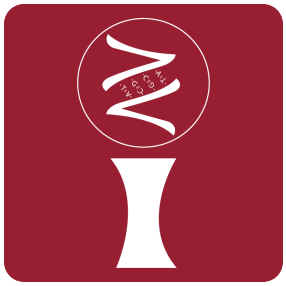
User Manual for



Intelligent Prediction and Association Tool

(Version 1.3)

Last updated on Aug 14, 2017

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1. Getting start

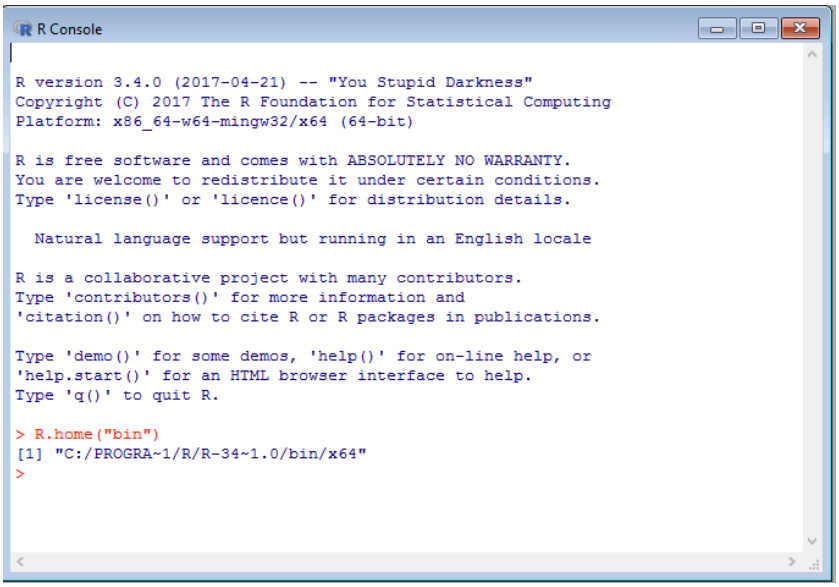
1.1 Operation environment

- The operation environment need to meet the following requirement:
  - Operation System: Windows or Mac OS X .
  - Java Runtime Environment (JRE): Version 8 or later.
  - R: Version 3.4.0 or later.

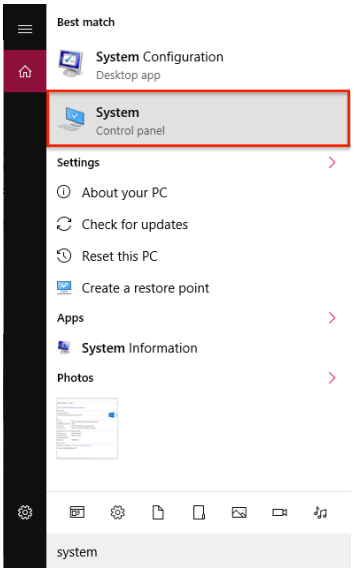
1.2 Windows users

1.2.1 Set up R environment

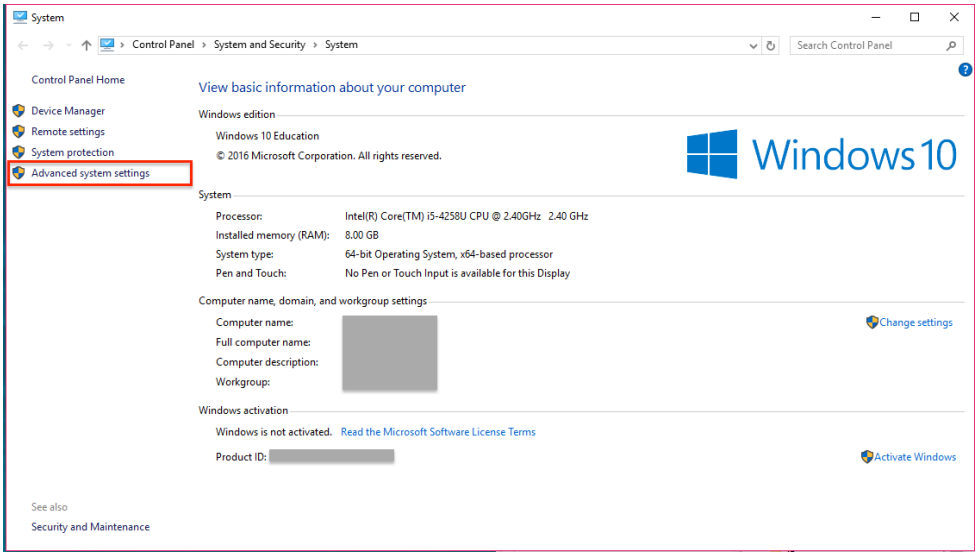
- If you can call R from the commnad-line window (cmd.exe) by typing "R" or "r", then you can skip to section 1.2.2. Otherwise, please follow the instruction below to get your system compatible with iPat.
- Open R software, and type `R.home("bin")` in the console. It will return a path to the executable R. Copy this path to the clipboard.



