

# LD\_SNPS\_RESISTIN

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```
library(tidyverse)
```

```
## -- Attaching packages ----- tidyverse 1.3.2 --
## v ggplot2 3.4.0      v purrr  1.0.1
## v tibble  3.1.8      v dplyr  1.0.10
## v tidyr   1.2.1      v stringr 1.5.0
## v readr   2.1.3      v forcats 0.5.2
```

```
## Warning: package 'stringr' was built under R version 4.2.3
```

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

```
library(pheatmap)
```

```
## Warning: package 'pheatmap' was built under R version 4.2.3
```

```
library(haplo.stats)
```

```
## Warning: package 'haplo.stats' was built under R version 4.2.3
```

```
## Loading required package: arsenal
```

```
## Warning: package 'arsenal' was built under R version 4.2.3
```

```
library(dplyr)
library(magrittr)
```

```
##
## Attaching package: 'magrittr'
##
## The following object is masked from 'package:arsenal':
##
##   set_attr
##
## The following object is masked from 'package:purrr':
##
```

```

##      set_names
##
## The following object is masked from 'package:tidyr':
##
##      extract

library(genetics)

## Warning: package 'genetics' was built under R version 4.2.3

## Loading required package: combinat
##
## Attaching package: 'combinat'
##
## The following object is masked from 'package:utils':
##
##      combn
##
## Loading required package: gdata

## Warning in system(cmd, intern = intern, wait = wait | intern,
## show.output.on.console = wait, : running command 'C:\WINDOWS\system32\cmd.exe /c
## ftype perl' had status 2

## Warning in system(cmd, intern = intern, wait = wait | intern,
## show.output.on.console = wait, : running command 'C:\WINDOWS\system32\cmd.exe /c
## ftype perl' had status 2

## gdata: read.xls support for 'XLS' (Excel 97-2004) files ENABLED.
##
## gdata: Unable to load perl libraries needed by read.xls()
## gdata: to support 'XLSX' (Excel 2007+) files.
##
## gdata: Run the function 'installXLSXsupport()'
## gdata: to automatically download and install the perl
## gdata: libraries needed to support Excel XLS and XLSX formats.
##
## Attaching package: 'gdata'
##
## The following objects are masked from 'package:dplyr':
##
##      combine, first, last
##
## The following object is masked from 'package:purrr':
##
##      keep
##
## The following object is masked from 'package:stats':
##
##      nobs
##
## The following object is masked from 'package:utils':

```

```
##
##      object.size
##
## The following object is masked from 'package:base':
##
##      startsWith
##
## Loading required package: gtools

## Warning: package 'gtools' was built under R version 4.2.3

## Loading required package: MASS

## Warning: package 'MASS' was built under R version 4.2.3

##
## Attaching package: 'MASS'
##
## The following object is masked from 'package:dplyr':
##
##      select
##
## Loading required package: mvtnorm
##
##
## NOTE: THIS PACKAGE IS NOW OBSOLETE.
##
##
##
## The R-Genetics project has developed an set of enhanced genetics
## packages to replace 'genetics'. Please visit the project homepage
## at http://rgenetics.org for informtion.
##
##
##
## Attaching package: 'genetics'
##
## The following object is masked from 'package:haplo.stats':
##
##      locus
##
## The following objects are masked from 'package:base':
##
##      %in%, as.factor, order
```

```
##STEP ONE-READING IN THE DATASET FROM THE LINK
```

```
fams <- read.delim("http://www.biostat.umn.edu/~cavanr/FMS_data.txt",header = T,
  sep = "\t")
```

