Introduction to Genomic Selection in R using the rrBLUP Package

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Learning Objectives

- Download the package and load the sample files
- Impute missing markers using A.mat()
- Define the training and validation populations
- Run mixed.solve() and determine accuracy of predictions

Overview of rrBLUP package

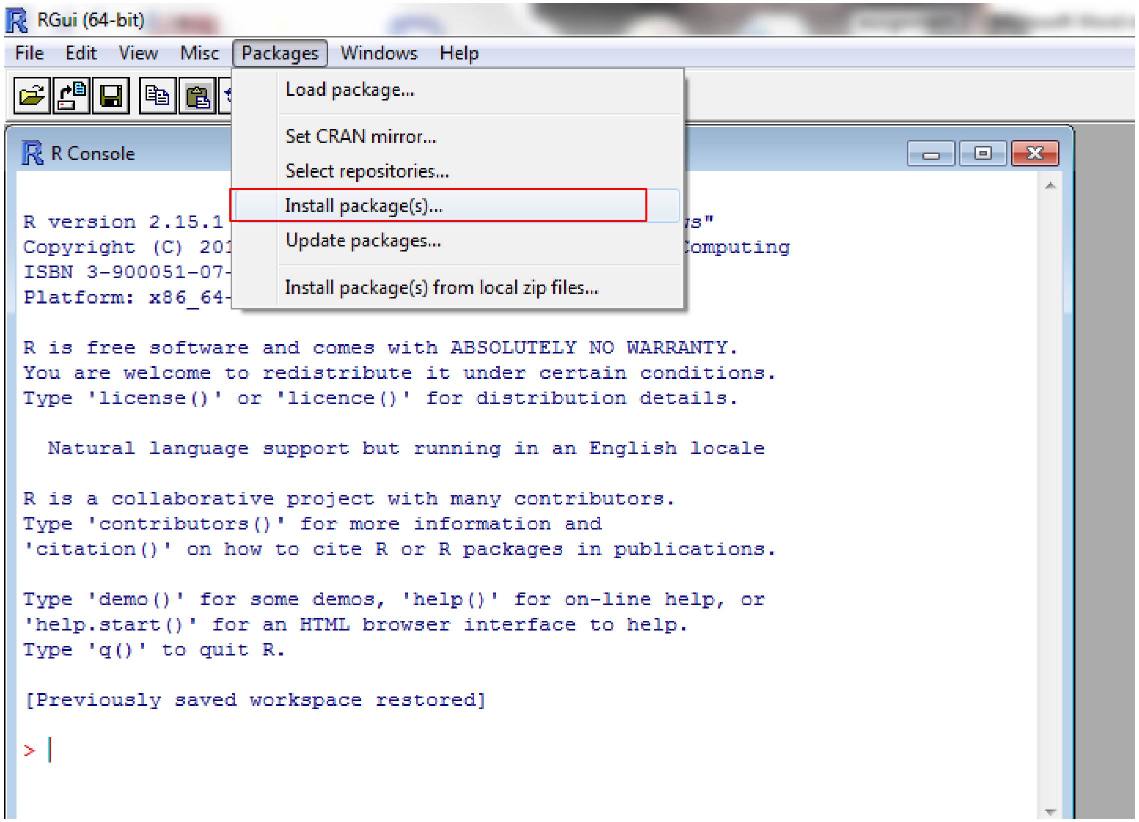
- Download from CRAN-version 4
  - Must use R version 2.14.1 or greater
- Uses ridge regression BLUP for genomic predictions
- Predicts marker effects through mixed.solve()
- A.mat() command can be used to impute missing markers
  - Mixed.sove does not allow NA marker values
- Define the training and validation populations

One Step vs. Two Step

- One step
  - Uses a mixed model analysis for the plot data
- Two step
  - Adjusted means are calculated across locations
  - Means are then used in ridge regression blup
- This webinar uses a two step approach
  - Computationally more efficient and faster

Install the rrBLUP Package

- Launch R->Packages->Install Package
- Select CRAN Mirror nearest you



```
R version 2.15.1                    s"
Copyright (C) 201                   Computing
ISBN 3-900051-07-
Platform: x86_64-

R is free software and comes with ABSOLUTELY NO WARRANTY.
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R is a collaborative project with many contributors.
Type 'contributors()' for more information and
'citation()' on how to cite R or R packages in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

[Previously saved workspace restored]

> 
```
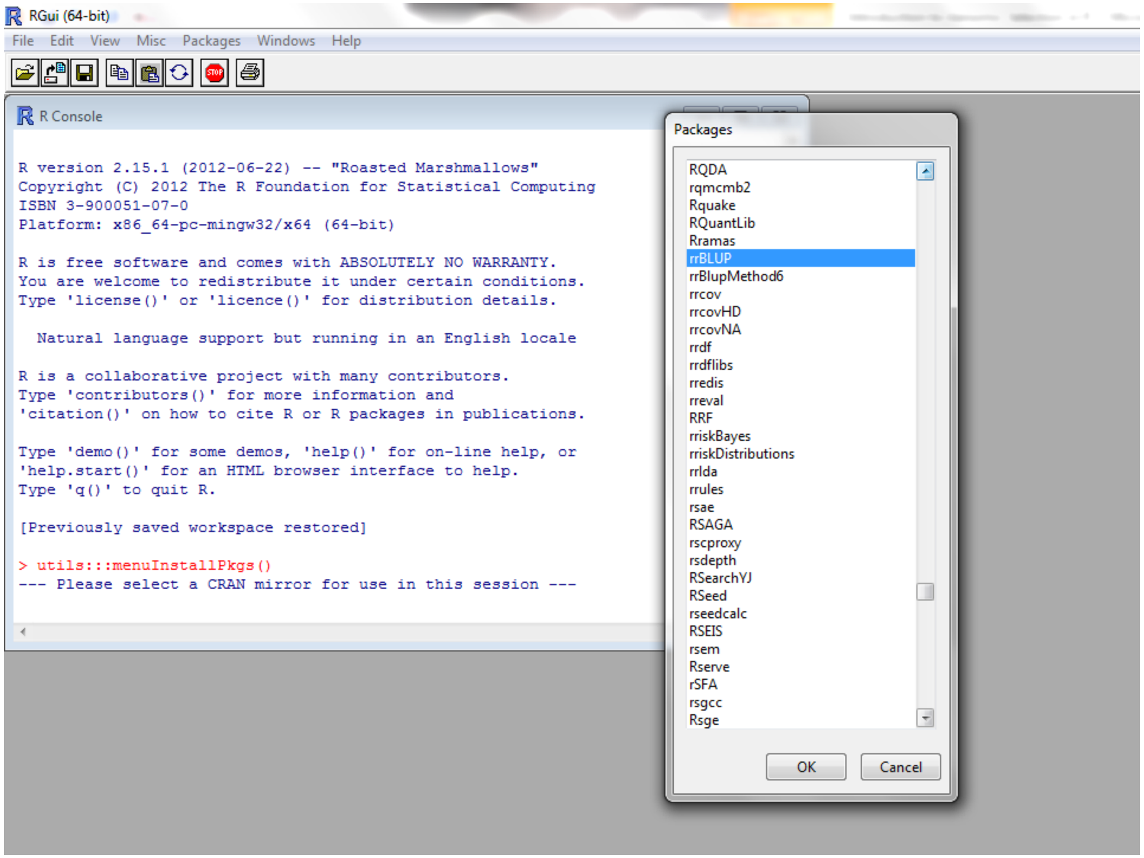

Install the rrBLUP Package

- Select the rrBLUP package



```
R version 2.15.1 (2012-06-22) -- "Roasted Marshmallows"
Copyright (C) 2012 The R Foundation for Statistical Computing
ISBN 3-900051-07-0
Platform: x86_64-pc-mingw32/x64 (64-bit)

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'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

[Previously saved workspace restored]

> utils:::menuInstallPkgs()
--- Please select a CRAN mirror for use in this session ---
```
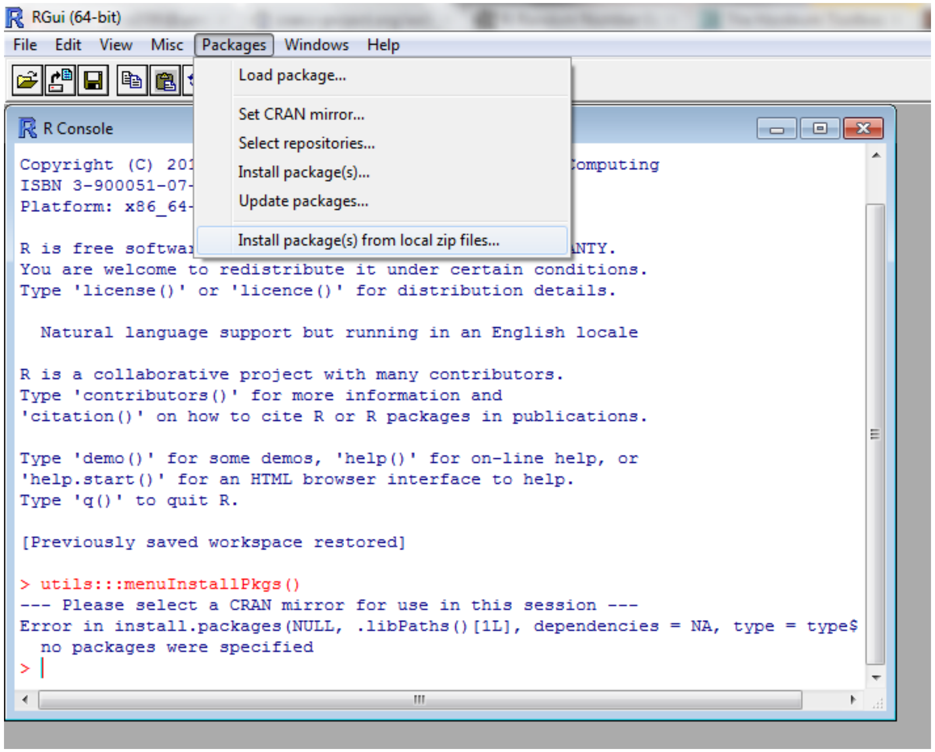

