Assignment 2.2 Part 1

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Understanding the data

Task 1

1. In the `sub_geno.tab` file, 0 represents homozygous reference, 1 represents heterozygous and 2 represents homozygous alternative. -1 represents missing data.
2. In the `sub_expr.tab` file, the rows are genes/transcripts and the columns are different samples and their gene expression values.
3. The `design.tab` file contains information about each sample used such as which population they belong to, organism, strain and other characteristics.

Task 2

1. Calculate the number of missing genotypes for each SNP across all individuals.

```
snps <- read.table("data/sub_geno.tab")
missing_count <- apply((snps == -1), 1, sum)
head(missing_count)
```

```
## snp_22_30772686 snp_22_34965577 snp_22_49436707 snp_22_30631851 snp_22_46215888
##               0               0               0               0               0
## snp_22_34153853
##               0
```

2. Calculate the minor allele frequency (MAF) for all SNPs across all individuals.

```
get_geno_frequency <- function(geno, snp_mat){
  boolean_matrix <- snp_mat == geno
  count <- apply(boolean_matrix, 1, sum)
  freq <- count/dim(snps)[2]
  return (freq)
}

get_maf <- function(snp_mat){
  genotypes <- c(0,1,2)
  #get matrix of genotype frequencies
  geno_freq <- sapply(genotypes, get_geno_frequency, snp_mat)
  colnames(geno_freq) <- genotypes
  allele_freq <- geno_freq + geno_freq[,2]/2
  allele_freq <- allele_freq[,-2]
  maf <- apply(allele_freq, 1, min)
  return (maf)
}
```

```r
maf <- get_maf(snps)
head(maf)
```

```
## snp_22_30772686 snp_22_34965577 snp_22_49436707 snp_22_30631851 snp_22_46215888
##     0.097402597     0.135281385     0.083333333     0.000000000     0.001082251
## snp_22_34153853
##     0.199134199
```

3. Filter our SNPs that have missing genotypes or a MAF<0.05 and use the filtered snps for the rest of the exercise.

```r
keep <- maf >= 0.05 & missing_count == 0
snps_filtered <- snps[keep,]
dim(snps_filtered)
```

```
## [1]  32 462
```

4. Calculate the MAF for africans and non-africans separately. Is there a difference?

```r
design <- read.table("data/design.tab", header = T, sep = "\t")

filter_snp_population <- function(pop, snp_mat, design_mat, inv=F){
  if(inv){
    cols <- design_mat$Source.Name[design_mat$Characteristics.population. != pop]
  } else{
    cols <- design_mat$Source.Name[design_mat$Characteristics.population. == pop]
  }
  snp_mat <- (snp_mat[, cols])
  return(snp_mat)
}

african_snps <- filter_snp_population("YRI", snps_filtered, design)
non_african_snps <- filter_snp_population("YRI", snps_filtered, design, inv = T)

african_maf <- get_maf(african_snps)
non_african_maf <- get_maf(non_african_snps)
print("African")
```

```
## [1] "African"
```

```r
print(head(african_maf))
```

```
## snp_22_30772686 snp_22_34965577 snp_22_49436707 snp_22_34153853 snp_22_21970216
##     0.002164502     0.049783550     0.004329004     0.076839827     0.067099567
## snp_22_48286671
##     0.000000000
```

```r
print("Non-African")
```

```
## [1] "Non-African"
```

```r
print(head(non_african_maf))
```

