

# data exploration 3-4

Marie Moriarty

2023-03-04

## Import MAGeCK data set

```
# Import MAGeCK data set
library(readr)
library(ggplot2)

mageck <- read_delim("mageckRRA.gene_summary.txt",
  delim = "\t", escape_double = FALSE,
  trim_ws = TRUE)

## Rows: 19672 Columns: 14
## -- Column specification -----
## Delimiter: "\t"
## chr (1): id
## dbl (13): num, neg|score, neg|p-value, neg|fdr, neg|rank, neg|goodsgrna, neg...
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.

# Reassign column names
colnames(mageck) <- c("id",
  "num",
  "neg.score",
  "neg.p_value",
  "neg.fdr",
  "neg.rank",
  "neg.goodsgrna",
  "neg.lfc",
  "pos.score",
  "pos.p_value",
  "pos.fdr",
  "pos.rank",
  "pos.goodsgrna",
  "lfc"
)

# Change `id` column to all lowercase characters
mageck$id <- tolower(mageck$id)

# Convert goodsgrna to factor
```

```

mageck$num <- as.factor(mageck$num)
mageck$neg.goodsgrna <- as.factor(mageck$neg.goodsgrna)
mageck$pos.goodsgrna <- as.factor(mageck$pos.goodsgrna)

# Delete neg.lfc
mageck <- mageck[,-8]

# view data summary
summary(mageck)

```

```

##      id          num      neg.score      neg.p_value
## Length:19672    1:   11  Min. :0.0000  Min. :0.0000002
## Class :character 2:   10  1st Qu.:0.1466  1st Qu.:0.3637900
## Mode  :character 3:  159  Median :0.4151  Median :0.6424600
##                  4:19490  Mean   :0.4624  Mean   :0.6012542
##                  8:    2  3rd Qu.:0.7843  3rd Qu.:0.8687800
##                           Max.  :1.0000  Max.  :1.0000000
##      neg.fdr      neg.rank      neg.goodsgrna  pos.score
## Min.  :0.000381  Min.   : 1  0:5337      Min.  :0.0000
## 1st Qu.:1.000000  1st Qu.: 4919 1:7395      1st Qu.:0.1667
## Median :1.000000  Median : 9836 2:4928      Median :0.4388
## Mean   :0.993202  Mean   : 9836 3:1693      Mean   :0.4731
## 3rd Qu.:1.000000  3rd Qu.:14754 4: 319       3rd Qu.:0.7905
## Max.   :1.000000  Max.   :19672        Max.  :1.0000
##      pos.p_value      pos.fdr      pos.rank      pos.goodsgrna
## Min.  :0.0000048  Min.  :0.001763  Min.   : 1  0:5157
## 1st Qu.:0.1433900 1st Qu.:0.591756  1st Qu.: 4919 1:7318
## Median :0.3493300  Median :0.731480  Median : 9836 2:4732
## Mean   :0.4113567  Mean   :0.714705  Mean   : 9836 3:1810
## 3rd Qu.:0.6704100  3rd Qu.:0.935922  3rd Qu.:14754 4: 655
## Max.   :1.0000000  Max.   :1.000000  Max.   :19672
##      lfc
## Min.  :-1.781400
## 1st Qu.:-0.151500
## Median :-0.004924
## Mean   : 0.026433
## 3rd Qu. : 0.154968
## Max.   : 3.622500

```

Since the data was already clean, after importing I only switched to more easily referenced variable names. I converted the sgRNA related columns to factor-type variables. I then printed the summary of the data set. One thing that I noticed was that there is a factor level in the `num` column saying that there were two observations that had 8 sgRNAs. These seem to be outliers, so they may need to be excluded before beginning the analysis, but I will consult Dr. Ge beforehand.

```

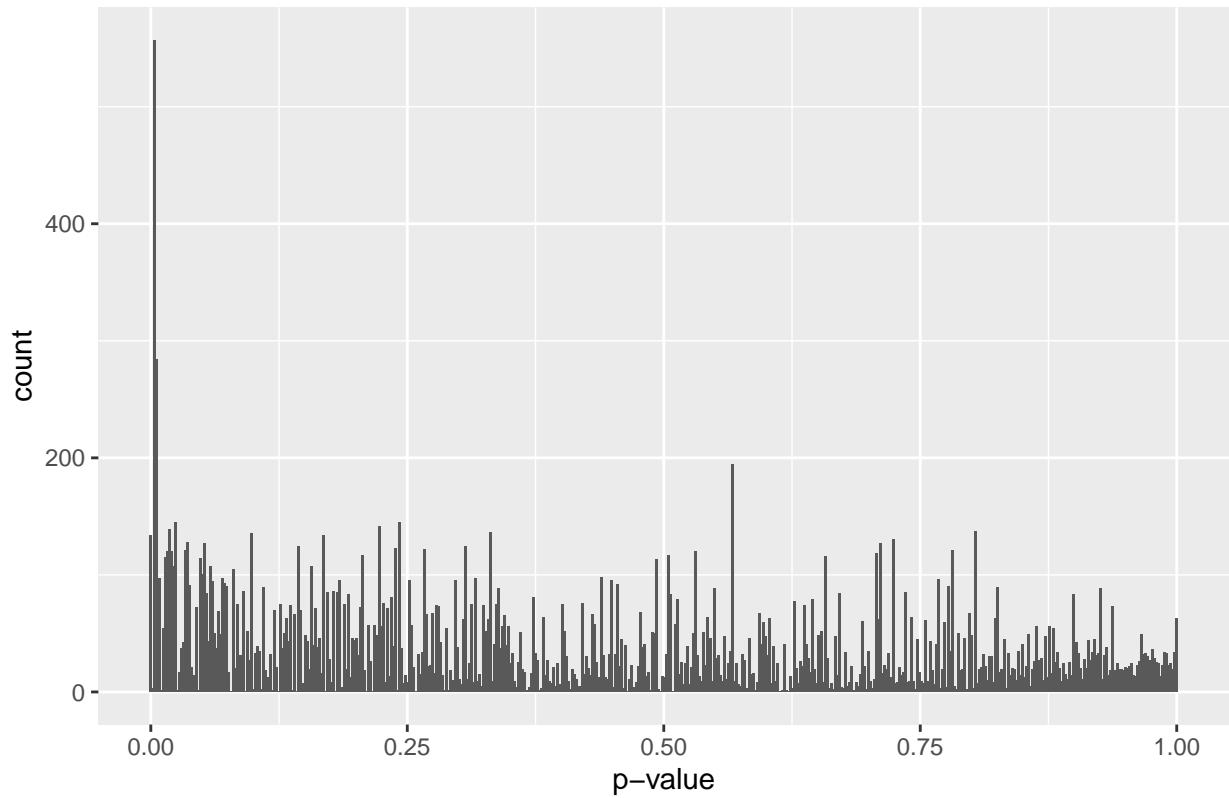
##### p-values

# Distribution of positive selection p-values
ggplot(mageck, aes(x = pos.p_value)) +
  geom_histogram(bins = 500) +

```

```
xlab("p-value") +  
ggtitle("Distribution of positive selection p-values")
```

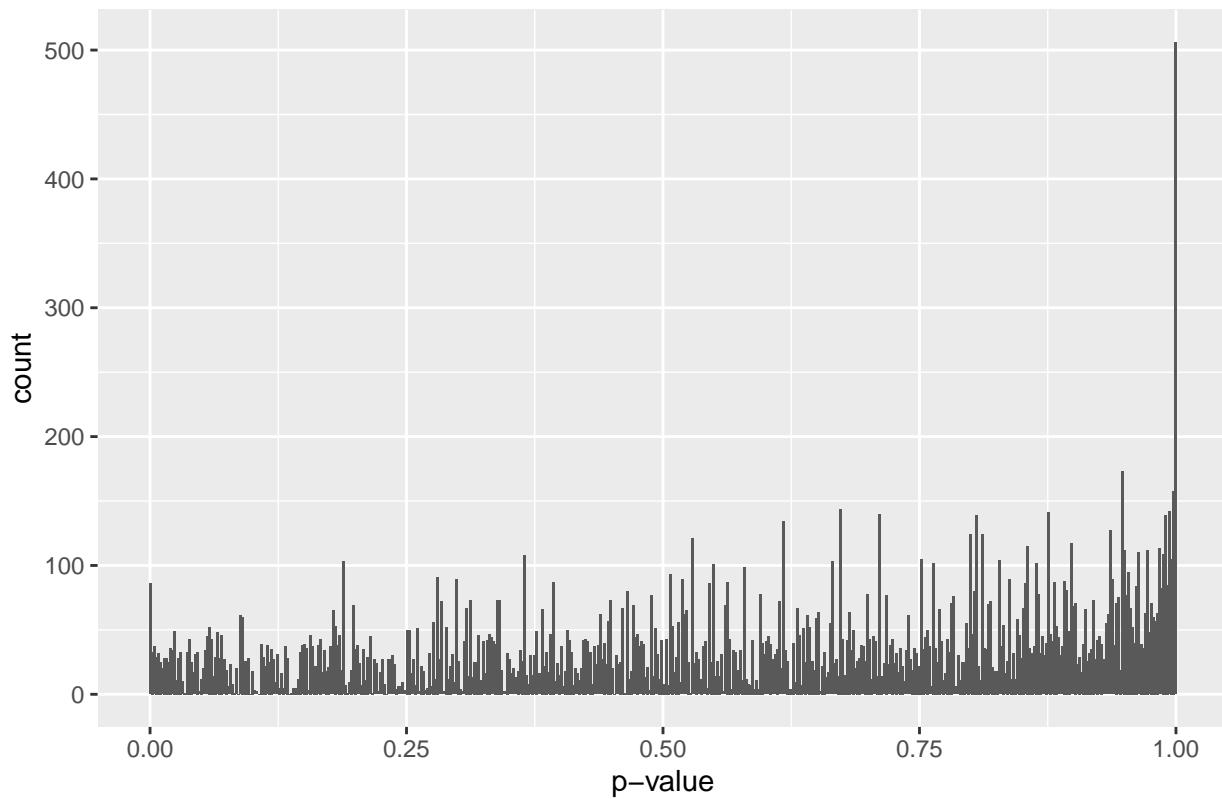
Distribution of positive selection p-values



