

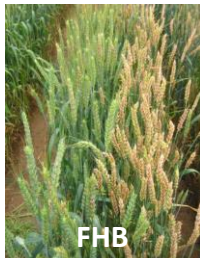
Using Galaxy to Conduct Genomic Selection Analyses

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Myself

Professor: The Ohio State University, wheat
 Assist, Assoc Prof: University of Arkansas, soybean
 Consultant with IGSS

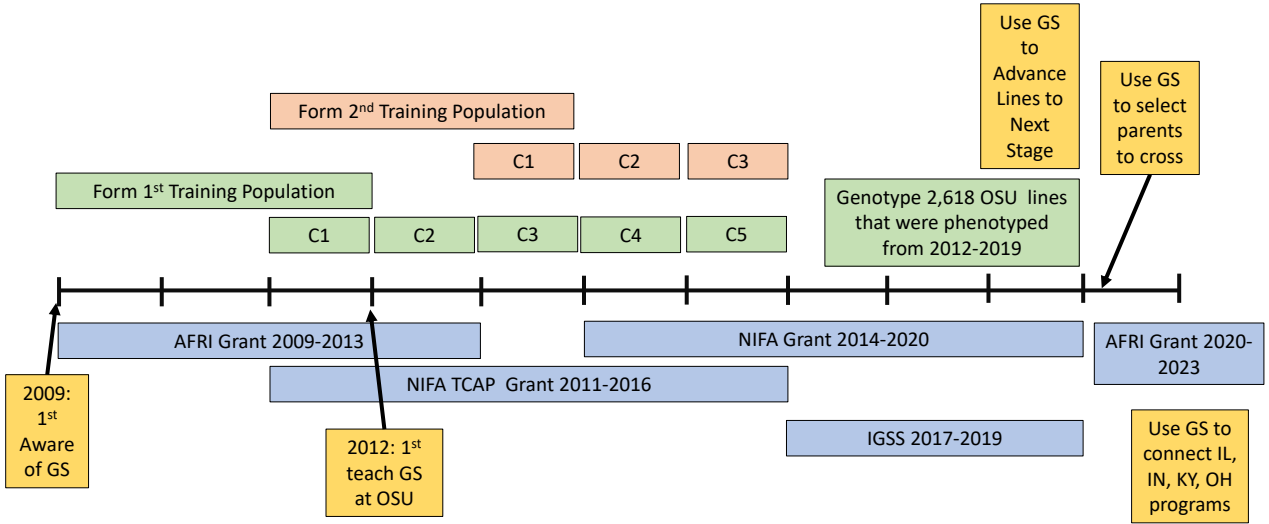
OSU, Michigan State University
 41 Years in Agriculture
 35 Years in Plant Breeding
 Worked with crops in 3 states, 13 Countries
 Wheat, Soybean
 Taught classes/workshops in 9 Countries
 Plant breeding, statistics, quantitative genetics, molecular breeding



