

# GWAS/GS exercise using GAPIT

## BIO373 at the University of Zurich

Yasuhiro Sato (lecturer)<sup>1</sup>   Katharina Jung (TA)  
Naoto-Benjamin Hamaya (TA)

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<sup>1</sup>[yasuhiro.sato@uzh.ch](mailto:yasuhiro.sato@uzh.ch)

# Dataset: Rice 44k genomes

- ▶ Data from Zhao et al. (2011) Nature Communications 2:467
- ▶ Data available at <http://www.ricediversity.org/data/>
- ▶ 34 agronomic traits were examined for 413 rice accessions



## ARTICLE

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# Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*

Keyan Zhao<sup>1,2</sup>, Chih-Wei Tung<sup>3</sup>, Georgia C. Eizenga<sup>4</sup>, Mark H. Wright<sup>1</sup>, M. Liakat Ali<sup>5</sup>, Adam H. Price<sup>6</sup>, Gareth J. Norton<sup>6</sup>, M. Rafiqul Islam<sup>7</sup>, Andy Reynolds<sup>1</sup>, Jason Mezey<sup>1</sup>, Anna M. McClung<sup>4</sup>, Carlos D. Bustamante<sup>1,2</sup> & Susan R. McCouch<sup>3</sup>

## What to do in this exercise

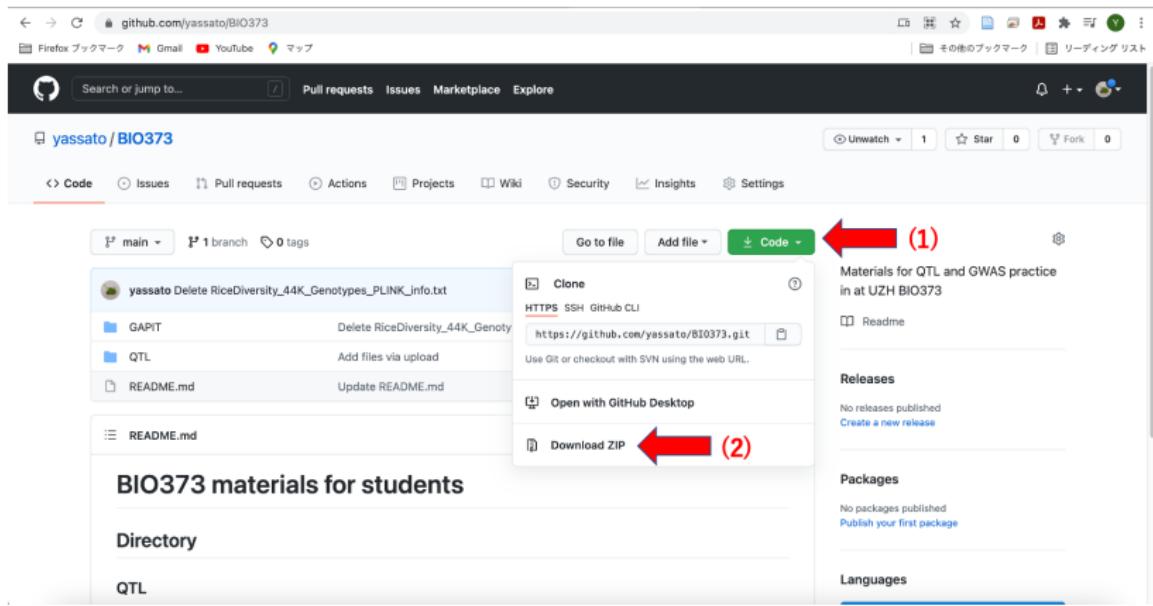
- ▶ GWAS example: Find genomic region associated with the seed length
- ▶ GS example: Predict flowering time between different study years
- ▶ Finally, you will be able to try GWAS/GS for traits of your interests!

## Source codes and input data

- ▶ RiceDiversity\_44K\_Genotypes\_PLINK\_imputed.txt.gz
- ▶ RiceDiversity\_44K\_Genotypes\_PLINK\_info.txt
- ▶ *This instruction PDF*
- ▶ All available at [https://github.com/yassato/BIO373\\_YS2023/tree/main/GAPIT](https://github.com/yassato/BIO373_YS2023/tree/main/GAPIT)

# Download materials (same as the QTL exercise)

- ▶ Download .zip from  
<https://github.com/yassato/BIO373> and unzip it  
(Of course, “git clone URL” works if you are good at Git)



# Set up the working directory

**Note: No support will be provided for your local environment (e.g., laptop)**

1. Access to RStudio server (<https://fgcz-genomics.uzh.ch>) and log-in with your B-fabric username and Password
2. Make and change your working directory with `mkdir GAPIT` from Terminal; and then `setwd("./GAPIT")` from R Console
3. Upload RiceDiversity\_44K\_Genotypes\_PLINK\_imputed.txt.gz and RiceDiversity\_44K\_Genotypes\_PLINK\_info.txt to the directory you made