Initial plots

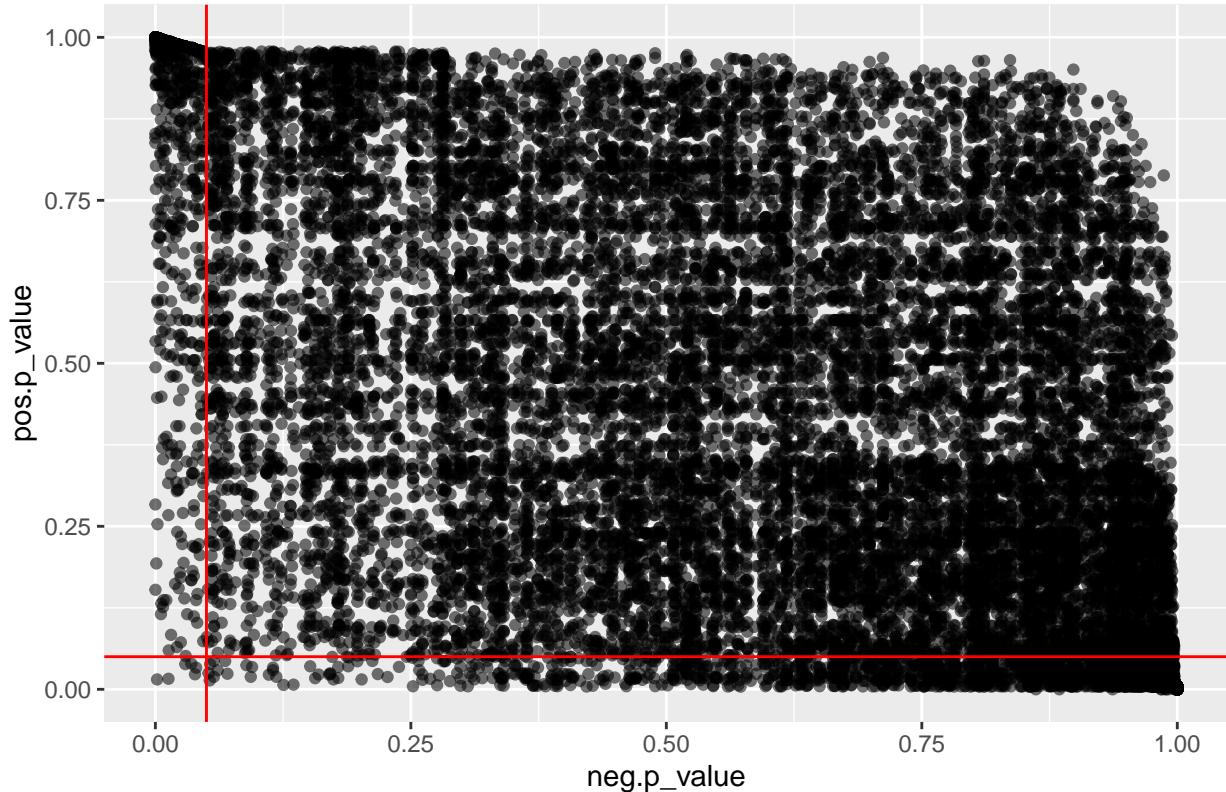
```
# Distribution of negative selection p-values  
ggplot(mageck, aes(x = neg.p_value)) +  
  geom_histogram(bins = 500) +  
  xlab("p-value") +  
  ggtitle("Distribution of negative selection p-values")
```

## Distribution of negative selection p–values



```
# Compare positive and negative p-values
ggplot(mageck, aes(x = neg.p_value,
                     y = pos.p_value,
                     alpha = 0.01)) +
  geom_point() +
  geom_vline(xintercept = 0.05,
             color = "red") +
  geom_hline(yintercept = 0.05,
             color = "red") +
  ggtitle("Comparison of positive and negative selection p-values") +
  theme(legend.position="none")
```

## Comparison of positive and negative selection p-values



Viewing the histogram of positive selection p-values, the data does not seem to show any obvious patterns, except for some higher frequencies as the values approach zero. For the negative selection p-values, we see more observations with p-values at or close to one. The scatter plot, showing both variables along with red lines marking a significance level of 0.05, shows far more significant p-values for the positive selection than the negative, with a large cluster of data points with both very low positive p-values and very high negative p-values.

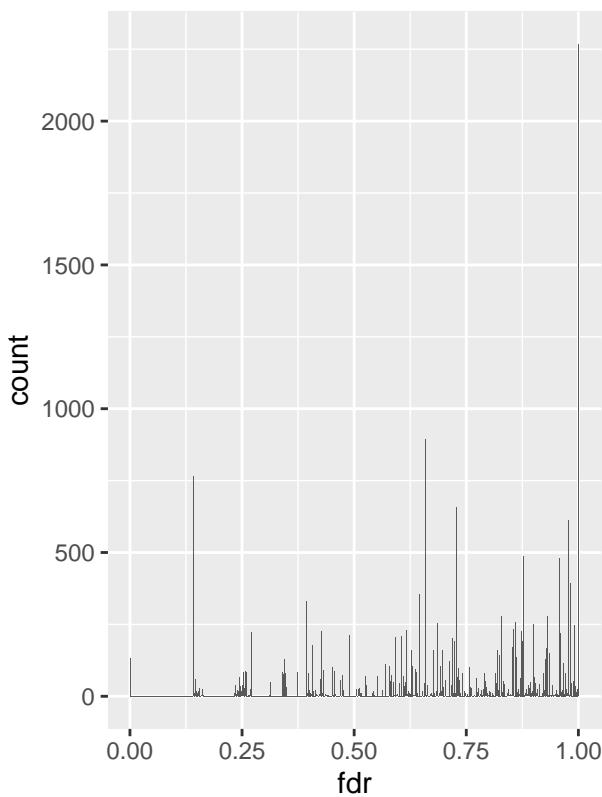
```
library(ggpubr)
##### False Discovery Rates

# Distribution of positive selection fdr
posfdr_plot <- ggplot(mageck, aes(x = pos.fdr)) +
  geom_histogram(bins = 500) +
  xlab("fdr") +
  ggtitle("Distribution of positive selection fdr values")

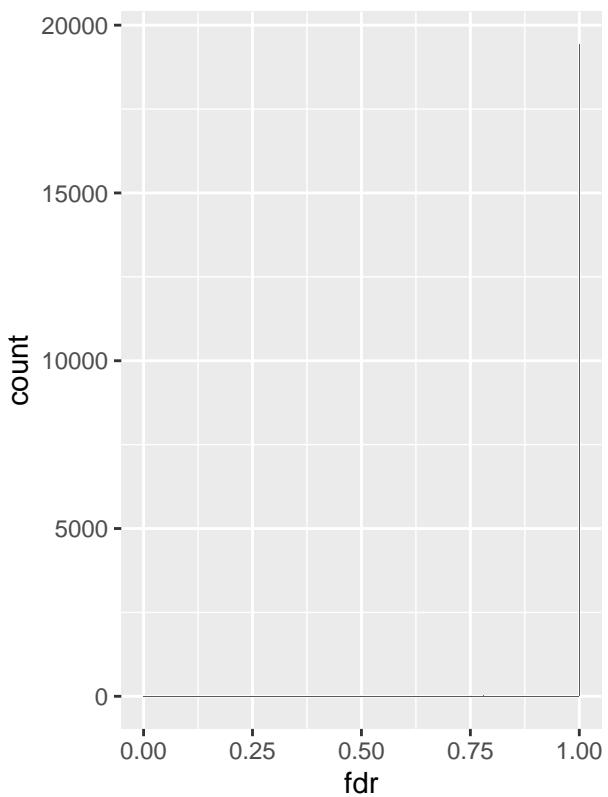
# Distribution of negative selection fdr
negfdr_plot <- ggplot(mageck, aes(x = neg.fdr)) +
  geom_histogram(bins = 500) +
  xlab("fdr") +
  ggtitle("Distribution of negative selection fdr values")

ggarrange(posfdr_plot, negfdr_plot)
```

