mat2)
mat2 <- join(mat2, gtf,by="Geneid")
row.names(mat2) <- mat2$Genesymbol
mat2 <- mat2[,-c(1:13)]
mat2 <- data.matrix(mat2)

hmcol <- colorRampPalette(brewer.pal(11, "RdBu"))(50)
df <- as.data.frame(colData(ddb)[,c("genotype", "condition")])

# scale = "row" converts to z-score scaling within rows
library(gplots)

heatmap.2(mat2, main="Heat map of overlapping genes", cexRow=0.6, labRow=NA,cexCol=0.8, col=hmcol, Rowv=T, Colv=T
, labCol=c("Control", NA,NA,NA,'   KOA',NA,NA,NA,'   KOB',NA,NA,NA), adjCol=c(-3,5,5),srtCol=0, scale="row", de
ndrogram="column", trace="none", margins=c(1, 5), key.title="Counts density", key.xlab="Counts", key.ylab="")
```

9. Plot the top 50 ordered by the mean of PC1 & using the rlDMatrix from WTvsKO study

```
row.names(KOA_KOB_values_combined_sortedPC) <- KOA_KOB_values_combined_sortedPC$Geneid
topgenes <- head(rownames(KOA_KOB_values_combined_sortedPC),50)
mat <- rlDMatrix[topgenes,]
mat <- mat - rowMeans(mat)
mat2 <- data.frame(mat)
mat2$Geneid <- row.names(mat2)
mat2 <- join(mat2, gtf,by="Geneid")
row.names(mat2) <- mat2$Genesymbol
mat2 <- mat2[,-c(1:13)]
mat2 <- data.matrix(mat2)

hmcol <- colorRampPalette(brewer.pal(11, "RdBu"))(50)
df <- as.data.frame(colData(ddb)[,c("genotype", "condition")])

library(gplots)
heatmap.2(mat2, main="Top 50 DEGs", cexRow=0.6, cexCol=0.8, col=hmcol, Rowv=T, Colv=F, labCol=c("Control", NA,NA,
NA,'   KOA',NA,NA,NA,'   KOB',NA,NA,NA), adjCol=c(-2,-45),srtCol=0, scale="row", dendrogram="none", trace="none",
margins=c(1, 5), key.title="Counts density", key.xlab="Counts", key.ylab="")
```

```
# Alternative plotting method
# pheatmap(mat2, annotation_col=df, fontsize_row = 5, fontsize_col = 7, cluster_cols = F,cluster_rows = F, main="
Top 50 DEGs !: P<0.05 & abs(Log2 Fold Change)>2")
```

10. Plot the expression of the top 50 genes on a bar plot - sorted by mean PC1 values

```
library(tibble)
library(tidyr)
library(ggplot2)
library(tidyverse)

KOA_KOB_values_combined_sortedPC_tb <- KOA_KOB_values_combined_sortedPC  %>%
  data.frame() %>%
  rownames_to_column(var="gene") %>%
  as_tibble()

top50_DEGs_genes <- KOA_KOB_values_combined_sortedPC_tb %>%
  arrange(-meanPC) %>%  #Arrange rows by padj values
  pull(gene) %>%        #Extract character vector of ordered genes
  head(n=50)
top50_DEG_genes <- merged_pcresults_sorted[top50_DEGs_genes,]
top50_DEG_genes <- data.frame(top50_DEG_genes)
top50_DEG_genesGeneid <- row.names(top50_DEG_genes)
top50_DEG_genes <- merge(top50_DEG_genes, gtf, by="Geneid")

#change the normalized counts into a tibble
normalized_counts_tb <- norm %>%
  data.frame() %>%
  rownames_to_column(var="gene") %>%
  as_tibble()

top50_DEG_norm <- normalized_counts_tb %>%
  filter(gene %in% top50_DEGs_genes)

gathered_top50_DEGs <- top50_DEG_norm %>%
  tidyr::gather(colnames(top50_DEG_norm)[2:13], key = "sample", value = "normalized_counts")

gathered_top50_DEGs <- inner_join(gathered_top50_DEGs, sample_info, by="sample")
```

```
## Warning: Column `sample` joining character vector and factor, coercing into
## character vector
```

```
names(gathered_top50_DEGs)[1] <- "Geneid"
gathered_top50_DEGs<- inner_join(gtf, gathered_top50_DEGs, by="Geneid")
```

```
## Warning: Column `Geneid` joining factor and character vector, coercing into
## character vector
```

```
gathered_top50_DEGs<- inner_join(merged_pcresults_sorted, gathered_top50_DEGs, by="Geneid")
```

```
## Warning: Column `Geneid` joining factor and character vector, coercing into
## character vector
```

```
#write.table(gathered_top20_DEGs, 'Differential Gene Expression/Top_50_DEGs.csv', row.names=F, quote=F, sep=",",
col.names=T)

#png('BarPlot_Top50_Overlapping_significant_genes.png', width=1200, height=820)
ggplot(gathered_top50_DEGs) +
  geom_point(aes(x = GeneSymbol.x, y = normalized_counts, color = condition)) +
  xlab("Genes") +
  ylab("Regularized log Counts") +
  ggtitle("Top 50 Overlapping Significant Genes (P<0.05 & abs(Log2FC) > 2)") +
  theme_bw() +
  theme(axis.text.x = element_text(angle = 45, hjust = 1, size=6)) +
  theme(plot.title = element_text(hjust = 0.5))
```

```
# dev.off()
```