ASSIGNMENT 11

Exercise 2.2.8

Question 1

1. In this question you will write an R loop to perform 1000 t-tests on randomly generated normal data with mean 0. Therefore the null hypothesis is always true but you will see that one still obtains small p values (therefore indicating falsely signifcant results or false positives).

```r
pvalues <- sapply(1:1000, function(i) {
  t.test(rnorm(20,0,1))$p.value
})
```

Question 2

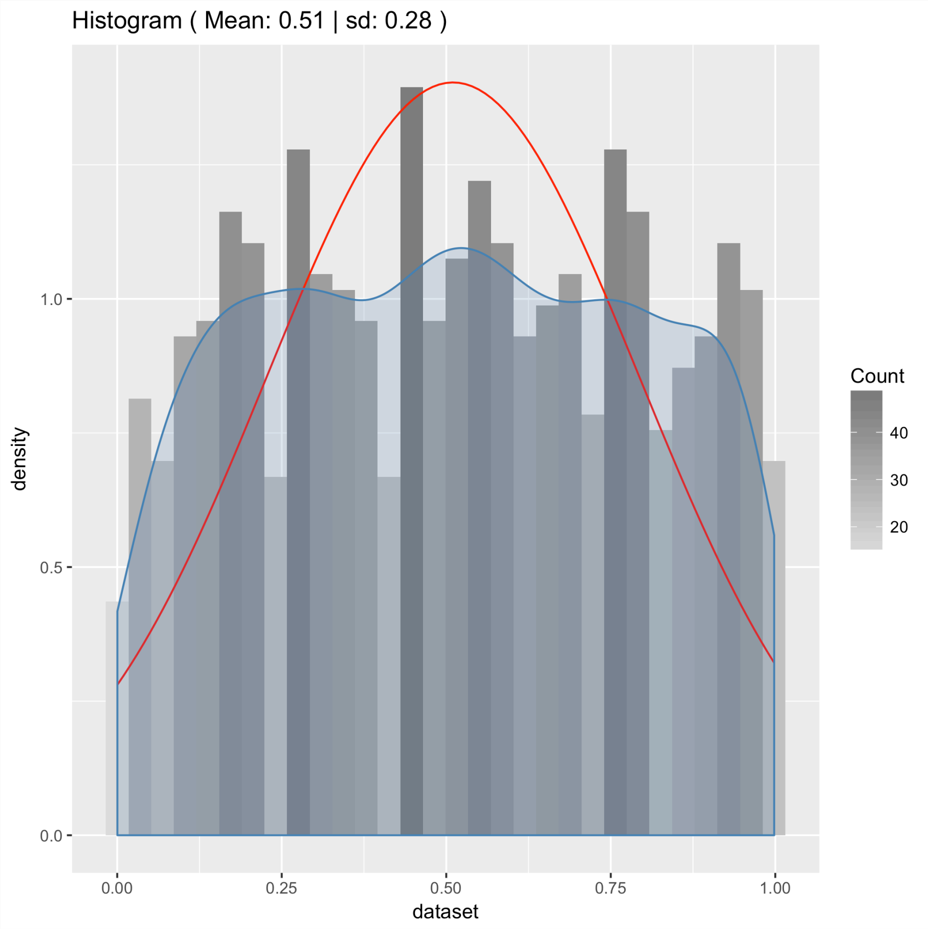
2. Using the which function, count the fraction of times you observe p values less than 0.1, 0.05, 0.01.

```r
length(which(pvalues<0.1))
# 102
length(which(pvalues<0.05))
# 50
length(which(pvalues<0.01))
# 10
```

Question 3

3. Make a histogram of the p values obtained. What distribution is this?

```r
library('ggplot2')
draw_histogram <- function(dataset) {
  dist_mean <- mean(dataset)
  dist_sd <- sd(dataset)
  gg <- ggplot(as.data.frame(dataset), aes(dataset))
  gg <- gg + geom_histogram(aes(y=..density.., fill=..count..))
  gg <- gg + scale_fill_gradient("Count", low="#DCDCDC", high="#7C7C7C")
  gg <- gg + stat_function(fun=dnorm, color="red",
                           args=list(mean=dist_mean,sd=dist_sd))
  # Adds a density plot on top
  gg <- gg + geom_density(alpha = 0.2, fill="steelblue", colour="steelblue")
  gg <- gg + ggtitle(paste("Histogram", "( Mean:", round(dist_mean,2), '|',
                           "sd:", signif(dist_sd,2), ")"))
  return(gg)
}

draw_histogram(pvalues)
ggsave('Assignment/histogram.png')
```