Distribution of positive selection fdr

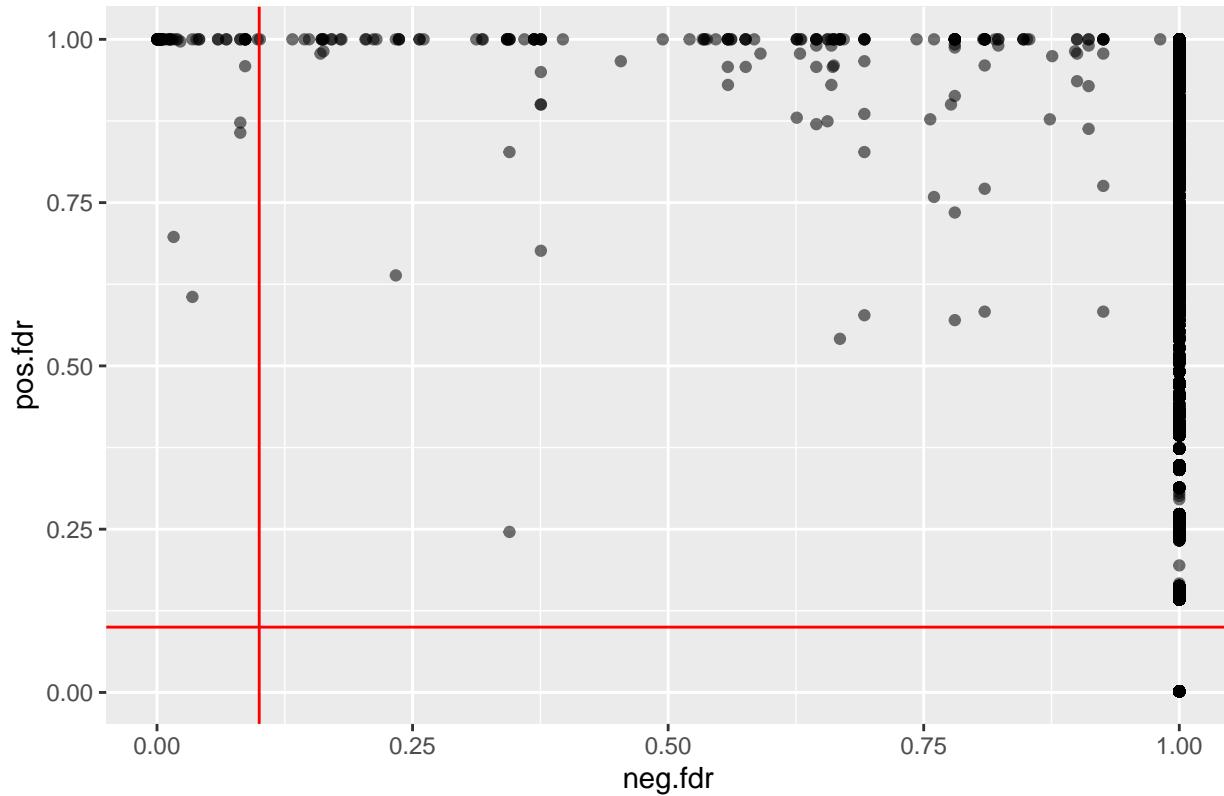


Distribution of negative selection fdr



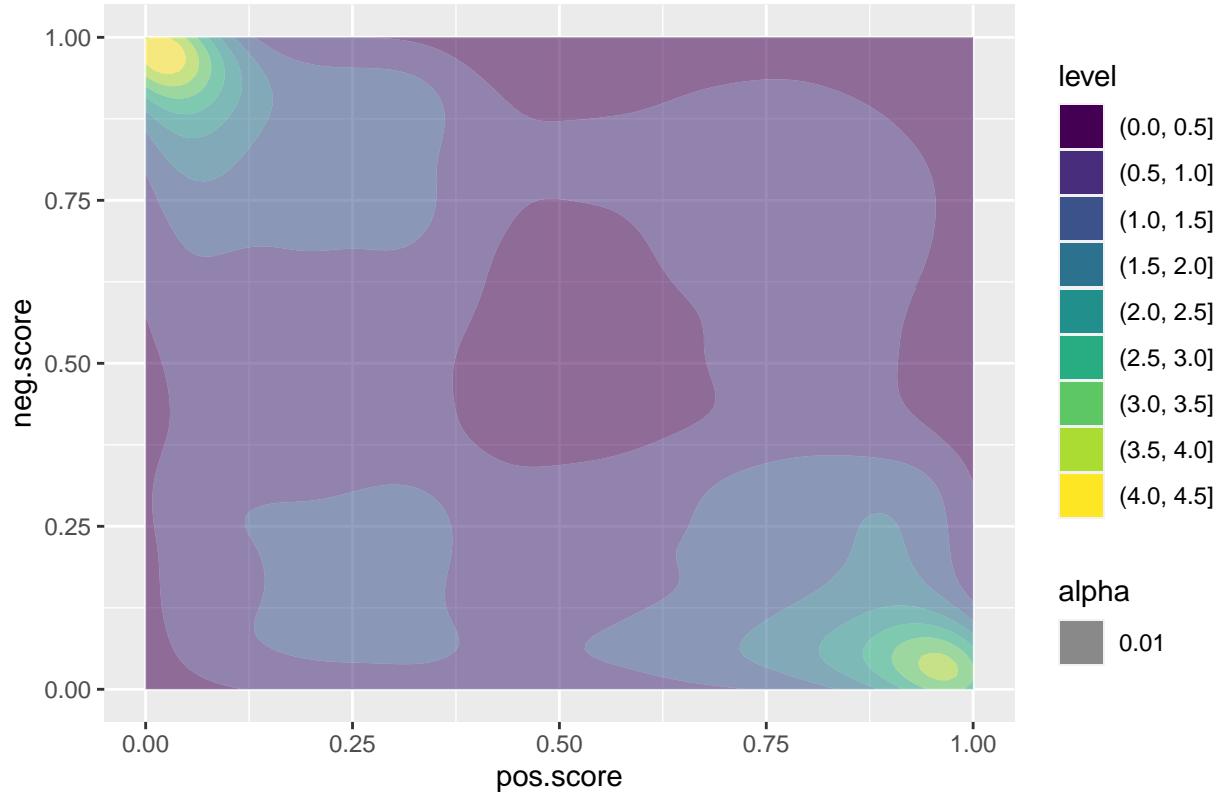
```
# # Distribution of positive selection p-values
# ggplot(mageck, aes(x = pos.p_value)) +
#   geom_histogram(bins = 500) +
#   xlab("p-value") +
#   ggtitle("Distribution of positive selection p-values")
#
# # Distribution of negative selection p-values
# ggplot(mageck, aes(x = neg.p_value)) +
#   geom_histogram(bins = 500) +
#   xlab("p-value") +
#   ggtitle("Distribution of negative selection p-values")
#
# Compare positive and negative fdr's
ggplot(mageck, aes(x = neg.fdr,
                    y = pos.fdr,
                    alpha = 0.01)) +
  geom_point() +
  geom_vline(xintercept = 0.1,
             color = "red") +
  geom_hline(yintercept = 0.1,
             color = "red") +
  ggtitle("Comparison of positive and negative selection fdr values") +
  theme(legend.position="none")
```

## Comparison of positive and negative selection fdr values



```
# Compare positive score and negative score
ggplot(mageck, aes(x = pos.score,
                    y = neg.score,
                    alpha = 0.01)) +
  geom_density_2d_filled() +
  ggtitle("Comparison of positive score and negative score")
```

## Comparison of positive score and negative score



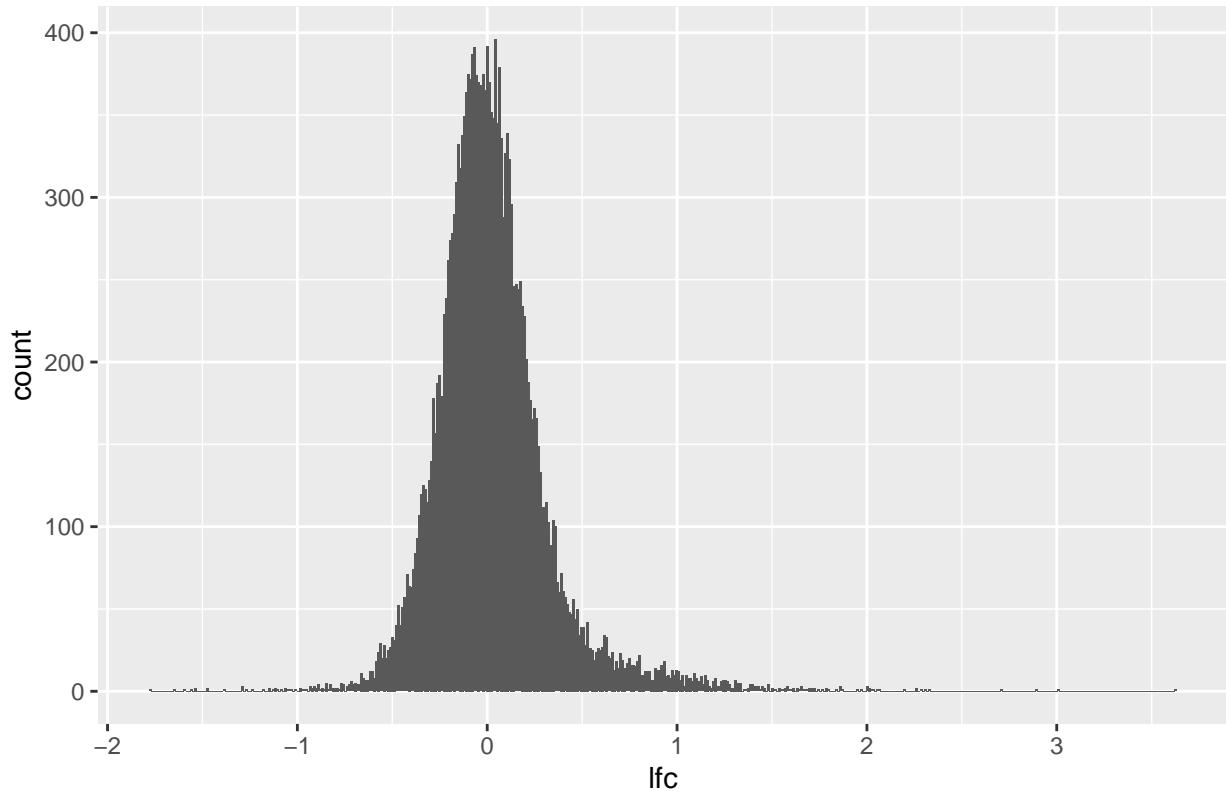
```
#sig_negs <- na.omit(filter(mageck$neg.fdr, mageck$neg.fdr != 1))
```

Looking at the positive and negative selection false discovery rate values, we see the majority of the positive values are closer to one, and surprisingly all but a handful of values for the negative selection are equal to one. We see this more clearly in the scatter plot, which includes significance lines at FDR = 0.1.

```
#### log fold change

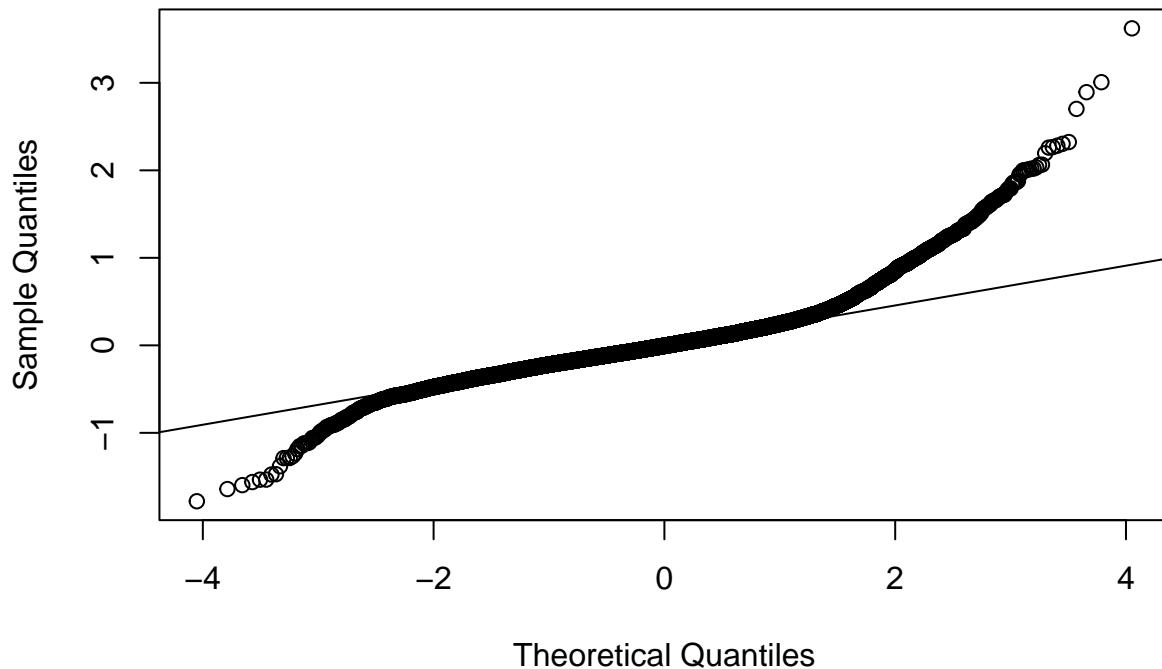
# Distribution of lfc (positive and negative values the same)
ggplot(mageck, aes(x = lfc)) +
  geom_histogram(bins = 500) +
  xlab("lfc") +
  ggtitle("Distribution of log fold change for all genes")
```

## Distribution of log fold change for all genes



```
# Check normality of variable  
qqnorm(mageck$lfc, main = "Normality of log fold change")  
qqline(mageck$lfc)
```