- Quality
- Other Diseases
- Agronomic Traits
- Biomass
- Diversity

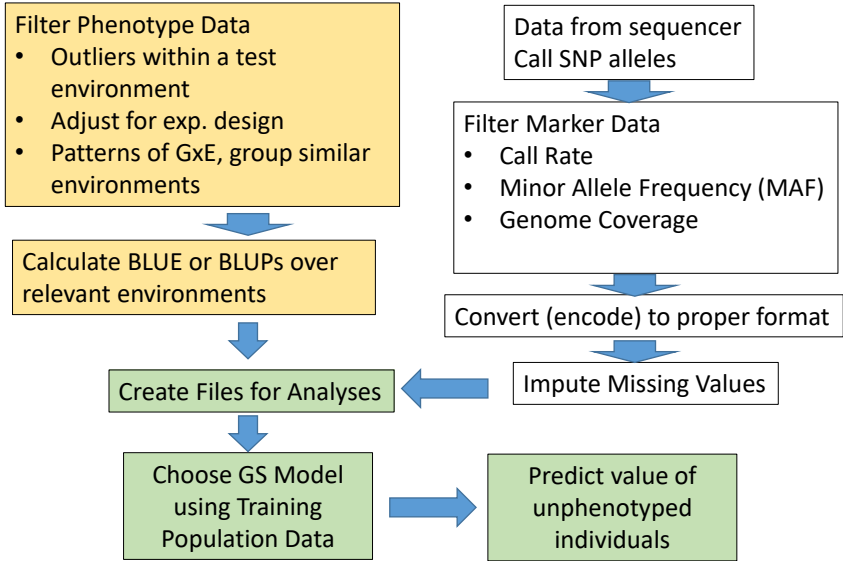
2

Genomic Selection in OSU Wheat Breeding: *Change is a Process*



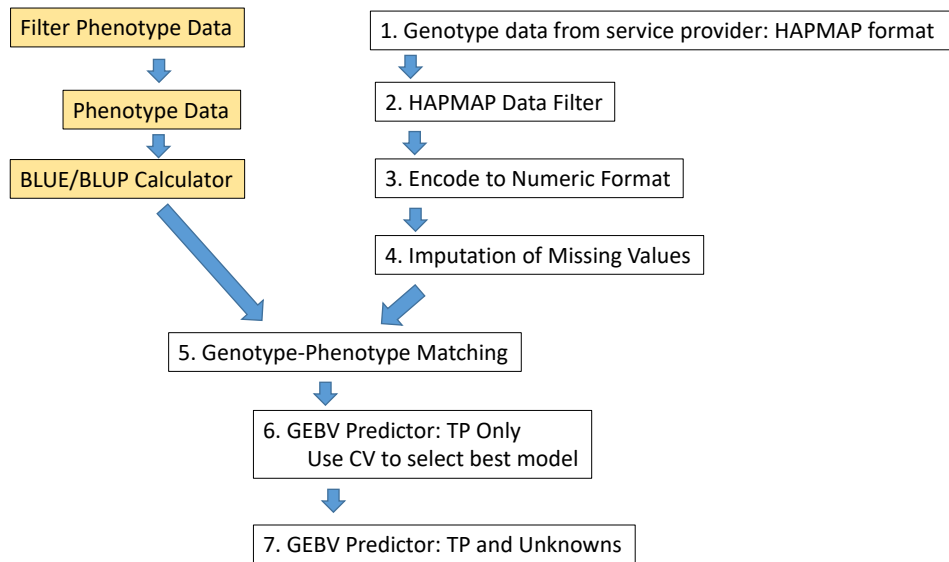
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GS Data and Analysis Steps



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GOBii, Galaxy for Genomic Selection



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Numeric Data Formats for GS Analyses

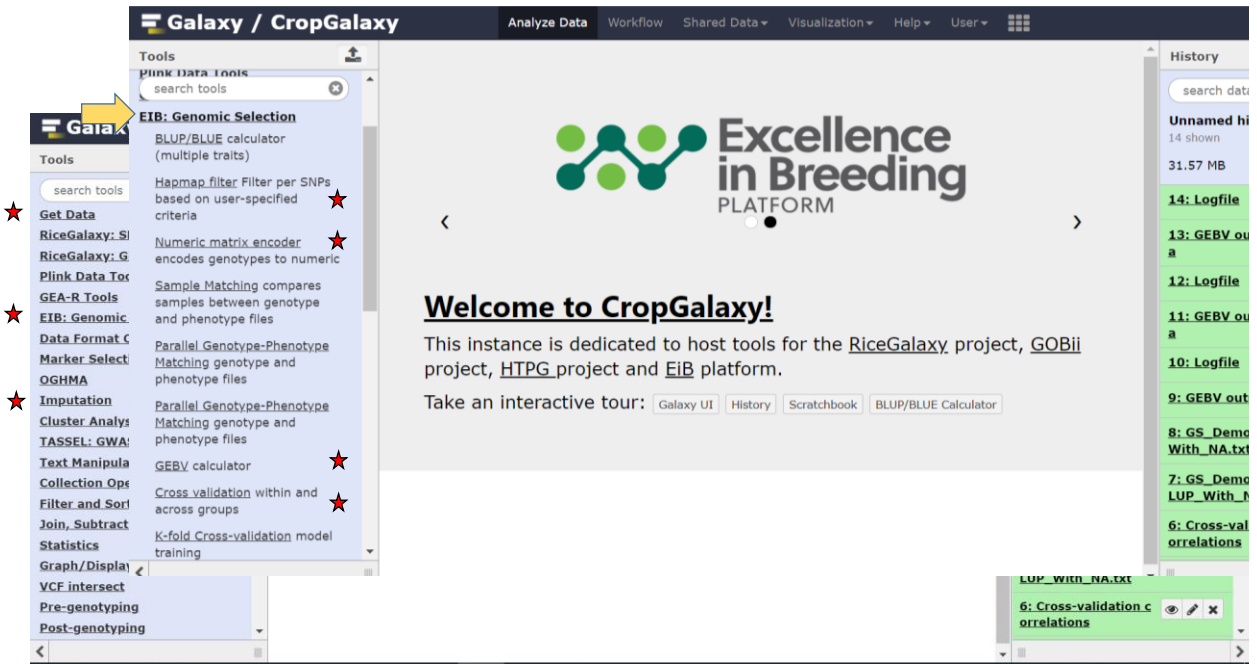
Source	Homozygous Reference Allele	Heterozygote	Homozygous SNP Allele
HAPMAP	AA	AC	CC
DARtseq 1-Row	0	2	1
Other 1-row	-1	0	1
Other 1-row	0	1	2

