PLANT BREEDING & GENOMICS

Use of GAPIT for Genome Wide Association Studies

Presented by

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Plant Breeding and Genomics

extension

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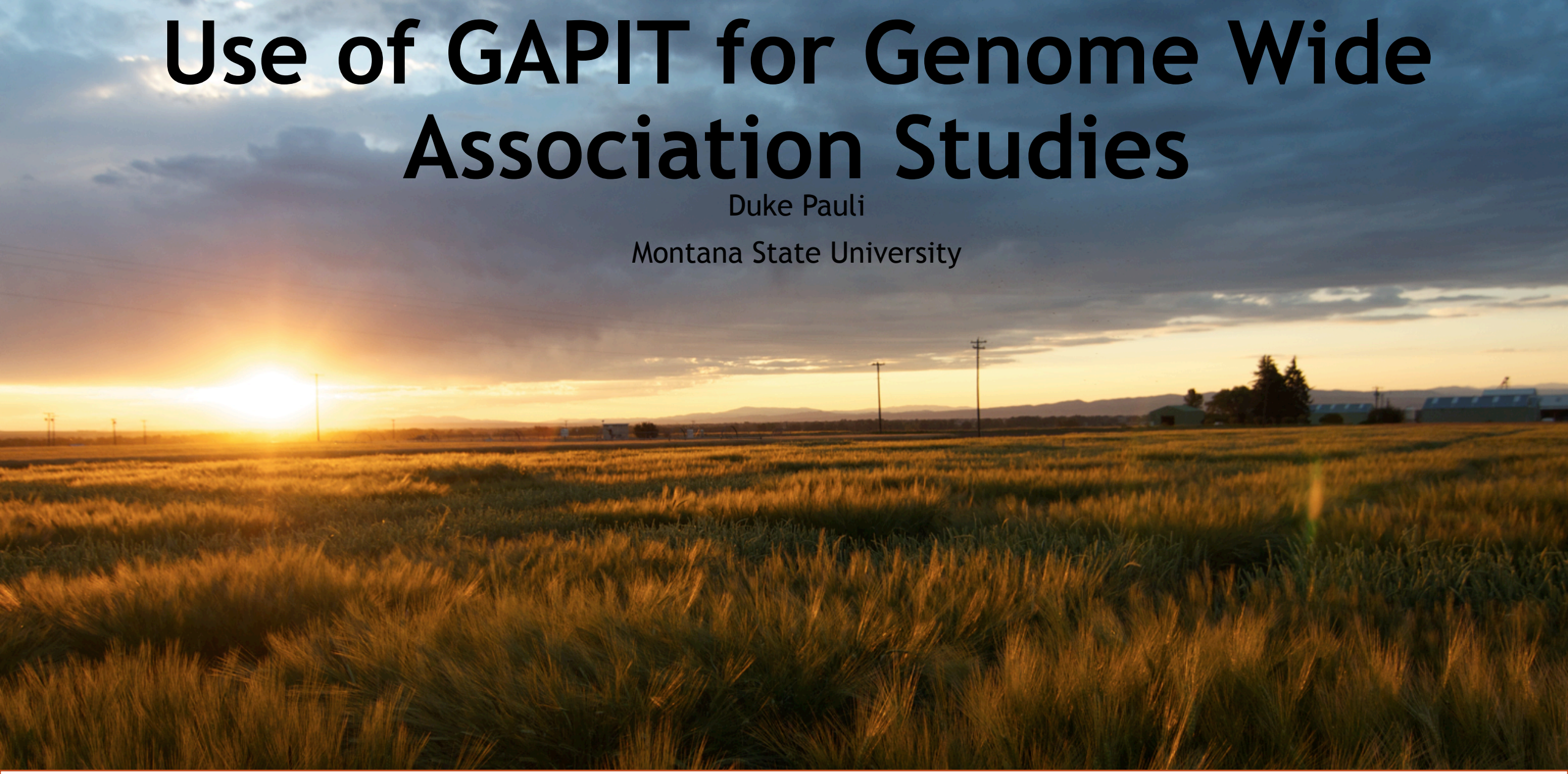


MONTANA STATE UNIVERSITY
Mountains & Minds

USDA
United States Department of Agriculture
National Institute of Food and Agriculture

Triticeae CAP
Coordinated Agricultural Project

Overview

- Part 1
  - Learning objectives
  - Introduction and background
  - Installing RStudio
- Part 2
  - Sample Files
  - Loading GAPIT
- Part 3
  - Loading files, checking
  - Doing the analysis
- Part 4
  - Output
  - Questions

Learning Objectives

- Install RStudio
- Load needed packages and source code for GAPIT
- Load sample files
- Running basic analyses
- Using the output

Disclaimer

- What I am assuming for today's tutorial
  - Have run R before, familiar with basic commands
  - Have an understanding of the principals of GWAS
- What I *will not* cover today
  - Installing and running R
  - The eXtension website has a great tutorial
  - What GWAS is and the theory behind it
  - Genomic prediction functions of GAPIT

What is GAPIT?

- Genome Association and Prediction Integrated Tool
- Statistical package that is run in the R software environment
- Developed by Alex Lipka and Zhiwu Zhang
- Alexander E. Lipka et al. (2012) GAPIT: Genome Association and Prediction Integrated Tool. Bioinformatics. doi: 10.1093/bioinformatics/bts444
- Uses statistical tools implemented in other programs like TASSEL

Why use GAPIT?

- Although there are programs that implement the same statistical methods, size of data sets can be a problem
- GAPIT can handle large data sets, file splitting
- Some of these programs have complex user interfaces, most of you are already familiar with R
- DOES NOT REQUIRE THAT MUCH CODE TO RUN AN ANALYSIS
- Do all data manipulations and analysis in one common environment
- Supporting information is good, excellent user manual
- Surprisingly easy to use
- Developers are extremely helpful
- Faster

Installing R studio

- Open source, integrated development environment (IDE)
- Nicer user interface for R
- To install, go to http://www.rstudio.com/ , select download now (green button)
- Select “Download RStudio Desktop”
- Select the version that is recommended for your system
- Follow setup wizard
- Has a script window, R console, displays objects that are currently in your workspace, and another display that shows graphs or packages

RStudio

File Edit Code View Plots Session Project Build Tools Help

Code for GAPIT webinar.R* | pheno

Source on Save | Run | Source

```
31
32 # If our data is in the HapMap format, you will only need the two files-the phenotypic a
33 # file. We will do this analysis first for the sake of time.
34 file.choose()
35 hapmap_geno<-read.table("D:\\GAPIT\\HapMap_genotypes.txt",header=F)
36 pheno<-read.table("D:\\GAPIT\\protein.txt",header=T)
37
38 # good idea to check our pehontype data to make sure the file strucutre is correct and h
39 # is distributed, checking for outliers
40
41 str(pheno) # gives us information on the object, in this case a data frame and other inf
42 hist(pheno$protein) # creates a histogram plot of our data, things look pretty good, we
43  #lines that are bit extreme but with 775 lines we would expect about 27 to be at least
44  # the mean.
45
46 #some basic statistics to look at
47
```

43:2 (Top Level) R Script

Console ~/

```
    %in%, as.factor, order

> library("compiler") #this library is already installed in R
> source("http://www.maizegenetics.net/images/stories/bioinformatics/GAPIT/gapit_functions.tx
> source("http://www.maizegenetics.net/images/stories/bioinformatics/GAPIT/emma.txt")
> hapmap_geno<-read.table("D:\\GAPIT\\HapMap_genotypes.txt",header=F)
> pheno<-read.table("D:\\GAPIT\\protein.txt",header=T)
> hist(pheno$protein) # creates a histogram plot of our data, things look pretty good, we hav
>
```

Workspace | History

Import Dataset

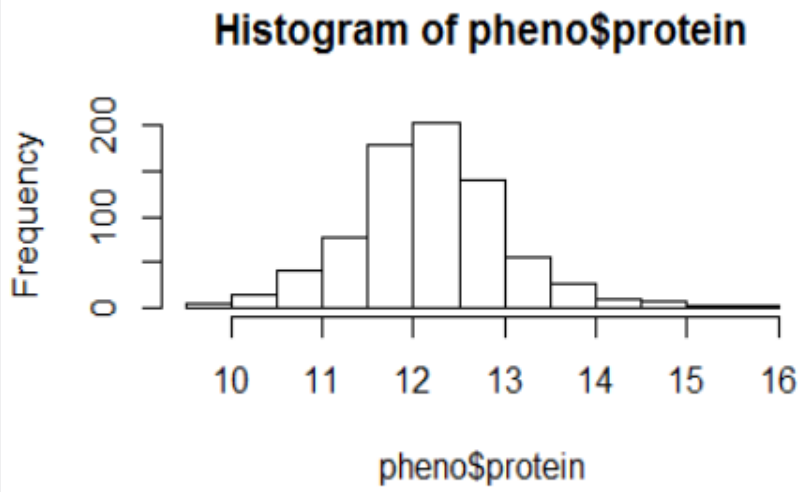
Data	
hapmap_geno	2360 obs. of 779 variables
pheno	768 obs. of 2 variab