## Normality of log fold change

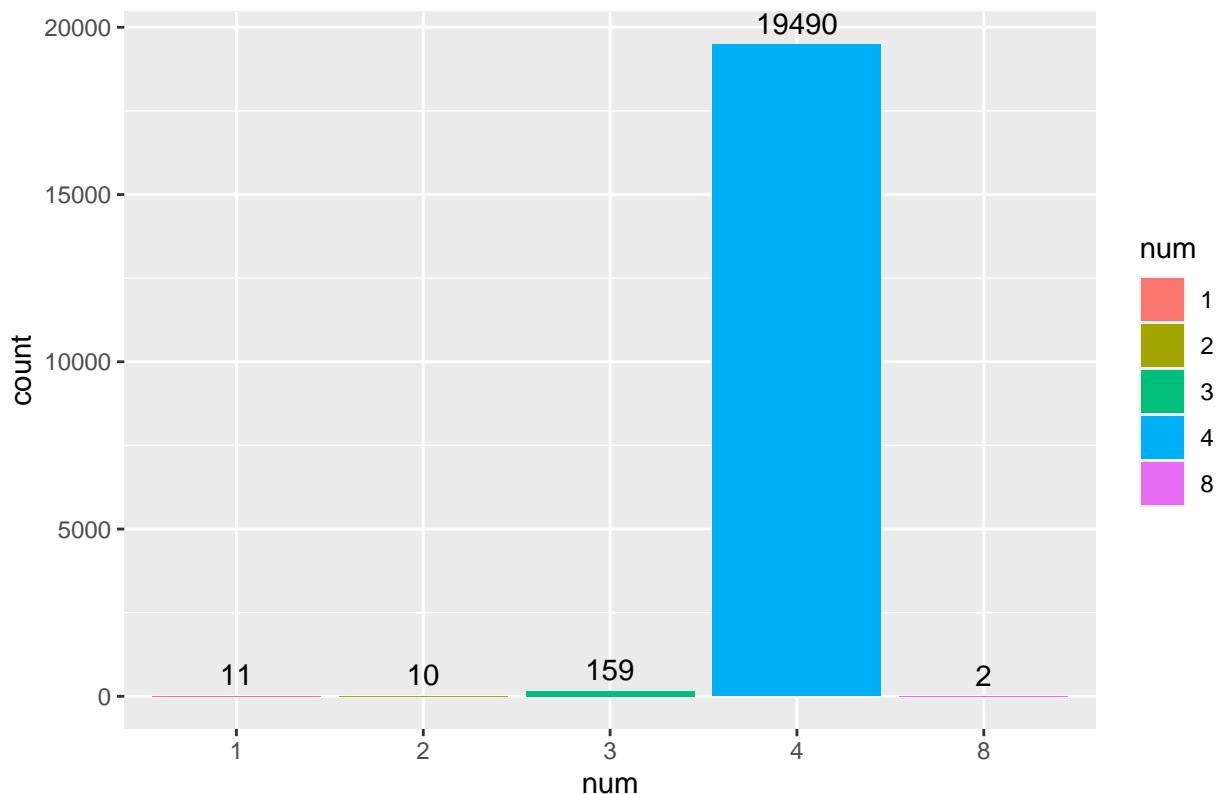


The log fold change variable (same for both positive and negative selections) appears to follow an approximately normal distribution, judging by the histogram. We validated this result using the Q-Q plot in the next figure.

```
### number of sgRNA's

ggplot(mageck, aes(num)) +
  geom_bar(aes(fill = num)) +
  geom_text(stat = 'count',
            aes(label = after_stat(count)),
            vjust = -0.5) +
  ggtitle("Distribution of number of guide RNAs used")
```

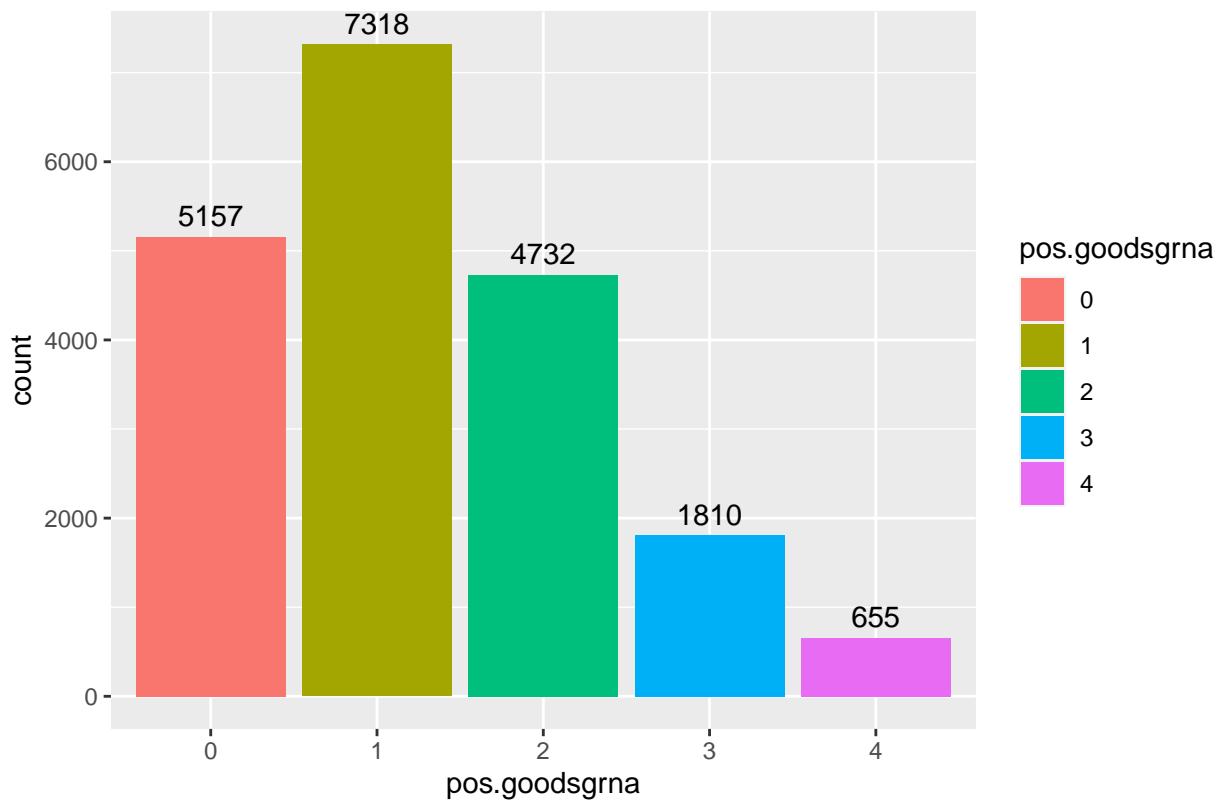
### Distribution of number of guide RNAs used



```
### goodsgrna

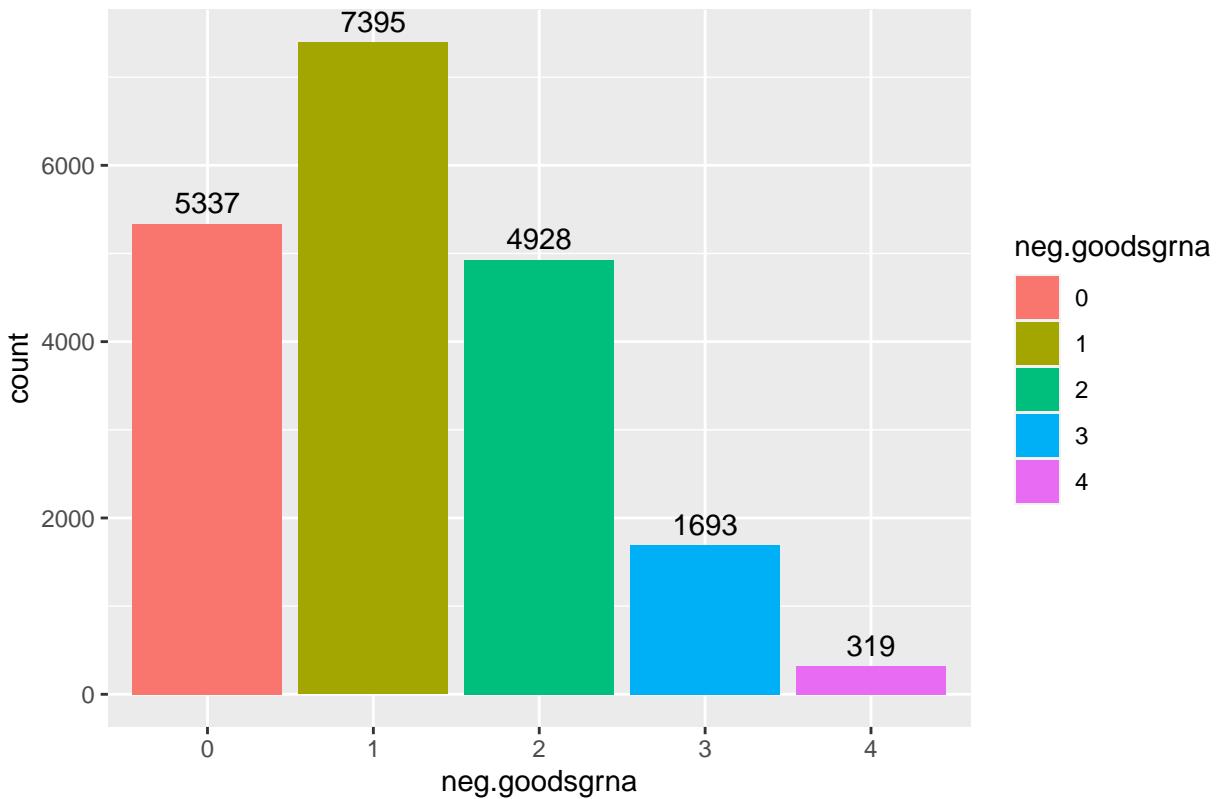
# positive selection
ggplot(mageck, aes(pos.goodsgrna)) +
  geom_bar(aes(fill = pos.goodsgrna)) +
  geom_text(stat = 'count',
            aes(label = after_stat(count)),
            vjust = -0.5) +
  ggtitle("Distribution of negative selection `goodsgrna`")
```

Distribution of negative selection `goodsgrna`



```
# negative selection
ggplot(mageck, aes(neg.goodsgrna)) +
  geom_bar(aes(fill = neg.goodsgrna)) +
  geom_text(stat = 'count',
            aes(label = after_stat(count)),
            vjust = -0.5) +
  ggtitle("Distribution of negative selection `goodsgrna`")
```