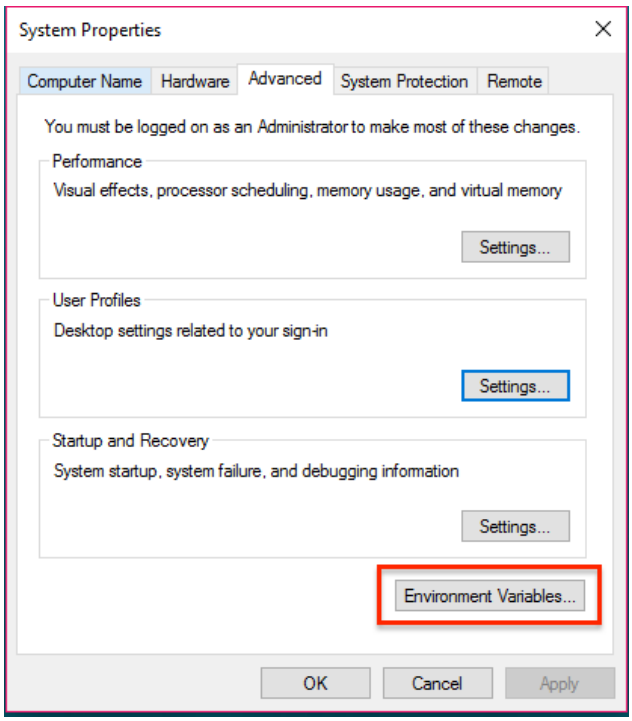
- Search keyword "system" from Windows, and open "System".



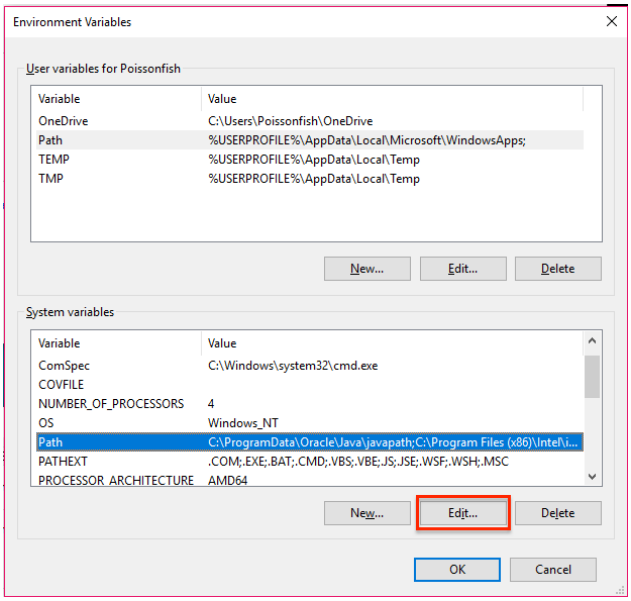
- Then select "Advanced system settings" at the left side of the panel.



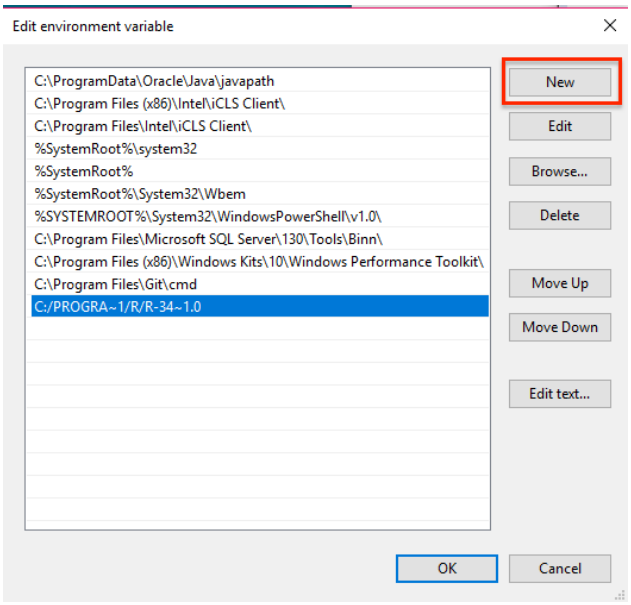
- And click "Environment Variable..." at the bottom-right area.