GS Uses numeric data Cant Have Missing Values

Be VERY CAREFUL

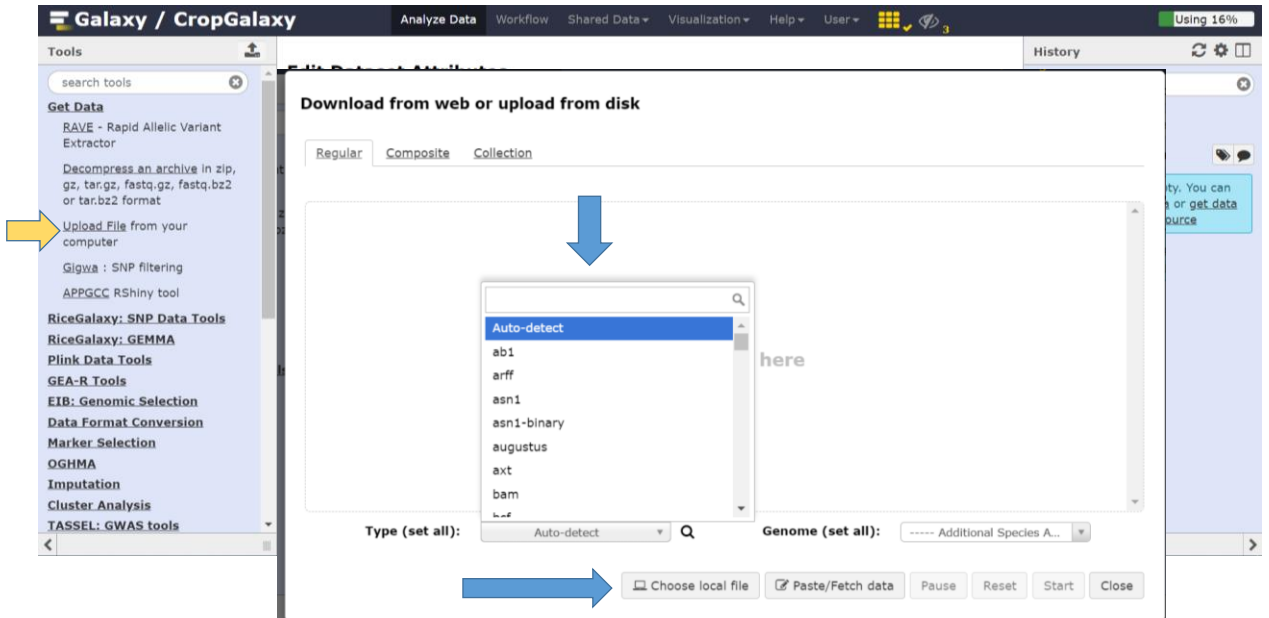
- Order of samples must be identical in genotype and phenotype files
- Order of markers must be identical in TP and PP data files
- Names and spellings must be identical in all files
- Designation of missing values need to be as specified in software

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1. Get Data



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2. HAPMAP filter

Objective: Filter out markers that you want to exclude from the analysis

Required: a HAPMAP file of marker data

- Tab delimited
- First rows begin with #. These are meta data not used in any analysis
- Header row identify each column starts with "rs#"
- Marker data starts in first row after the header row: 1 row per marker
- First 11 columns contain data describing each marker
- SNP data begins in column 12: there is one column for each sample
- Example, 1st marker is 0100001151, the SNP is C or A, first four individuals are homozygous CC

rs#	alleles	chrom	pos	strand	assembl	center	protLSI	assayLSI	panelLSI	QCcode	IRIS_313-1	IRIS_313-1	IRIS_313-1	IRIS_313-1	
#											dnarun_barcode				
34 other meta data (#) rows											project_test_load	test_load	test_load	test_load	
#											dnasample_ref_sample				
10100001151	C/A			+							CC	CC	CC	CC	
10100001173	C/T			+							CC	CC	CC	CC	
10100001178	G/T			+							GG	GG	GG	GG	
10100001203	T/C			+							CC	CC	CC	CC	
10100001248	G/A			+							GG	GG	GG	GG	
10100001249	A/C			+							CC	CC	CC	CC	
10100001266	G/A			+							AA	AA	AA	AA	

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2. Hapmap Filter

Markers with >25% missing data are deleted

Markers with MAF <12.5% are deleted

Markers with >80% heterozygous calls are deleted

Output file looks identical to input files, but with fewer markers

Example: HAPMAP_filter_example file has 583 markers
Output file (Hapmap_data_filter_on_data_132) has 146 markers

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3. Encode

Objective: Convert HAPMAP character data to numeric data

Required: a HAPMAP file of marker data

- Same as shown before
- Data should have been filtered before encoding
- **Homozygotes for minor allele converts to "0"**
- **Heterozygotes convert to "1"**
- **Heterozygotes for major allele converts to "2"**

Note: Encoder can handle alternative HAPMAP formats

Example for one marker:

The frequency of the "C" allele is 0.8, thus it is the major allele
The frequency of the "A" allele is 0.2, thus it is the minor allele
Missing data is encoded as "NA"