Functions

```
GAPIT(Y = NULL, G = NULL, GD = NULL, GM = NULL, KI
NULL, Z = NULL, CV = NULL, CV.Inheritance = NULL,
NULL, GK = NULL, group.from = 30, group.to = 1e+06
group.by = 10, DPP = 1e+05, kinship.cluster =
"average", kinship.group = "Mean", kinship.algorit
```

Files | Plots | Packages | Help

Zoom | Export | Clear All



Running GAPIT

- USE THE MANUAL!!!!
- It is like an R vignette but a thousand times better
- Has all the code, can copy and paste in to R
- Describes the formatting of data for input
- Lists all the options for doing analyses
- Describes the output that is generated
- Explains more advanced uses of the program, more example code

Sample Files

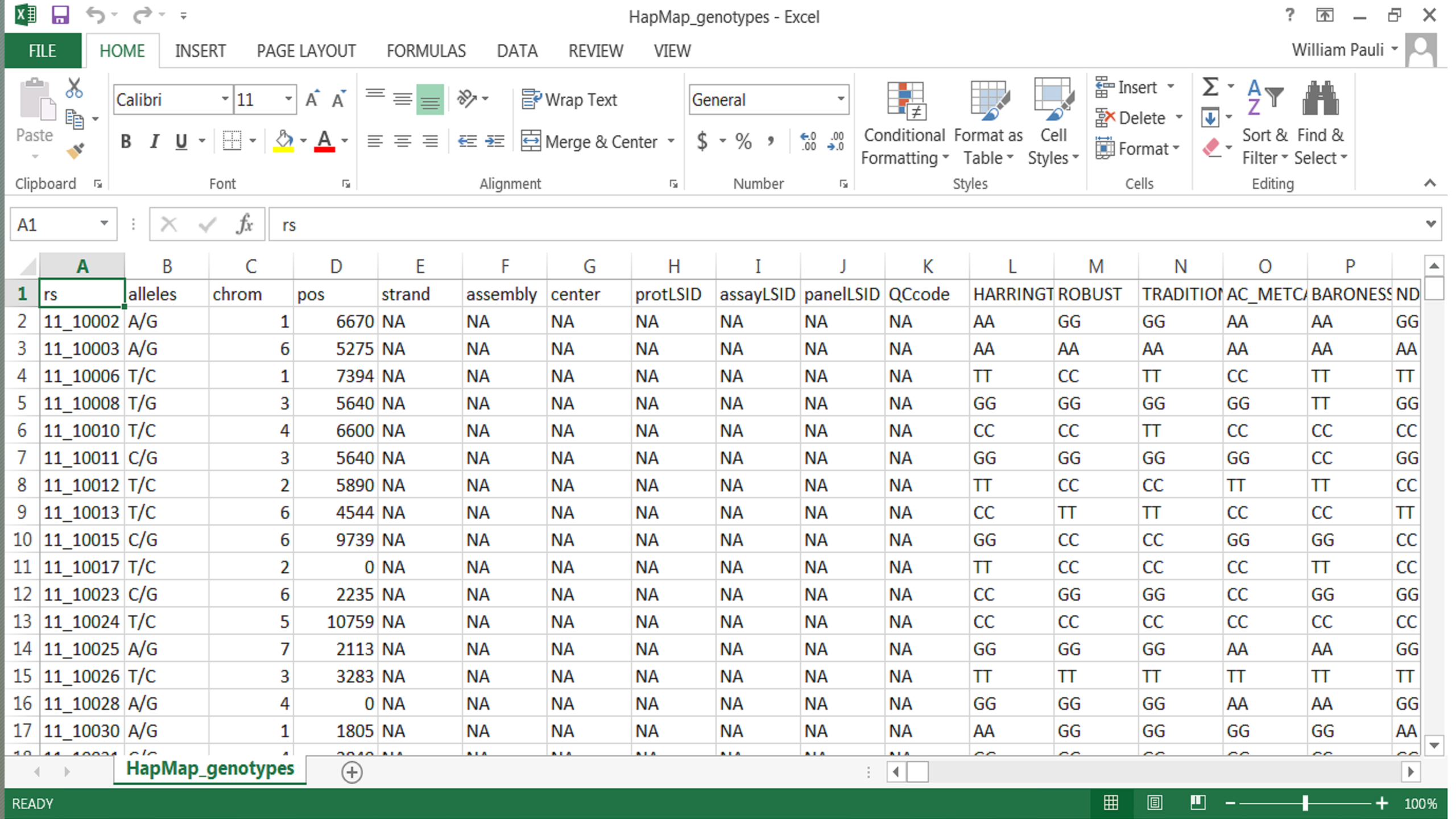
- Barley data set generated for the Barley CAP
- All data available from http://triticeaetoolbox.org/barley/
- Data from replicated yield trial, 768 lines
- Trait is grain protein content, important in malting/brewing
- GAPIT handles multiple traits

Sample Files

- Do an analysis using HapMap formatted genotype data
- Again, check manual for formatting
- Two files
  - HapMap_Genotypes – data in HapMap format
  - Protein – protein content of grain in %
- 2359 markers
- Missing data was set at 20%
  - (Markers missing more than 20% were removed)

HapMap Format

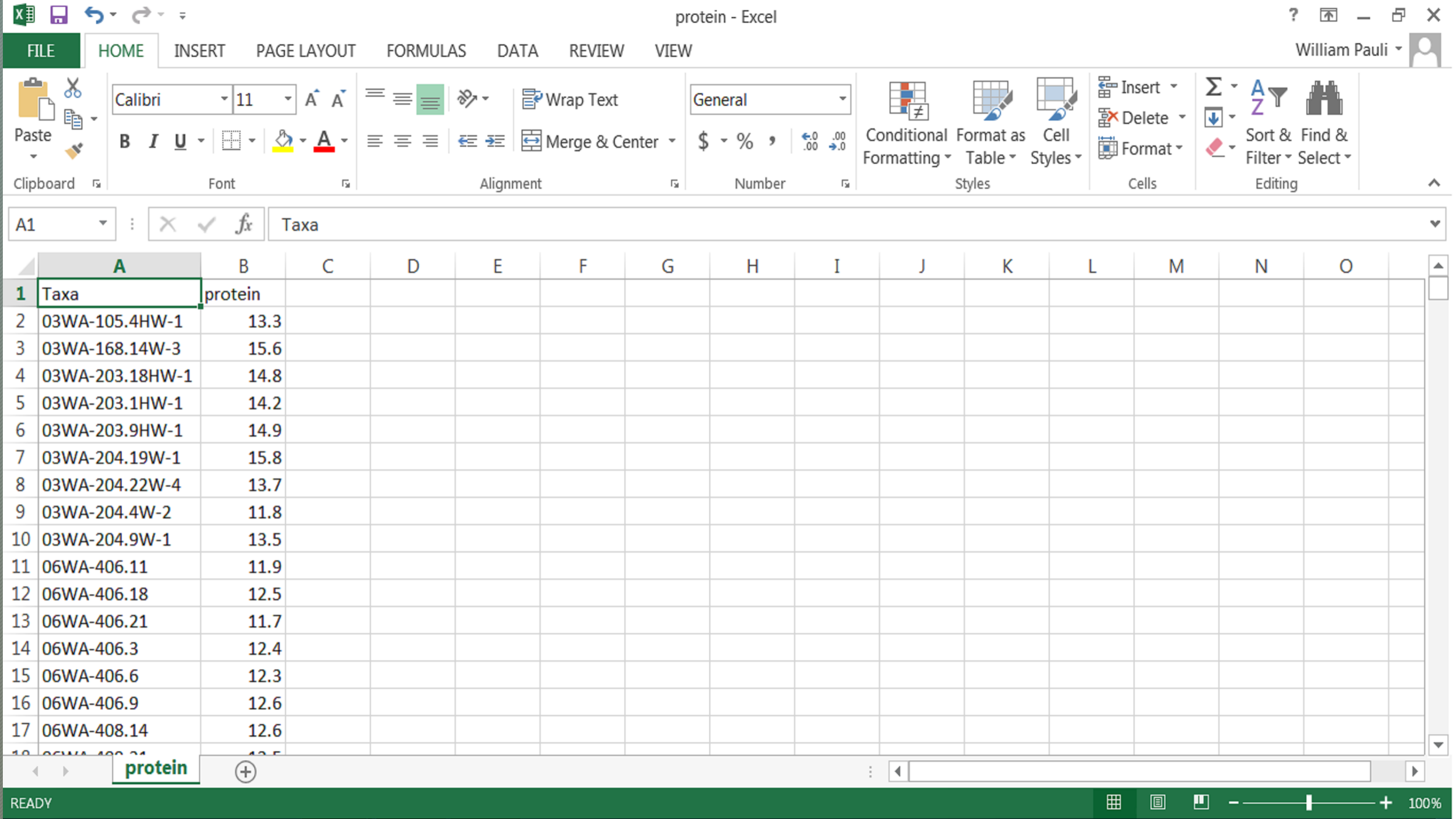
- Genetic map and marker data in one file
- Make sure there are no # signs in column headers, typically show up in assembly column
- Columns that are required for GAPIT are rs, chrom, pos – everything else can be filled in NA with regard to assembly, center, protLSID etc.
- In the import process make sure header=F