```
#Vieing the first five rows and 10columns of the dataset
```

```
fams[1:5,1:10]
```

```
##      id acdc_rs1501299 ace_id actn3_r577x actn3_rs540874 actn3_rs1815739
## 1 FA-1801          CA      DD          CC          GG          CC
## 2 FA-1802          CA      ID          CT          GA          TC
## 3 FA-1803          CA      ID          CT          GA          TC
## 4 FA-1804          CC      DD          CT          GA          TC
## 5 FA-1805          CA      ID          CC          GG          CC
##      actn3_1671064 ardb1_1801253 adrb2_1042713 adrb2_1042714
## 1          AA          <NA>          GA          CG
## 2          GA          <NA>          GA          CC
## 3          GA          <NA>          GA          CG
## 4          GA          <NA>          AA          CC
## 5          AA          <NA>          GA          CG
```

#### ##SELECTING OUT COLUMNS WITH RESISTIN GENES

```
fams_restn <- fams[grepl("^resistin", names(fams))]
head(fams_restn)
```

```
##      resistin_c30t resistin_c398t resistin_g540a resistin_c980g resistin_c180g
## 1          CC          CC          GG          GG          CC
## 2          CC          TT          AA          CG          GG
## 3          CC          CC          GG          CG          CC
## 4          CC          CC          GA          CG          CG
## 5          CT          CC          GG          CC          CC
## 6          CC          CC          GG          CG          CC
##      resistin_a537c
## 1          AA
## 2          AA
## 3          AA
## 4          AA
## 5          AA
## 6          AA
```

#### ##Counting the number of generated columns

```
restn_col <- ncol(fams_restn)
cat("The columns with resistin gene are:", restn_col)
```

```
## The columns with resistin gene are: 6
```

#### ##IDENTIFYING UNIQUE SNPS IN THE COLUMNS

##### ##STEP ONE CREATE A VECTOR

```
restn_snp <- unlist(fams_restn)
head(restn_snp)
```

```
## resistin_c30t1 resistin_c30t2 resistin_c30t3 resistin_c30t4 resistin_c30t5
##      "CC"          "CC"          "CC"          "CC"          "CT"
## resistin_c30t6
##      "CC"
```

#### ##STEP TWO:IDENTIFYING UNIQUE VALUES IN THE VVECTOR

```
num_snps_restn <- length(unique(restn_snp))  
cat("The total number of snps in the restin genes is :",num_snps_restn)
```

```
## The total number of snps in the restin genes is : 9
```

#### ##CREATING A GENOTYPE OBJECT FOR OUR GENES OF THE RESISTIN GENE

```
geno_restn <- as.data.frame(lapply(fams_restn, genotype, sep=""))  
geno_restn[1:5,]
```