The screenshot shows the RStudio Server interface. The browser tab is 'BIO373/GAPIT at main · yasato'. The RStudio window has 'Terminal' selected in the tabs. The terminal pane shows the following R code:

```
R 4.1.2 --> /GAPIT/
5 090414-A09   5   1   5   1.8817708 18.49218 89.37386 91.25563
6 090105-A02   7   1   6   8.8639442 20.66431 89.37386 98.23780
> pred <- predOrder(pred$Taxa)
> y <- pOrder(p$HgID)
> cor.test(pred$Prediction, y$Year06Flowering.time.at.Aransas)

Pearson's product-moment correlation

data: pred$Prediction and y$Year06Flowering.time.at.Aransas
t = 49.431, df = 335, p-value < 2.2e-16
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.9234637 0.9494873
sample estimates:
cor
0.9377791

>
> res <- lm(y$Year07Flowering.time.at.Aransas ~ pred$Prediction)
> plot(pred$Prediction,
+       y$Year07Flowering.time.at.Aransas)
```

The RStudio environment pane shows the global environment with 'res' and 'y' loaded. The file tree pane shows several files in the 'GAPIT' directory, including 'RiceDiversity\_44K\_Genotypes\_PLINK\_info.txt.gz' and 'RiceDiversity\_44K\_Genotypes\_PLINK\_imputed.txt.gz'. A red arrow points to the 'Upload' button in the file tree pane.

# First of all, load Genomic Association and Prediction Integrated Tool (GAPIT)

- ▶ Install GAPIT source code and its dependency
- ▶ Wait ca. 15 min. to install everything
- ▶ Some packages are not installed but they are negligible
- ▶ Try twice when it fails

```
# clean up your workplace
rm(list=ls())

# select "1:All" if this asks something about dependent packages
install.packages("devtools")
BiocManager::install("snpStats")
devtools::install_github("SFUStatgen/LDheatmap")
devtools::install_github("jiabowang/GAPIT3@078fe28",force=TRUE)
[REDACTED] # load GAPIT3 package
```

# Load and see phenotype data

```
url <- "http://www.ricediversity.org/data/sets/44kgwas/RiceDiversity_44K_Phenotypes_34traits_PLINK.txt"
p <- read.table(url, sep="\t", header=TRUE)
nrow(p) # no. of plants
```

```
## [1] 413
```

```
head(p)
```

```
##      HybID NSFTVID Flowering.time.at.Arkansas Flowering.time.at.Faridpur
## 1 081215-A05      1          75.08333            64
## 2 081215-A06      3          89.50000            66
## 3 081215-A07      4          94.50000            67
## 4 081215-A08      5          87.50000            70
## 5 090414-A09      6          89.08333            73
## 6 090414-A10      7         105.00000           NA
##   Flowering.time.at.Aberdeen FT.ratio.of.Arkansas.Aberdeen
## 1                      81          0.9269547
## 2                      83          1.0783133
## 3                      93          1.0161290
## 4                     108          0.8101852
## 5                     101          0.8820132
## 6                     158          0.6645570
##   FT.ratio.of.Faridpur.Aberdeen Culm.habit Leaf.pubescence Flag.leaf.length
## 1             0.7901235       4.0            1        28.37500
## 2             0.7951807       7.5            0        39.00833
## 3             0.7204301       6.0            1        27.68333
## 4             0.6481481       3.5            1        30.41667
## 5             0.7227723       6.0            1        36.90833
## 6               NA          3.0            1        36.99000
##   Flag.leaf.width Awn.presence Panicle.number.per.plant Plant.height
## 1     1.2833333          0            3.068053      110.9167
## 2     1.0000000          0            4.051785      143.5000
## 3     1.5166667          0            3.124565      128.0833
## 4     0.8916667          0            3.697178      153.7500
```

# Read genotype data and marker information

```
g <- read.table("RiceDiversity_44K_Genotypes_PLINK_imputed.txt.gz",
                 header=TRUE, sep="\t")
gm <- read.table("RiceDiversity_44K_Genotypes_PLINK_info.txt.gz",
                 header=TRUE, sep="\t")
nrow(g) # no. of plants
```

```
## [1] 413
```

```
ncol(g[,-1]) # no. of SNPs
```

```
## [1] 36901
```

```
head(gm) # marker info
```

```
##           ID CHROM   POS
## 1 id1000001     1 13147
## 2 id1000003     1 73192
## 3 id1000005     1 74969
## 4 id1000007     1 75852
## 5 id1000008     1 75953
## 6 id1000011     1 91016
```

## (1) Genome-wide association study (GWAS)

## Aim: Looking for genomic region underlying the length of rice grains

- ▶ Indica cultivars have long grains, while Japonica have round-shaped grains
- ▶ *Grain Size 3 (GS3)* is known to regulate the seed length in rice (Wang et al. 2011)
- ▶ Can we detect the known loci with GWAS?