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
1	rs	alleles	chrom	pos	strand	assembly	center	protLSID	assayLSID	panelLSID	QCcode	HARRINGT	ROBUST	TRADITION	AC_METC/	BARONESS	ND
2	11_10002	A/G	1	6670	NA	NA	NA	NA	NA	NA	NA	AA	GG	GG	AA	AA	GG
3	11_10003	A/G	6	5275	NA	NA	NA	NA	NA	NA	NA	AA	AA	AA	AA	AA	AA
4	11_10006	T/C	1	7394	NA	NA	NA	NA	NA	NA	NA	TT	CC	TT	CC	TT	TT
5	11_10008	T/G	3	5640	NA	NA	NA	NA	NA	NA	NA	GG	GG	GG	GG	TT	GG
6	11_10010	T/C	4	6600	NA	NA	NA	NA	NA	NA	NA	CC	CC	TT	CC	CC	CC
7	11_10011	C/G	3	5640	NA	NA	NA	NA	NA	NA	NA	GG	GG	GG	GG	CC	GG
8	11_10012	T/C	2	5890	NA	NA	NA	NA	NA	NA	NA	TT	CC	CC	TT	TT	CC
9	11_10013	T/C	6	4544	NA	NA	NA	NA	NA	NA	NA	CC	TT	TT	CC	CC	TT
10	11_10015	C/G	6	9739	NA	NA	NA	NA	NA	NA	NA	GG	CC	CC	GG	GG	CC
11	11_10017	T/C	2	0	NA	NA	NA	NA	NA	NA	NA	TT	CC	CC	CC	TT	CC
12	11_10023	C/G	6	2235	NA	NA	NA	NA	NA	NA	NA	CC	GG	GG	CC	GG	GG
13	11_10024	T/C	5	10759	NA	NA	NA	NA	NA	NA	NA	CC	CC	CC	CC	CC	CC
14	11_10025	A/G	7	2113	NA	NA	NA	NA	NA	NA	NA	GG	GG	GG	AA	AA	GG
15	11_10026	T/C	3	3283	NA	NA	NA	NA	NA	NA	NA	TT	TT	TT	TT	TT	TT
16	11_10028	A/G	4	0	NA	NA	NA	NA	NA	NA	NA	GG	GG	GG	AA	AA	GG
17	11_10030	A/G	1	1805	NA	NA	NA	NA	NA	NA	NA	AA	GG	GG	GG	GG	AA

Phenotype File

- Straightforward text file
- Taxa used for header of entries
- GAPIT can run multiple phenotypes, i.e. analyze multiple traits in one analysis
- One phenotype file with multiple columns for different phenotypes
- Missing data coded as NA or NaN
- During data import use header=T


protein - Excel
FILE
HOME
INSERT
PAGE LAYOUT
FORMULAS
DATA
REVIEW
VIEW
William Pauli
Paste
Calibri
11
Wrap Text
General
Merge & Center
Conditional Formatting
Format as Table
Cell Styles
Insert
Delete
Format
Sort & Filter
Find & Select
Clipboard
Font
Alignment
Number
Styles
Cells
Editing
A1
Taxa
protein
03WA-105.4HW-1 13.3
03WA-168.14W-3 15.6
03WA-203.18HW-1 14.8
03WA-203.1HW-1 14.2
03WA-203.9HW-1 14.9
03WA-204.19W-1 15.8
03WA-204.22W-4 13.7
03WA-204.4W-2 11.8
03WA-204.9W-1 13.5
06WA-406.11 11.9
06WA-406.18 12.5
06WA-406.21 11.7
06WA-406.3 12.4
06WA-406.6 12.3
06WA-406.9 12.6
06WA-408.14 12.6
protein
READY
100%

Running GAPIT

- Need to install some other packages that GAPIT uses
- GAPIT uses code from the Buckler lab website for running the package
- Using source code allows them to update the package continuously without having to constantly install new versions of the package
- Uses EMMA which also must be loaded
- Install.packages puts them into your library
- Library loads them for use in your current session
- ***Important if GAPIT isn't loading check website for updated code
  - http://www.maizegenetics.net/gapit

RStudio

File Edit Code View Plots Session Project Build Tools Help

Go to file/function

Code for GAPIT webinar.R* | pheno

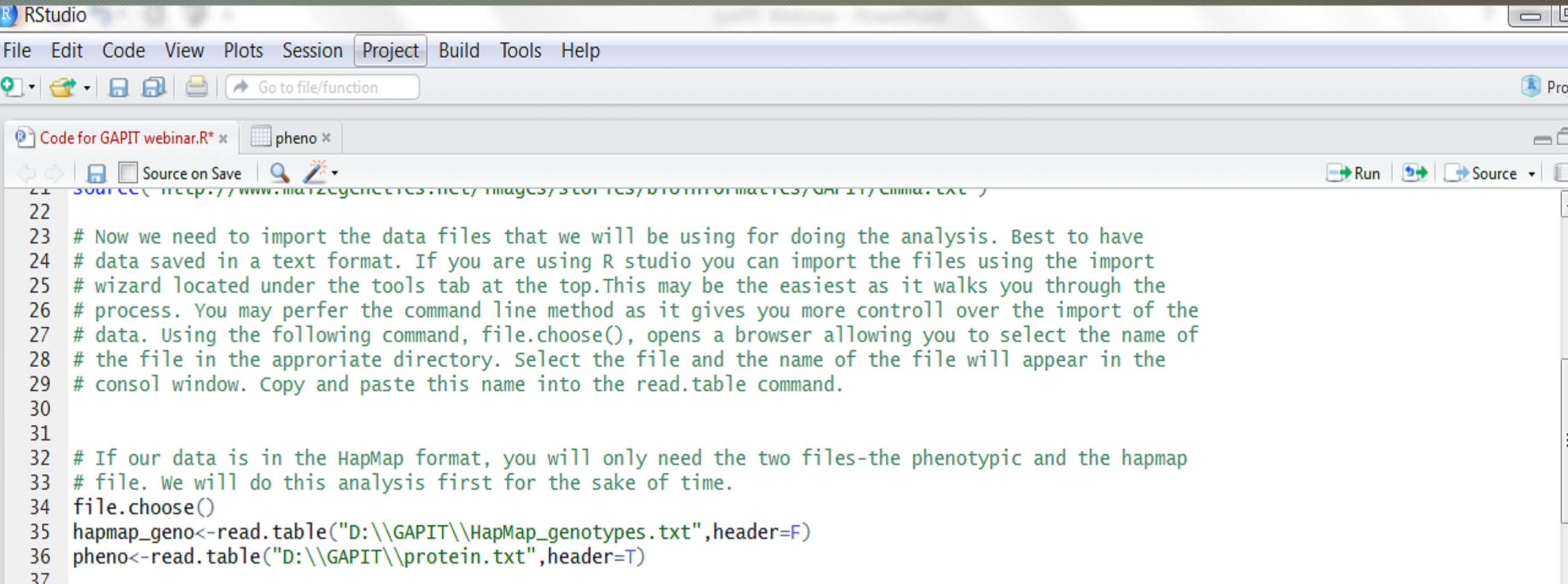
Source on Save | Run | Source

```
1  # to run GAPIT we need to access some tools that are located in other packages. The following lines of
2  # code will install the required packages in to your R library and then will import them so that GAPIT
3  # may access them. The easiest way to do this is to highlight all of the following code and select run.
4  # Once you have done this, you will only need to use the library functions to access them at future dates.
5
6  source("http://www.bioconductor.org/biocLite.R")
7  biocLite("multtest")
8
9  install.packages("gplots")
10 install.packages("LDheatmap")
11 install.packages("genetics")
12
13 library(multtest)
14 library("gplots")
15 library("LDheatmap")
16 library("genetics")
17 library("compiler") #this library is already installed in R
18
19 #these lines of code install the actual GAPIT functions from the maize genetics website
20 source("http://www.maizegenetics.net/images/stories/bioinformatics/GAPIT/gapit_functions.txt")
21 source("http://www.maizegenetics.net/images/stories/bioinformatics/GAPIT/emma.txt")
```

Upload Files

- A few ways...
- Use the import wizard in RStudio
  - Under Tools tab, then import data set, will walk you through it
- Manually, need to know the address of the file
- Use file.choose()
  - Opens a file browser, navigate to the file, double click it
  - Displays file name in console
  - Copy that and use in the read.table statement
    - E.g. Example_data<-read.table("D;\\GAPIT\\protein.txt",header=T)

Uploading Files



Doing the analyses

- Know your data
- Do a quick check to make sure files are properly loaded
  - Use the str() command to get information about the data object
- Plot the phenotype data
- Look at basic descriptive statistics
- Helps insure that data is properly loaded

Checking phenotype data

RStudio

File Edit Code View Plots Session Project Build Tools Help

Go to file/function

Code for GAPIT webinar.R* × pheno ×

Source on Save Run Sourc

```
38 # good idea to check our pehontype data to make sure the file strucutre is correct and how the data
39 # is distributed, checking for outliers
40
41 str(pheno) # gives us information on the object, in this case a data frame and other information
42 hist(pheno$protein) # creates a histogram plot of our data, things look pretty good, we have a
43  #lines that are bit extreme but with 775 lines we would expect about 27 to be at least 3 sd's away from
44  # the mean.
45
46 #some basic statistics to look at
47 mean(pheno$protein) # 12.23
48 range(pheno$protein) # 9.5 to 15.8
49 sd(pheno$protein) # .88
50 which(is.na(pheno$protein)) # look for lines with missing data, there should be none which is confimred
```