Install the rrBLUP Package

- Install the package by a zip file
- http://cran.r-project.org/web/packages/rrBLUP/index.html
- Packages->install package from local zip files



```
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Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

[Previously saved workspace restored]

> utils:::menuInstallPkgs()
--- Please select a CRAN mirror for use in this session ---
Error in install.packages(NULL, .libPaths()[1L], dependencies = NA, type = type$
  no packages were specified
> 
```
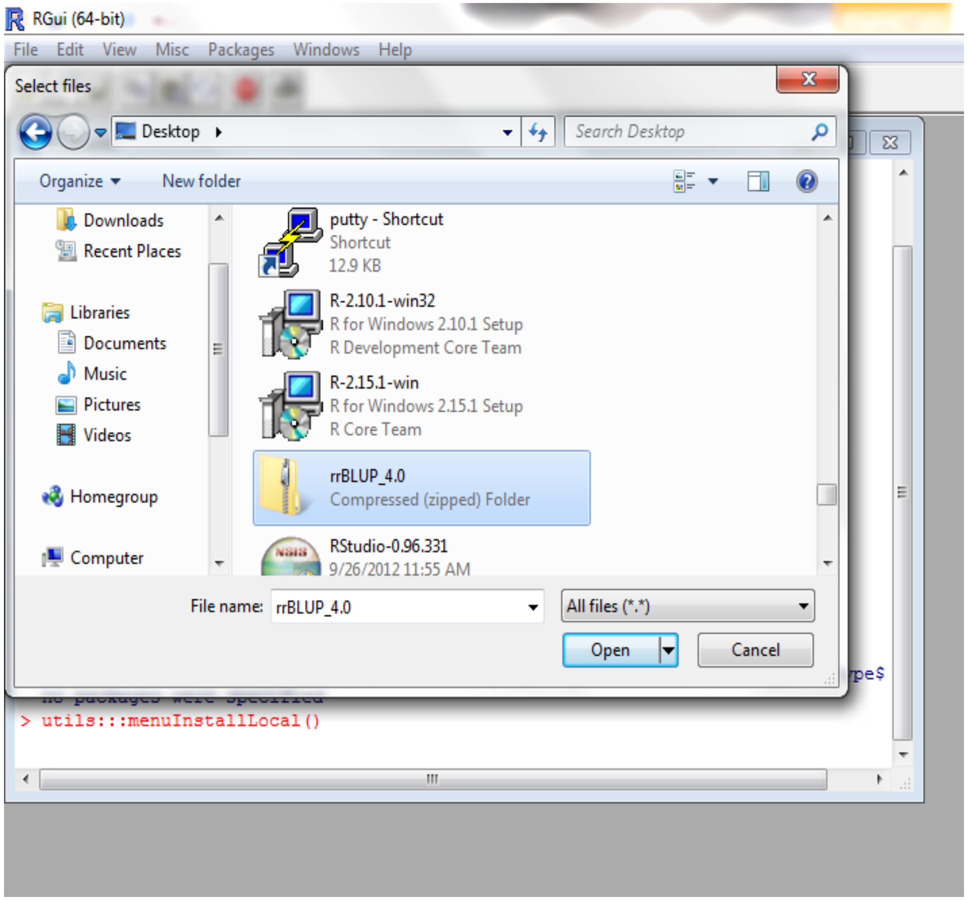

Install the rrBLUP Package

- Select the package from saved location



putty - Shortcut
Shortcut
12.9 KB

R-2.10.1-win32
R for Windows 2.10.1 Setup
R Development Core Team

R-2.15.1-win
R for Windows 2.15.1 Setup
R Core Team

rrBLUP_4.0
Compressed (zipped) Folder

RStudio-0.96.331
9/26/2012 11:55 AM

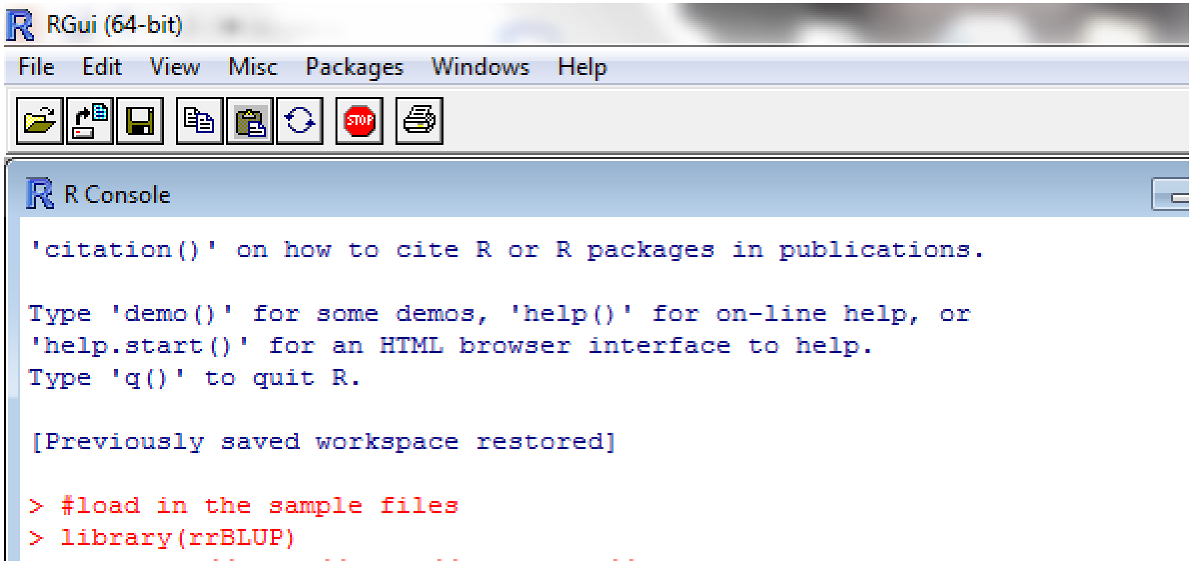
File name: rrBLUP_4.0

All files (*.*)

```
no packages were specified
> utils:::menuInstallLocal()
```

Install the rrBLUP Package

- Now that the package is installed, the library must be loaded every time R is opened



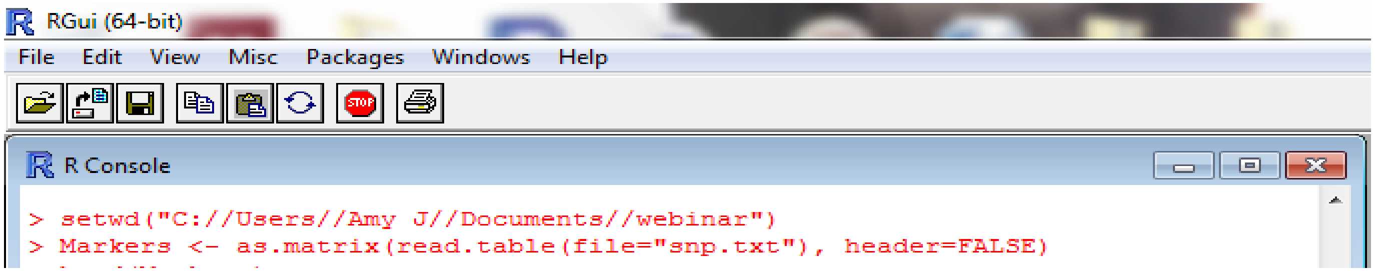
```
'citation()' on how to cite R or R packages in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

[Previously saved workspace restored]

> #load in the sample files
> library(rrBLUP)
```

Sample Files

- Files downloaded from the Hordeum Toolbox http://hordeumtoolbox.org/
- University of Minnesota barley breeding program preliminary yield trail-St. Paul location in 2009
- Phenotypic traits-yield, plant height and heading date
- 1178 markers, 164 NA markers
- 1 = homozygous for parent 1, 0 = heterozygous, and -1 homozygous for parent 2
  - Markers must be in the {-1,0,1} format for rrBLUP

Load the Sample Files

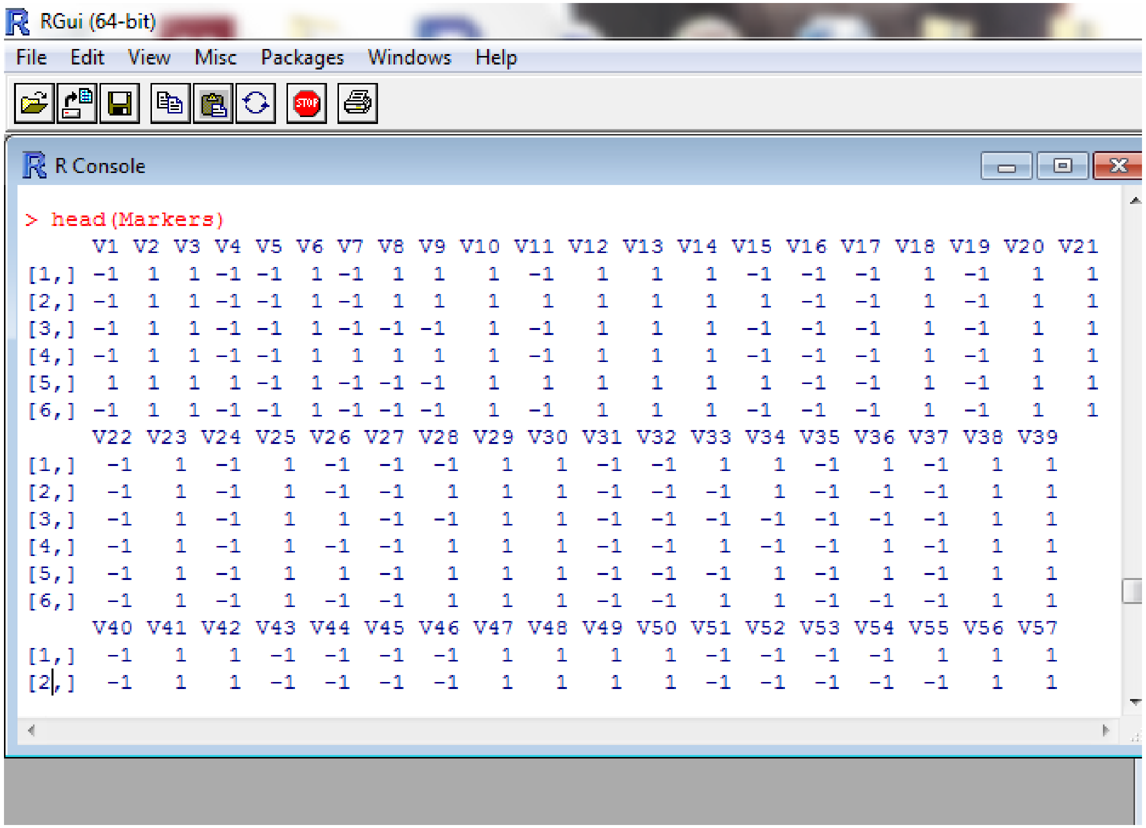
- Setwd()-Set the working directory to the location of the sample files
- Read.table command used for .txt files
- Read.csv command used for .csv files
- Header=F since sample marker file does not have a header with marker names



```
> setwd("C://Users//Amy J//Documents//webinar")
> Markers <- as.matrix(read.table(file="snp.txt"), header=FALSE)
```