## Run GWAS with a general linear model (GLM) or mixed linear model (MLM)

- ▶ It takes several minutes. Wait.
- ▶ When finished, output files appear in the current directory
- ▶ Warning messages occur but the program still works
- ▶ **Note: When you run GAPIT twice, the second run may not work. In such a case, log-out once and retry from data loading.**

```
myGAPIT <- GAPIT( # warnings occur but it still works
  Y=p[,c("HybID", "Seed.length")],
  GD=g,
  GM=gm,
  SNP.MAF=0.05, # cut-off minor alleles at 0.05
  Inter.Plot=TRUE, # option to make interactive plots
  model=c("GLM", "MLM"),
  kinship.algorithm="VanRaden",
  Multiple_analysis=TRUE)
```

# GWAS is done. Let us see a trait diagnosis first

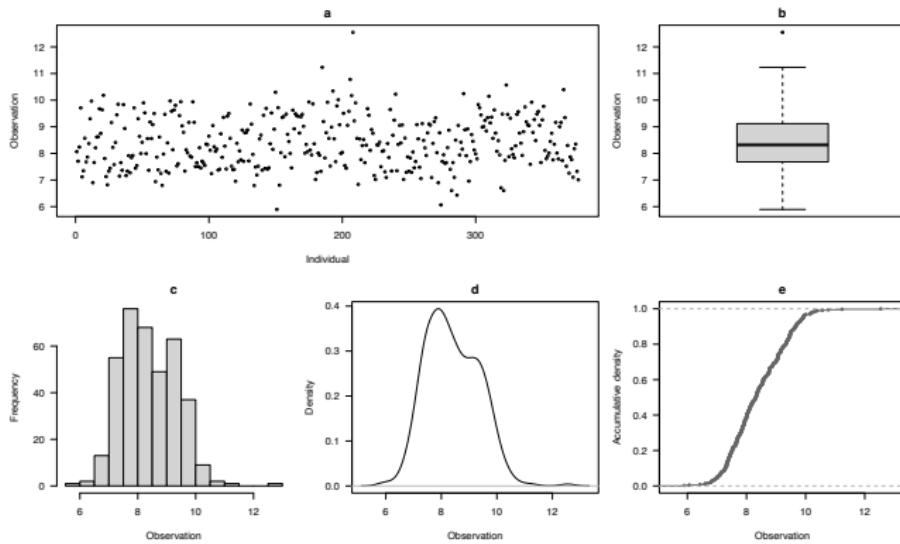


Figure 1: “GAPIT.Phenotype.View.Seed.length.pdf”

- ▶ The seed length looks normally distributed

... and also check heritability in the seed length

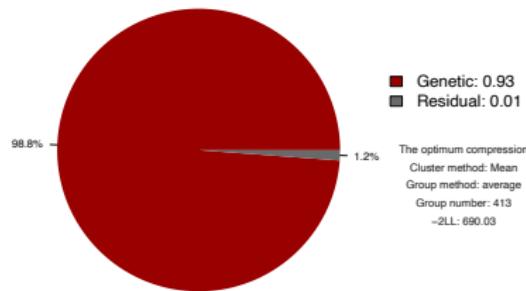


Figure 2: "GAPIT.Association.Optimum.MLM.Seed.length.pdf"

- ▶ Heritability,  $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$ ,
- ▶ where  $\sigma_g^2$ : genetic variance;  $\sigma_e^2$ : residual variance
- ▶  $h^2$  (%) is calculated as  
$$100 * (\sigma_g^2 / (\sigma_g^2 + \sigma_e^2)) = 100 * (0.93 / (0.93 + 0.01)) = 98.8\%$$

Check LD to see what kbp we should refer around the SNPs.