Distribution of negative selection `goodsgrna`

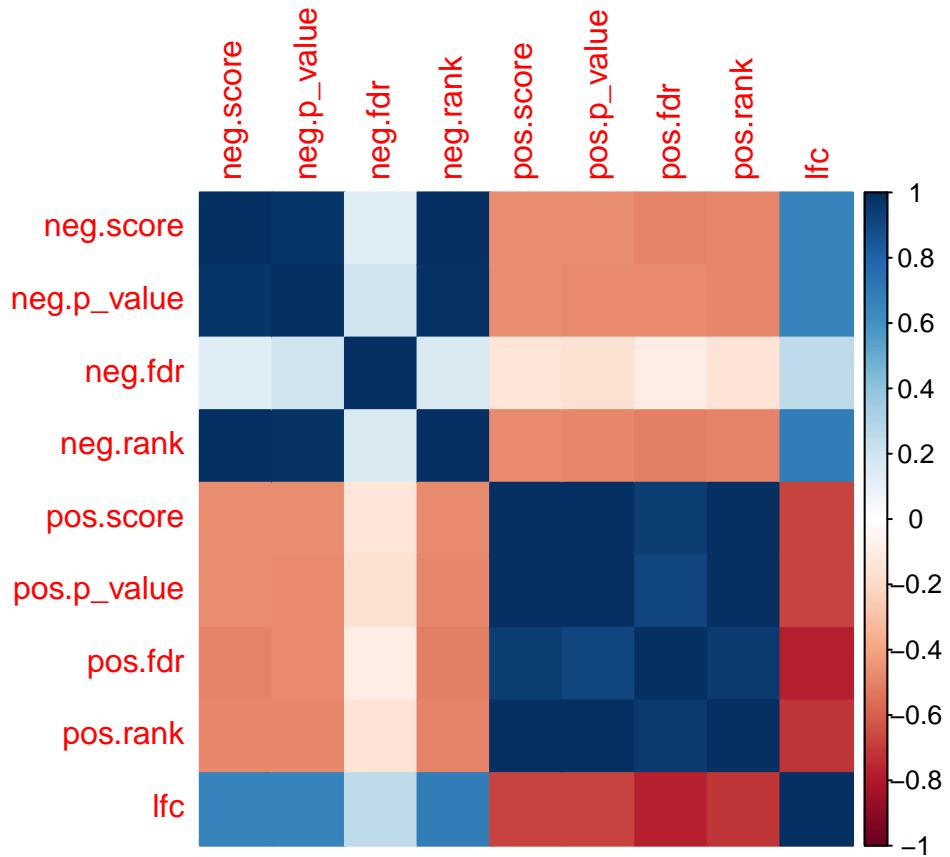


I created bar plots showing the `num` column (the number of targeting sgRNAs for each gene) and the positive and negative selection `goodsgrna` columns (the number of “good” sgRNAs, i.e. those whose ranking fell below a set FDR cutoff). We see that for almost every gene four sgRNA’s were used (perhaps the rest could even be considered outliers). The number of “good” sgRNA’s followed similar distributions for both positive and negative, with the majority of genes having 1.

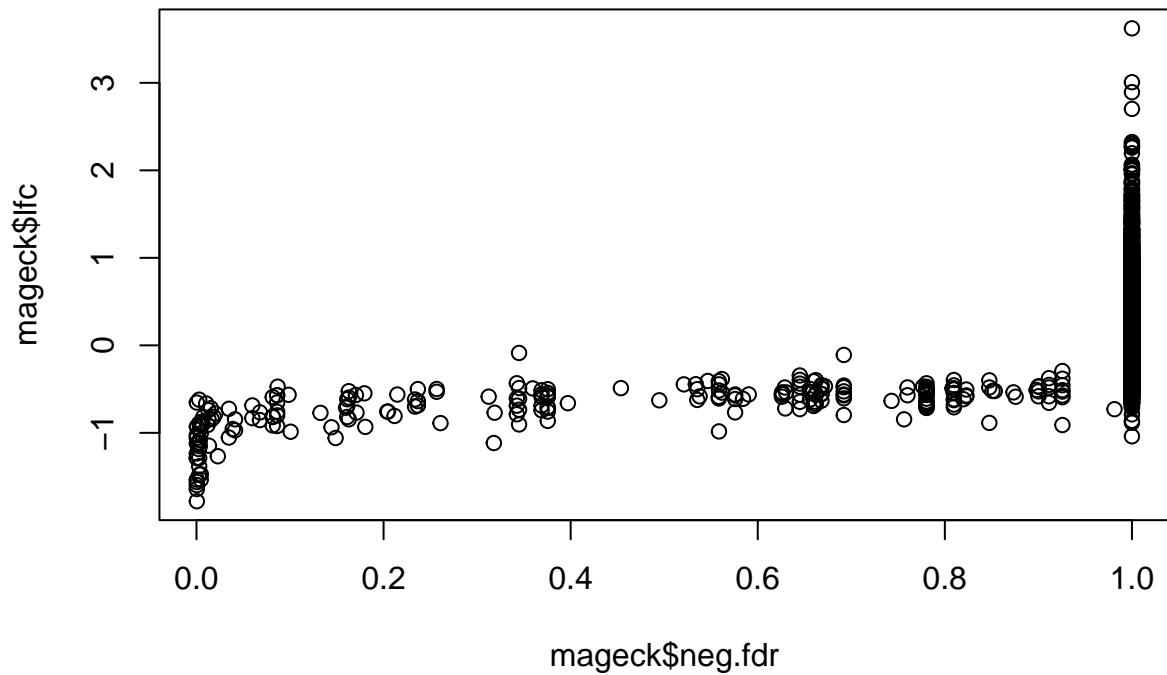
```
# Create correlation plot
library(corrplot)

## corrplot 0.92 loaded

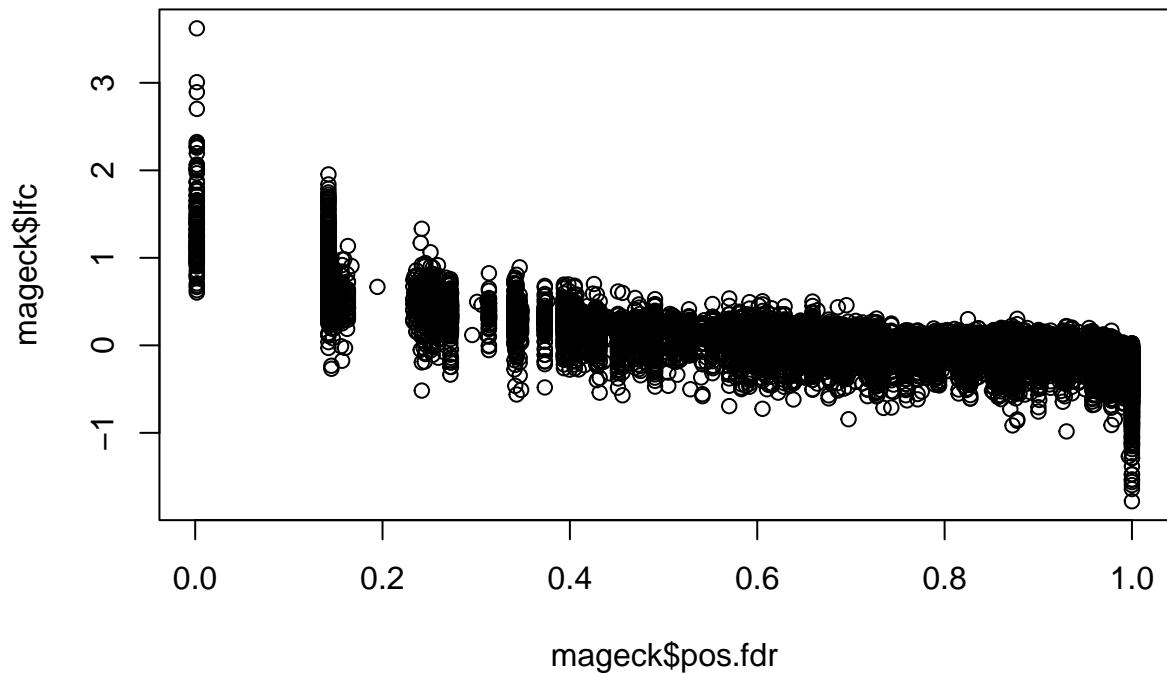
cor.crispr <- cor(mageck[,-c(1,2,7,12)])
corrplot(cor.crispr,
         method = "color")
```



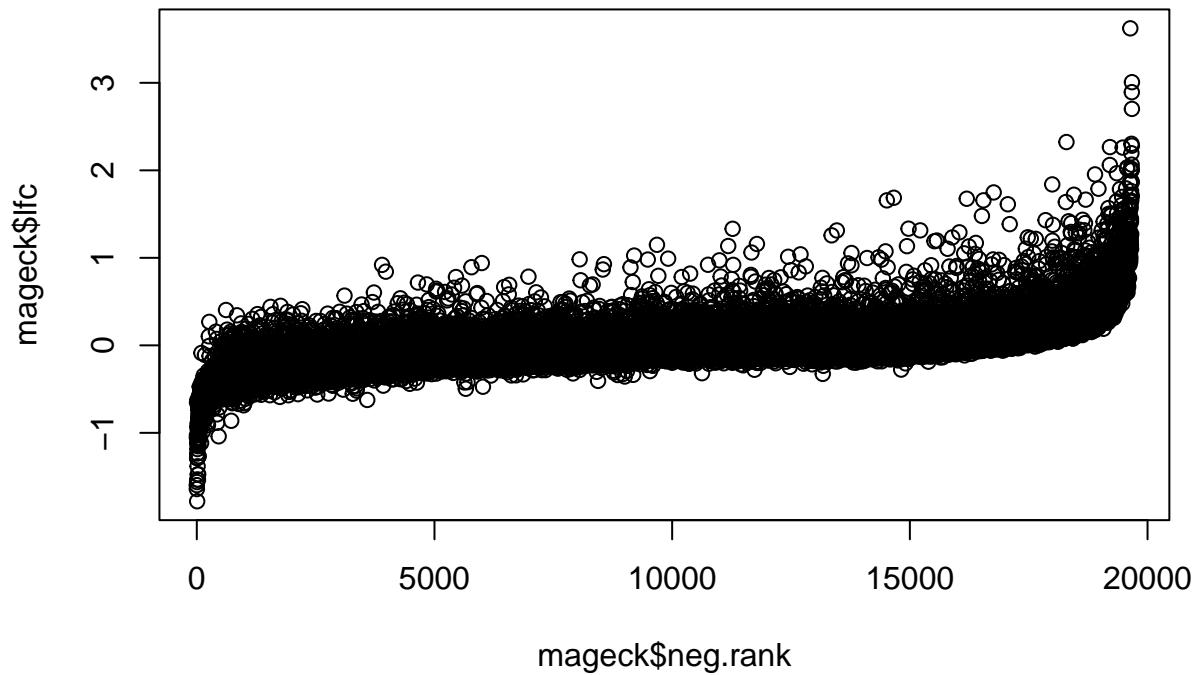
```
# Plot lfc with pos and neg fdr
plot(mageck$neg.fdr,
      mageck$lfc)
```