RStudio

File Edit Code View Plots Session Project Build Tools Help

Go to file/function

Project: (None)

Code for GAPIT webinar.R | pheno

Source on Save | Run | Source

```
27 # data. Using the following command, file.choose(), opens a browser allowing
28 # the file in the approriate directory. Select the file and the name of the
29 # consol window. Copy and paste this name into the read.table command.
30
31
32 # If our data is in the HapMap format, you will only need the two files-the
33 # file. We will do this analysis first for the sake of time.
34 file.choose()
35 hapmap_geno<-read.table("D:\\GAPIT\\HapMap_genotypes.txt",header=F)
36 pheno<-read.table("D:\\GAPIT\\protein.txt",header=T)
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```

43:2 (Top Level) R Script

Console ~/

```
> source("http://www.maizegenetics.net/images/stories/bioinformatics/GAPIT/emma.tx
> hapmap_geno<-read.table("D:\\GAPIT\\HapMap_genotypes.txt",header=F)
> pheno<-read.table("D:\\GAPIT\\protein.txt",header=T)
> str(pheno) # gives us information on the object, in this case a data frame and c
information
'data.frame': 768 obs. of  2 variables:
 $ Taxa   : Factor w/ 768 levels "03WA-105.4HW-1",..: 1 2 3 4 5 6 7 8 9 10 ...
 $ protein: num  13.3 15.6 14.8 14.2 14.9 15.8 13.7 11.8 13.5 11.9 ...
> hist(pheno$protein) # creates a histogram plot of our data, things look pretty g
>
```

Workspace | History

Import Dataset

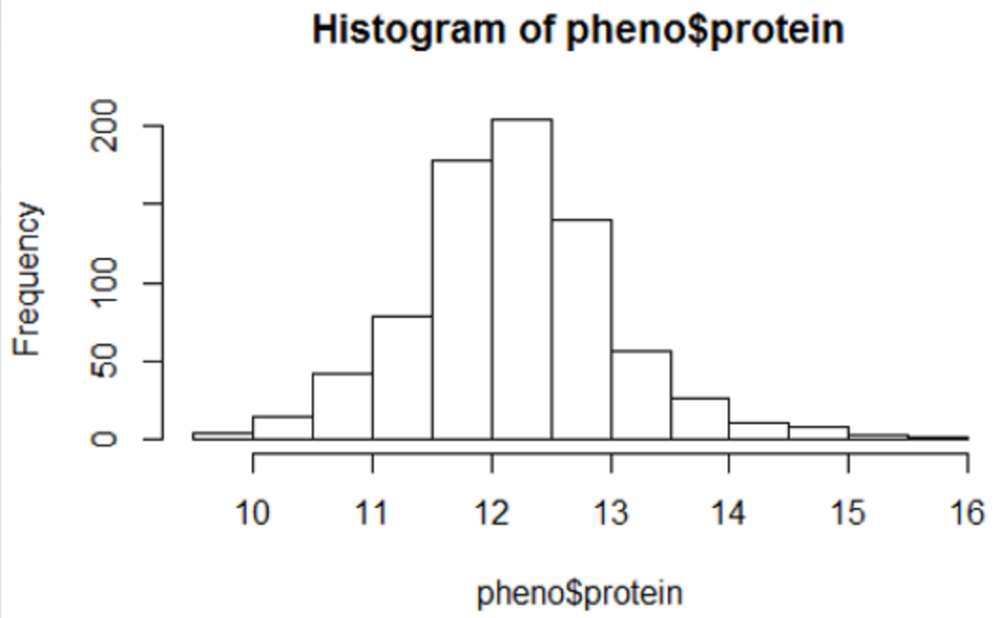
Data	
hapmap_geno	2360 obs. of 779 variables
pheno	768 obs. of 2 variables

Functions

```
GAPIT(Y = NULL, G = NULL, GD = NULL, GM = NULL, KI = NULL, Z
= NULL  CV = NULL  CV.Inheritance = NULL  GP = NULL  GK =
```

Files | Plots | Packages | Help

Zoom | Export | Clear All



GAPIT Function

- To run GAPIT using the HapMap formatted data need 2 files
  - G = HapMap formatted genotype file
  - Y = phenotype file
- The number of input parameters is large, refer to the manual
- We will do a basic analysis accounting for population structure, with and without using compression, using P3D
- When data is in HapMap format GAPIT can impute missing data using
  - Major allele (SNP.impute="Major")
  - Minor allele (SNP.impute="Minor")
  - Heterozygote (SNP.impute="Middle")

Code for analysis