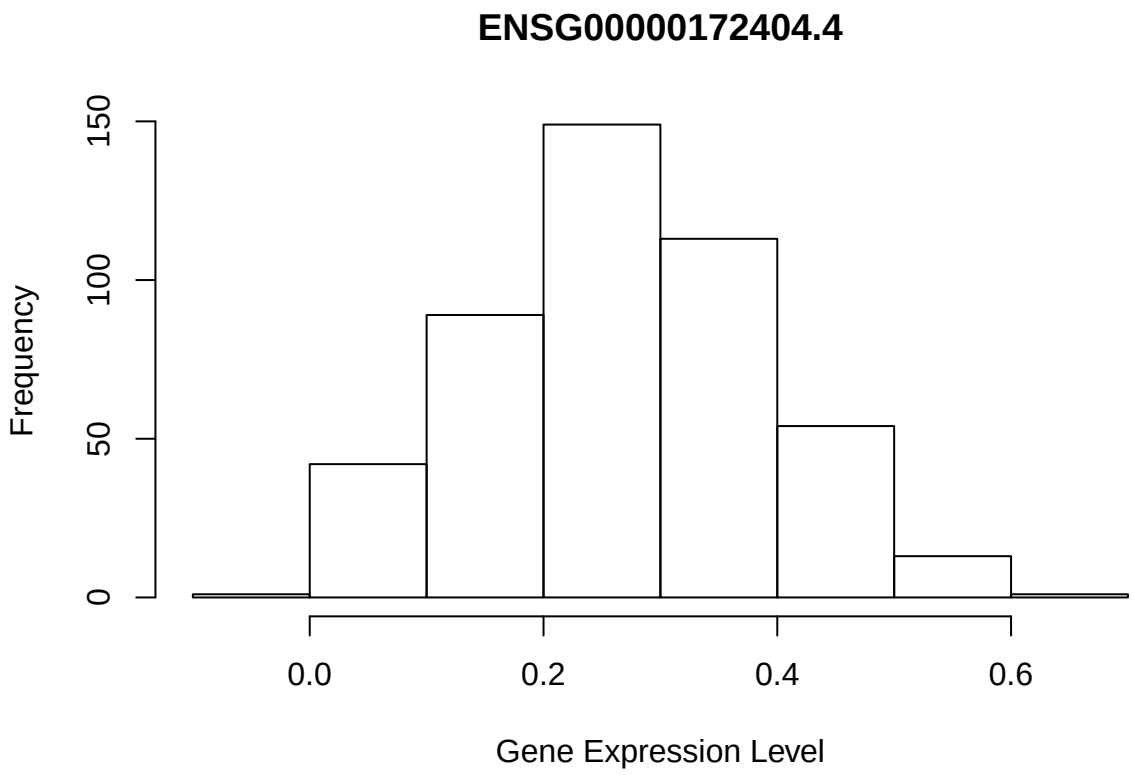
```
## snp_22_30772686 snp_22_34965577 snp_22_49436707 snp_22_34153853 snp_22_21970216
##      0.09523810      0.08549784      0.07900433      0.12229437      0.00000000
## snp_22_48286671
##      0.05735931
```

Yes, there is a difference between the two groups.

Task 3

1. Plot the distribution of expression levels across all samples for the ENSG00000172404.4 gene.

```
gene_expr <- read.table("data/sub_expr.tab")
gene1 <- c("ENSG00000172404.4")
hist(as.matrix(gene_expr[gene1,]), main=gene1, xlab="Gene Expression Level")
```



2. Plot the expression levels of ENSG00000172404.4 against the genotypes of snp_22_41256802 and snp_22_45782142

```
library(tidyverse)
```