Load the Sample Files

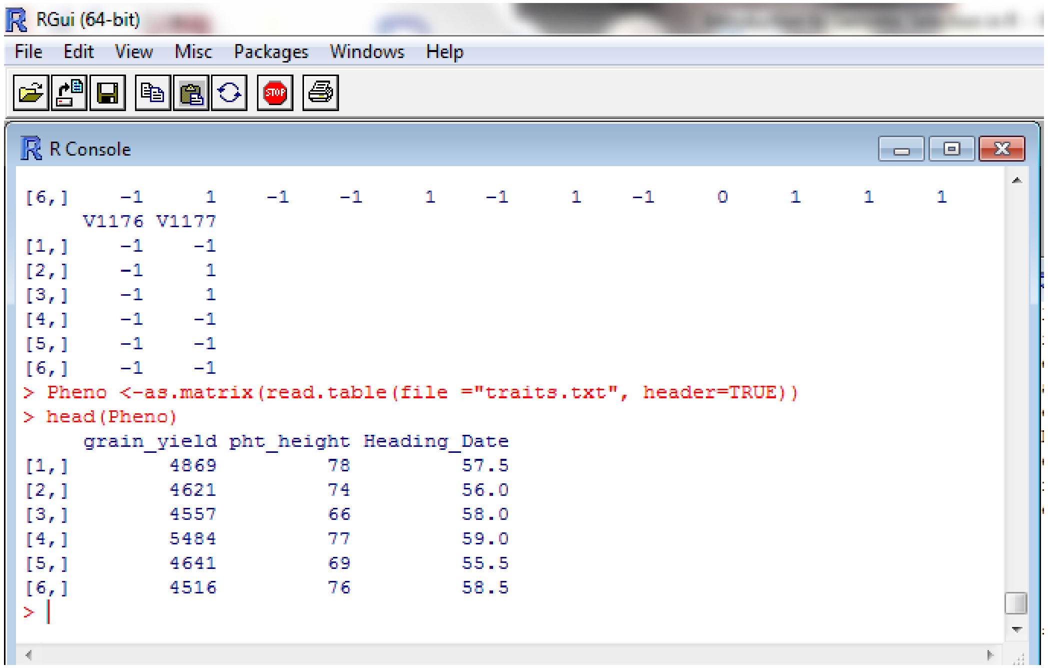
- head() command used to see the first 5 lines of a file
- Useful to see if data was loaded correctly



```
> head(Markers)
     V1 V2 V3 V4 V5 V6 V7 V8 V9 V10 V11 V12 V13 V14 V15 V16 V17 V18 V19 V20 V21
[1,] -1  1  1 -1 -1  1 -1  1  1   1  -1   1   1   1  -1  -1  -1   1  -1   1   1
[2,] -1  1  1 -1 -1  1 -1  1  1   1   1   1   1   1   1  -1  -1   1  -1   1   1
[3,] -1  1  1 -1 -1  1 -1 -1 -1   1  -1   1   1   1  -1  -1  -1   1  -1   1   1
[4,] -1  1  1 -1 -1  1  1  1  1   1  -1   1   1   1  -1  -1  -1   1  -1   1   1
[5,]  1  1  1  1 -1  1 -1 -1 -1   1   1   1   1   1   1  -1  -1   1  -1   1   1
[6,] -1  1  1 -1 -1  1 -1 -1 -1   1  -1   1   1   1  -1  -1  -1   1  -1   1   1
     V22 V23 V24 V25 V26 V27 V28 V29 V30 V31 V32 V33 V34 V35 V36 V37 V38 V39
[1,]  -1   1  -1   1  -1  -1  -1   1   1  -1  -1   1   1  -1   1  -1   1   1
[2,]  -1   1  -1   1  -1  -1   1   1   1  -1  -1  -1   1  -1  -1  -1   1   1
[3,]  -1   1  -1   1   1  -1  -1   1   1  -1  -1  -1  -1  -1  -1  -1   1   1
[4,]  -1   1  -1   1  -1  -1   1   1   1  -1  -1   1  -1  -1   1  -1   1   1
[5,]  -1   1  -1   1   1  -1   1   1   1  -1  -1  -1   1  -1   1  -1   1   1
[6,]  -1   1  -1   1  -1  -1   1   1   1  -1  -1   1   1  -1  -1  -1   1   1
     V40 V41 V42 V43 V44 V45 V46 V47 V48 V49 V50 V51 V52 V53 V54 V55 V56 V57
[1,]  -1   1   1  -1  -1  -1  -1   1   1   1   1  -1  -1  -1  -1   1   1   1
[2,]  -1   1   1  -1  -1  -1  -1   1   1   1   1  -1  -1  -1  -1  -1   1   1
```

Load the Sample Files

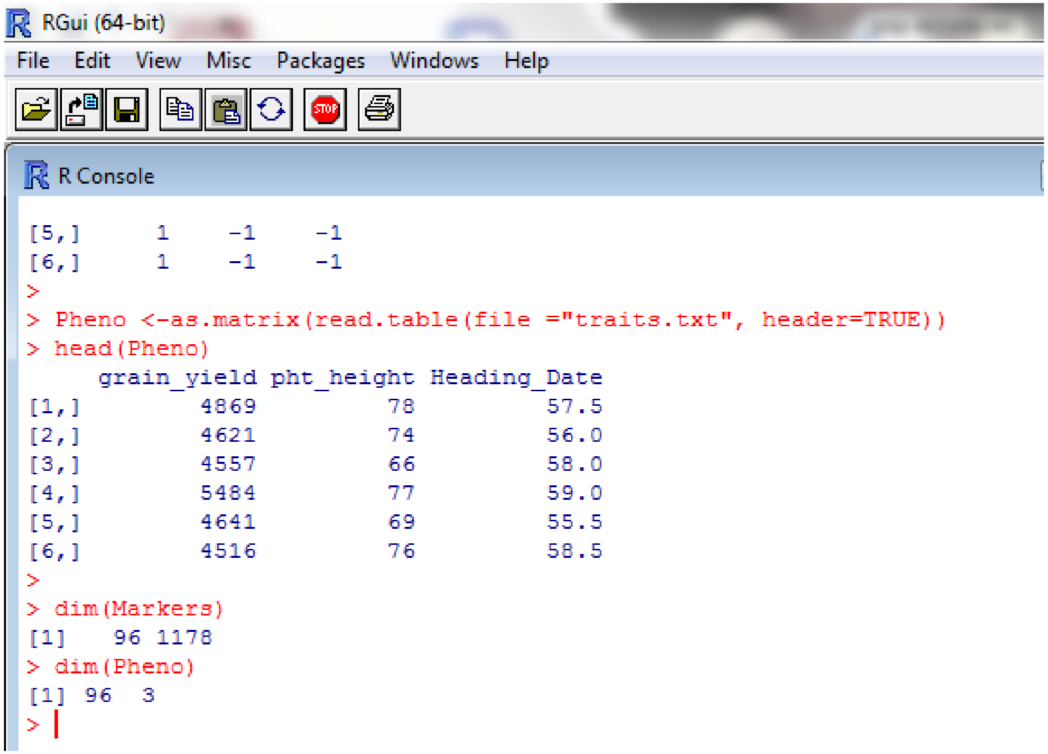
- Load the phenotype file and use the head command to see the first five lines
  - Header=T since phenotype files have column names
- Markers and phenotypes must be in matrix format



```
[6,]    -1     1    -1    -1     1    -1     1    -1     0     1     1     1
     V1176 V1177
[1,]    -1    -1
[2,]    -1     1
[3,]    -1     1
[4,]    -1    -1
[5,]    -1    -1
[6,]    -1    -1
> Pheno <-as.matrix(read.table(file ="traits.txt", header=TRUE))
> head(Pheno)
     grain_yield pht_height Heading_Date
[1,]        4869         78         57.5
[2,]        4621         74         56.0
[3,]        4557         66         58.0
[4,]        5484         77         59.0
[5,]        4641         69         55.5
[6,]        4516         76         58.5
> 
```

Load the Sample Files

- Determine the size of the matrices
- dim() command gives the number of rows and columns
- 96 observations and 1178 markers, 3 traits



```
[5,]     1    -1    -1
[6,]     1    -1    -1
>
> Pheno <-as.matrix(read.table(file ="traits.txt", header=TRUE))
> head(Pheno)
     grain_yield pht_height Heading_Date
[1,]        4869         78         57.5
[2,]        4621         74         56.0
[3,]        4557         66         58.0
[4,]        5484         77         59.0
[5,]        4641         69         55.5
[6,]        4516         76         58.5
>
> dim(Markers)
[1]   96 1178
> dim(Pheno)
[1] 96  3
> 
```

Learning Objectives

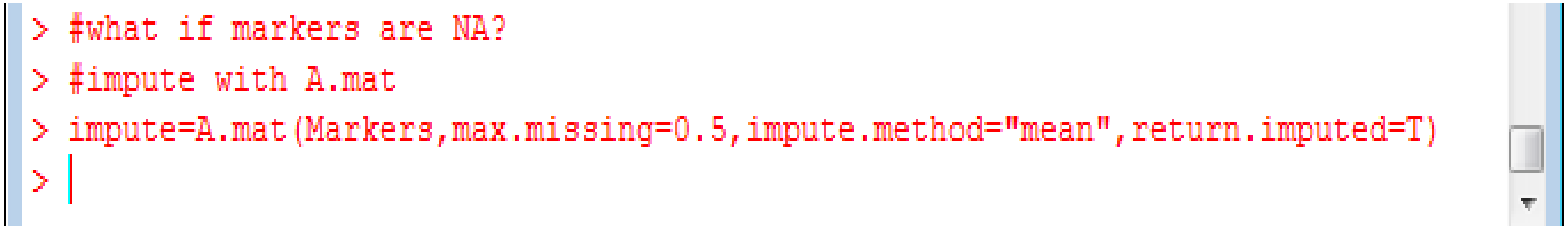
- Download the package and load the sample files
- **Impute missing markers using A.mat()**
- Define the training and validation populations
- Run mixed.solve() and determine accuracy of predictions

Impute Missing Markers

- rrBLUP mixed.solve() does not allow for missing markers
- Imputed value is the population mean for that marker
- Useful for SNP data since level of missing data is low
    - In the sample files 164 markers are missing out of 1178 (0.14%)
- A.mat also calculates the additive relationship matrix

Impute Missing Markers

- max.missing-maximum proportion of missing data
  - If 50% of markers are missing data then markers are not imputed
- impute method- imputes the mean of the markers
- return.imputed-prints out the imputed results if set to TRUE



```
> #what if markers are NA?
> #impute with A.mat
> impute=A.mat(Markers,max.missing=0.5,impute.method="mean",return.imputed=T)
> 
```