```
##   resistin_c30t resistin_c398t resistin_g540a resistin_c980g resistin_c180g  
## 1           C/C           C/C           G/G           G/G           C/C  
## 2           C/C           T/T           A/A           C/G           G/G  
## 3           C/C           C/C           G/G           C/G           C/C  
## 4           C/C           C/C           G/A           C/G           C/G  
## 5           C/T           C/C           G/G           C/C           C/C  
##   resistin_a537c  
## 1           A/A  
## 2           A/A  
## 3           A/A  
## 4           A/A  
## 5           A/A
```

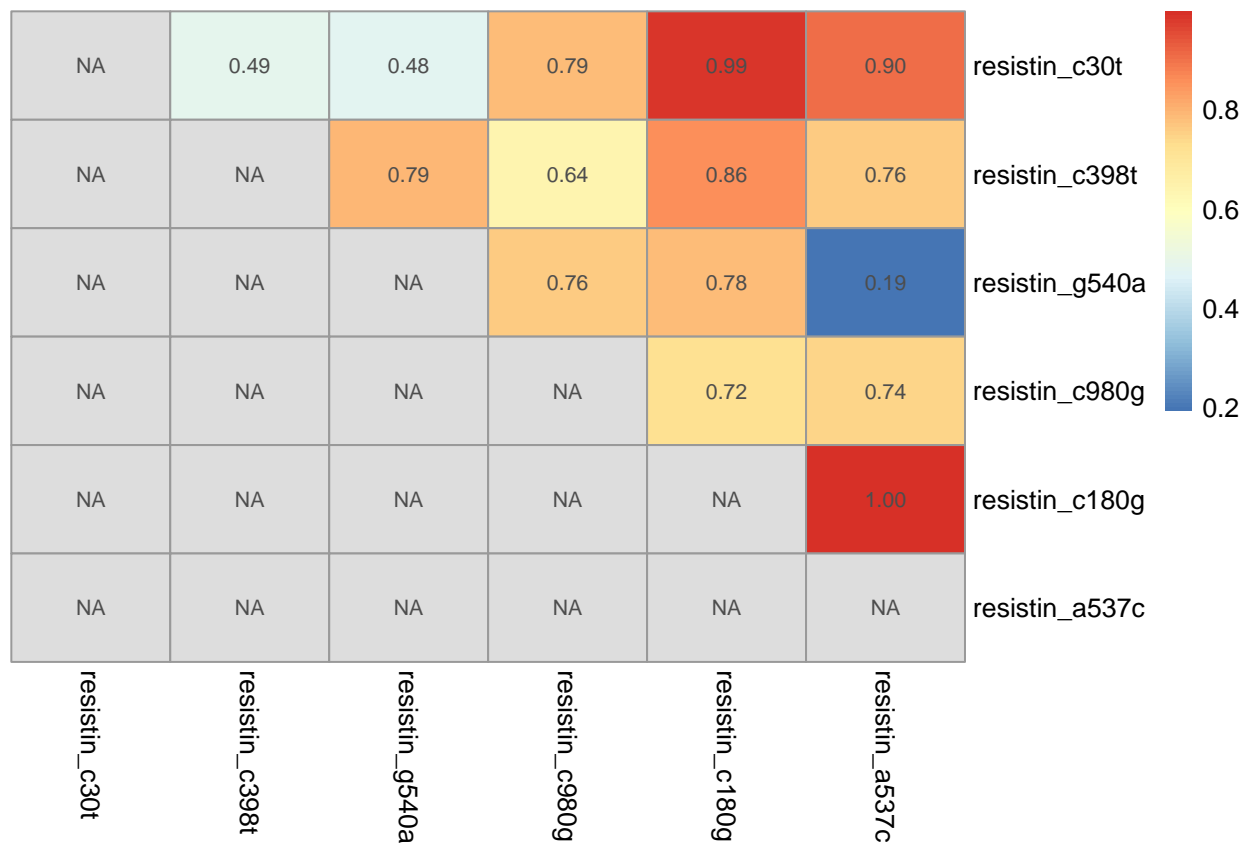
#### ##Calculating D' OF THE SNPS IN THE RESISTIN GENE

```
restn_D <- LD(geno_restn)$`D`  
restn_D
```

```
##           resistin_c30t resistin_c398t resistin_g540a resistin_c980g  
## resistin_c30t           NA      0.4855449      0.4810285      0.7877728  
## resistin_c398t           NA           NA      0.7944463      0.6403467  
## resistin_g540a           NA           NA           NA      0.7586427  
## resistin_c980g           NA           NA           NA           NA  
## resistin_c180g           NA           NA           NA           NA  
## resistin_a537c           NA           NA           NA           NA  
##           resistin_c180g resistin_a537c  
## resistin_c30t      0.9850864      0.9021448  
## resistin_c398t      0.8581316      0.7633220  
## resistin_g540a      0.7833792      0.1929656  
## resistin_c980g      0.7183456      0.7441364  
## resistin_c180g           NA      0.9983006  
## resistin_a537c           NA           NA
```

#### ##GENERATING A HEATMAP TO SHOW THE D' OF SNPS