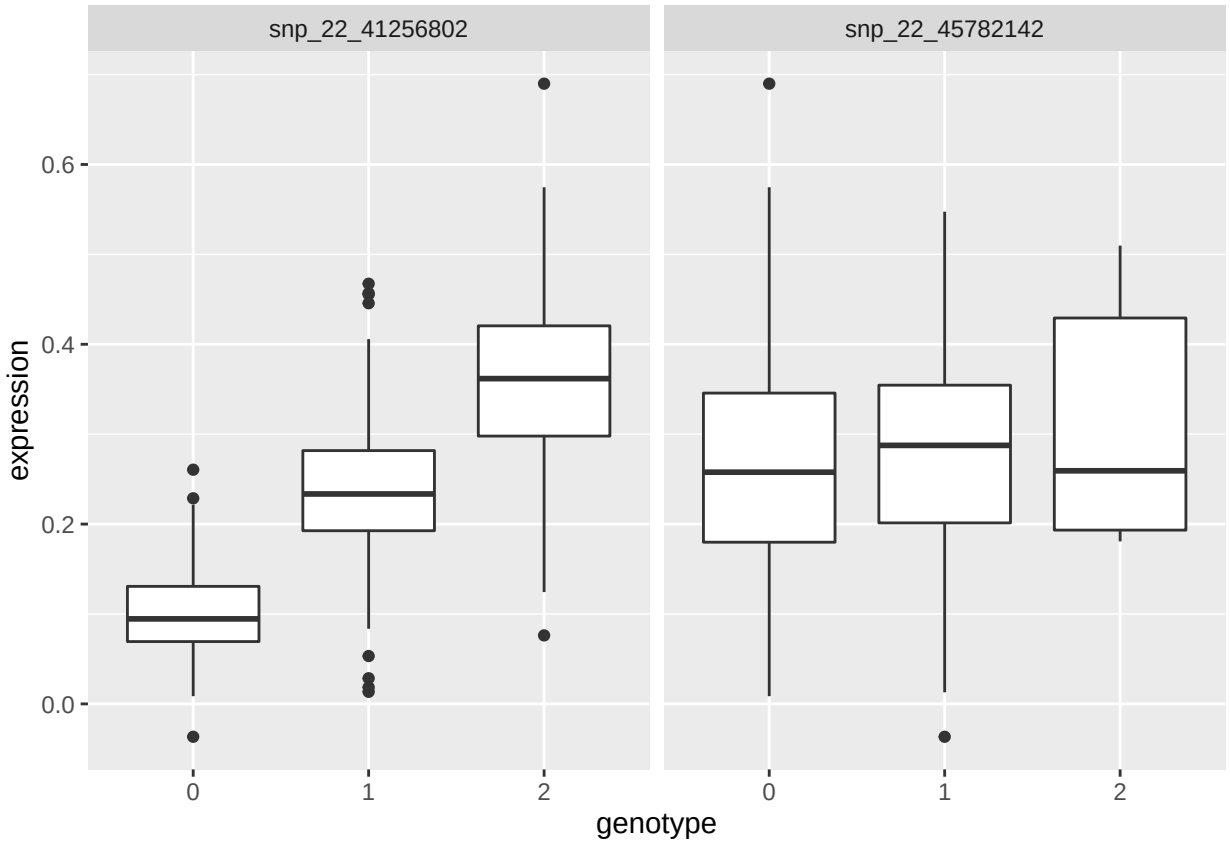
```
## -- Attaching packages ------------------------------------------------ tidyverse 1.3.0 --
```

```
## v ggplot2 3.3.2     v purrr   0.3.4
## v tibble  3.0.3     v dplyr   1.0.0
## v tidyr   1.1.0     v stringr 1.4.0
## v readr   1.3.1     v forcats 0.5.0
```

```
## -- Conflicts --------------------------------------------------- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

```
snps1 <- c("snp_22_41256802", "snp_22_45782142")
gene1_col <- t(gene_expr[gene1,])
snps1_col <- t(snps_filtered[snps1,])
df <- cbind(snps1_col, gene1_col)
colnames(df) <- c(snps1, "expression")
df <- df %>%
```

```
  as_tibble %>%
  gather(-expression, key="SNP", value ="genotype")
df$genotype <- as.factor(df$genotype)
df %>%
  ggplot(aes(x=genotype, y=expression)) + geom_boxplot() +
  facet_wrap(~SNP)
```



Task 4

1. Do a linear regression of all sample genotypes on sample gene expression for snp_22_41256802 on ENSG00000172404.4

```
lm_snp_a <- lm(gene1_col ~ snps1_col[,1])
summary(lm_snp_a)
```

```
##
## Call:
## lm(formula = gene1_col ~ snps1_col[, 1])
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.28348 -0.04934 -0.00143  0.04950  0.33024
##
## Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.109393   0.007824   13.98   <2e-16 ***
```

```
## snps1_col[, 1] 0.125135   0.005391   23.21   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.08216 on 460 degrees of freedom
## Multiple R-squared:  0.5394, Adjusted R-squared:  0.5384
## F-statistic: 538.7 on 1 and 460 DF,  p-value: < 2.2e-16
```

2. Do a linear regression of all sample genotypes on sample gene expression for snp_22_45782142 on ENSG00000172404.4