Impute Missing Markers

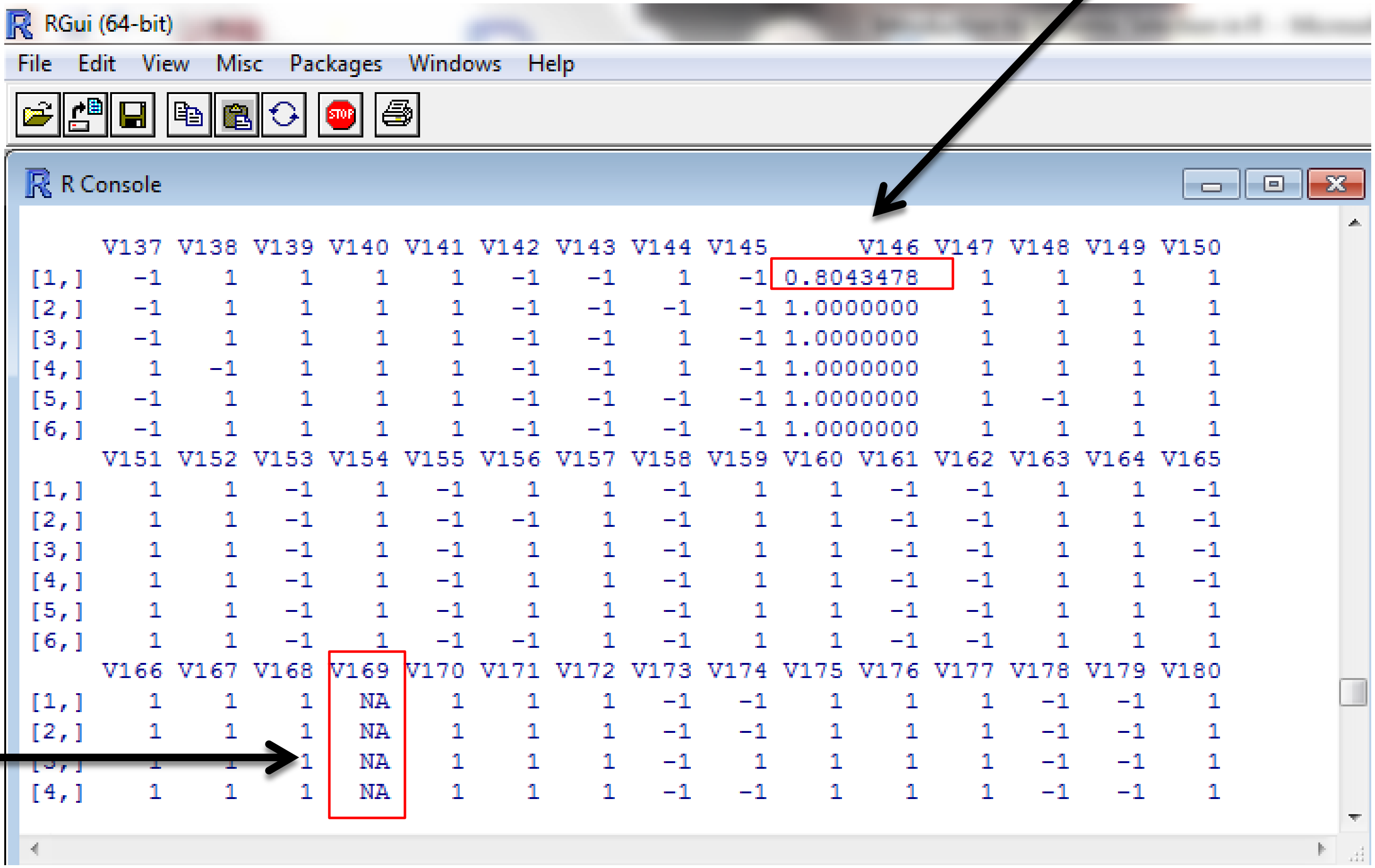
-
  ```
  >impute=A.mat(Markers,max.missing=0.5,impute.method="mean",return.imputed=T)
  ```

-
  ```
  > Markers_impute=impute$imputed
  ```
  - Rename imputed marker matrix as Markers_impute
- impute$imputed-returns the imputed marker matrix
- impute$A-returns the additive relationship matrix

Impute Missing Markers

- >impute$imputed

Imputed marker value

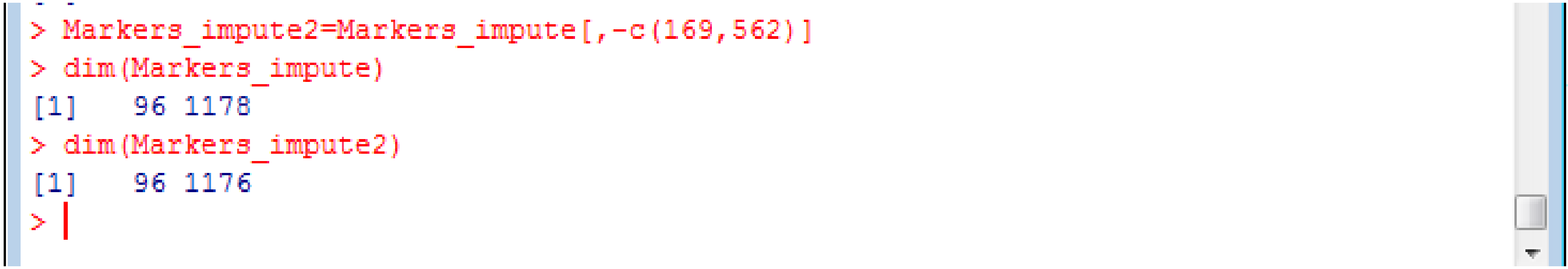


```
     V137 V138 V139 V140 V141 V142 V143 V144 V145      V146 V147 V148 V149 V150
[1,]   -1    1    1    1    1   -1   -1    1   -1 0.8043478    1    1    1    1
[2,]   -1    1    1    1    1   -1   -1   -1   -1 1.0000000    1    1    1    1
[3,]   -1    1    1    1    1   -1   -1    1   -1 1.0000000    1    1    1    1
[4,]    1   -1    1    1    1   -1   -1    1   -1 1.0000000    1    1    1    1
[5,]   -1    1    1    1    1   -1   -1   -1   -1 1.0000000    1   -1    1    1
[6,]   -1    1    1    1    1   -1   -1   -1   -1 1.0000000    1    1    1    1
     V151 V152 V153 V154 V155 V156 V157 V158 V159 V160 V161 V162 V163 V164 V165
[1,]    1    1   -1    1   -1    1    1   -1    1    1   -1   -1    1    1   -1
[2,]    1    1   -1    1   -1   -1    1   -1    1    1   -1   -1    1    1   -1
[3,]    1    1   -1    1   -1    1    1   -1    1    1   -1   -1    1    1   -1
[4,]    1    1   -1    1   -1    1    1   -1    1    1   -1   -1    1    1   -1
[5,]    1    1   -1    1   -1    1    1   -1    1    1   -1   -1    1    1    1
[6,]    1    1   -1    1   -1   -1    1   -1    1    1   -1   -1    1    1    1
     V166 V167 V168 V169 V170 V171 V172 V173 V174 V175 V176 V177 V178 V179 V180
[1,]    1    1    1   NA    1    1    1   -1   -1    1    1    1   -1   -1    1
[2,]    1    1    1   NA    1    1    1   -1   -1    1    1    1   -1   -1    1
[3,]    1    1    1   NA    1    1    1   -1    1    1    1    1   -1   -1    1
[4,]    1    1    1   NA    1    1    1   -1   -1    1    1    1   -1   -1    1
```

Marker value left NA if more than 50% missing data

Impute Missing Markers

- Remove markers that had more than 50% missing data
  - NA values are not allowed in mixed.solve
  - Two markers in the SNP file must be removed
  - Column 169 and 562
  - New dimensions show 2 less columns
- Use Markers_impute2 as marker matrix for estimating marker effects



```
> Markers_impute2=Markers_impute[,-c(169,562)]
> dim(Markers_impute)
[1]   96 1178
> dim(Markers_impute2)
[1]   96 1176
> 
```

Learning Objectives

- Download the package and load the sample files
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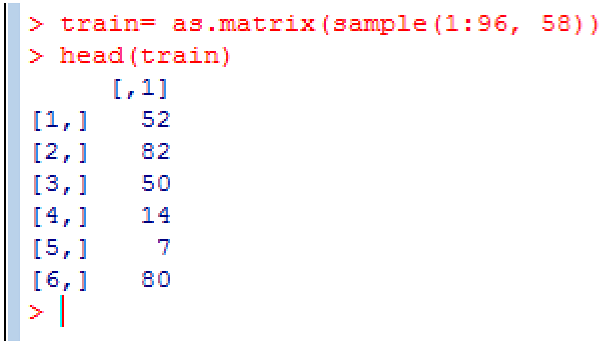
- **Define the training and validation populations**
- Run mixed.solve() and determine accuracy of predictions

Training and Validation Populations

- Training population-genotyped and phenotyped
- Validation population-phenotype values estimated based on marker effects calculated from training population
- Code is set that 60% of the total population is the training population
    - 40% validation population

Training and Validation Populations

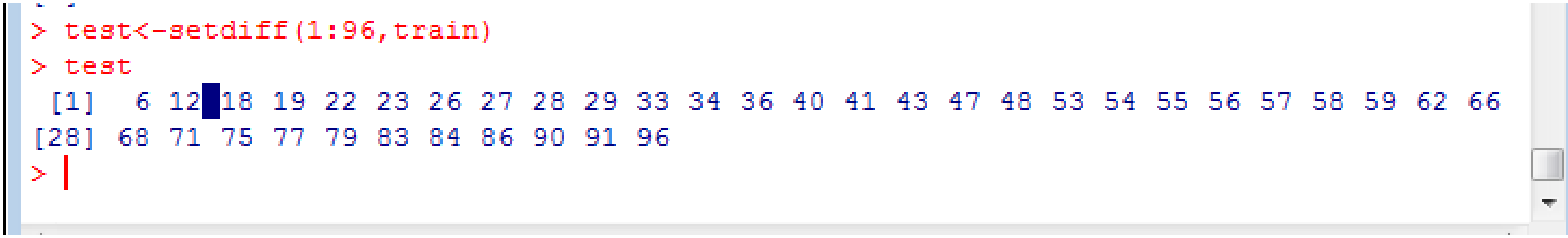
- 58 (60% of total population of 96) random numbers sampled to determine which individuals are in the training population
- Individuals are the row numbers for the phenotypes and marker matrices
- Sampled numbers will be different every time the code is run and will affect the correlation accuracy



```
> train= as.matrix(sample(1:96, 58))
> head(train)
     [,1]
[1,]   52
[2,]   82
[3,]   50
[4,]   14
[5,]    7
[6,]   80
> 
```