Note: Encoder can also produce -1, 0, 1 scoring

IND1	IND2	IND3	IND4	IND5	IND6	IND7	IND8	IND9	IND10	IND11	
AA	CC	CC	CC	CC	CC	AC	CC	AC	CC	NN	HAPMAP
0	2	2	2	2	2	1	2	1	2	NA	Encoded

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3. Numeric Encoder

The screenshot shows the Galaxy web interface for the 'Numeric matrix encoder' tool. The tool is configured with the following settings:

- Genotype data file:** 76: Hapmap filter on data 75 (Annotated: Filtered HAPMAP file)
- Data type:** Two-letter nucleotide (Annotated: There are additional formats that can be encoded)
- Select coding scheme to use:** (0,1,2,NA) (Annotated: Format of output data)

The tool description states: "This tool transforms genotype data to numeric by using a desired numerical scheme. It takes a tab delimited GOBII-hapmap format as input." The datatypes are listed as:

- Dominant datatype - alleles are coded as 0 or 1.
- IUPAC datatype - alleles appear as:

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Output file from Encoding:

Still in a HAPMAP format, just numeric scoring

#	dnarun_barcode																					
34 addition # rows																						
#	dnasample_ref_sample																					
rs#	alleles	chrom	pos	strand	seml	center	rot	LSI	say	LSI	anel	LSI	Ccode	IRIS_313	11806	IRIS_313	11644	IRIS_313	11515	IRIS_313	9944	
10100002469	A/G			+										0	0	0	0	0	0	0	0	0
10100011130	A/G			+										0	NA	0	0	0	0	0	0	1
10100011334	T/C			+										0	0	0	0	2	0	2	2	2
10100021546	T/A			+										NA	1	0	1	2	1	2	2	2

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4. Naïve Imputation

Objective: Replace missing genotypes (“NA”) with a numeric value

Missing marker values (NA) within a row are replaced with either the mean or the mode of the values in that row

Required: a Filtered and Encoded HAPMAP file of marker data. Generated by #3

Tab delimited

#	dnarun_barcode																					
34 addition # rows																						
#	dnasample_ref_sample																					
rs#	alleles	chrom	pos	strand	seml	center	rot	LSI	say	LSI	anel	LSI	Ccode	IRIS_313	11806	IRIS_313	11644	IRIS_313	11515	IRIS_313	9944	
10100002469	A/G			+										0	0	0	0	0	0	0	0	0
10100011130	A/G			+										0	NA	0	0	0	0	0	0	1
10100011334	T/C			+										0	0	0	0	2	0	2	2	2
10100021546	T/A			+										NA	1	0	1	2	1	2	2	2

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5. Genotype-Phenotype Matching

Objective: Ensure the phenotype and genotype files contain data on the same lines and in the same order

The number of samples in each file type must be identical

The names of the samples must be identical

The order of the samples must be identical

Pheno File

GID	Trait1
AAA	100
BBB	97
CCC	68
DDD	58
EEE	92
FFF	102
GGG	85
HHH	76
III	48
JJJ	96
etc	105

Genotype File

GID	M1	M2	M3	M4
AAA	0	2	2	0
BBB	2	2	2	0
CCC	0	0	1	0
DDD	2	2	0	0
EEE	2	0	1	2
FFF	2	0	2	2
GGG	1	0	0	2
HHH	0	0	0	0
III	0	0	0	0
JJJ	0	2	0	0
etc	2	2	2	0

Genotype File in format for the analysis

M1	M2	M3	M4
0	2	2	0
2	2	2	0
0	0	1	0
2	2	0	0
2	0	1	2
2	0	2	2
1	0	0	2
0	0	0	0
0	0	0	0
0	2	0	0
2	2	2	0



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5. Matching Genotype and Phenotype Files

The screenshot shows the Galaxy/CropGalaxy interface. In the 'Tools' panel on the left, the 'Sample_Matching' tool is highlighted with a red box. A blue arrow points from a yellow box containing the text 'In Development DO NOT USE!' to the 'Sample_Matching' tool. A green notification box at the top right of the interface displays a checkmark and the message: '1 job has been successfully added to the queue - resulting in the following datasets: 83: Imputed Data. You can check the status of queued jobs and view the resulting data by refreshing the History pane. When the job has been run the status will change from 'running' to 'finished' if completed successfully or 'error' if problems were encountered.'

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6. GEBV Calculator: GS Accuracy by Cross Validation

Objective: Run various prediction models on the training population data only to assess which model is best

Required Files:

Filtered, Encoded, imputed genotype data file with column of sample names removed
 Phenotype file, Tab delimited