```
lm_snp_b <- lm(gene1_col ~ snps1_col[,2])
summary(lm_snp_b)
```

```
##
## Call:
## lm(formula = gene1_col ~ snps1_col[, 2])
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.31360 -0.08515 -0.00588  0.07998  0.42486
##
## Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.265040   0.006332  41.856   <2e-16 ***
## snps1_col[, 2] 0.011987   0.012425   0.965    0.335
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1209 on 460 degrees of freedom
## Multiple R-squared:  0.002019,   Adjusted R-squared:  -0.0001502
## F-statistic: 0.9308 on 1 and 460 DF,  p-value: 0.3352
```

3. Make sense of the results (Understand what the values represent) Gene expression levels can be somewhat explained by the first SNP but not the second. This is because the first SNP has an R^2 value of 0.5394 and very small p value (<2e-16) for its coefficient meaning the model explains around half the variation in the data and the association is significant. In contrast the second SNP has an R^2 value of 0.002019 meaning the model explains almost no variation in the data and a large p value 0.335 meaning the association is not significant.

Question 4 Do a linear regression for snp_22_43336231 on ENSG00000100266.11

Without covariates

```
gene2 <- "ENSG00000100266.11"
snp2 <- "snp_22_43336231"

gene2_col <- t(gene_expr)[,gene2]
gene2_snp <- t(snps_filtered)[,snp2]
lm_no_cov <- lm(gene2_col ~ gene2_snp)
summary(lm_no_cov)
```

```
##
## Call:
## lm(formula = gene2_col ~ gene2_snp)
##
```

```
## Residuals:
##     Min      1Q  Median      3Q     Max
## -34.367  -5.791  -0.774   4.563  41.890
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  23.8641     0.5297   45.05  < 2e-16 ***
## gene2_snp     3.3238     0.6121    5.43 9.13e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 8.746 on 460 degrees of freedom
## Multiple R-squared:  0.06024,    Adjusted R-squared:  0.0582
## F-statistic: 29.49 on 1 and 460 DF,  p-value: 9.131e-08
```

Using the genotype PCs from pc_cvrt.tab as covariates

```
pc <- read.table("data/pc_cvrt.tab")
lm_pc <- lm(gene2_col ~ gene2_snp + pc$PC1 + pc$PC2 + pc$PC3 + pc$PC4 + pc$PC5)
summary(lm_pc)
```

```
##
## Call:
## lm(formula = gene2_col ~ gene2_snp + pc$PC1 + pc$PC2 + pc$PC3 +
##     pc$PC4 + pc$PC5)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -33.129  -5.400  -0.454   4.568  43.137
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 23.521911   0.543181  43.304  < 2e-16 ***
## gene2_snp    3.941343   0.660838   5.964 4.94e-09 ***
## pc$PC1       0.012720   0.004472   2.844  0.00465 **
## pc$PC2       0.026296   0.014024   1.875  0.06142 .
## pc$PC3      -0.034836   0.014238  -2.447  0.01480 *
## pc$PC4       0.004344   0.015497   0.280  0.77934
## pc$PC5       0.007566   0.016014   0.472  0.63681
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 8.623 on 455 degrees of freedom
## Multiple R-squared:  0.09625,    Adjusted R-squared:  0.08433
## F-statistic: 8.076 on 6 and 455 DF,  p-value: 2.643e-08
```

Separately for african and non-africans without covariates. Hint: Use the information in the design.tab

```
get_pop_gene <- function(genes, pop, gene_mat, design_mat, inv = F){
  genes_table <- filter_snp_population(pop, gene_mat, design_mat, inv)
  genes <- t(genes_table)[,genes]
  return(genes)
}
```

```
#make african model
gene2_africa <- get_pop_gene(gene2, "YRI", gene_expr, design)
snp2_africa <- t(african_snps)[,snp2]
lm_africa <- lm(gene2_africa ~ snp2_africa)
summary(lm_africa)
```