Training and Validation Populations

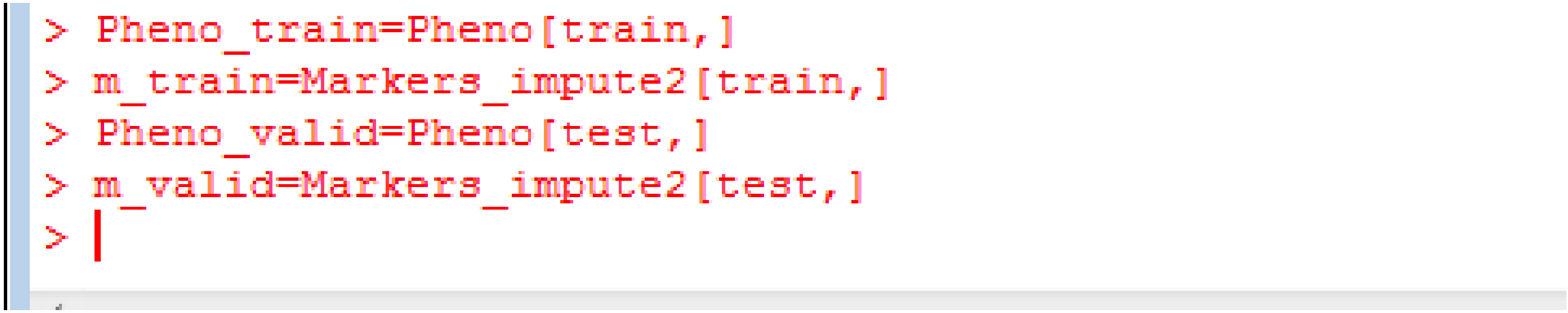
- Validation population is 40% of the total population
- setdiff() command determines the numbers that are not in the training population and will be part of the validation population



```
> test<-setdiff(1:96,train)
> test
 [1]  6 12 18 19 22 23 26 27 28 29 33 34 36 40 41 43 47 48 53 54 55 56 57 58 59 62 66
[28] 68 71 75 77 79 83 84 86 90 91 96
> 
```

Training and Validation Populations

- Pheno_train and m_train are the phenotype and marker matrices for the values in the training population
- Pheno_valid and m_valid will be the validation populations

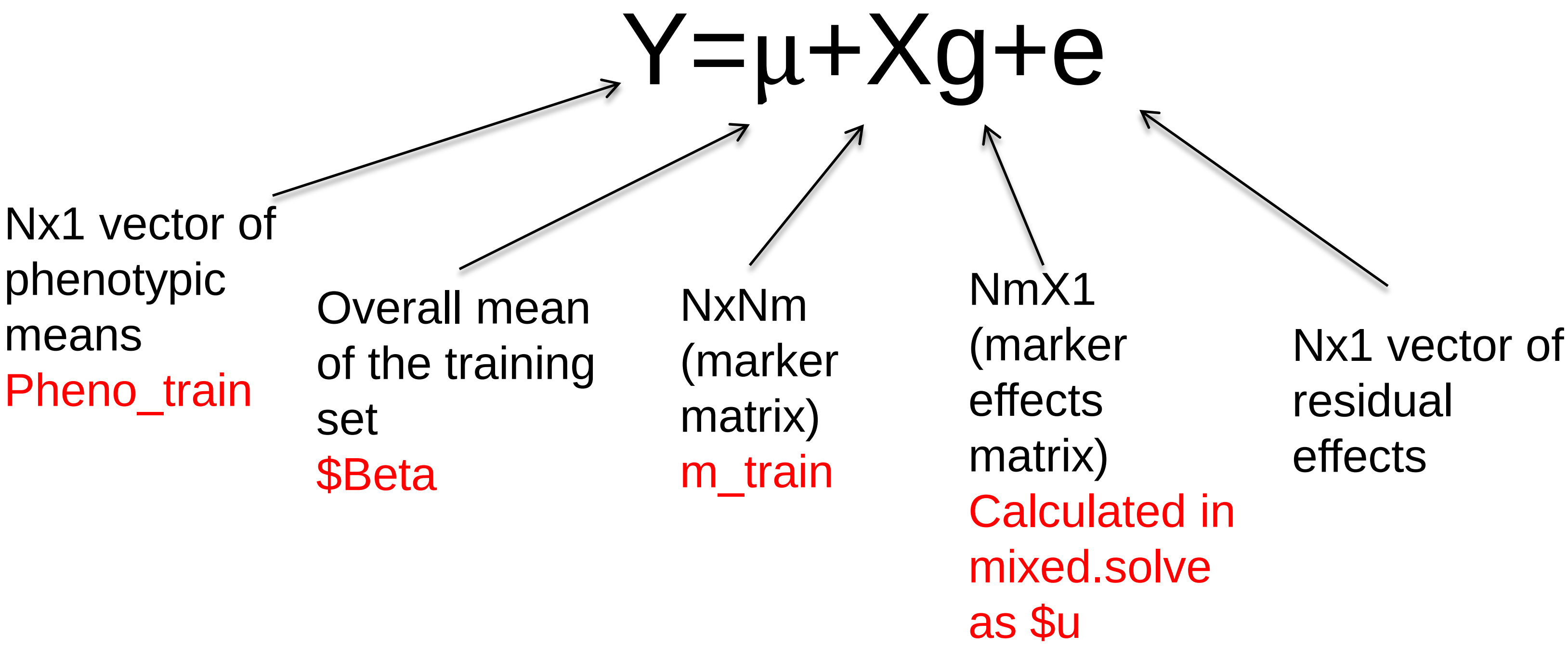


```
> Pheno_train=Pheno[train,]
> m_train=Markers_impute2[train,]
> Pheno_valid=Pheno[test,]
> m_valid=Markers_impute2[test,]
> 
```

Learning Objectives

- Download the package and load the sample files
- Impute missing markers using A.mat()
- Define the training and validation populations
- **Run mixed.solve() and determine accuracy of predictions**

Run mixed.solve



$$Y=\mu+Xg+e$$

Nx1 vector of phenotypic means
Pheno_train

Overall mean of the training set
$Beta

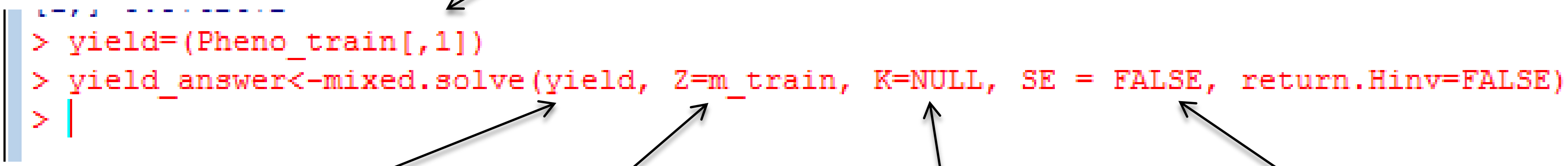
NxNm (marker matrix)
m_train

NmX1 (marker effects matrix)
Calculated in mixed.solve as $u

Nx1 vector of residual effects

Run mixed.solve

Yield is the first column of the pheno_train matrix



```
> yield=(Pheno_train[,1])
> yield_answer<-mixed.solve(yield, Z=m_train, K=NULL, SE = FALSE, return.Hinv=FALSE)
> 
```

Vector of observations

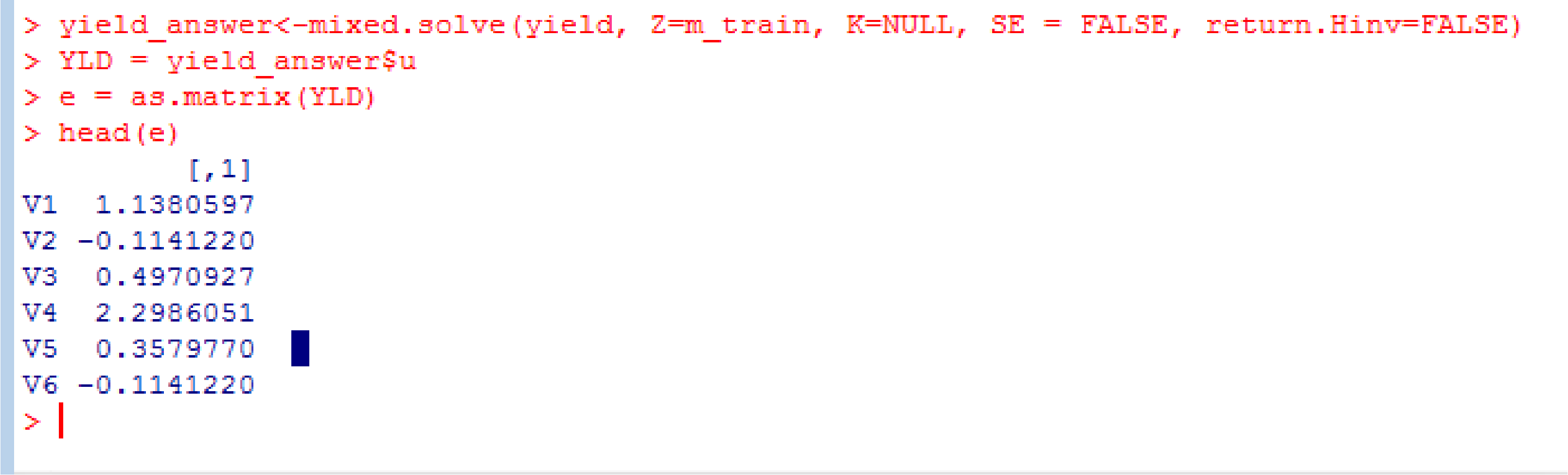
Design matrix of random effects (Markers)

K matrix is the identity matrix

Standard errors are not calculated

Run mixed.solve

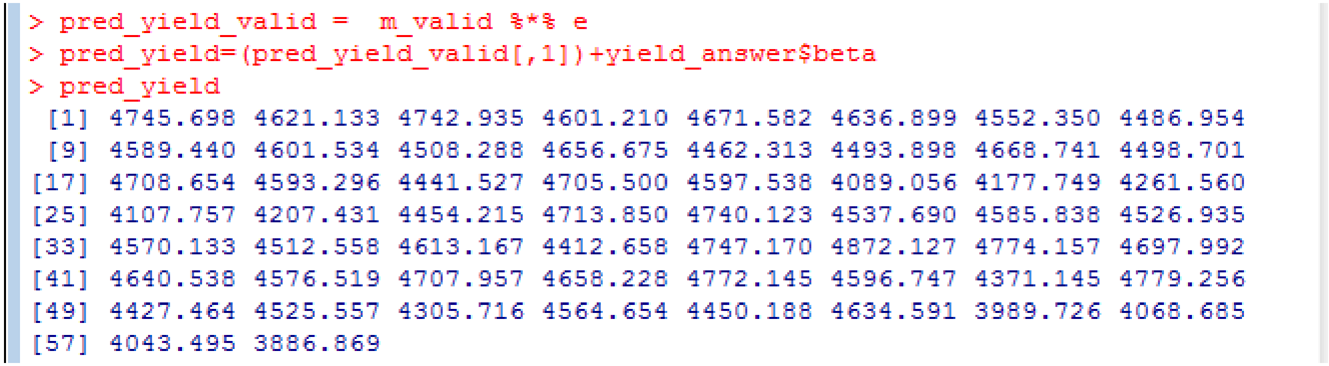
- Yield_answer$u is the output of the marker effects
- head(e) shows the marker effects for the first five markers



```
> yield_answer<-mixed.solve(yield, Z=m_train, K=NULL, SE = FALSE, return.Hinv=FALSE)
> YLD = yield_answer$u
> e = as.matrix(YLD)
> head(e)
          [,1]
V1  1.1380597
V2 -0.1141220
V3  0.4970927
V4  2.2986051
V5  0.3579770
V6 -0.1141220
> 
```