- The pop-up windows will display two set of system variables. Highligh the system variable "Path" at the bottom list, and click "Edit".

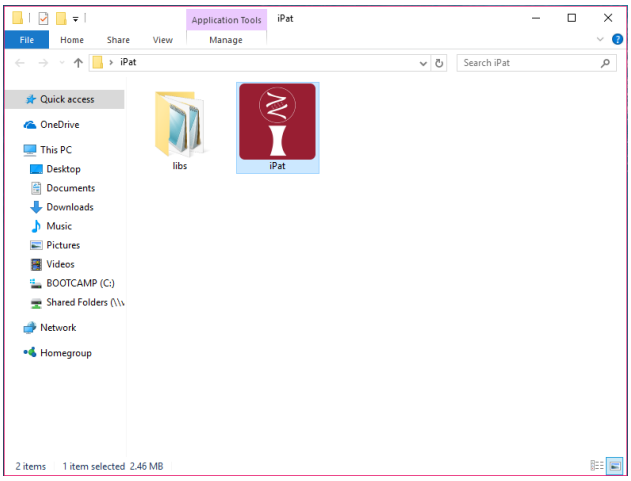


- Almost there. Click "new" and paste the path you got from the clipboard, then click "OK" to save the configuration.



1.2.2 Extract iPat.exe from iPat.zip

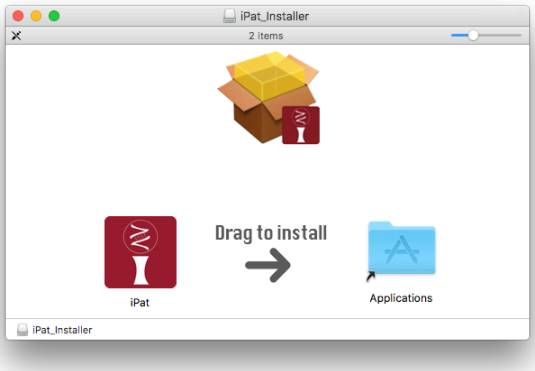
- Download iPat.zip and decompress it. You will then get a folder named "iPat", which contains a executable file "iPat.exe" and a folder "libs".
- It's noted that users are alway required to place "iPat.exe" and the folder "libs" in the same folder (directory) so that iPat can function normally.



- Double click 'iPat.exe' to launch iPat.

1.3 Mac OS users

- Download iPat_Installer.dmg and mount it on Mac.
- Follow the instruction to install iPat.



- Double click 'iPat.app' to launch iPat.

2. Interface

2.1 *Import files*

- At beginning, iPat will show nothing but an icon "iPat" at the top of screen.
- Users can import files simply by dragging and dropping.

2.2 *Create a project*

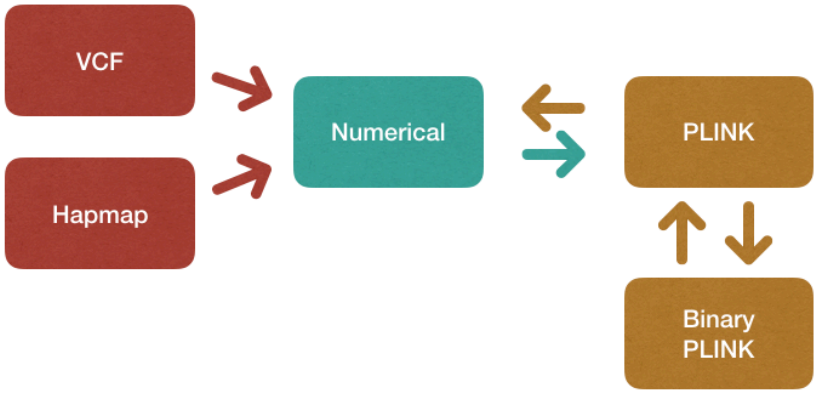
- After importing the files, double clicking on anywhere in iPat to create a new project (a gear icon).
- Build a project by dragging a files over the project icon. A dashline will be shown between the file and project, which mean this file has been already included in this project. The below two are examples for a valid project.