```
##
## Call:
## lm(formula = gene2_africa ~ snp2_africa)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -15.0137  -4.1504  -0.3292   5.0336  19.5839
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  26.3095     0.7353  35.781   <2e-16 ***
## snp2_africa  -0.7181     2.8319  -0.254      0.8
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 6.699 on 87 degrees of freedom
## Multiple R-squared:  0.0007385,  Adjusted R-squared:  -0.01075
## F-statistic: 0.0643 on 1 and 87 DF,  p-value: 0.8004
```

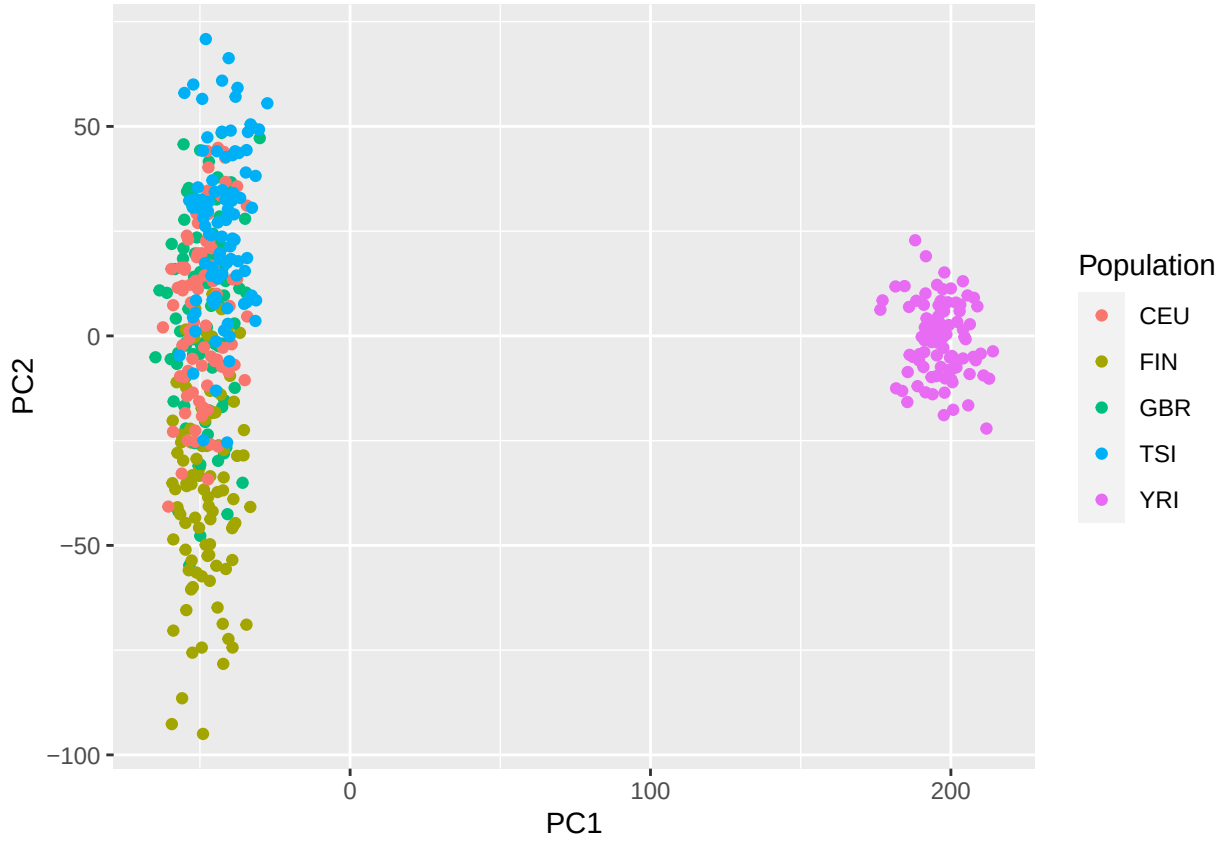
```
#make non african model
gene2_nonafrica <- get_pop_gene(gene2, "YRI", gene_expr, design, T)
snp2_nonafrica <- t(non_african_snps)[,snp2]
lm_nonafrica <- lm(gene2_nonafrica ~ snp2_nonafrica)
summary(lm_nonafrica)
```