```
Analysis1<- GAPIT(
Y = name of phenotype file,
G = name of genotype HapMap File,
SNP.impute = method of imputation,
PCA.total = number of principal components to use to control for population structure,
Major.allele.zero=T  reports allele effect with respect to the minor allele,
Group.from = 768,
Group.to = 768,
Group.by = 1)
```

- Last three lines turn compression off
- Set working directory where you want files to be saved (Lots of output)
  - In RStudio – Session tab, Set working directory, Choose directory
  - In R, File, Change directory
  - Manually with setwd("folder location")

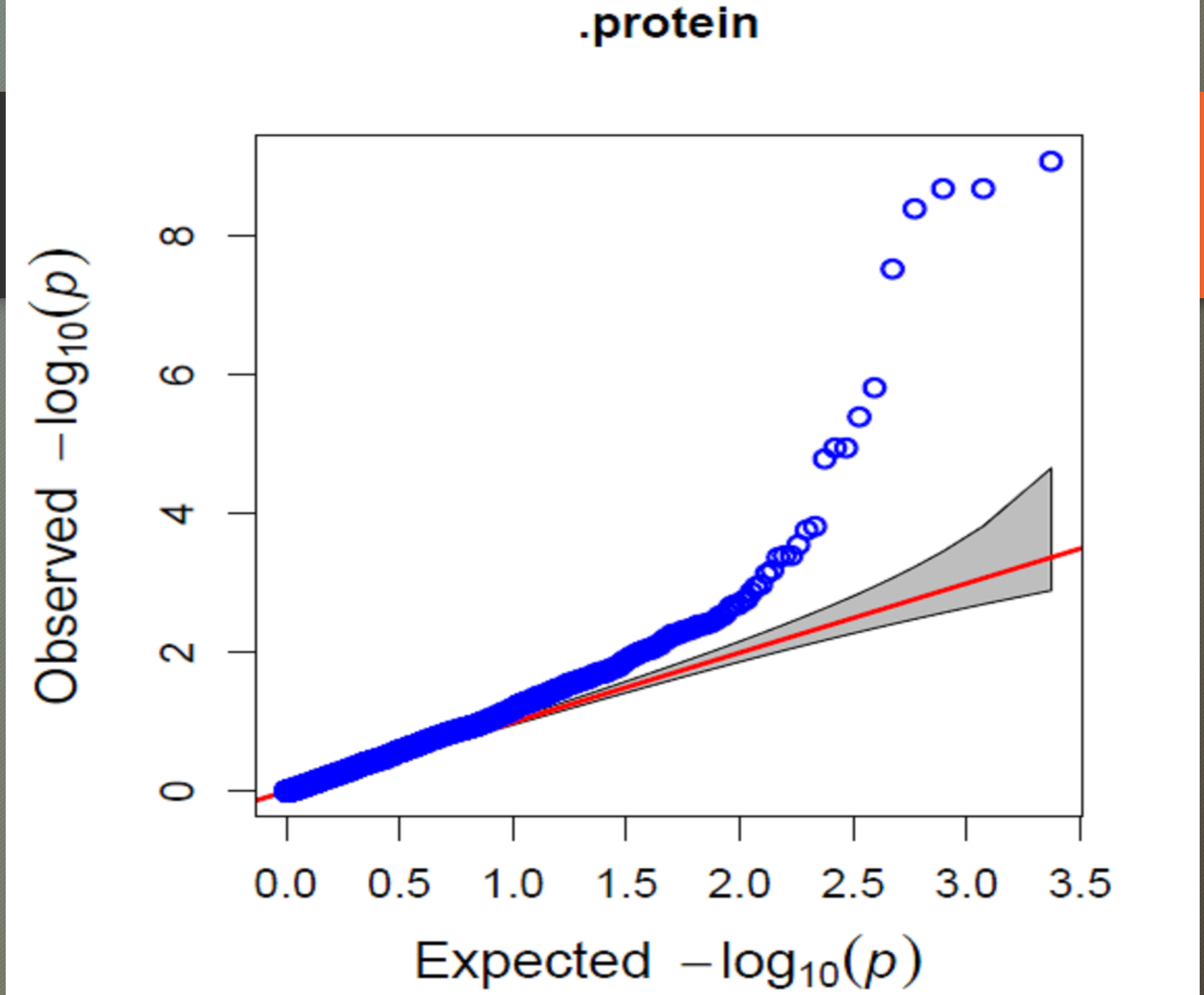
```
51
52 # the following is a very basic analysis. Make sure to change directory to where the results
53 # will be saved. In R studio go to session at the top and select "set working directory"
54 # and select "choose directory"- this will allow you to browse to the correct folder in which you
55 # would like to save the results. Once you locate the correct folder, highlight it and hit select.
56
57 # first analysis where compression is not used in the model
58 analysis1<-GAPIT(
59   Y=pheno,
60   G=hapmap_geno,
61   SNP.impute="Major",
62   PCA.total=3,
63   Major.allele.zero=T,
64   group.from=768,
65   group.to=768,
66   group.by=1)
67
68 # now we use compression to see how that effects the outcome of the analysis
69 analysis2<-GAPIT(
70   Y=pheno,
71   G=hapmap_geno,
72   SNP.impute="Major",
73   PCA.total=3,
74   Major.allele.zero=T)
```

file.G	NULL	User	The common name of file for genotype in hapmap format
file.GD	NULL	User	The common name of file for genotype map for numeric format
file.GM	NULL	User	The common name of file for genotype data in numeric format
file.path	NULL	User	Path for genotype files
file.from	0	>0	The first genotype files named sequentially
file.to	0	>0	The last genotype files named sequentially
group.by	10	>0	The grouping interval of compression
group.from	1	>1	The starting number of groups of compression
group.to	10000000	>1	The ending number of groups of compression
kinship.algorithm	VanRaden	Loiselle and EMMA	Algorithm to derive kinship from genotype