Pheno File

GID	Trait1
AAA	100
BBB	97
CCC	68
DDD	58
EEE	92
FFF	102
GGG	85
HHH	76
III	48
JJJ	96
etc	105

Genotype File

M1	M2	M3	M4
0	2	2	0
2	2	2	0
0	0	1	0
2	2	0	0
2	0	1	2
2	0	2	2
1	0	0	2
0	0	0	0
0	0	0	0
0	2	0	0
2	2	2	0

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Sample Data Files

Pheno_for_Demo 190 samples

Sample.Name.in.GBS.Data	Season	GY_blue	GY_blup	GY_PEV	GY_pegReliability	GY_means
MARS-WEMA_270039	Main	6.715	6.975	0.05	0.176	6.715
MARS-WEMA_270040	Main	5.959	6.841	0.05	0.176	5.959
MARS-WEMA_270041	Main	6.925	7.012	0.05	0.176	6.925
MARS-WEMA_270042	Main	6.925	7.127	0.05	0.176	7.576
MARS-WEMA_270043	Main	8.101	7.22	0.05	0.176	8.101
MARS-WEMA_270044	Main	7.335	7.084	0.05	0.176	7.335
MARS-WEMA_270045	Main	8.015	7.205	0.05	0.176	8.015
MARS-WEMA_270046	Main	7.234	7.066	0.05	0.176	7.234
MARS-WEMA_270047	Main	6.87	7.002	0.05	0.176	6.87
MARS-WEMA_270048	Main	7.43	7.101	0.05	0.176	7.43
MARS-WEMA_270049	Main	7.178	7.056	0.05	0.176	7.178
MARS-WEMA_270050	Main	6.555	6.946	0.05	0.176	6.555
MARS-WEMA_270051	Main	6.491	6.935	0.05	0.176	6.491
MARS-WEMA_270052	Main	6.811	6.992	0.05	0.176	6.811
MARS-WEMA_270053	Main	7.82	7.17	0.05	0.176	7.82
MARS-WEMA_270054	Main	6.384	6.916	0.05	0.176	6.384
MARS-WEMA_270055	Main	6.384	6.993	0.05	0.176	6.817
MARS-WEMA_270056	Main	7.374	7.091	0.05	0.176	7.374
MARS-WEMA_270057	Main	7.186	7.058	0.05	0.176	7.186

Geno_for_demo 190 markers (columns)

rs145689	rs150837	rs150103	rs150977	rs148542	rs148897	rs148817	rs145887	rs151228	rs145371
1	0	1	0	0	0	0	2	1	2
1	0	1	0	1	0	0	1	1	1
0	1	1	0	2	0	1	0	1	0
1	0	1	1	1	0	0	1	2	1
0	1	1	0	2	1	0	1	0	0
0	1	2	1	1	1	0	1	2	2
2	1	1	1	0	2	1	2	2	1
1	0	1	2	0	2	2	1	1	1
0	1	1	1	0	1	1	1	1	1
1	1	1	0	0	0	1	1	1	1
0	2	1	1	0	2	1	0	2	1
0	1	0	1	1	1	1	2	1	1
0	0	0	1	0	0	1	1	1	1
1	1	0	1	2	2	1	0	1	1
0	1	1	2	0	2	1	2	0	1
2	0	1	2	1	0	2	0	2	2
1	1	1	1	0	1	0	2	1	1
2	1	0	1	0	1	1	1	0	2
1	1	2	1	0	1	1	2	1	2

↑
 No Identifier Column

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6. GS Accuracy via CV

Galaxy / CropGalaxy Analyze Data Workflow Shared Data Visualization Help User

Tools

- Parallel_Genotype-Phenotype Matching genotype and phenotype files
- Parallel_Genotype-Phenotype Matching genotype and phenotype files
- Parallel_Genotype-Phenotype Matching genotype and phenotype files
- GEBV calculator
- Cross_validation within and across groups
- K-fold_Cross-validation model training
- Split_datasets_by_grouping_factor
- Merge_multiple_GEBV_results

Data Format Conversion

- Marker Selection
- OGHMA
- Imputation
- Cluster Analysis
- TASSEL: GWAS tools

GEBV calculator (Galaxy Version 1.5.0)

Input phenotype File
85: Phenotype_for_Demo.txt

Column Header Containing the Sample Names
c1: Sample.Name.in.GBS.Data

Column headers for phenotypes

- Column Header Containing the First Phenotype**
c4: GY_blup
- Column Header Containing the Last Phenotype**
c4: GY_blup

Response Type
gaussian

Lower Bound Vector (a) Column
(default to NULL, integer) Only required for censored outcomes

History

- Unnamed history
- 84: Genotype_for_Demo.txt
- 83: Imputed Data
- 81: Encoded data in hapmap format
- 76: Hapmap filter on data 75
- 75: HAPMAP_filter_example.txt

Annotations:

- Column with sample identifier
- Select Traits
- Gaussian for continuous data

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6. GS Accuracy via CV (cont)

Galaxy / CropGalaxy Analyze Data Workflow Shared Data Visualization Help User

Tools

- Parallel_Genotype-Phenotype Matching genotype and phenotype files
- Parallel_Genotype-Phenotype Matching genotype and phenotype files
- Parallel_Genotype-Phenotype Matching genotype and phenotype files
- GEBV calculator
- Cross_validation within and across groups
- K-fold_Cross-validation model training
- Split_datasets_by_grouping_factor
- Merge_multiple_GEBV_results