```
##
## Call:
## lm(formula = gene2_nonafrica ~ snp2_nonafrica)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -34.922  -5.727  -0.700   4.583  42.142
##
## Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)     22.8046     0.6598  34.562  < 2e-16 ***
## snp2_nonafrica   4.1310     0.6911   5.978 5.32e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 9.075 on 371 degrees of freedom
## Multiple R-squared:  0.08785,    Adjusted R-squared:  0.08539
## F-statistic: 35.73 on 1 and 371 DF,  p-value: 5.321e-09
```

Make a dotplot of PC1 vs PC2 and color the dots by population

```
library(tidyverse)
pc_df <- data.frame(PC1=pc$PC1, PC2=pc$PC2, Population=design$Characteristics.population.)
```

```r
pc_df %>%
  #gather(-Population, key="PC", value="Value") %>%
  ggplot(aes(x=PC1, y=PC2, col=Population)) +
  geom_point()
```



Question 5

1. There is no difference since both models have very low R^2 values meaning both models fit the data very poorly. The additional covariates added in the second model (the principal components) also have weak associations meaning they are not useful tot he model.
2. While both models do not fit the data very well due to their low R^2 values, the non African model has a better fit. In addition, the p value for the non African model is very low indicating a a significant association for non African SNPs. This association is not present for the African model. 3 We are including the principal components of the gene expression data.

Task 6 Do a linear regression on 1st snp on 1st gene, 2nd snp on 2nd gene etc.

1. Create a matrix containing the gene_id, snp_id, effect size, t.value and p.value

```r
gene_t <- as.data.frame(t(gene_expr))
snps_filtered_t <- as.data.frame(t(snps_filtered))

get_lm_values <- function(expr, snps){
  model <- summary(lm(expr ~ snps))
  return(model$coefficients[2,])
}
```

```
get_lm_matrix <- function(expr, snps, fun){
  lm_matrix <- mapply(fun, expr, snps)
  lm_matrix <- as.data.frame(t(lm_matrix))
  lm_matrix$gene_id <- rownames(lm_matrix)
  lm_matrix$snp_id <- colnames(snps)
  rownames(lm_matrix) <- NULL
  lm_matrix <- lm_matrix[,c(5,6,1,3,4)]
  colnames(lm_matrix) <- c("gene_id", "snp_id", "effect_size", "t.value", "p.value")
  return(lm_matrix)
}

lm_matrix <- get_lm_matrix(gene_t, snps_filtered_t, get_lm_values)
head(lm_matrix)
```

```
##              gene_id          snp_id   effect_size     t.value   p.value
## 1 ENSG00000185386.10 snp_22_30772686  0.0151891271  0.11471394 0.9087219
## 2  ENSG00000203606.3 snp_22_34965577 -0.0026938729 -0.43502718 0.6637467
## 3  ENSG00000069998.8 snp_22_49436707  0.0303004155  0.05279978 0.9579144
## 4  ENSG00000240293.1 snp_22_34153853  0.0005987039  0.12815676 0.8980809
## 5  ENSG00000232926.1 snp_22_21970216 -0.0123130207 -1.20403852 0.2291939
## 6 ENSG00000100151.11 snp_22_48286671 -0.0689291239 -0.37137433 0.7105297
```

2. Do a multiple testing correction on the resulting p.values using fdr.

```
lm_matrix$p.adj <- p.adjust(lm_matrix$p.value, method = "fdr")
lm_matrix %>%
  filter(p.adj < 0.05)
```

```
##              gene_id          snp_id effect_size   t.value      p.value
## 1  ENSG00000205853.5 snp_22_32778467 -0.09675995 -4.362069 1.591656e-05
## 2 ENSG00000186716.14 snp_22_23454881 -0.73348131 -2.635860 8.676019e-03
## 3  ENSG00000172404.4 snp_22_41256802  0.12513490 23.209894 1.853078e-79
## 4 ENSG00000075234.12 snp_22_46686404  3.02798811 14.751187 1.335992e-40
## 5 ENSG00000100266.11 snp_22_43336231  3.32381025  5.430240 9.130826e-08
## 6  ENSG00000128408.7 snp_22_45782142 -0.27686405 -3.974314 8.193048e-05
##          p.adj
## 1 1.273325e-04
## 2 4.627210e-02
## 3 5.929849e-78
## 4 2.137587e-39
## 5 9.739548e-07
## 6 5.243551e-04
```

3. Do the same but now include the genotype PCs from pc_cvrt.tab as covariates.