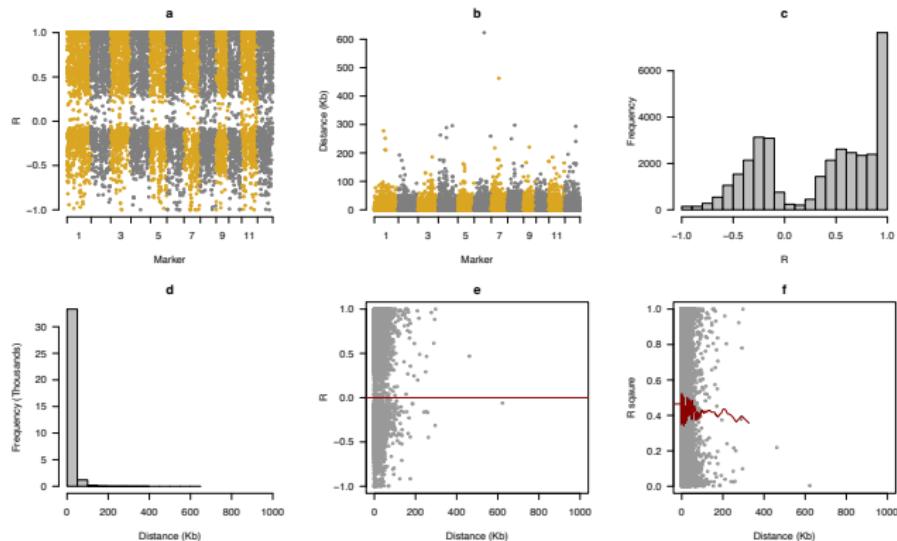


Figure 3: "GAPIT.Genotype.Density\_R\_sqaure.pdf"

- (b) The length of linkage disequilibrium is at most 600 kbp.

# Compare the marker density with the LD length

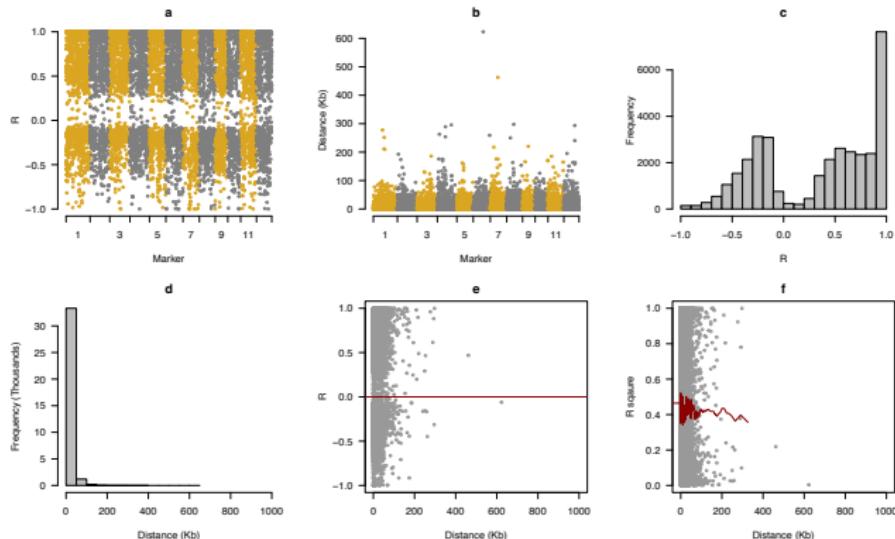


Figure 4: “GAPIT.Genotype.Density\_R\_sqare.pdf”

- ▶ (d) The marker intervals are much shorter than the length of LD,
- ▶ indicating that marker density was enough

# Manhattan plot of the general linear model (GLM)

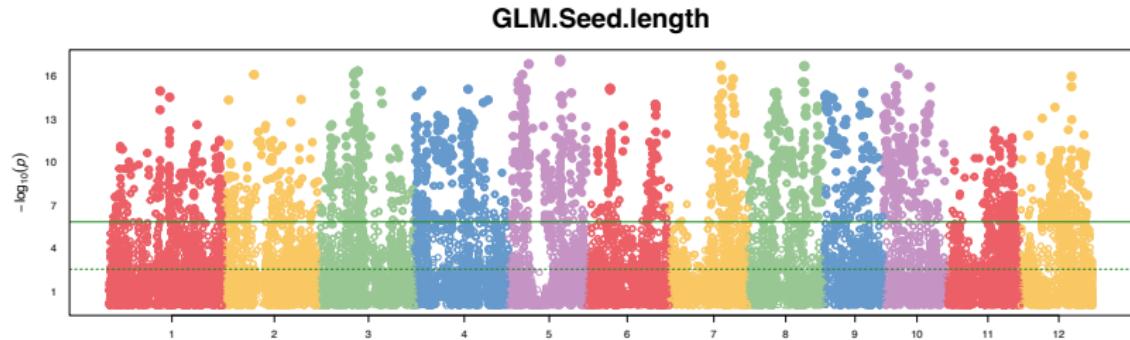


Figure 5: The file name  
“GAPIT.Association.Manhattan\_Geno.GLM.Seed.length.pdf”

- ▶ Is everything significant?? Very difficult to find key variants. . .