Run mixed.solve

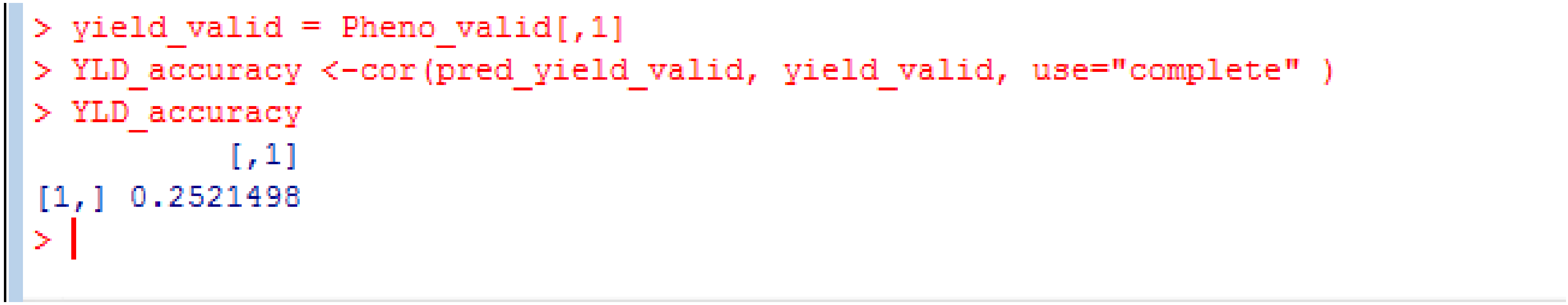
- m_valid*e = marker validation matrix times the marker effects
- Pred_yield=predicted yield based on the marker effects of the training population with the grand mean added in



```
> pred_yield_valid =  m_valid %*% e
> pred_yield=(pred_yield_valid[,1])+yield_answer$beta
> pred_yield
 [1] 4745.698 4621.133 4742.935 4601.210 4671.582 4636.899 4552.350 4486.954
 [9] 4589.440 4601.534 4508.288 4656.675 4462.313 4493.898 4668.741 4498.701
[17] 4708.654 4593.296 4441.527 4705.500 4597.538 4089.056 4177.749 4261.560
[25] 4107.757 4207.431 4454.215 4713.850 4740.123 4537.690 4585.838 4526.935
[33] 4570.133 4512.558 4613.167 4412.658 4747.170 4872.127 4774.157 4697.992
[41] 4640.538 4576.519 4707.957 4658.228 4772.145 4596.747 4371.145 4779.256
[49] 4427.464 4525.557 4305.716 4564.654 4450.188 4634.591 3989.726 4068.685
[57] 4043.495 3886.869
```

Determine Correlation Accuracy

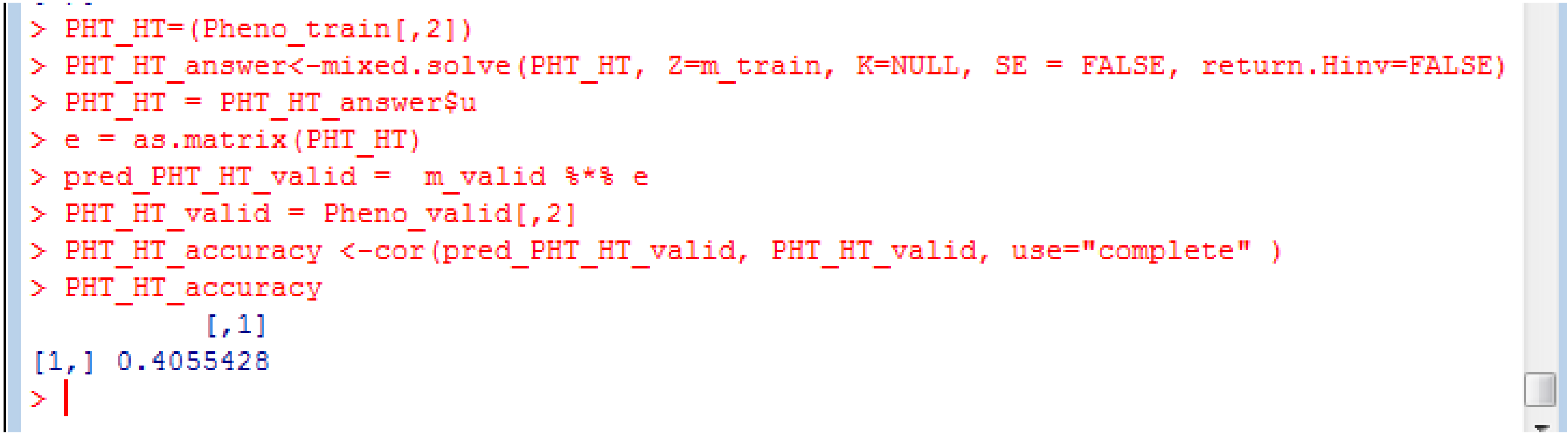
- Correlation between the predicted yield values and the observed yield values
- Accuracy will change slightly each time due to different individuals sampled for the training and validation populations



```
> yield_valid = Pheno_valid[,1]
> YLD_accuracy <-cor(pred_yield_valid, yield_valid, use="complete" )
> YLD_accuracy
          [,1]
[1,] 0.2521498
> 
```

Determine Correlation Accuracy

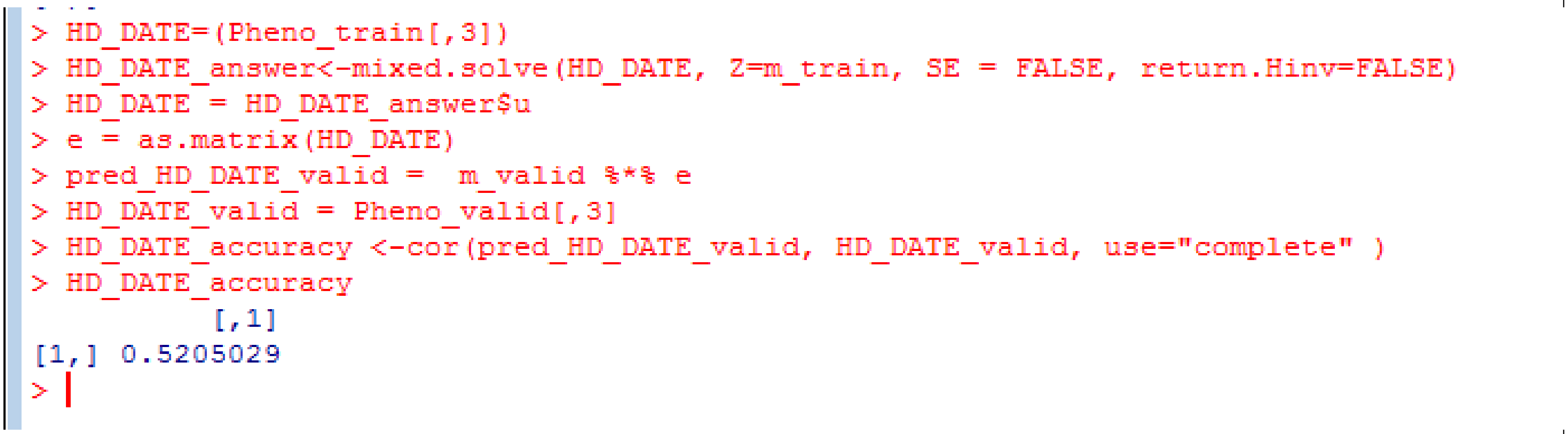
- Plant Height



```
> PHT_HT=(Pheno_train[,2])
> PHT_HT_answer<-mixed.solve(PHT_HT, Z=m_train, K=NULL, SE = FALSE, return.Hinv=FALSE)
> PHT_HT = PHT_HT_answer$u
> e = as.matrix(PHT_HT)
> pred_PHT_HT_valid =  m_valid %*% e
> PHT_HT_valid = Pheno_valid[,2]
> PHT_HT_accuracy <-cor(pred_PHT_HT_valid, PHT_HT_valid, use="complete" )
> PHT_HT_accuracy
          [,1]
[1,] 0.4055428
> 
```