kinship.cluster	average	complete, ward, single, mcquitty, median, and centroid	Clustering algorithm to group individuals based on their kinship
kinship.group	Mean	Max, Min, and Median	Method to derive kinship among groups
LD.chromosome	NULL	User	Chromosome for LD analysis
LD.location	NULL	User	Location (center) of SNPs for LD analysis
PCA.scaling	None	Scaled, Centered.and.scaled	Scale and/or center and scale the SNPs before conducting PCA
SNP.FDR	1	>0 and <1	Threshold to filter SNP on FDR
SNP.MAF	0	>0 and <1	Minor Allele Frequency to filter SNPs in GWAS reports
SNP.effect	Add	Dom	Genetic model
SNP.P3D	TRUE	FALSE	Logic variable to use P3D or not for testing SNPs
SNP.fraction	1	>0 and <1	Fraction of SNPs sampled to estimate kinship and PCs
SNP.test	TRUE	FALSE	Logic variable to test SNPs or not

Output

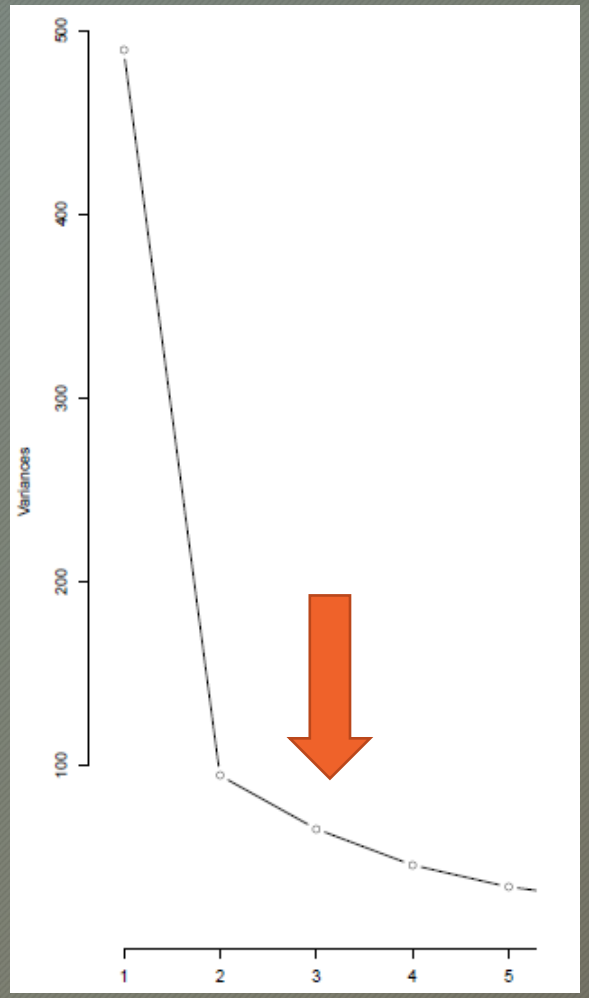
- QQ plot
- PCA graph
- Allelic Effects Estimate
- BLUPs
- GWAS results
- Manhattan Plots
  - Genome-wide
  - Chromosome specific
- There is more output than this
- Model Selection (BIC)

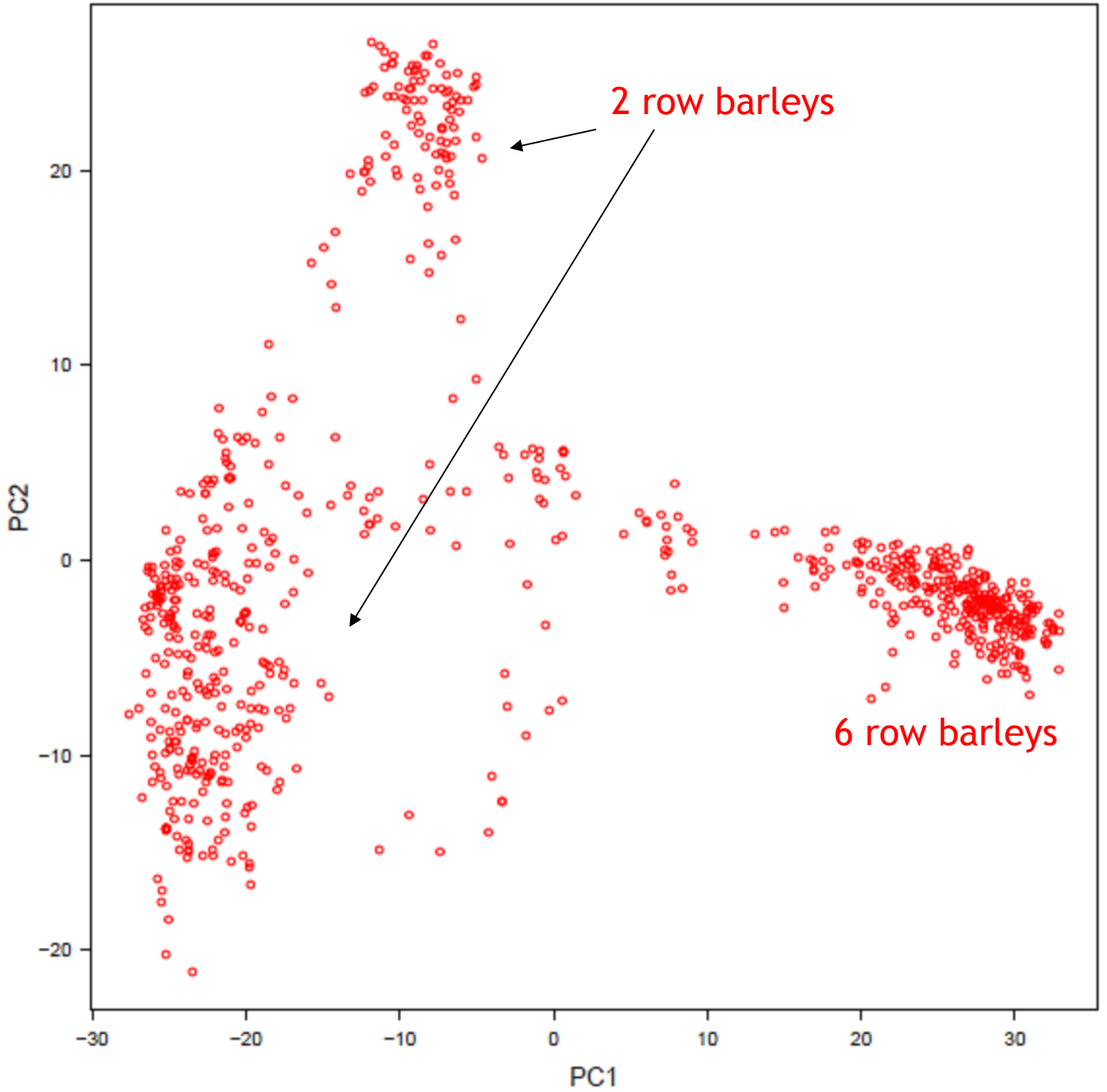
QQ Plot


.protein
Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$
0
2
4
6
8
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

Population Str.

Scree Plot





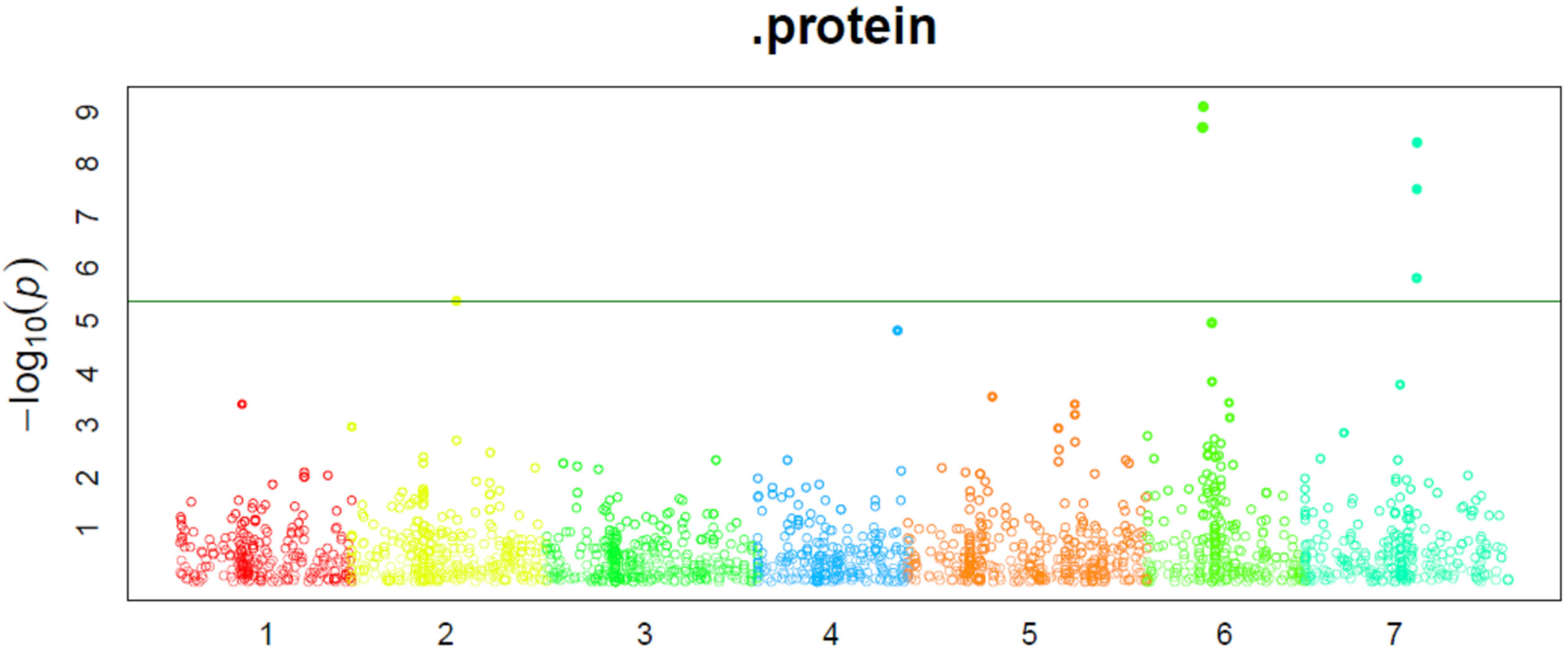
GWAS Results

	A	B	C	E	F	G	H	I
1	SNP	Chromoso	Position	P.value	maf	nobs	Rsquare.of.Model.without.SNP	Rsquare.of.Model.with.SNP	FDR_Adjusted_P-values
2	12_10811	6	4544	7.96E-10	0.160156	768	0.453308974	0.481035038	1.60E-06
3	12_10199	6	4544	2.03E-09	0.172526	768	0.453308974	0.479661509	1.60E-06
4	12_10575	6	4544	2.03E-09	0.172526	768	0.453308974	0.479661509	1.60E-06
5	12_20685	7	9179	4.00E-09	0.049479	768	0.453308974	0.478672367	2.36E-06
6	12_11437	7	9179	2.97E-08	0.048177	768	0.453308974	0.475758998	1.40E-05
7	12_30301	7	9179	1.52E-06	0.041667	768	0.453308974	0.470114965	0.000599332
8	11_20340	2	8592	3.99E-06	0.435547	768	0.453308974	0.46875265	0.001345092
9	12_11353	6	5275	1.12E-05	0.061198	768	0.453308974	0.467302678	0.002932349
10	12_30032	6	5275	1.12E-05	0.061198	768	0.453308974	0.467302678	0.002932349