- A valid project must include a certain number of required files, **no less, no more.** Otherwise iPat won't work and will return an error message. Valid datasets for each format can be found from the table below:

Format	**File 1 (required)**	**File 2 (required)**	**File 3 (required)**	**File 4 (required)**
Hapmap	Genotype (.hmp)	Phenotype (.txt)	None	None
Numeric	Genotype (.dat)	Phenotype (.txt)	Map information (.map) (Only required for GWAS)	None
VCF	Genotype (.vcf)	Phenotype (.txt)	None	None
PLINK	Genotype (.ped)	Phenotype (.txt)	Map information (.map)	None
PLINK (binary)	Genotype (.bed)	Phenotype (.txt)	Map information (.bim)	Individual information (.fam)

2.3 *File formats*

- iPat mainly work with files in numerical format, but it can also work fine with Hapmap, VCF and PLINK format. iPat will recognize the format of input files and do a format conversion automatically if needed.



2.3.1 Phenotype

- Phenotype data for every formats except PLINK must contain **sample names** in the first column and **traits names** as the header:

taxa	trait 1	trait 2
sample1		
sample2		
sample3		

- Phenotype data for PLINK must contain **sample and family names** in the first 2 columns and **traits names** as the header:

FID	SID	trait 1	trait 2
family 1	sample1		
family 2	sample2		
family 3	sample3		

2.3.2 Hapmap

- Genotype data, the header is requried to be provided:

rs	alleles	chrom	pos	strand	assembly	center	protLSID	assayLSID	panel	QCcode	sample 1	sample 2	sample 3
marker 1	A/C	1	157104	+	AGPv1	Panzea	NA	NA	maize282	NA	CC	CC	AA
marker 2	C/G	1	1947984	+	AGPv1	Panzea	NA	NA	maize282	NA	GG	GG	CC

2.3.3 Numeric

- Genotype data, samples are recorded in rows. The header and sample names can be omitted:

taxa	marker 1	marker 2	marker 3
sample1	0	0	1
sample2	0	0	0
sample3	1	0	0

- Map information, the header is requried to be provided:

SNP	Chromosome	Position
marker 1	1	157104
marker 2	1	1947984
marker 3	1	2914066

2.3.4 VCF

- Genotype data, the header is requried to be provided:

CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	sample 1	sample 2	sample 3
1	157104	marker 1	A	C	.	PASS	.	GT	0/0	1/1	0/0
1	1947984	marker 2	C	G	.	PASS	.	GT	0/0	1/1	1/1

2.3.5 PLINK (the header should be removed)

- Genotype data (.ped). Missing value can be filled as "0":

Family ID	Sample ID	Paternal ID	Maternal ID	Sex	Affection	marker 1	marker 2	marker 3
FAM1	NA06985	0	0	1	1	A A	T T	A A
FAM1	NA06991	0	0	1	1	A A	T T	A A
0	NA06993	0	0	1	1	C T	C C	T T

- Map information (.map):

Chromosome	Marker ID	Genetic distance	Physical Position
1	marker 1	0	157104
1	marker 2	0	1947984
1	marker 3	0	2914066

2.3.6 Binary PLINK (the header should be removed)

- Genotype data (.bed):

  Please follow the instruction from here

- FAM file:

Family ID	Sample ID	Paternal ID	Maternal ID	Sex	Affection
FAM1	NA06985	0	0	1	1
FAM1	NA06991	0	0	1	1

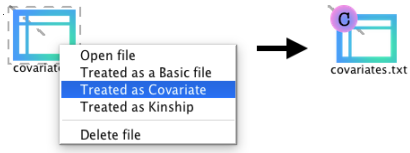
- BIM file:

Chromosome	Marker ID	Genetic distance	Physical Position	Allele 1	Allele 2
1	marker 1	0	157104	A	C
1	marker 2	0	1947984	A	T

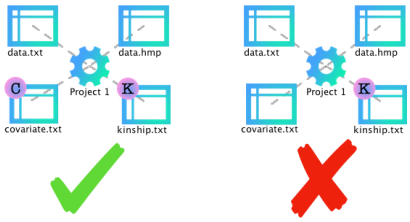
2.4 *Covariates and kinship*

2.4.1 Add additional information to iPat

- Covariates provided by users will be treated as **fixed effect** in the selected model except in BGLR.
- It's **optional** that users can add **user-define** covariates or kinship into the project. Right clicking on the file to tell iPat what type of file it is. (i.e. covariates, kinship or a basic required file)



- Be aware that apart from the basic required file (i.e. phenotype and genotype), optional files must be properly labeled in a project.