Determine Correlation Accuracy

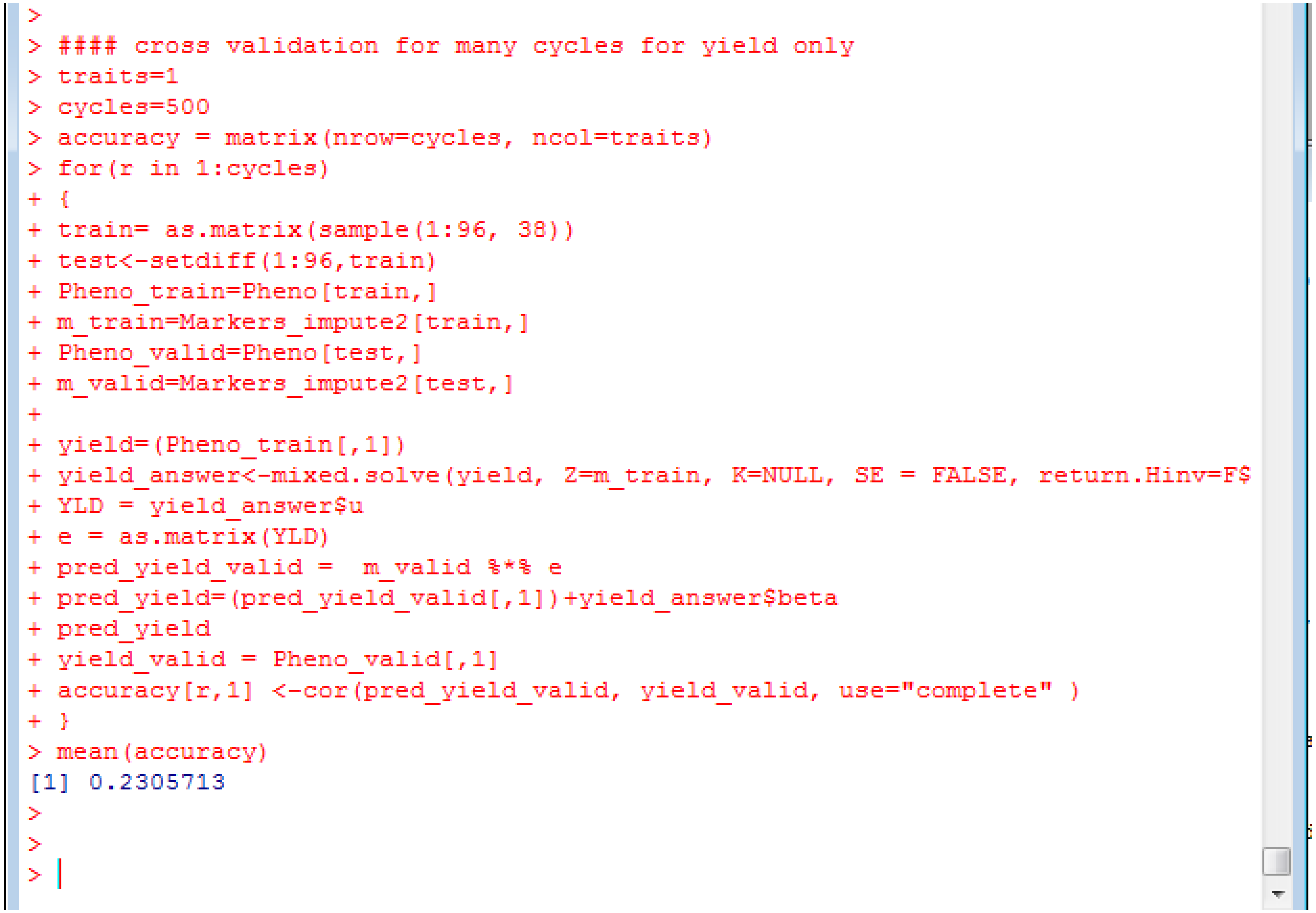
- Heading Date



```
> HD_DATE=(Pheno_train[,3])
> HD_DATE_answer<-mixed.solve(HD_DATE, Z=m_train, SE = FALSE, return.Hinv=FALSE)
> HD_DATE = HD_DATE_answer$u
> e = as.matrix(HD_DATE)
> pred_HD_DATE_valid =  m_valid %*% e
> HD_DATE_valid = Pheno_valid[,3]
> HD_DATE_accuracy <-cor(pred_HD_DATE_valid, HD_DATE_valid, use="complete" )
> HD_DATE_accuracy
          [,1]
[1,] 0.5205029
> 
```

Determine Correlation Accuracy

- Correlation accuracy with 500 iterations



```
>
> #### cross validation for many cycles for yield only
> traits=1
> cycles=500
> accuracy = matrix(nrow=cycles, ncol=traits)
> for(r in 1:cycles)
+ {
+ train= as.matrix(sample(1:96, 38))
+ test<-setdiff(1:96,train)
+ Pheno_train=Pheno[train,]
+ m_train=Markers_impute2[train,]
+ Pheno_valid=Pheno[test,]
+ m_valid=Markers_impute2[test,]
+
+ yield=(Pheno_train[,1])
+ yield_answer<-mixed.solve(yield, Z=m_train, K=NULL, SE = FALSE, return.Hinv=F$
+ YLD = yield_answer$u
+ e = as.matrix(YLD)
+ pred_yield_valid =  m_valid %*% e
+ pred_yield=(pred_yield_valid[,1])+yield_answer$beta
+ pred_yield
+ yield_valid = Pheno_valid[,1]
+ accuracy[r,1] <-cor(pred_yield_valid, yield_valid, use="complete" )
+ }
> mean(accuracy)
[1] 0.2305713
>
>
> |
```

Determine Correlation Accuracy

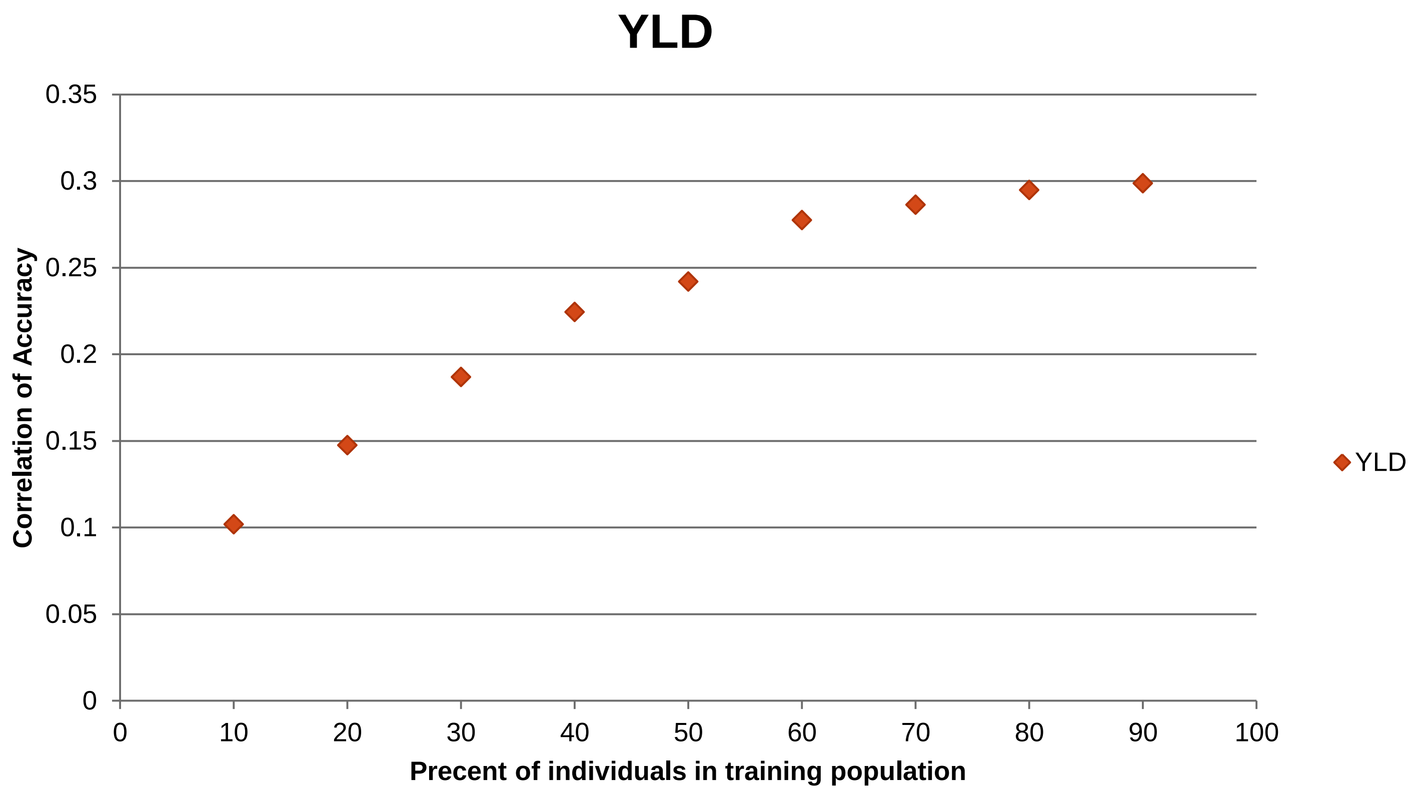
- Correlation accuracy is different for each trait

- Values will be different every time it is run since different lines will be included in the training or validation sets

- Accuracy is affected by training size, validation size, number of markers and heritability