Data Format Conversion

- Marker Selection
- OGHMA
- Imputation
- Cluster Analysis
- TASSEL: GWAS tools

Incidence Matrix and Model

1: Incidence Matrix and Model

Matrix File
84: Genotype_for_Demo.txt

Model
Bayesian Ridge Regression

Needs Transposition
Yes No

Weights Vector Column
(default to NULL, integer) A vector of weights

Number of Iterations
1500

Burn-In
500

Thinning
5

SaveAt

Annotations:

- Genotype Data File
- Choose GS model
- Choose "No" if marker data is set up as in example (eg one column per marker)

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6. GS Accuracy via CV (cont)

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Output

GS Accuracy

Randomization Number	GY_blup correlation
1	0.125
2	0.189
3	0.244
4	0.19
5	0.223
6	0.203
7	0.157
8	0.157
9	0.189
10	0.169

Randomization Number	RR Blups	Bayes A	Bayes B	Bayes C	Bayes LASSO
1	0.125	0.194	0.118	0.108	0.173
2	0.189	0.188	0.109	0.123	0.174
3	0.244	0.200	0.077	0.127	0.076
4	0.190	0.191	0.144	0.145	0.137
5	0.223	0.093	0.078	0.149	0.155
6	0.203	0.214	0.137	0.212	0.176
7	0.157	0.087	0.131	0.130	0.081
8	0.157	0.132	0.142	0.189	0.184
9	0.189	0.084	0.116	0.129	0.174
10	0.169	0.208	0.145	0.168	0.210
Average	0.185	0.159	0.120	0.148	0.154
Stdev	0.035	0.054	0.025	0.033	0.044

RR Blup gives highest Average Accuracy. Select it to predict value of unphenotyped individuals

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7. Predict value of unphenotyped individuals

Objective: Run best model on data from training population and genotype data for unphenotyped individuals

Sample.Name.in.GBS.Data	Season	GY_blue	GY_blup	GY_PEV	pevReliab	GY_means	rs145689	rs150837	rs150103	rs150977	rs148542	rs148897	rs148817	rs145887	rs151228	rs145371
MARS-WEMA_270039	Main	6.715	6.975	0.05	0.176	6.715	1	0	1	0	0	0	0	2	1	2
MARS-WEMA_270040	Main	5.959	6.841	0.05	0.176	5.959	1	0	1	0	1	0	0	1	1	1
MARS-WEMA_270041	Main	6.925	7.012	0.05	0.176	6.925	0	1	1	0	2	0	1	0	1	0
MARS-WEMA_270042	Main	6.925	7.127	0.05	0.176	7.576	1	0	1	1	1	0	0	1	2	1
MARS-WEMA_270043	Main	8.101	7.22	0.05	0.176	8.101	0	1	1	0	2	1	0	1	0	0
MARS-WEMA_270044	Main	7.335	7.084	0.05	0.176	7.335	0	1	2	1	1	1	0	1	2	2
MARS-WEMA_270045	Main	8.015	7.205	0.05	0.176	8.015	2	1	1	1	0	2	1	2	2	1
MARS-WEMA_270046	Main	7.234	7.066	0.05	0.176	7.234	1	0	1	2	0	2	2	1	1	1
MARS-WEMA_270047	Main	6.87	7.002	0.05	0.176	6.87	1	1	1	0	1	1	1	1	1	1
MARS-WEMA_270048	Main	7.43	7.101	0.05	0.176	6.811	1	0	0	0	0	1	1	1	1	1
MARS-WEMA_270049	Main	7.178	7.056	0.05	0.176	6.811	1	1	0	2	1	0	2	1	1	1
MARS-WEMA_270050	Main	6.555	6.946	0.05	0.176	6.811	0	1	1	1	1	1	2	1	1	1
MARS-WEMA_270051	Main	6.491	6.935	0.05	0.176	6.491	0	1	0	1	0	0	1	1	1	1
MARS-WEMA_270052	Main	6.811	6.992	0.05	0.176	6.811	1	1	0	1	2	2	1	0	1	1
MARS-WEMA_270053	Main	7.82	7.17	0.05	0.176	7.82	0	1	1	2	0	2	1	2	0	1
MARS-WEMA_270054	Main	6.384	6.916	0.05	0.176	6.384	2	0	1	2	1	0	2	0	2	2
MARS-WEMA_270055	Main	6.384	6.993	0.05	0.176	6.817	1	1	1	1	0	1	0	2	1	1
MARS-WEMA_270056	Main	7.374	7.091	0.05	0.176	7.374	2	1	0	1	0	1	1	1	0	2
MARS-WEMA_270057	Main	7.186	7.058	0.05	0.176	7.186	1	1	2	1	0	1	1	2	1	2
UnPheno 1	NA	NA	NA	NA	NA	NA	0	0	1	0	1	0	2	2	1	1
UnPheno 2	NA	NA	NA	NA	NA	NA	2	0	0	1	1	1	2	2	0	1
UnPheno 3	NA	NA	NA	NA	NA	NA	0	1	0	0	0	1	1	2	0	2
UnPheno 4	NA	NA	NA	NA	NA	NA	1	0	0	1	2	0	1	2	0	1
UnPheno 5	NA	NA	NA	NA	NA	NA	1	1	2	1	1	1	0	2	2	1