```
pheatmap(restn_D, cluster_cols = FALSE, cluster_rows = FALSE, display_numbers = TRUE)
```



##From the heatmap, it shows that the SNP at resistin\_c180g is in high LD with resistin\_a537c  
 ## Also resistin\_c30t is in high LD with resistin\_c180g.

###CALCULATING OF HARDY WEINBERG EQUILLIBRIUM(USING CHISQUARE AND FISCHER'S EXACT TEST)

```
#chi_pval <- vector() #create empty vector
```

```
#for (col in geno_restn){
  #chiq <- HWE.chisq(col)
  # chi_pval <- c(chi_pval, chisq$p.value)
#}
#sort(chi_pval)
```

##naming p-values

```
#names(chi_pval)=colnames(geno_restn) ##assigning names with those in the genotype object
#sort(chi_pval)
```

```
#sum(chi_pval<0.05)##finding total pvalues <0.5
```

```
#names(chi_pval[chi_pval<0.05])##getting names of columns with pvalues < 0.5
```

##CALCULATING THE FISCHER'S EXACT

```

ext_pval <- vector()

for (col in geno_restn){
  exact <- HWE.exact(col)
  ext_pval <- c(ext_pval,exact$p.value)
}
sort(ext_pval)

## [1] 0.1166305 0.3153145 0.4105245 1.0000000 1.0000000 1.0000000

sum(ext_pval<0.05)

## [1] 0

names(ext_pval)=colnames(geno_restn)
sort(ext_pval)

## resistin_g540a resistin_c980g resistin_c180g resistin_a537c resistin_c30t
##      0.1166305      0.3153145      0.4105245      1.0000000      1.0000000
## resistin_c398t
##      1.0000000

names(ext_pval[ext_pval<0.05])

## character(0)

##Adjusting using Bonferoni to cater for multiple testing

##Adjusting chi-square values
#set.seed(100)
#adj_pva <- p.adjust(chi_pval, method ="bonferroni")
#sort(adj_pva)
#sum(adj_pva<0.05)
#names(adj_pva[adj_pva<0.05])

##Adjusting exact p-values
set.seed(42)#for reproducibility
aj_val <- p.adjust(ext_pval,method = "bonferroni")
sort(aj_val)

## resistin_g540a resistin_c30t resistin_c398t resistin_c980g resistin_c180g
##      0.699783      1.000000      1.000000      1.000000      1.000000
## resistin_a537c
##      1.000000

sum(aj_val<0.05)

## [1] 0

```

```
names(aj_val[aj_val<0.05])
```

```
## character(0)
```

```
##CALCULATING MINOR ALLELE FREQUENCY(MAF) OF SNPS
```

```
##We first identify missing values
```

```
miss_gen <- data.frame(summary(is.na(geno_restn))[3,])
```

```
names(miss_gen) <- c("Missing values")
```

```
miss_gen
```

```
##           Missing values
## resistin_c30t  TRUE :664
## resistin_c398t TRUE :662
## resistin_g540a TRUE :661
## resistin_c980g TRUE :660
## resistin_c180g TRUE :658
## resistin_a537c TRUE :657
```

```
##Regardless, we take it that our allele frequencies will remain the same even if we had got the missing
```

```
##Calculating the MINOR ALLELE FREQUENCY(MAF) FOR AT EACH SNP
```

```
round(summary(geno_restn$resistin_c30t)$"allele.freq",1)
```

```
##      Count Proportion
## C      1445          1
## T        21          0
## NA     1328         NA
```

```
round(summary(geno_restn$resistin_c398t)$"allele.freq",1)
```

```
##      Count Proportion
## C      1165          0.8
## T       305          0.2
## NA     1324         NA
```

```
round(summary(geno_restn$resistin_g540a)$"allele.freq",1)
```

```
##      Count Proportion
## G      1025          0.7
## A       447          0.3
## NA     1322         NA
```

```
##MAF gives us a picture of frequency of a variation within a population
```

Note that the `echo = FALSE` parameter was added to the code chunk to prevent printing of the R code that generated the plot.