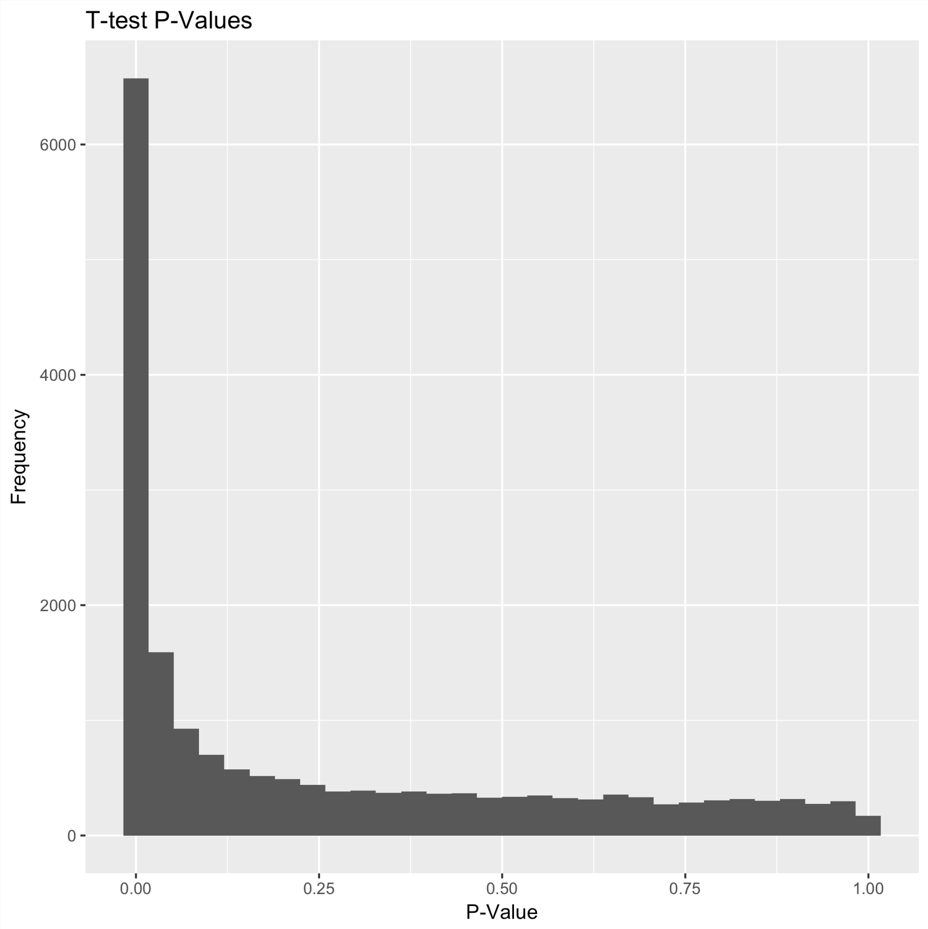
As shown by the above histogram, the data seems to follow a continuous uniform distribution.

Exercise 2.2.12

Question 1

1. Repeat the analysis above to examine the genes that are differentially expressed after 24 hour exposure to cadmium. Provide a histogram of the t-test p values for this dataset.

```r
library("GEOquery")
library('ggplot2')

gds      <- getGEO(filename='GDS3420_full.soft')
geData   <- Table(gds)
geData2  <- geData[which( geData['Platform_ORF'] != "" ),]

# Identify Samples for each factor
hrs4         <- unlist( strsplit( Meta(gds)$sample_id[1], ',' ) )
hrs24        <- unlist( strsplit( Meta(gds)$sample_id[2], ',' ) )

# separate the data for the two factors
geData_hrs4  <- data.matrix(geData2[, names(geData2) %in% hrs4])
geData_hrs24 <- data.matrix(geData2[, names(geData2) %in% hrs24])

top_hits <- function (dataMatrix, genes, identifiers, hits_type='upregulated',
                      fc=2, pvalue=2.57e-6) {
  numNA      <- apply(dataMatrix, MARGIN=1, function(x) sum(is.na(x)) )
  indices    <- which(numNA <= 5)

  dataMatrix <- dataMatrix[indices, ]
  genenames <- genes[indices, ]
  symbols    <- identifiers[indices]

  foldchange <- apply(dataMatrix, MARGIN=1, function(x){
    median( exp(as.numeric(x)), na.rm=T)
  })

  tresults <- apply(dataMatrix, MARGIN=1, function(x){
    t.test(as.numeric(x))$p.value
  })

  results <- data.frame(pvalue=tresults, FC=foldchange,
                        id=genenames, symbol=symbols)

  gg <- ggplot(results, aes(tresults)) + geom_histogram()
  gg <- gg + scale_fill_gradient("Count", low="#DCDCDC", high="#7C7C7C")
  gg <- gg + ggtitle("T-test P-Values") + xlab('P-Value') + ylab('Frequency')

  if (hits_type == 'upregulated') {
    hits <- results[ which( results$pvalue < pvalue & results$FC > fc ), ]
  } else if (hits_type == 'downregulated') { #
    hits <- results[ which( results$pvalue < pvalue & results$FC < fc ), ]
  } else {
    cat("ERROR: The hit_type variable in the top_hits() function must be 'upregulated' or 'downregulated'.", file=stderr())
    quit(save = "no", status = 1, runLast = FALSE)
  }
  hits <- hits[order(hits$FC, decreasing=T),]

  return (list(hits=hits, histogram=gg))
}

results_hrs4  <- top_hits(geData_hrs4, geData2["Gene ID"], geData2[, 'IDENTIFIER'])
results_hrs24 <- top_hits(geData_hrs24, geData2["Gene ID"], geData2[, 'IDENTIFIER'])

results_hrs24$histogram
ggsave('Assignment/results_hrs24.png')
```



Question 2

2. How many genes are significantly up-regulated?

```
results_hrs24$hits
dim(results_hrs24$hits)
# [1] 99  4
```

As shown above, there 99 hits that are significantly up-regulated.

Question 3

3. How many genes are significantly down regulated ($FC < 0.5$)

```
down_regulated_hrs24 <- top_hits(geData_hrs24, geData2["Gene ID"],
                                 geData2[, 'IDENTIFIER'], 'downregulated', 0.5)
down_regulated_hrs24$hits
dim(down_regulated_hrs24$hits)
# [1] 6 4
```

As show above, there are 6 genes that are significantly down regulated.