Training Population Data

Add phenotypic and genotypic data from UnPhenotyped Individuals whose value you want to predict

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7. Predict value of unphenotyped individuals

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Output

Sample.Name	GY_blue_Observed	GY_blue_Predicted
MARS-WEMA_270039	6.72	6.95
MARS-WEMA_270040	5.96	6.77
MARS-WEMA_270041	6.93	7.06
MARS-WEMA_270042	6.93	6.91
...
MARS-WEMA_293000	7.28	7.05
MARS-WEMA_293001	6.33	6.83
MARS-WEMA_293002	7.28	7.11
MARS-WEMA_293003	6.60	6.41
MARS-WEMA_293004	6.54	7.24
UnPheno 1	NA	7.05
UnPheno 2	NA	6.83
UnPheno 3	NA	7.11
UnPheno 4	NA	6.41
UnPheno 5	NA	7.24

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Predictions with and across groups of data

The screenshot shows the Galaxy / CropGalaxy interface. The left sidebar contains a list of tools, with 'Cross_validation within and across groups' highlighted by a yellow arrow. The main panel displays the configuration for this tool, titled 'Cross validation within and across groups (Galaxy Version 1.2.0)'. The configuration includes the following fields:

- Input Genotypes File:** 87: Phenotype_for_Demo_UnPheno.txt (required) The TAB file containing the data matrix of the genotypes
- Input Phenotypes File:** 87: Phenotype_for_Demo_UnPheno.txt (required) The TAB file containing the data matrix of the phenotypes
- Sample Name Column:** c1: Sample.Name.in.GBS.Data (required)
- First Phenotype Column:** c2: Season (required)
- Number of Phenotypes:** 3 (required, integer)
- Group Memberships File:** 87: Phenotype_for_Demo_UnPheno.txt (required) The TAB file containing the data vector of the group memberships
- Main Option:** within groups (required, string) Admits within groups or across groups
- Number of Folds to Perform Cross Validation with:** (field partially visible)

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Example: Same set of lines, phenotyped in three environments

GID	GY_ENV_1	GY_ENV_2	GY_ENV_3
49319	7.7	8.5	3.5
49446	NA	8.5	7.2
49359	7.0	8.7	4.2
49322	7.3	NA	3.8
49413	7.4	8.8	4.6
49339	6.8	8.9	3.4
49315	7.3	8.9	3.9
49397	7.4	9.0	4.2
49444	7.8	9.0	4.9
49340	6.9	9.0	NA



GID	GROUP	GY
49319	1	7.7
49446	1	NA
49359	1	7.0
49322	1	7.3
49413	1	7.4
49339	1	6.8
49315	1	7.3
49397	1	7.4
49444	1	7.8
49340	1	6.9
49319	2	8.5
49446	2	8.5
49359	2	8.7
49322	2	NA
49413	2	8.8
49339	2	8.9
49315	2	8.9
49397	2	9.0
49444	2	9.0
49340	2	9.0
49319	3	3.5
49446	3	7.2
49359	3	4.2
49322	3	3.8
49413	3	4.6
49339	3	3.4
49315	3	3.9
49397	3	4.2
49444	3	4.9
49340	3	NA

CV within and across groups

CV within groups

Use G1 data to predict value of G1 lines
Use G2 data to predict value of G2 lines
Use G3 data to predict value of G3 lines

CV across groups

Use G2&3 data to predict value of G1 lines
Use G1&3 data to predict value of G2 lines
Use G1&2 data to predict value of G3 lines

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Example: Three sets of lines, phenotyped in same environment

GID	GROUP	GY
A1	1	7.7
A2	1	NA
A3	1	7.0
A4	1	7.3
A5	1	7.4
A6	1	6.8
A7	1	7.3
A8	1	7.4
A9	1	7.8
A10	1	6.9
B1	2	8.5
B2	2	8.5
B3	2	8.7
B4	2	NA
B5	2	8.8
B6	2	8.9
B7	2	8.9
B8	2	9.0
B9	2	9.0
B10	2	9.0
C1	3	3.5
C2	3	7.2
C3	3	4.2
C4	3	3.8
C5	3	4.6
C6	3	3.4
C7	3	3.9
C8	3	4.2
C9	3	4.9
C10	3	NA