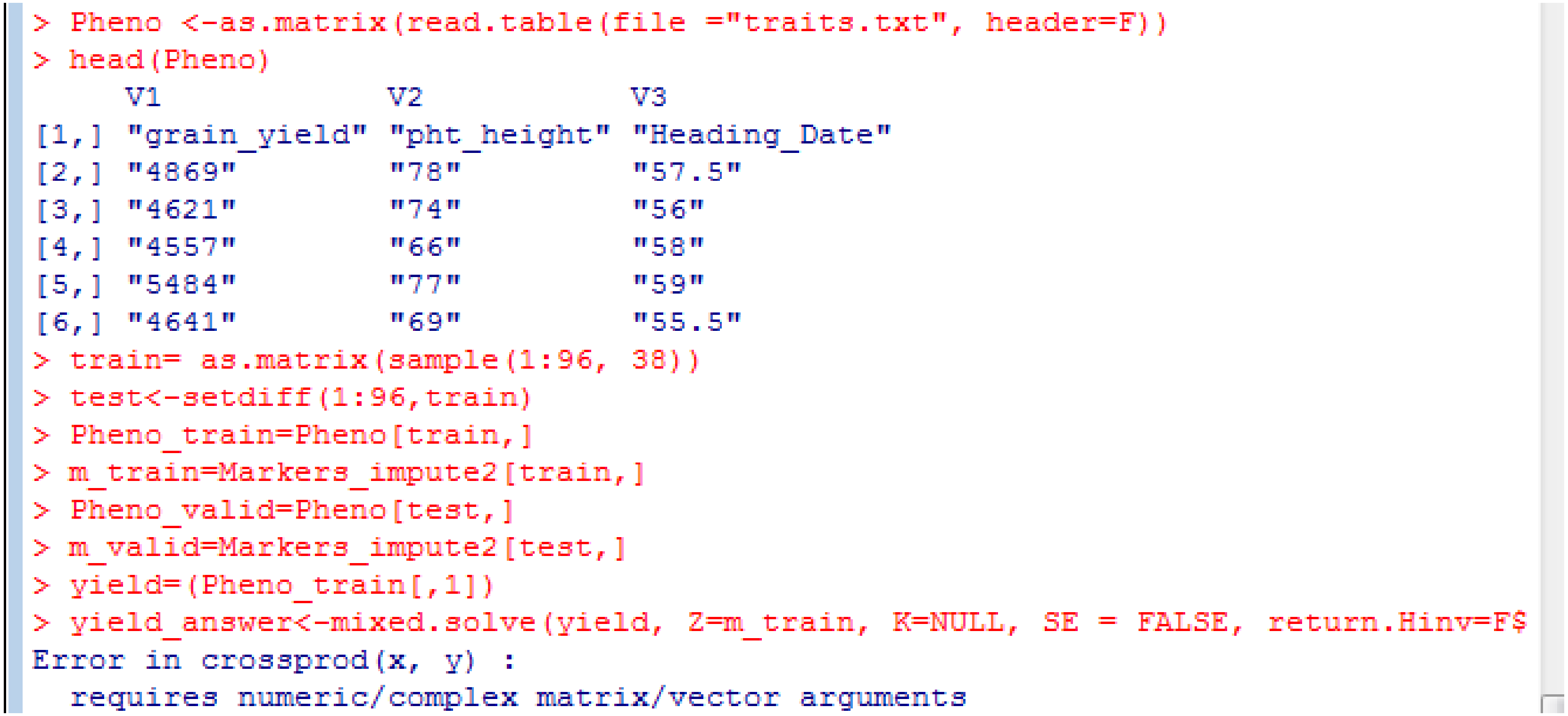
Determine Correlation Accuracy

- Effects of training population size on accuracy



Common Errors

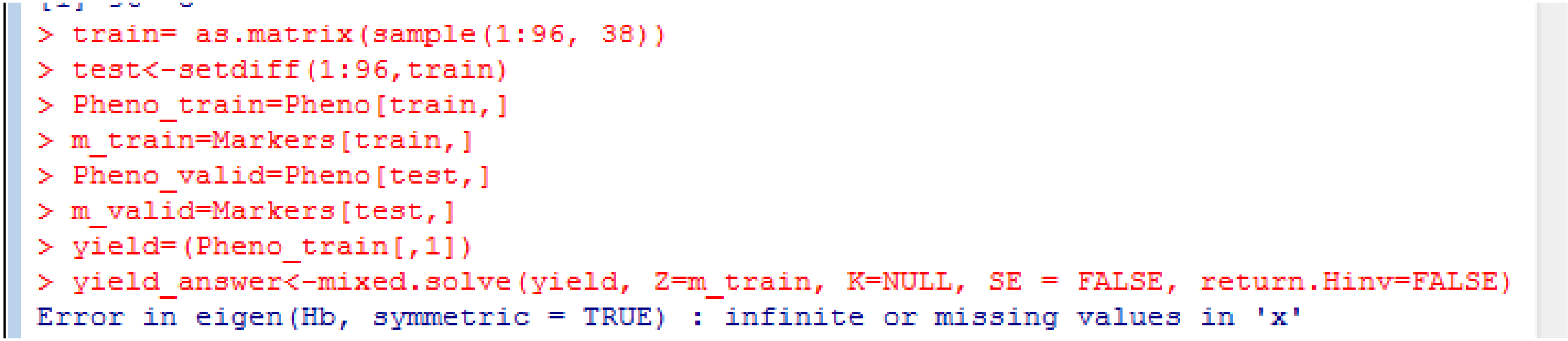
- Headers incorrectly input



```
> Pheno <-as.matrix(read.table(file ="traits.txt", header=F))
> head(Pheno)
     V1            V2           V3            
[1,] "grain_yield" "pht_height" "Heading_Date"
[2,] "4869"        "78"         "57.5"        
[3,] "4621"        "74"         "56"          
[4,] "4557"        "66"         "58"          
[5,] "5484"        "77"         "59"          
[6,] "4641"        "69"         "55.5"        
> train= as.matrix(sample(1:96, 38))
> test<-setdiff(1:96,train)
> Pheno_train=Pheno[train,]
> m_train=Markers_impute2[train,]
> Pheno_valid=Pheno[test,]
> m_valid=Markers_impute2[test,]
> yield=(Pheno_train[,1])
> yield_answer<-mixed.solve(yield, Z=m_train, K=NULL, SE = FALSE, return.Hinv=F$
Error in crossprod(x, y) : 
  requires numeric/complex matrix/vector arguments
```

Common Errors

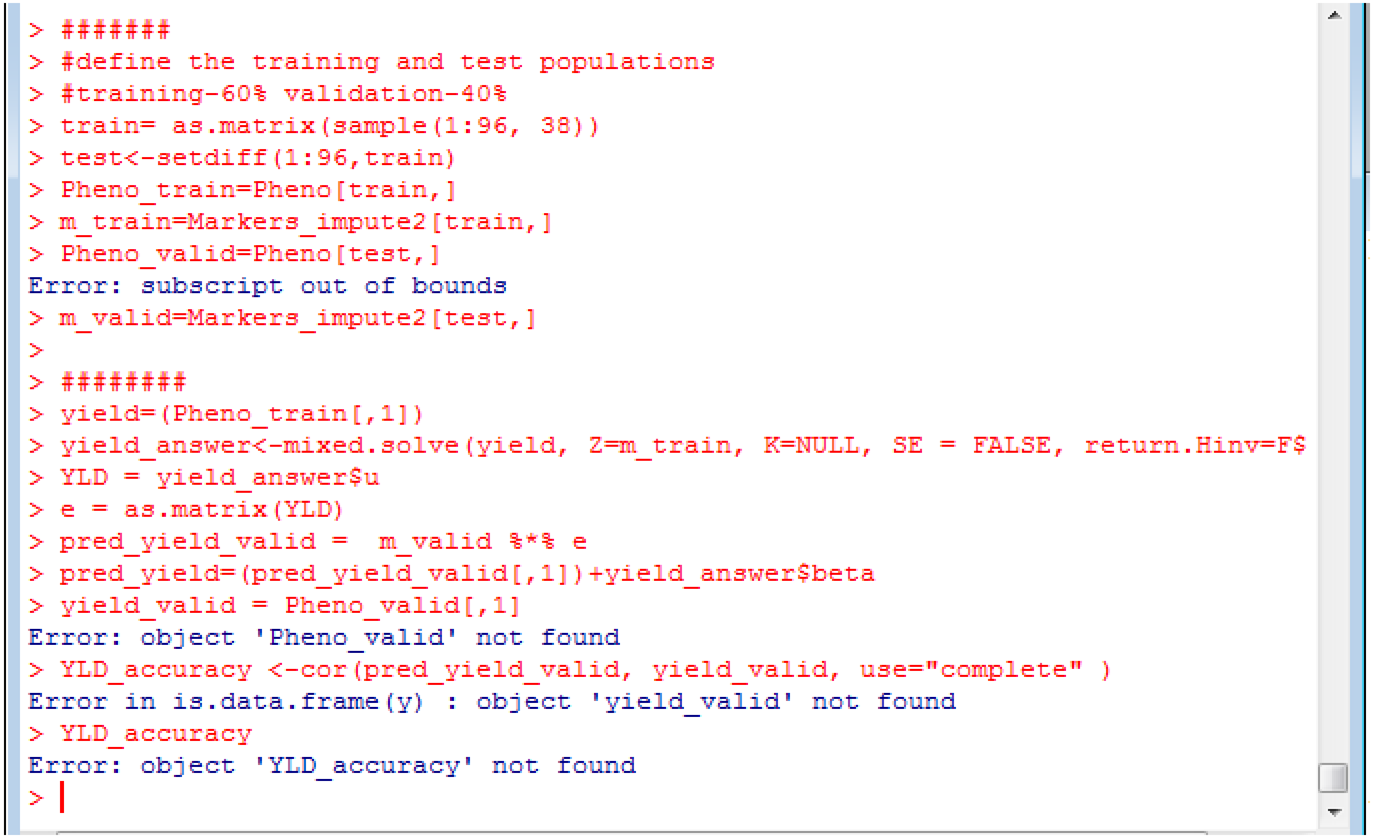
- NA Markers



```
> train= as.matrix(sample(1:96, 38))
> test<-setdiff(1:96,train)
> Pheno_train=Pheno[train,]
> m_train=Markers[train,]
> Pheno_valid=Pheno[test,]
> m_valid=Markers[test,]
> yield=(Pheno_train[,1])
> yield_answer<-mixed.solve(yield, Z=m_train, K=NULL, SE = FALSE, return.Hinv=FALSE)
Error in eigen(Hb, symmetric = TRUE) : infinite or missing values in 'x'
```

Common Errors

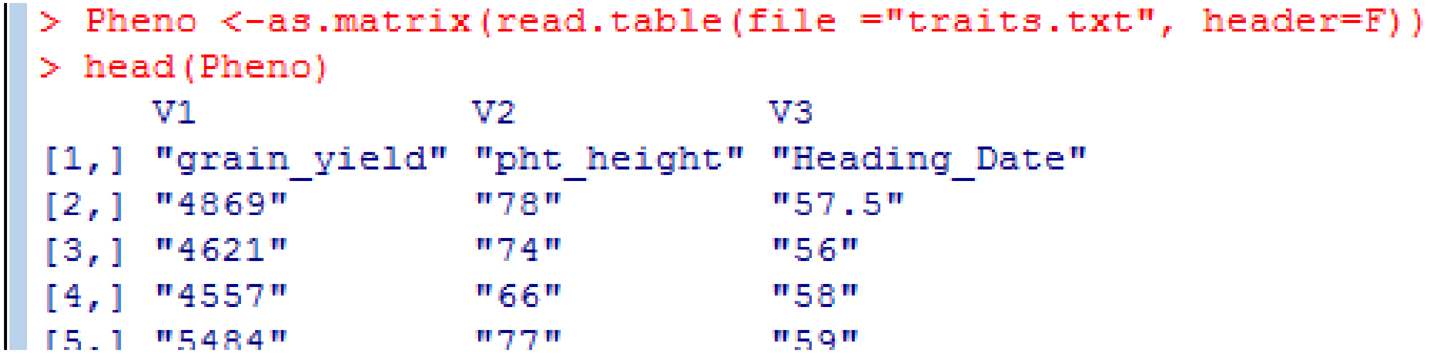
- Incorrect matrix dimensions
  - Removed one individual from phenotype matrix



```
> #######
> #define the training and test populations
> #training-60% validation-40%
> train= as.matrix(sample(1:96, 38))
> test<-setdiff(1:96,train)
> Pheno_train=Pheno[train,]
> m_train=Markers_impute2[train,]
> Pheno_valid=Pheno[test,]
Error: subscript out of bounds
> m_valid=Markers_impute2[test,]
>
> ########
> yield=(Pheno_train[,1])
> yield_answer<-mixed.solve(yield, Z=m_train, K=NULL, SE = FALSE, return.Hinv=F$
> YLD = yield_answer$u
> e = as.matrix(YLD)
> pred_yield_valid =  m_valid %*% e
> pred_yield=(pred_yield_valid[,1])+yield_answer$beta
> yield_valid = Pheno_valid[,1]
Error: object 'Pheno_valid' not found
> YLD_accuracy <-cor(pred_yield_valid, yield_valid, use="complete" )
Error in is.data.frame(y) : object 'yield_valid' not found
> YLD_accuracy
Error: object 'YLD_accuracy' not found
> |
```

Common Errors

- Read in values as characters instead of numeric
  - Quotes around values



```
> Pheno <-as.matrix(read.table(file ="traits.txt", header=F))
> head(Pheno)
     V1            V2           V3
[1,] "grain_yield" "pht_height" "Heading_Date"
[2,] "4869"        "78"         "57.5"
[3,] "4621"        "74"         "56"
[4,] "4557"        "66"         "58"
[5,] "5484"        "77"         "59"
```

Resources

- rrBLUP reference manual
  - http://cran.r-project.org/web/packages/rrBLUP/rrBLUP.pdf
- rrBLUP vingettes
  - http://cran.r-project.org/web/packages/rrBLUP/vignettes/vignette.pdf
- Endelman, J.B. 2011. Ridge regression and other kernels for genomic selection with R package rrBLUP. Plant Genome 4:250-255. doi: 10.3835/plantgenome2011.08.0024

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Dataset

TCAP Hordeum's

Toolbox

Questions?