11	11_10697	4	11466	1.56E-05	0.013021	768	0.453308974	0.466836667	0.003680907
12	11_10003	6	5275	0.000146	0.221354	768	0.453308974	0.463730878	0.031397203
13	11_10256	7	7785	0.000171	0.03776	768	0.453308974	0.463519898	0.033560523
14	11_21239	5	6934	0.000282	0.227865	768	0.453308974	0.462832961	0.051170938
15	11_20714	6	6704	0.000383	0.132161	768	0.453308974	0.462415252	0.060326767
16	11_10095	5	13716	0.000403	0.231771	768	0.453308974	0.462345234	0.060326767
17	12_10938	1	5060	0.000409	0.029297	768	0.453308974	0.462325767	0.060326767

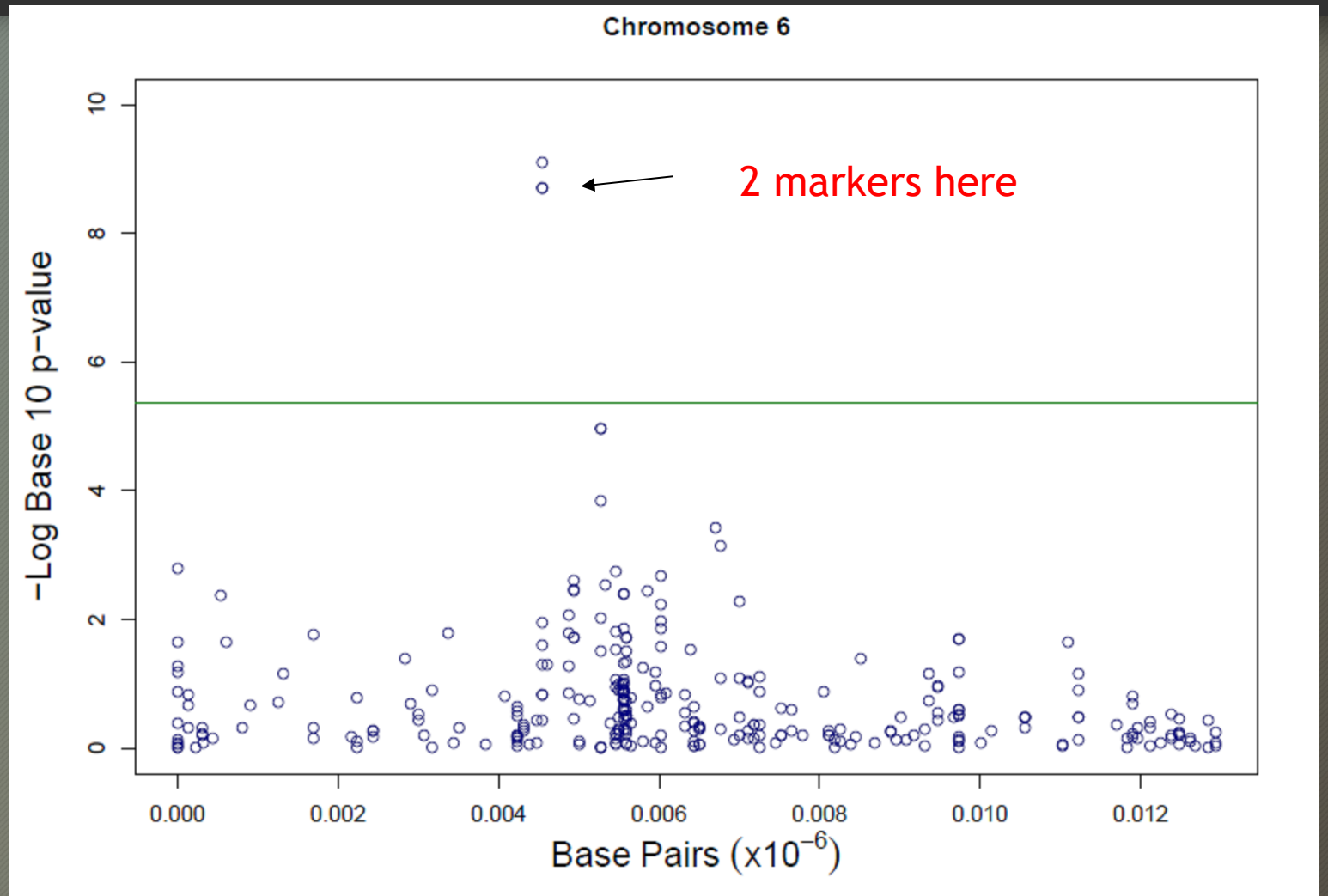
Allelic Effects Estimate

	A	B	C	D	E	F
1	SNP	Chromoso	Position	Allelic Effect Estimate		
2	11_10002	1	6670	0.002848		
3	11_10003	6	5275	-0.23801		
4	11_10006	1	7394	-0.03255		
5	11_10008	3	5640	0.129514		
6	11_10010	4	6600	-0.04677		
7	11_10011	3	5640	0.100203		
8	11_10012	2	5890	-0.27811		
9	11_10013	6	4544	0.155822		
10	11_10015	6	9739	0.016424		
11	11_10017	2	0	-0.09578		
12	11_10023	6	2235	0.012274		
13	11_10024	5	10759	0.045846		

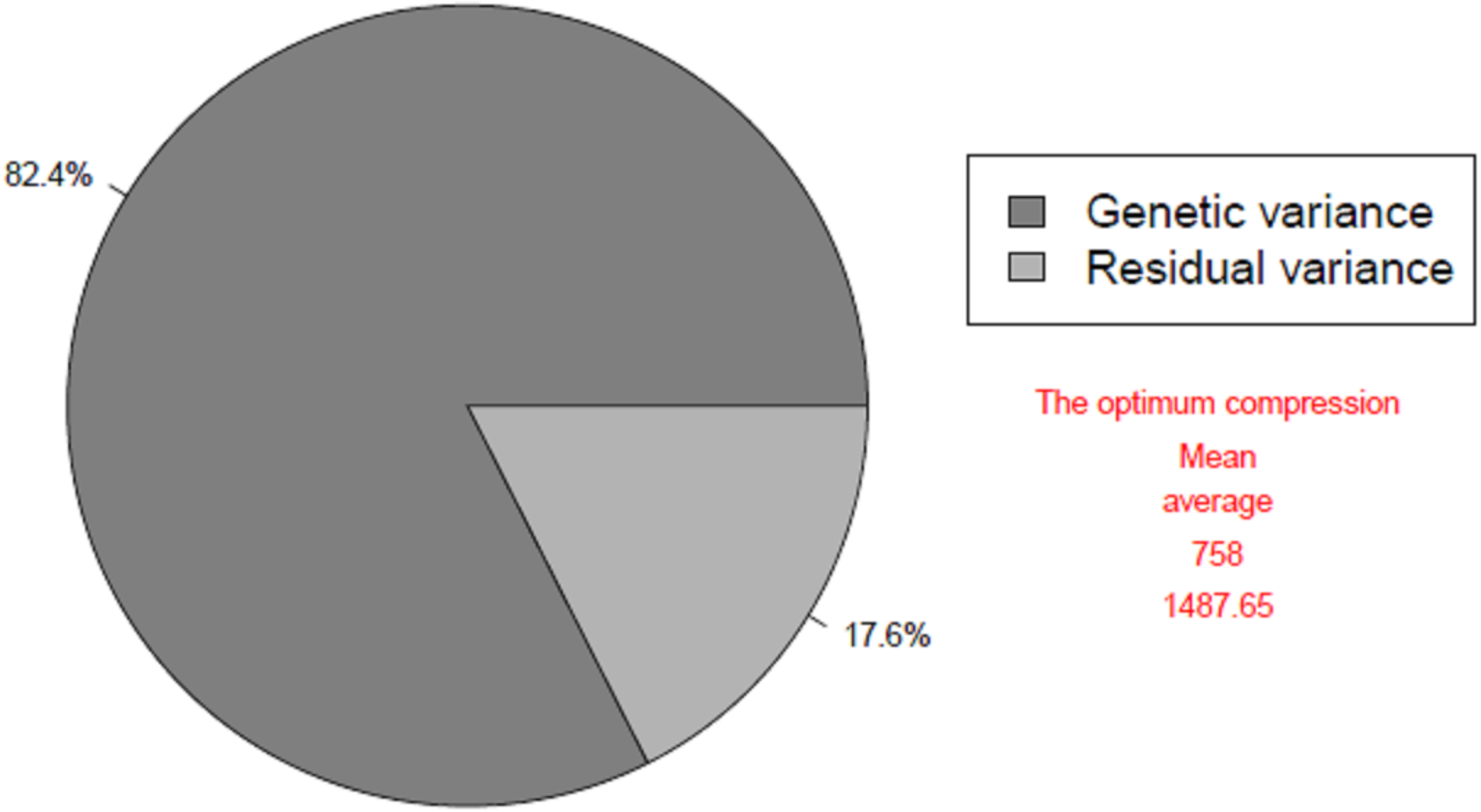
Genome-wide Manhattan Plot



Chromosome Specific Manhattan Plot



Optimal Compression Parameters



- Optimal method to calculate group kinship is “Mean”
- Optimal clustering method is “Average”
- Number of groups is 758
- -2*log likelihood is 1487.65
- Heritability is 0.824

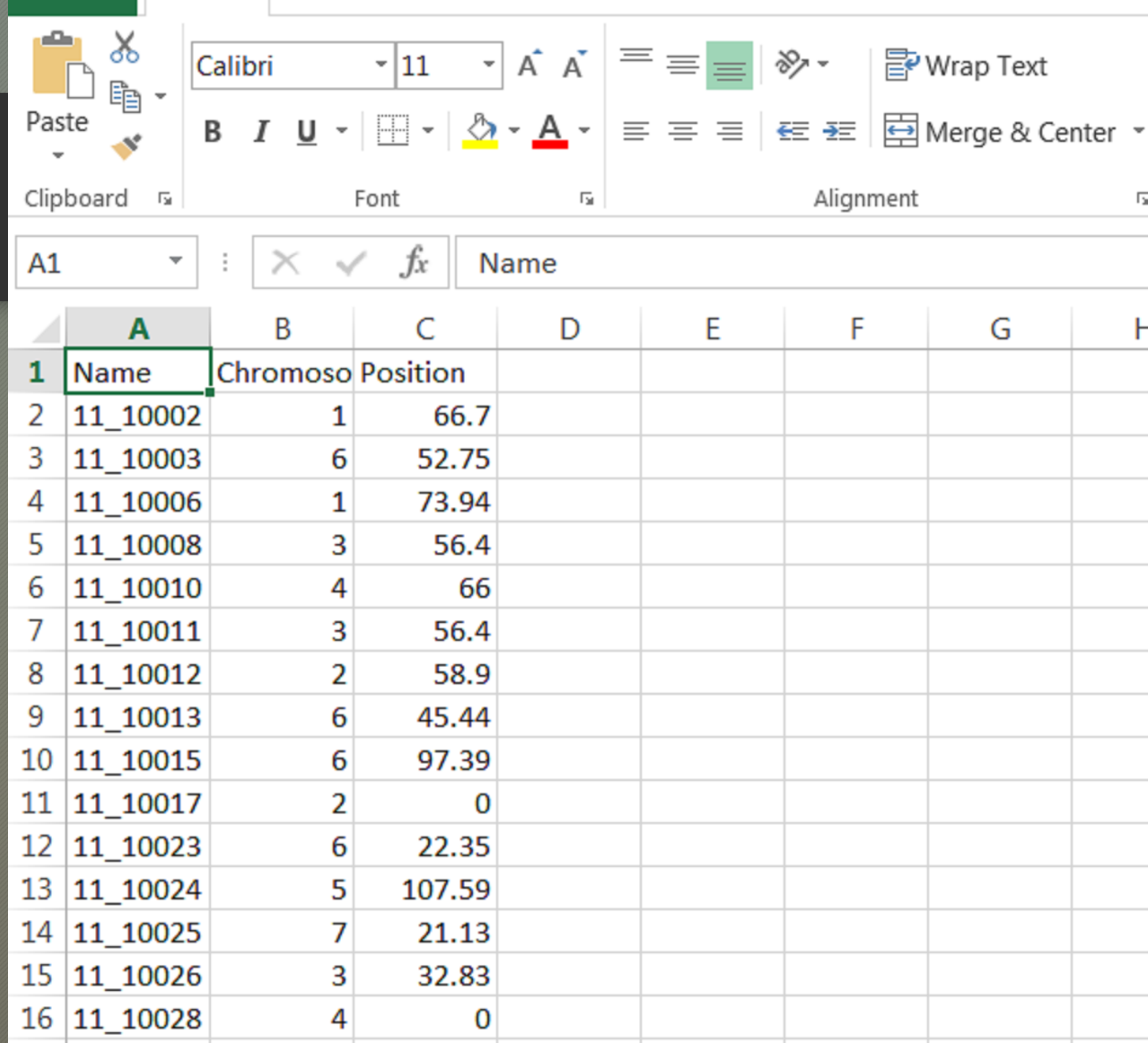
Your data

- Most of the time the data is not in HapMap form, what do you do?
- Build your own HapMap file, code SNPs as AA, AC, CC
- GAPIT does not impute marker data for numerically formatted genotype files
  - Impute in another program like TASSEL and import that genotype file
  - Impute with R, use marker average for missing data etc.
- If you use a numeric format you need to provide a genetic map file that has marker information
- In the GAPIT function the G = genotype file is changed to GD = genotype file and you must also include GM = genetic map file

Code for numeric data

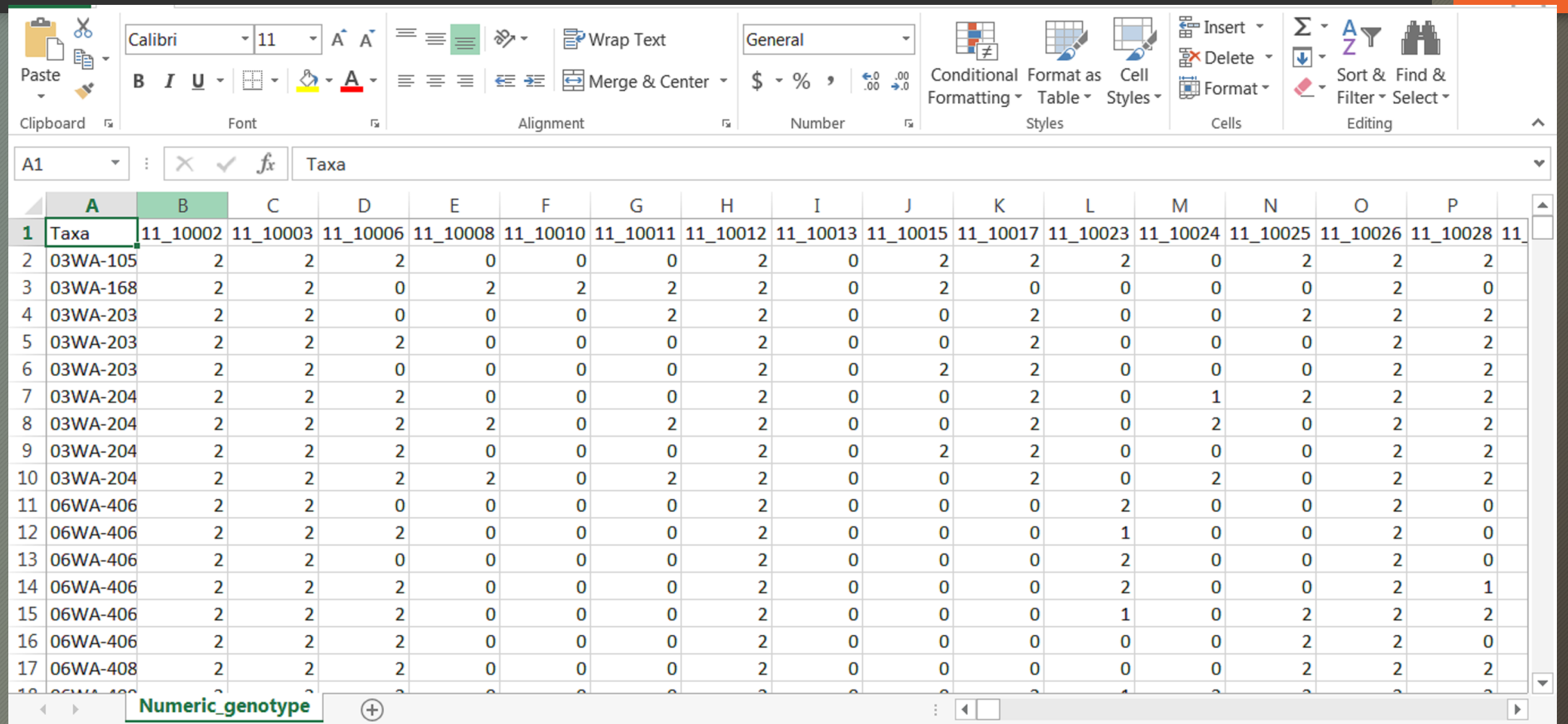
```
83
84  analysis2<-GAPIT(
85    Y=pheno,
86    GD=numeric_geno,
87    GM=genetic_map,
88    SNP.impute="Major",
89    PCA.total=3,