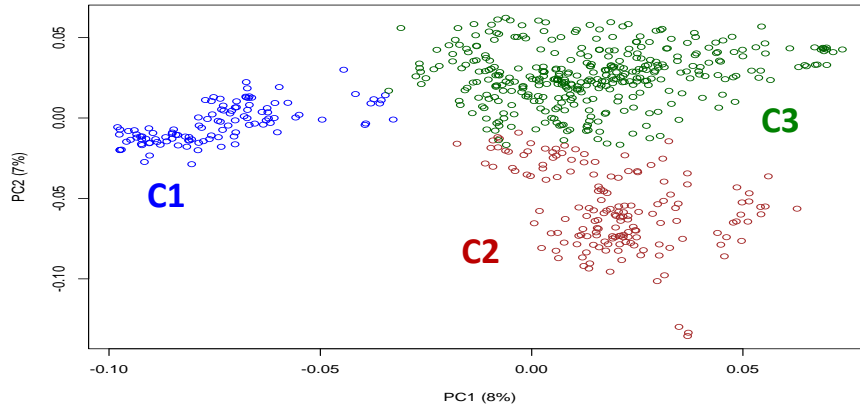
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Your Practicum Files and Objectives

576 Winter Wheat Lines
Genotyped with 8,703 GBS SNPs
All Pheno. for 5 Fusarium Head Blight Traits



346 Winter Wheat Lines
1,451 GBS SNPs
307 Pheno. 39 need to be predicted

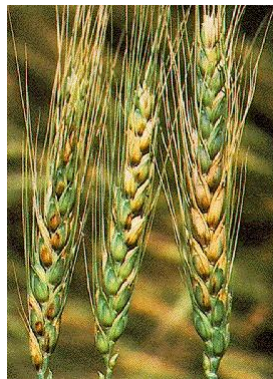


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- **Incidence (INC)** : Percentage of spikes showing symptoms
- **Severity (SEV)**: % of florets showing symptoms within an infected spike
- **Index (IND)**: % of symptomatic florets in the plot (IND=INC*SEV/100)
- **Fusarium Damaged Kernels (FDK)**: % of kernels showing symptoms
- **Deoxynivalenol (DON)**: concentration of DON in grain

SEV
25% 50% 65%

INC 5% 95%
SEV 5% 80%
IND 0.3% 75%



34

GS_Demo_FHB_BLUP (307 rows)
 GS_Demo_FHB_BLUP_With_NA (346 rows)

NAME	GROUP	IND	FDK	ISK	DON	PC1
IL95-4162	3	-0.16	-0.26	-0.23	-0.09	-0.40
IL02-18228	3	-0.65	-0.86	-0.91	-0.74	-1.76
PEMBROKE	3	-0.05	0.30	0.28	0.11	0.30
MO080104	3	-0.56	-0.58	-0.62	-0.66	-1.39
IL79-002DH	3	-0.40	-0.52	-0.51	-0.62	-1.21
TRUMAN	2	-0.50	-0.48	-0.54	-0.55	-1.18
O128A1-2	3	-0.15	0.55	0.32	-0.15	0.23
JENSEN	1	0.74	1.03	0.91	0.70	1.92
OH10-143-23	3	0.08	0.94	0.39	0.34	1.11

2 Data Files to Upload

- 2 for Phenotypes
- 2 for Genotypes
- 1 for grouping

GS_Demo_FHB_GENO (307 rows)
 GS_Demo_FHB_GENO_With_NA (346 rows)

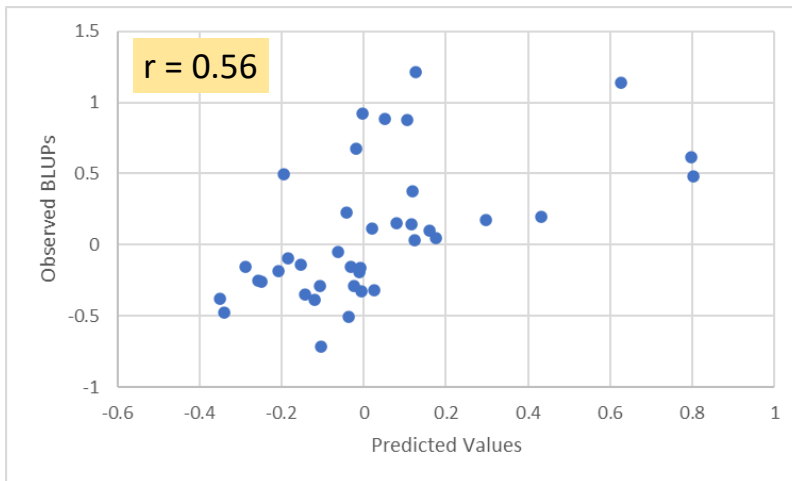
TPM59	TPM383	TPM1663	TPM2559	TPM3009	TPM3622
0	0	0	0	0	1
0	1	0	1	2	1
0	0	0	0	0	0
0	0	1	0	0	0
0	0	0	0	2	0
0	1	0	1	0	1
1	0	1	1	2	1
0	0	0	0	0	1
0	0	0	1	2	1

GS_Demo_FHB_Group (346 rows)

GROUP
3
3
3
3
3
2
3
1
3

35

Results for IND



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