Quantile-quantile (QQ) plot also shows inflated  $p$ -values

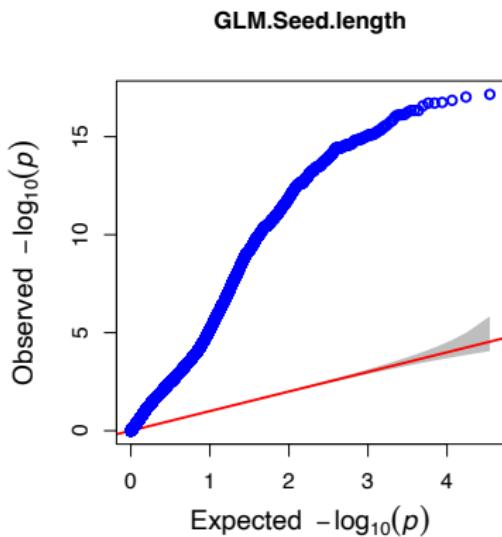


Figure 6: "GAPIT.Association.QQ.GLM.Seed.length.pdf"

- ▶ Blue dots: Observed  $-\log_{10}(p\text{-values})$
- ▶ Red line: Expected  $-\log_{10}(p\text{-values})$  when they are random

## Heatmap of the kinship matrix shows two clusters

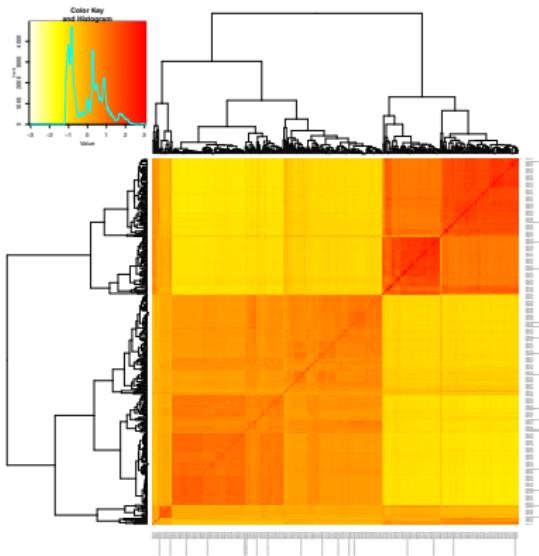


Figure 7: “GAPIT.Genotype.Kin\_VanRaden.pdf”

- ▶ Here we see a complex kinship structure
- ▶ A mixed linear model is worth trying to correct it

## Manhattan plot of the mixed linear model (MLM)

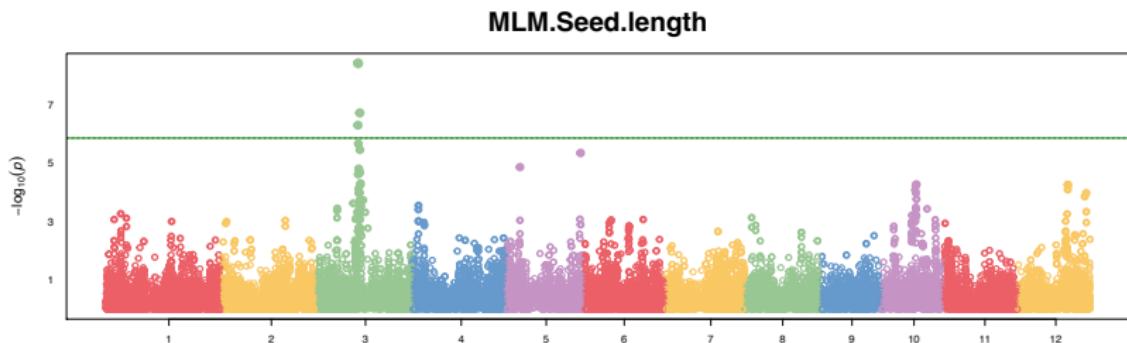


Figure 8: “GAPIT.Association.Manhattan\_Geno.MLM.Seed.length.pdf”

- ▶ We can find a peak on the chromosome 3!