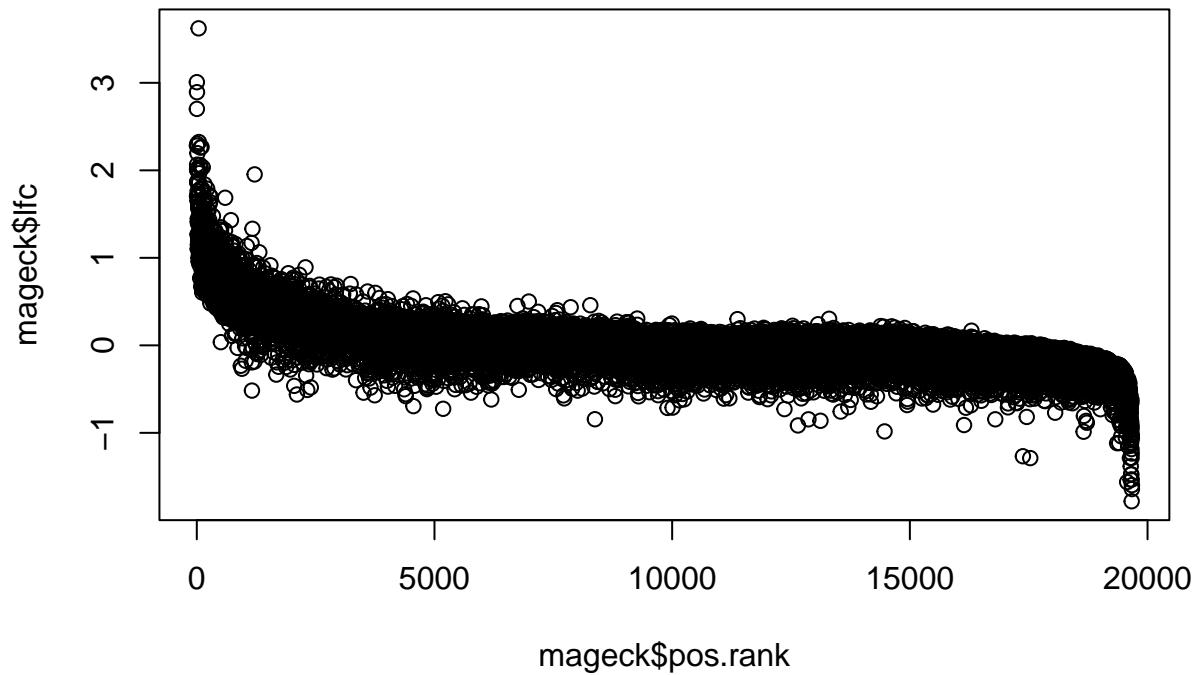
```
plot(mageck$pos.fdr,  
      mageck$lfc)
```



```
plot(mageck$neg.rank,  
      mageck$lfc)
```

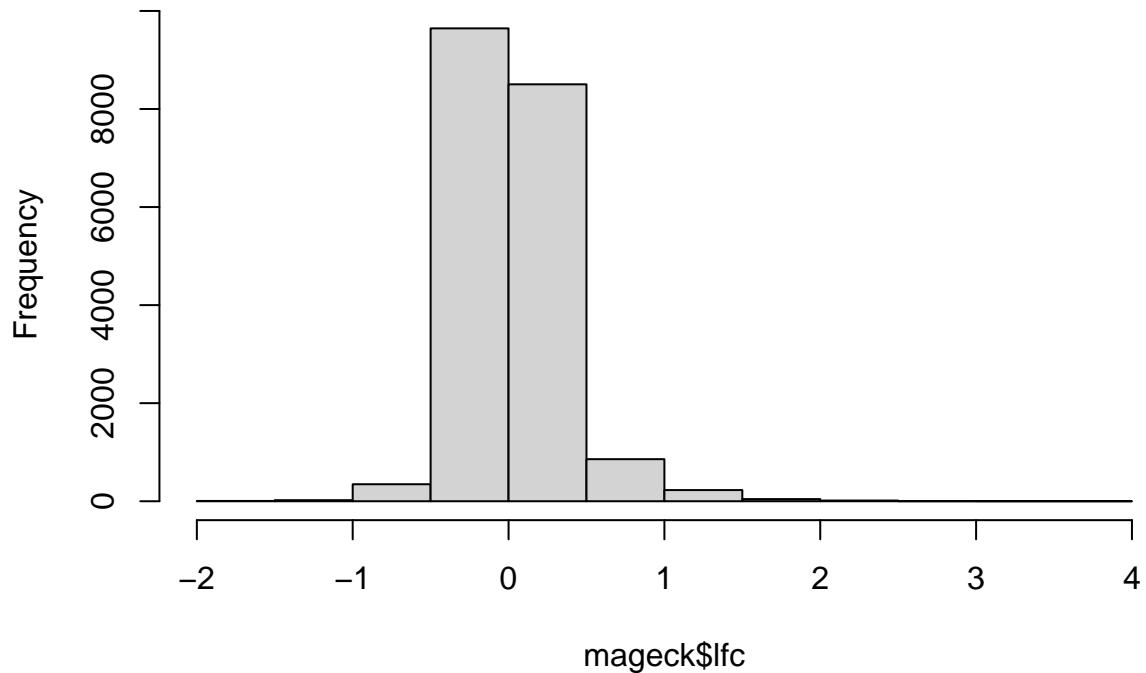


```
plot(mageck$pos.rank,  
      mageck$lfc)
```



```
hist(mageck$lfc)
```

## Histogram of mageck\$lfc



Note the white strips corresponding to the negative `fdr` correlations, since most of the observations are at or close to 1, there is essentially no linear correlation between it and any other variable.

```
# Code adapted from https://stackoverflow.com/questions/6602881/text-file-to-list-in-r

# Read in pathways data as list and split elements into strings
gmt <- scan("m2.cp.v2022.1.Mm.symbols.gmt", what = "", sep = "\n")
pathways <- strsplit(gmt, "[[:space:]]+")

# Assign first entry to names of each list element
names(pathways) <- sapply(pathways, `[[`, 1)

# save urls to separate list for reference
source <- sapply(pathways, `[[`, 2)

# Change gene names to lowercase to reduce errors
pathways <- lapply(pathways[-1], tolower)

# Remove two beginning reference rows
pathways <- lapply(pathways, `[[`, -c(1:2))

# Preview data set
head(pathways)
```

## Create list of all gene sets

```
## $BIOCARTA_CSK_PATHWAY
## [1] "cd4"      "cd3d"     "zap70"    "prkacb"   "csk"      "prkar2b"   "prkar1a"
## [8] "crebbp"   "cd3e"     "cd247"    "prkar2a"   "adcy1"    "lck"      "prkar1b"
## [15] "cd3g"     "ptprc"    #
##
## $BIOCARTA_SRCRPTP_PATHWAY
## [1] "csk"      "cdc25b"   "prkcb"    "prkca"    "ptpra"    "ccnb1"    "cdk1"     "cdc25c"
## [9] "cdc25a"   "grb2"    #
##
## $BIOCARTA_ARAP_PATHWAY
## [1] "arfgap1"  "cyth1"    "arfgap3"  "gbf1"     "cyth2"    "asap1"    "arap1"
## [8] "cyth3"    "gpld1"    "clta"     "chmp4c"   "arf1"    #
##
## $BIOCARTA_AGR_PATHWAY
## [1] "cdc42"    "rapsn"   "dvl1"     "chrna1"   "sp1"     "dag1"     "mapk3"
## [8] "egfr"     "musk"    "mapk8"    "pak4"     "pak3"    "lama3"   "git2"
## [15] "mapk1"    "cttn"    "acta1"    "pak2"     "chrm1"   "lama2"   "lama4"
## [22] "nrg3"    "pak1"    "arhgef6" "itgb1"    "agrn"    "jun"     "dmd"
## [29] "lama1"   "itga1"   "utrn"    #
##
## $BIOCARTA_AKAP95_PATHWAY
## [1] "prkag1"   "prkacb"  "prkar2b"  "ddx5"     "prkar2a"  "ncapd2"   "ccnb1"
## [8] "ppp2ca"   "cdk1"    "akap8"    #
##
## $BIOCARTA_AKT_PATHWAY
## [1] "fasl"     "ghr"     "chuk"     "bad"      "akt1"     "pdpk1"
## [7] "ikbkg"    "foxo1"   "casp9"    "nfkbia"   "foxo4"    "rela"
## [13] "nfkb1"   "pik3r1"  "ppp2ca"   "pik3ca"   "ikbkb"   "ywhah"
## [19] "foxo3"   "hsp90aa1" "gh"      #
```

This code chunk imports a file containing a collection of mouse gene sets which will be used for the GSEA analysis. The data needed to be reformatted so that we could separate the gene set names and source urls from the actual list of genes for each set. Finally we ended up with a list object where each element contains a gene set, with a list of all the genes in that set in order.