```
get_lm_values_covariate <- function(expr, snps){
  model <- summary(lm(expr ~ snps + pc$PC1 + pc$PC2 + pc$PC3 + pc$PC4 + pc$PC5))
  return(model$coefficients[2,])
}

lm_matrix_cov <- get_lm_matrix(gene_t, snps_filtered_t, get_lm_values_covariate)
lm_matrix_cov$p.adj <- p.adjust(lm_matrix_cov$p.value, method = "fdr")
lm_matrix_cov %>%
  filter(p.adj < 0.05)
```

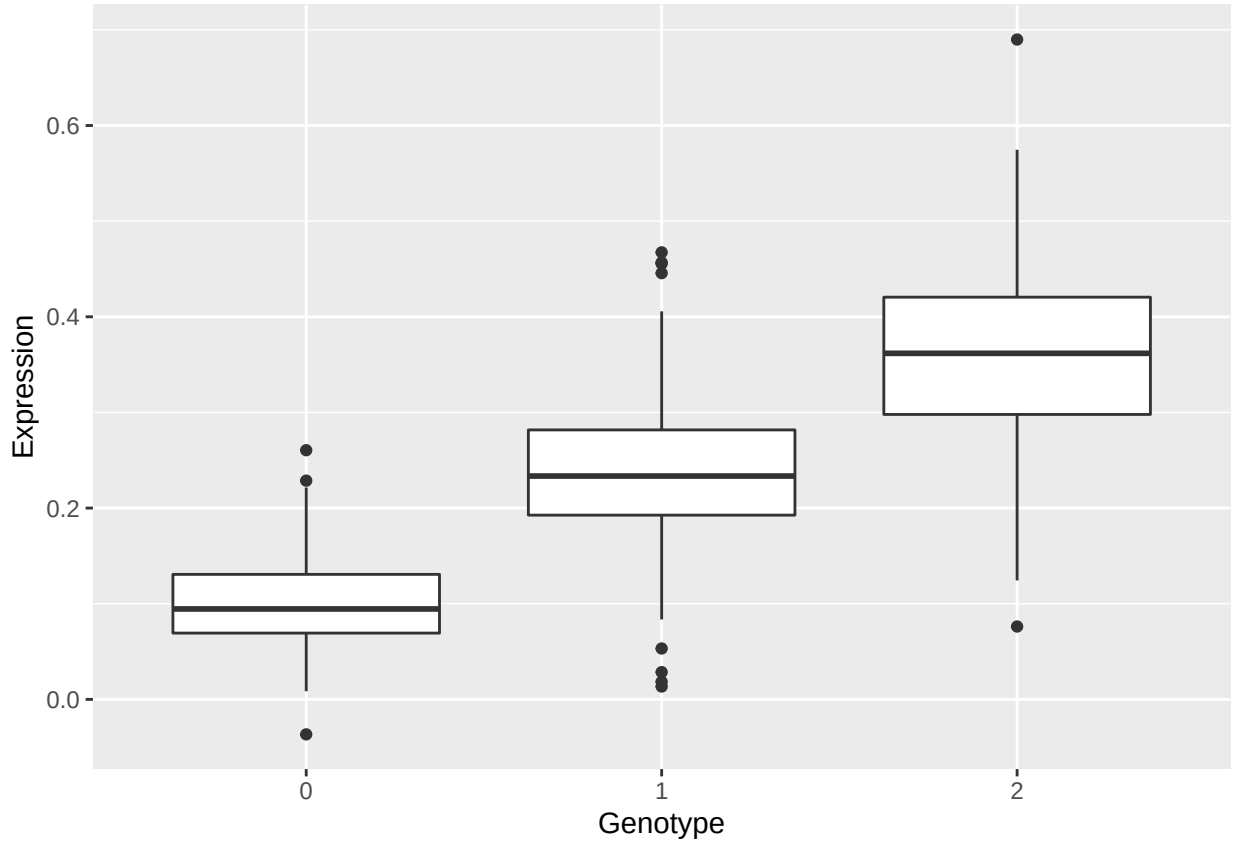
```
##              gene_id          snp_id effect_size   t.value      p.value
```