## QQ plot of the mixed linear model

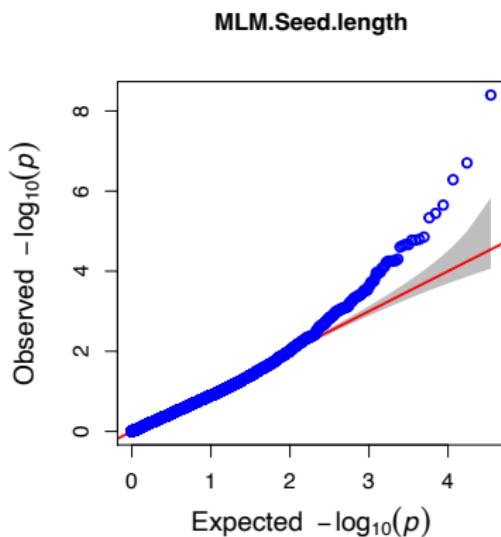


Figure 9: “GAPIT.Association.QQ.MLM.Seed.length.pdf”

- ▶ Only for top-scoring SNPs,  $-\log_{10}(p\text{-values})$  are higher than expected

# GWAS works well. Check the position of top-scoring SNPs

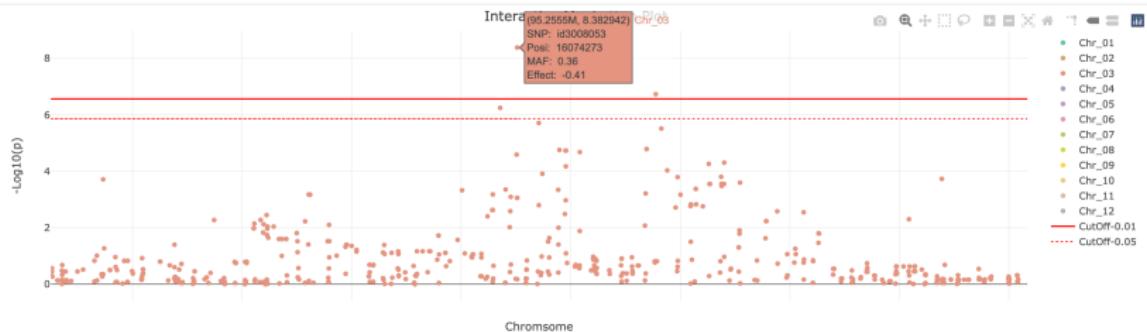
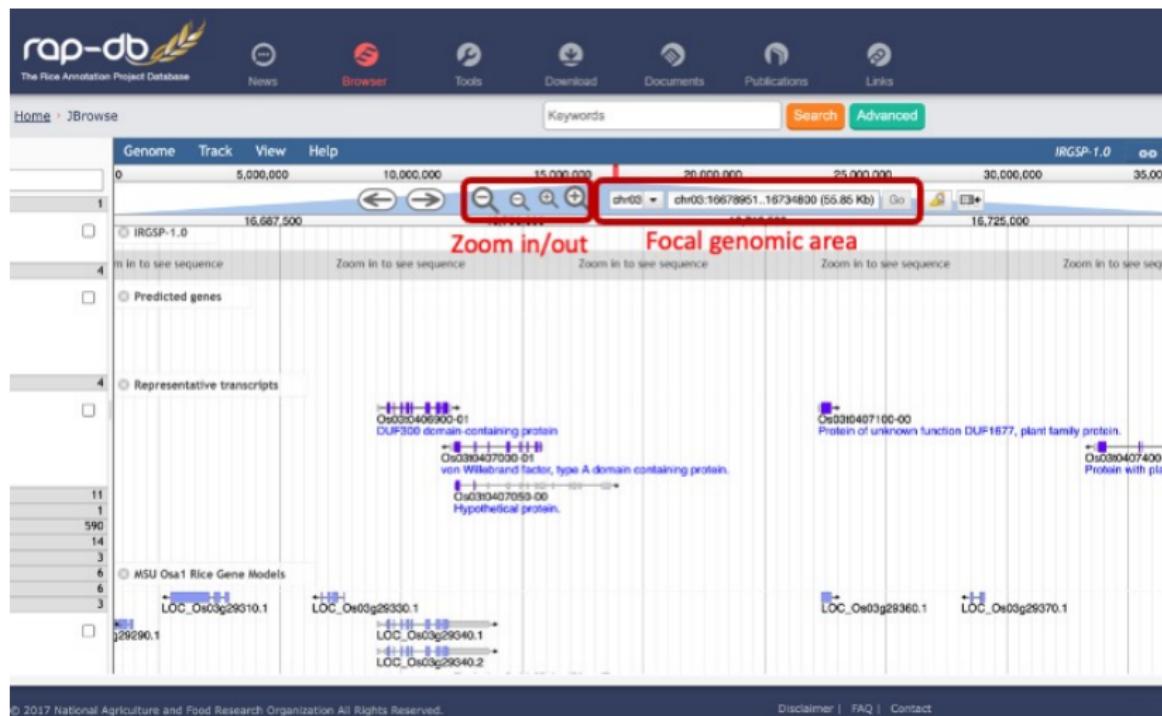


Figure 10:  
“GAPIT.Association.Interactive\_Manhattan.MLM.Seed.length.html”

- ▶ Open “Interactive.Manhattan.MLM.Seed.length.html”
- ▶ You can find 2 significant SNPs on the chromosome 3
- ▶ The position info appears when you put your cursor on a SNP

# What genes are located nearby? Check the database

- ▶ Access RAP-DB website at <https://rapdb.dna.affrc.go.jp/>
- ▶ Look for and click “JBrowse”



## Short exercise: Let's find the *GS3* locus.

By using RAP-DB,

1. Input significant SNP positions  $\pm$  3 bp as a focal genomic area and “Go”  
e.g., chr03:16706777..16706779
2. Zoom in/out  $\pm$  the average LD length near the SNP
3. Find the locus ID “Os03t0407400-01” (= *GS3*) and click!