## Add selection column

```
library(ggridges)

selection <- NULL

# Sort values based on p-values to see which selection p-value is lower
for (i in 1:length(mageck$id)){
  if (mageck$neg.p_value[i] > mageck$pos.p_value[i]){
    selection[i] <- "positive"
  } else {
    selection[i] <- "negative"
  }
}
```

```

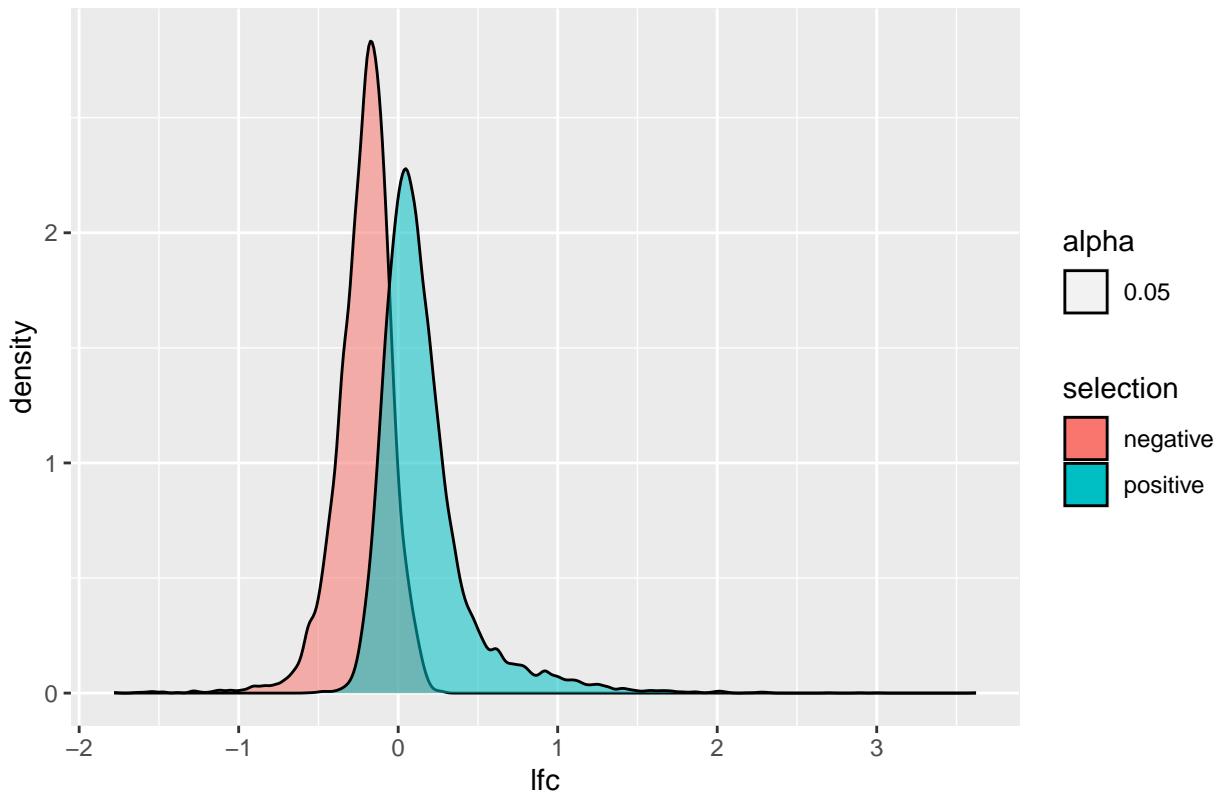
# Append to mageck data frame as factor
mageck$selection <- as.factor(selection)
summary(mageck$selection)

## negative positive
##      6901    12771

# Compare lfc across selections
ggplot(mageck, mapping = aes(x = lfc,
                               group = selection,
                               fill = selection,
                               alpha = 0.05)) +
  geom_density() +
  ggtitle("Log fold change distribution for positive and negative selections")

```

Log fold change distribution for positive and negative selections



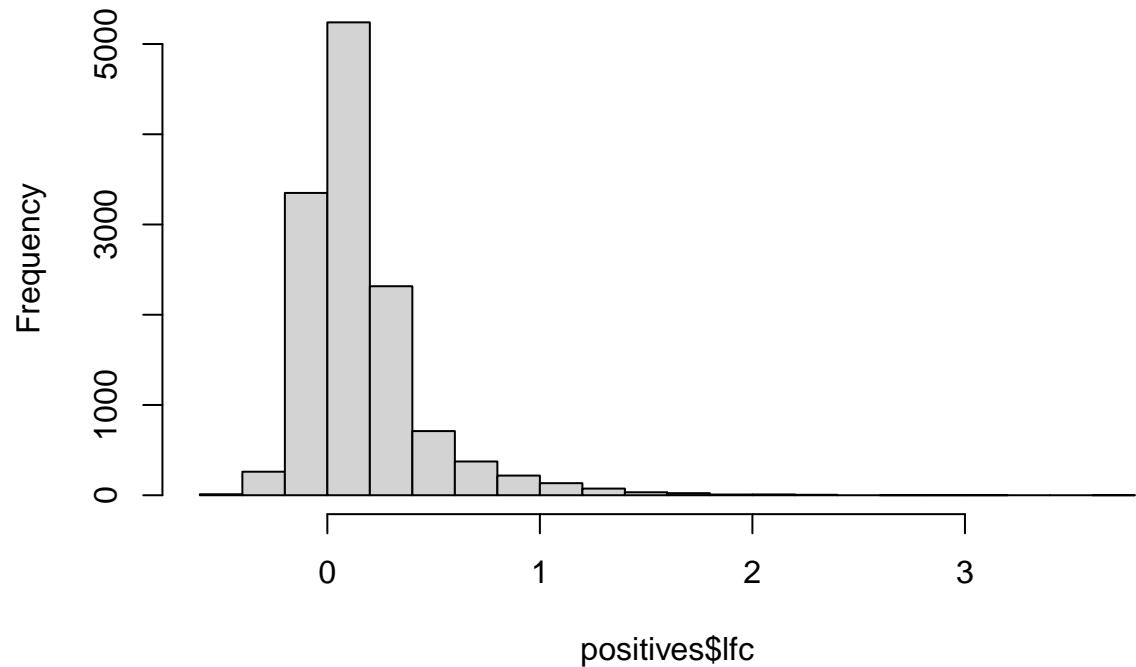
```

# Create separate data frames for each positive and negative selection
# and remove selection variable from each
positives <- mageck[mageck$selection == "positive", -14]
negatives <- mageck[mageck$selection == "negative", -14]

# Compare lfc in both groups
hist(positives$lfc)

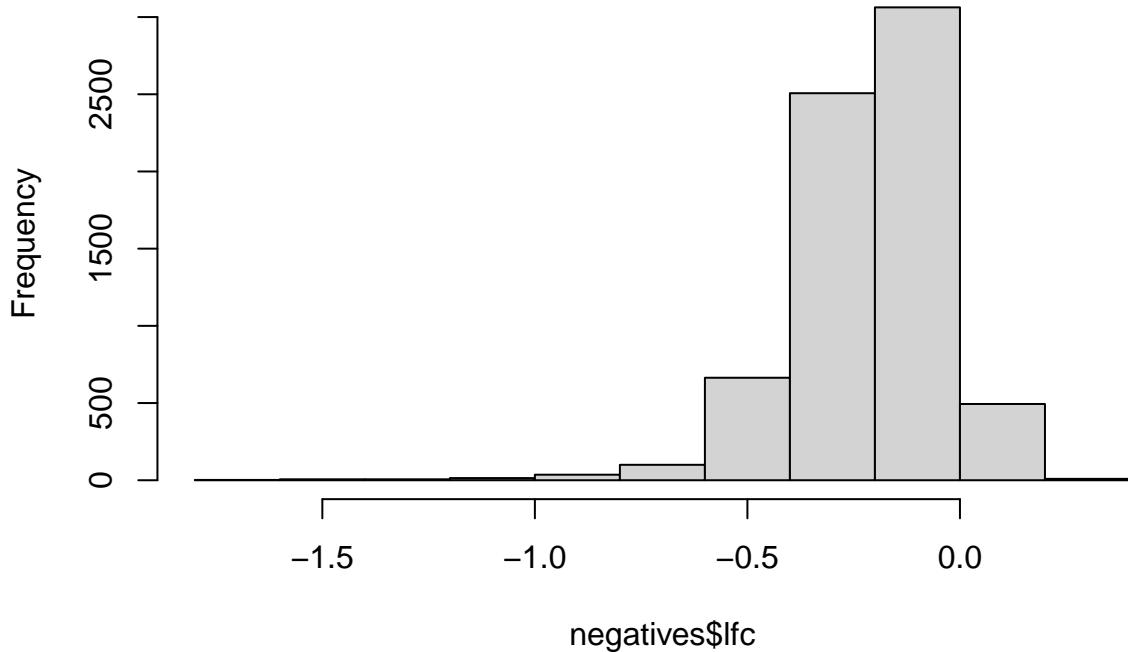
```