90    Major.allele.zero=T)
91
```

Genetic Map



	A	B	C	D	E	F	G	H
1	Name	Chromoso	Position					
2	11_10002	1	66.7					
3	11_10003	6	52.75					
4	11_10006	1	73.94					
5	11_10008	3	56.4					
6	11_10010	4	66					
7	11_10011	3	56.4					
8	11_10012	2	58.9					
9	11_10013	6	45.44					
10	11_10015	6	97.39					
11	11_10017	2	0					
12	11_10023	6	22.35					
13	11_10024	5	107.59					
14	11_10025	7	21.13					
15	11_10026	3	32.83					
16	11_10028	4	0					

Numeric Genotype Format



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
1	Taxa	11_10002	11_10003	11_10006	11_10008	11_10010	11_10011	11_10012	11_10013	11_10015	11_10017	11_10023	11_10024	11_10025	11_10026	11_10028	11_
2	03WA-105	2	2	2	0	0	0	2	0	2	2	2	0	2	2	2	
3	03WA-168	2	2	0	2	2	2	2	0	2	0	0	0	0	2	0	
4	03WA-203	2	2	0	0	0	2	2	0	0	2	0	0	2	2	2	
5	03WA-203	2	2	2	0	0	0	2	0	0	2	0	0	0	2	2	
6	03WA-203	2	2	0	0	0	0	2	0	2	2	0	0	0	2	2	
7	03WA-204	2	2	2	0	0	0	2	0	0	2	0	1	2	2	2	
8	03WA-204	2	2	2	2	0	2	2	0	0	2	0	2	0	2	2	
9	03WA-204	2	2	2	0	0	0	2	0	2	2	0	0	0	2	2	
10	03WA-204	2	2	2	2	0	2	2	0	0	2	0	2	0	2	2	
11	06WA-406	2	2	0	0	0	0	2	0	0	0	2	0	0	2	0	
12	06WA-406	2	2	2	0	0	0	2	0	0	0	1	0	0	2	0	
13	06WA-406	2	2	0	0	0	0	2	0	0	0	2	0	0	2	0	
14	06WA-406	2	2	2	0	0	0	2	0	0	0	2	0	0	2	1	
15	06WA-406	2	2	2	0	0	0	2	0	0	0	1	0	2	2	2	
16	06WA-406	2	2	2	0	0	0	2	0	0	0	0	0	2	2	0	
17	06WA-408	2	2	2	0	0	0	2	0	0	0	0	0	2	2	2	

Numeric_genotype

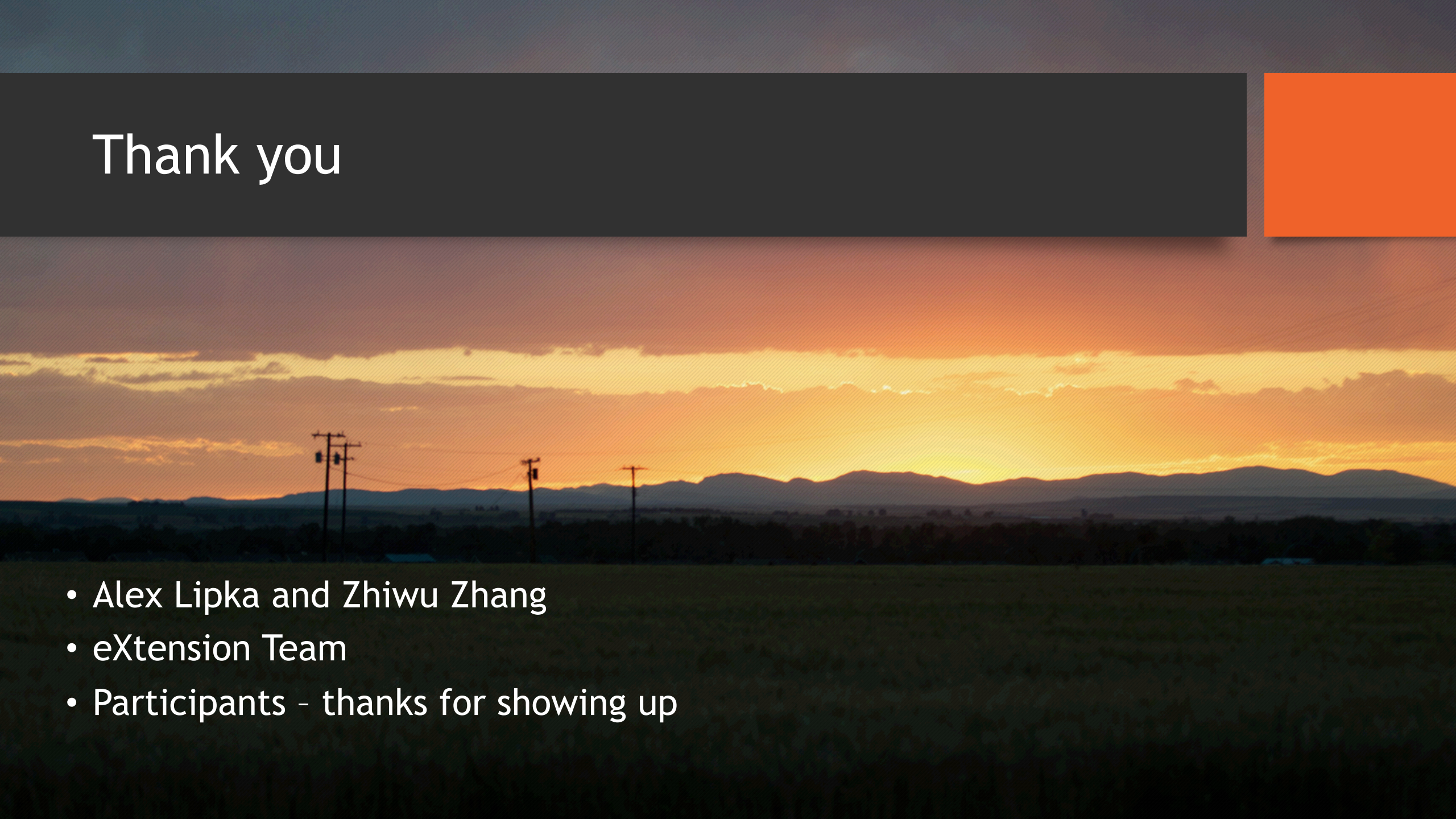
Problems

- Consult the manual, example code for nearly all situations that might arise
- Check the website, check the date on the user manual because that is updated as well as the code. The updated manual reflects changes to the code
- Tutorial and example data sets
- Contact the makers – very friendly and EXTREMELY helpful

Getting the most out of GAPIT

- Adjust analysis parameters
- Run several analyses
- Again, consult the model for more advanced features
- There is more example code in the provided script file that uses more of the options for running an analysis

Thank you



- Alex Lipka and Zhiwu Zhang
- eXtension Team
- Participants – thanks for showing up

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