### Point of (biological) interpretation

Which SNPs can you find *GS3* locus nearby?

How far is the *GS3* from the significant SNP?

What family of proteins does the *GS3* encode?

## (2) Genomic selection (GS)

## Aim: Prediction of the flowering time in rice cultivars

- ▶ Flowering time, or heading date in rice, was recorded at Arkansas on 2006 and 2007 (Zhao et al. 2011)
- ▶ Genotypes were same but environment should be different between years
- ▶ The flowering time of some accessions were unavailable
- ▶ Can we predict the flowering time only on the basis of genotypes?

# Estimate a trait value of each plant with gBLUP

- When finished, results are stored in “myGAPIT\_BLUP” object.

```
# gBLUP for the flowering time 2006 at Arkansas
myGAPIT_BLUP <- GAPIT( # warnings occur but it still works
  Y=p[,c("HybID","Year06Flowering.time.at.Arkansas")],
  GD=g,
  GM=gm,
  SNP.MAF=0.05,
  model="gBLUP",
  kinship.algorithm="VanRaden",
  file.output=FALSE)
```

```
## [1] "----- Welcome to GAPIT -----"
## [1] "gBLUP"
## [1] "-----Processing traits-----"
## [1] "Phenotype provided!"
## [1] "The 1 model in all."
## [1] "MLM"
## [1] "GAPIT.DP in process..."
## [1] "GAPIT will filter marker with MAF setting !!"
## [1] "The markers will be filtered by SNP.MAF: 0.05"
## maf_index
## FALSE TRUE
## 2150 34751
## [1] "Calculating kinship..."
## [1] "Number of individuals and SNPs are 413 and 34751"
## [1] "Calculating kinship with VanRaden method..."
## [1] "subtracting P..."
## [1] "Getting X'X..."
## [1] "Adjusting..."
## [1] "Calculating kinship with VanRaden method: done"
## [1] "kinship calculated"
```

## We get BLUP, PEV, BLUE, and predicted trait values

- ▶ BLUP: Best Linear Unbiased Predictor shows trait variance around mean
- ▶ PEV: prediction error variance of BLUP
- ▶ BLUE: Best Linear Unbiased Estimator shows mean differences of traits
- ▶ BLUP + BLUE = Prediction

```
# load results of genomic prediction
pred <- myGAPIT_BLUP$Pred
head(pred)
```

##	Taxa	Group	RefInf	ID	BLUP	PEV	BLUE	Prediction
## 1	081215-A05		1	1	-10.3304078	11.01517	89.3738566605352	79.04345
## 2	081215-A06		2	1	0.7679973	21.42044	89.3738566605352	90.14185
## 3	081215-A07		3	1	-1.9286126	20.80244	89.3738566605352	87.44524
## 4	081215-A08		4	1	0.4137206	15.23512	89.3738566605352	89.78758
## 5	090414-A09		5	1	1.8817708	18.49218	89.3738566605352	91.25563
## 6	090105-A02		7	1	8.8639442	20.66431	89.3738566605352	98.23780

Of course, predicted flowering time is well correlated with observed values

```
# align predicted and observed traits following the taxa name
pred <- pred[order(pred$Taxa),]
y <- p[order(p$HybID),]

# calculate Pearson's correlation between predicted and observed flowering
cor(pred$Prediction, y$Year06Flowering.time.at.Arkansas)

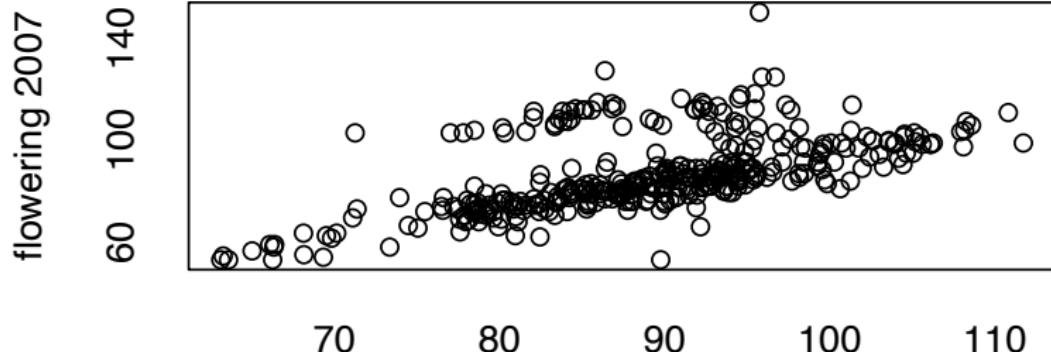
## 
## Pearson's product-moment correlation
##
## data: pred$Prediction and y$Year06Flowering.time.at.Arkansas
## t = 49.431, df = 335, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## 0.9234637 0.9494873
## sample estimates:
## cor
## 0.9377791
```