**Histogram of positives\$lfc**

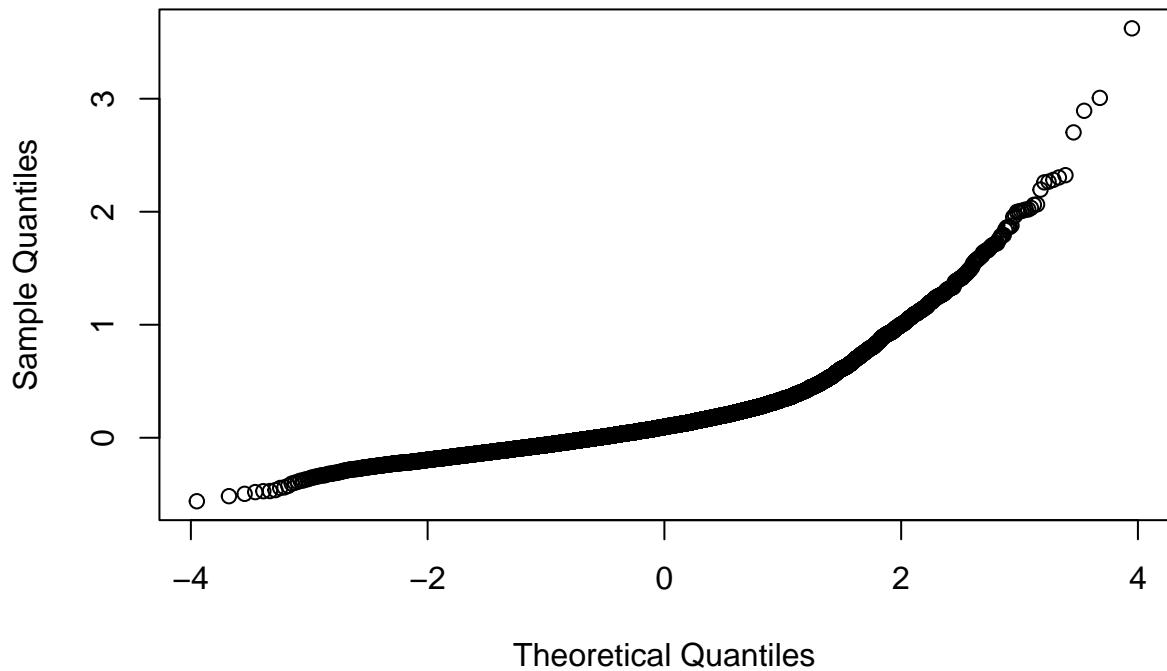


```
hist(negatives$lfc)
```

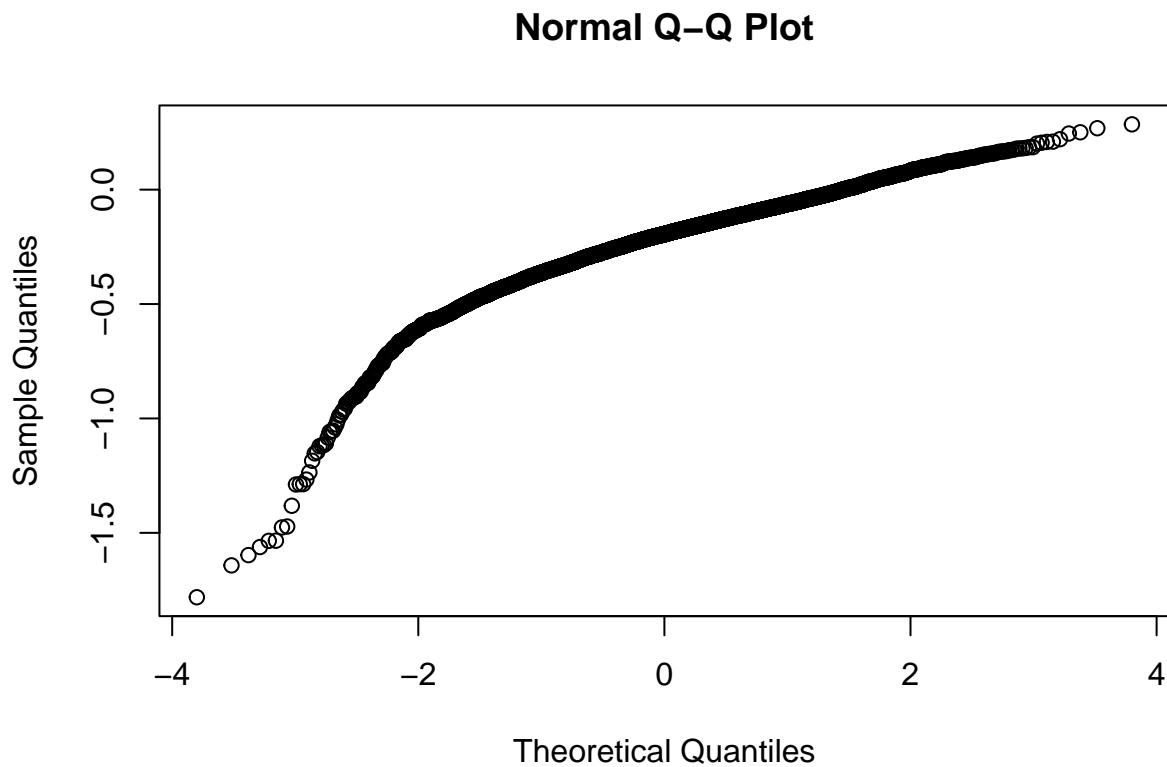
## Histogram of negatives\$lfc



### Normal Q-Q Plot



```
qqnorm(negatives$lfcc)
```



```
t.test(negatives$lfc, positives$lfc)

##
##  Welch Two Sample t-test
##
## data:  negatives$lfc and positives$lfc
## t = -112.07, df = 19370, p-value < 2.2e-16
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.3784137 -0.3654046
## sample estimates:
## mean of x  mean of y
## -0.2150097  0.1568995
```

We sorted every observation based on whether it had a lower negative or positive p-value (none of them were equal), creating a column indicating which selection they belonged to, and separate data frames to hold the information for each selection for use in more in depth analysis of each group. There were approximately twice as many observations in the positive category as in the negative one.

We then observed how the lfc changed between the two groups using the density plot. It appears that the log fold change for the positive group was largely positive, and largely negative for the negative group. This confirms our expectations. This was again confirmed using a t-test, with the results given above.

Remember significant positive selection genes are those whose knockout leads to cholesterol accumulation, and significant negatives are those whose knockout caused cholesterol depletion.

```

lfc_sort <- mageck[order(mageck$lfc, decreasing = TRUE),]

mageck_lfc_sort <- as.vector(lfc_sort$lfc)
names(mageck_lfc_sort) <- lfc_sort$id

```

## GSEA

```

library(BiocManager)
library(fgsea)

SampleES <- fgsea::fgseaSimple(
  pathways = pathways[1:4],
  stats = mageck_lfc_sort,
  nperm = 1000,
  minSize = 5,
  scoreType = "std",
  gseaParam = 1
)

```

```

## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are ties in
## The order of those tied genes will be arbitrary, which may produce unexpected results.

```

```

Sample2ES <- fgseaSimple(
  pathways = pathways[1:10],
  stats = mageck_lfc_sort,
  nperm = 1000,
  minSize = 5,
  scoreType = "std",
  gseaParam = 1
)

```

```

## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are ties in
## The order of those tied genes will be arbitrary, which may produce unexpected results.

```

Sample2ES

	pathway	pval	padj	ES	NES
## 1:	BIOCARTA_CSK_PATHWAY	0.47116969	0.6081469	0.4026875	0.9964732
## 2:	BIOCARTA_SRCRPTP_PATHWAY	0.54733219	0.6081469	0.4194077	0.9280997
## 3:	BIOCARTA_ARAP_PATHWAY	0.15458937	0.3091787	-0.4881491	-1.2905602
## 4:	BIOCARTA_AGR_PATHWAY	0.14878398	0.3091787	0.4397666	1.2886549
## 5:	BIOCARTA_AKAP95_PATHWAY	0.01721170	0.1011804	0.7369954	1.6308836
## 6:	BIOCARTA_AKT_PATHWAY	0.52941176	0.6081469	-0.3103427	-0.9676959
## 7:	BIOCARTA_AT1R_PATHWAY	0.23259259	0.3876543	0.4272135	1.1943751
## 8:	BIOCARTA_ACE2_PATHWAY	0.96476510	0.9647651	0.2238476	0.5354424
## 9:	BIOCARTA_ASBCCELL_PATHWAY	0.03667482	0.1222494	-0.6663941	-1.6501128
## 10:	BIOCARTA_DNAFRAGMENT_PATHWAY	0.02023609	0.1011804	0.7069828	1.5970789
	nMoreExtreme	size		leadingEdge	
## 1:	285	15	crebbp,cd247,prkar2b,zap70,prkar2a,prkacb,...		
## 2:	317	9		cdk1,cdc25b	

```

## 3:          63   12           arap1,cyth1,arfgap3,arf1
## 4:         103   30           pak2,cdc42,musk,chrna1,jun,lama2,...
## 5:          9    9           ddx5,cdk1,ncapd2,ppp2ca,prkar2b,prkar2a,...
## 6:         179   21           akt1,nfkbia,ikbkg,rela,fasl
## 7:         156   25           map2k4,jun,map3k1,hras,ptk2b,calm3,...
## 8:         574   13           ace,ren1,agtrap,col4a2,cma1,agtr2
## 9:          14   10           il10,cd40lg,fasl,cd4,il4
## 10:         11   10           hmgb1,top2a,top2b,casp3

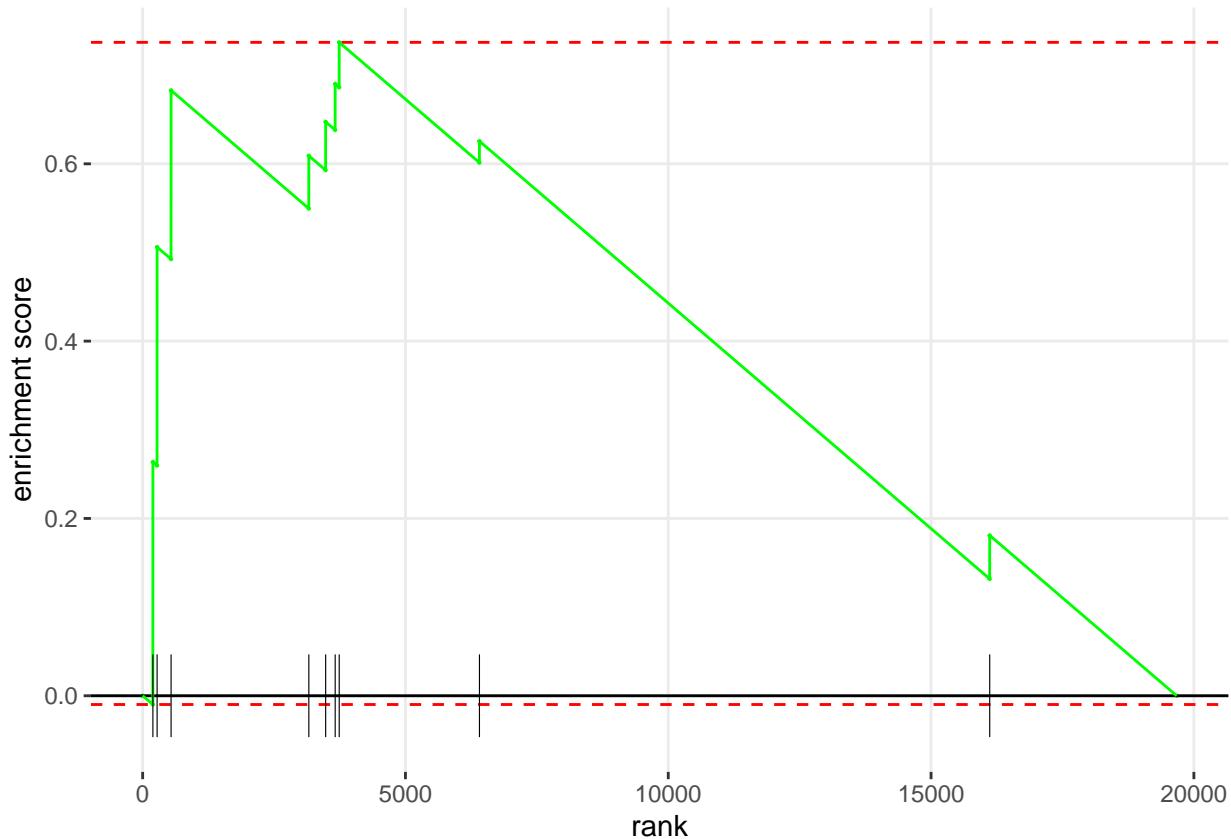
```

### Enrichment plots

```

sample_plot <- fgsea:::plotEnrichment(
  pathway = pathways[[5]],
  stats = mageck_lfc_sort,
  gseaParam = 1
)
sample_plot

```



### Code for gene look-up

```

## Lookup by gene code
if (length(mageck[mageck$id == "musk",]) != 0){

```

```
print(mageck[mageck$id=="musk",])
} else {
  print("No results found")
}

## # A tibble: 1 x 14
##   id      num    neg.score neg.p~1 neg.fdr neg.r~2 neg.g~3 pos.s~4 pos.p~5 pos.fdr
##   <chr> <fct>     <dbl>    <dbl>    <dbl> <dbl> <dbl>    <dbl>    <dbl>
## 1 musk  4        0.906    0.951     1    17155 0       0.0267  0.0207  0.264
## # ... with 4 more variables: pos.rank <dbl>, pos.goodsgrna <fct>, lfc <dbl>,
## #   selection <fct>, and abbreviated variable names 1: neg.p_value,
## #   2: neg.rank, 3: neg.goodsgrna, 4: pos.score, 5: pos.p_value
```