```
## 1  ENSG00000205853.5 snp_22_32778467  -0.1014790 -4.476520 9.601900e-06
## 2 ENSG00000186716.14 snp_22_23454881  -0.8623192 -2.997211 2.873675e-03
## 3  ENSG00000172404.4 snp_22_41256802   0.1382878 22.657865 1.207675e-76
## 4 ENSG00000075234.12 snp_22_46686404   3.6745686 14.935637 2.479445e-41
## 5 ENSG00000100266.11 snp_22_43336231   3.9413429  5.964158 4.942791e-09
## 6  ENSG00000128408.7 snp_22_45782142  -0.3092542 -4.407607 1.305208e-05
##          p.adj
## 1 7.681520e-05
## 2 1.532627e-02
## 3 3.864559e-75
## 4 3.967112e-40
## 5 5.272310e-08
## 6 8.353331e-05
```

4. Plot the most significant hit.

```
sig_row <- lm_matrix_cov[lm_matrix_cov$p.adj == min(lm_matrix_cov$p.adj),]
df <- data.frame(Expression = gene_t[,sig_row$gene_id],
                 Genotype = as.factor(snps_filtered_t[,sig_row$snp_id]))

df %>%
  ggplot(aes(x=Genotype, y=Expression)) + geom_boxplot()
```



Question 6

1. 32 tests were performed in a and 32 tests were performed in c.
2. Since multiple tests are being performed, it is possible to get a false positive by obtaining a significant

p value by chance. The false positives are corrected using a multiple test correction. In eQTL analysis, a huge number of significance tests are performed so many false positives can occur, so it is essential to make sure to check for false positives.

3. No both models produced the same number of significant hits.

Task 7

```
library(MatrixEQTL)
snps <- SlicedData$new()
snps$CreateFromMatrix(as.matrix(snps_filtered)) #filt_geno is your filtered genotype matrix
genes <- SlicedData$new()
genes$CreateFromMatrix(as.matrix(gene_expr)) #expr is the unchanged expression matrix
snp_pos <- read.table("data/sample_geno.pos",sep="\t",header=T)
snp_pos <- snp_pos[snp_pos$snp %in% row.names(snps_filtered),]
gene_pos <- read.table("data/sample_expr.pos",sep="\t",header=T)
all(colnames(snps) == colnames(genes))
```

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

```
eQTL <- Matrix_eQTL_main(snps, genes, output_file_name=NULL,
output_file_name.cis=NULL,
pvOutputThreshold.cis=1, pvOutputThreshold=1,
snpspos=snp_pos, genepos=gene_pos,
cisDist = 0)
```

```
## Matching data files and location files
## 32 of 32 genes matched
## 32 of 32 SNPs matched
## Task finished in 0.01 seconds
## Reordering SNPs
## Task finished in 0.14 seconds
## Reordering genes
## Task finished in 0.13 seconds
## Processing covariates
## Task finished in 0.02 seconds
## Processing gene expression data (imputation, residualization)
## Task finished in 0 seconds
## Creating output file(s)
## Task finished in 0 seconds
## Performing eQTL analysis
## 100.00% done, 0 cis-eQTLs, 1,024 trans-eQTLs
## No significant associations were found.
## Task finished in 0.01 seconds
##
```

Question 7

1. 1024 tests were performed.
2. MatrixeQTL found no significant hits while the analysis in Task 6 yielded 6 significant hits. This is caused by the fact that we only perform 32 tests while MatrixeQTL performs 1024 tests. The additional tests result in a a harsher correction for multiple testing resulting a higher p adjusted value which is why no significant hits were found.