# Predicted flowering time is correlated with those observed on 2007

```
# perform a linear regression to estimate the slope and intercept
res <- lm(y$Year07Flowering.time.at.Arkansas~pred$Prediction)

# plot the results
plot(pred$Prediction,
      y$Year07Flowering.time.at.Arkansas,
      ylab="flowering 2007", xlab="predicted",
      main=paste("r =",round(sqrt(summary(res)$r.squared),2)))
abline(res)
```

$$r = 0.58$$



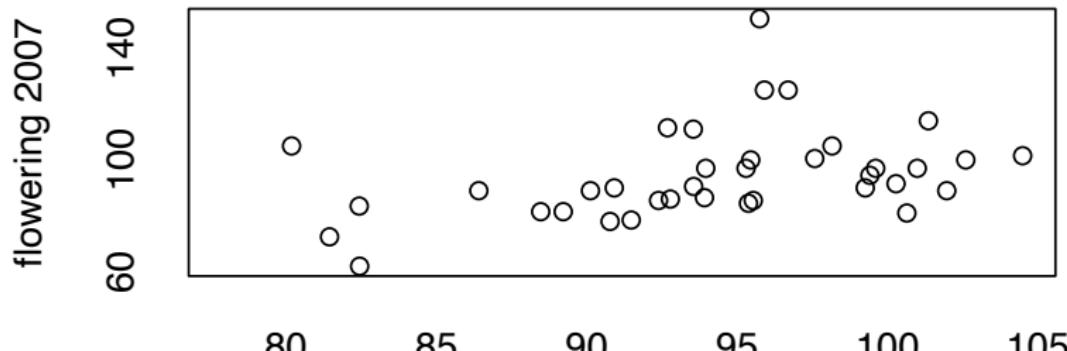
# Predicted flowering time is correlated with those of missing accessions

```
NA06 <- is.na(y$Year06Flowering.time.at.Arkansas)

# perform a linear regression to estimate the slope and intercept
res <- lm(y$Year07Flowering.time.at.Arkansas[NA06] ~ pred$Prediction[NA06])

# plot the results
plot(pred$Prediction[NA06],
      y$Year07Flowering.time.at.Arkansas[NA06],
      ylab="flowering 2007", xlab="predicted",
      main=paste("r =", round(sqrt(summary(res)$r.squared), 2)))
abline(res)
```

$$r = 0.37$$



### (3) Exercise

- ▶ Q1. Try GWAS of the flowering time at Aberdeen. How high is the heritability of this trait? At which chromosome can you find a peak?
- ▶ Q2. Find *HEADING DATE1* (*Hd1*: locus ID “Os06g0275000”). How distant is this gene from the top-scoring SNP? What is the ortholog of *Hd1* in *Arabidopsis thaliana*?
- ▶ Q3. Try gBLUP of the flowering time at Aberdeen. How large is the correlation between the predicted flowering time and observed one at Arkansas?
- ▶ Q4. More? You can test any traits of your interests!

## (4) GWAS group work (30 min. incl. a break)

- ▶ Select 1 interesting trait for 1 group
- 1. Report its heritability,
- 2. perform MLM, and report  $-\log_{10}(p)$  of the most significant SNP,
- 3. and list up 2 interesting candidate genes (specified by the code like Os03txxxxx) <200 kb near the most significant SNP.
- ▶ Send the result of 2., 3., and 4. to me ([yasuhiro.sato@uzh.ch](mailto:yasuhiro.sato@uzh.ch)). 1 email from a representative is ok. Please add your group no. to the email title.

# References

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- ▶ Zhao, Keyan, Chih-Wei Tung, Georgia C. Eizenga, Mark H. Wright, M. Liakat Ali, Adam H. Price, Gareth J. Norton, et al. (2011). Genome-Wide Association Mapping Reveals a Rich Genetic Architecture of Complex Traits in *Oryza sativa*. *Nature Communications* 2(1):467. <https://doi.org/10.1038/ncomms1467>.