- The file labeled “C” stands for a covariate file, while files labeled “K” is identified as a kinship file by iPat. For the example of a valid project below (Left one), the file "covariate.txt" and "kinship.txt" are treated as covariates and a kinship in this project, respectively. Each project can contain **one** covariate files and **one** single kinship.

2.4.2 Covariates

- Demo format for a covarate file. The header is required to be provided:

PC1	PC2	PC3
-1.8942149	-4.91532916	0.8674568
1.6858820	-5.08378277	-0.4069675
0.2579269	-6.29547725	2.6867939

2.4.3 Kinship

- Demo format for a kinship file. Taxa name is required while the header can be omitted:

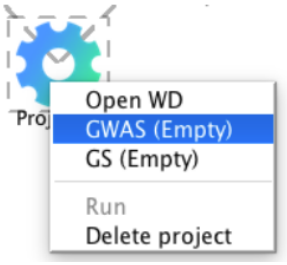
taxa	sample 1	sample 2	sample 3
sample 1	2.00000000	0.22883683	0.22932180
sample 2	0.22883683	2.00000000	0.24496455
sample 3	0.22932180	0.24496455	2.00000000

- If there is no user-define kinship, a kinship will be generated by the selected package:

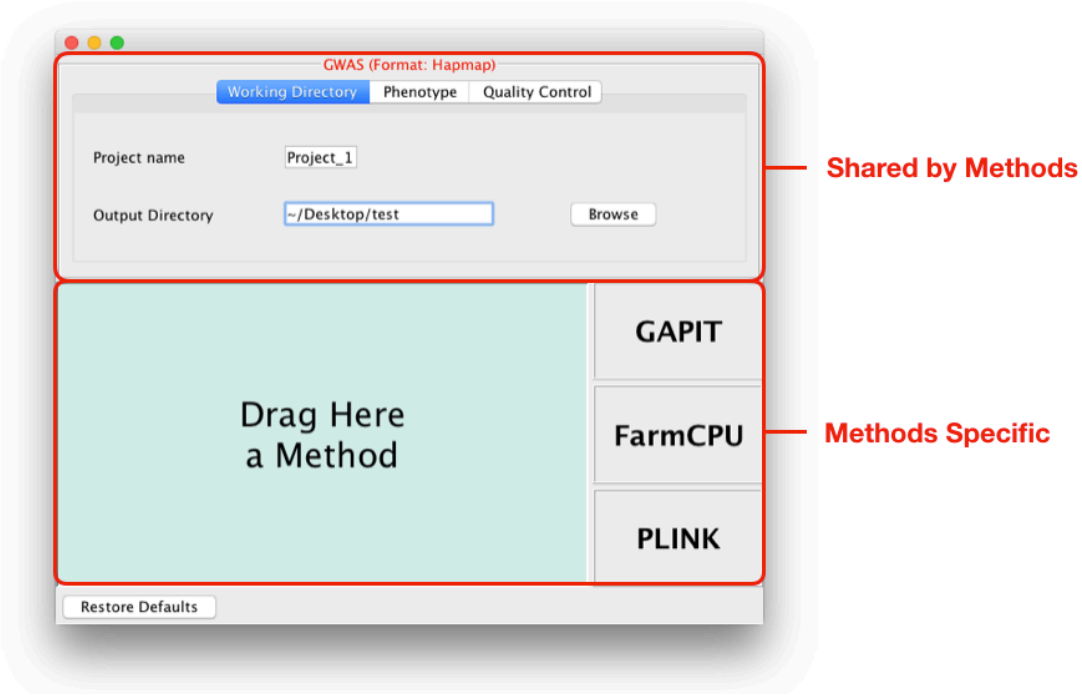
Package	Kinship algorithm
GAPIT	VanRaden (VanRaden, 2008), Loiselle (Loiselle *et al.*, 1995) or EMMA (Kang *et al.*, 2008)
FarmCPU	FARM-CPU (Liu *et al.*, 2016)
PLINK	User-provided
rrBLUP	VanRaden (VanRaden, 2008)
BGLR	User-provided

2.5 *Define Your Analysis*

- After linking every files needed in the project, right click on the project and choose either GWAS or GS to open a configuration panel.



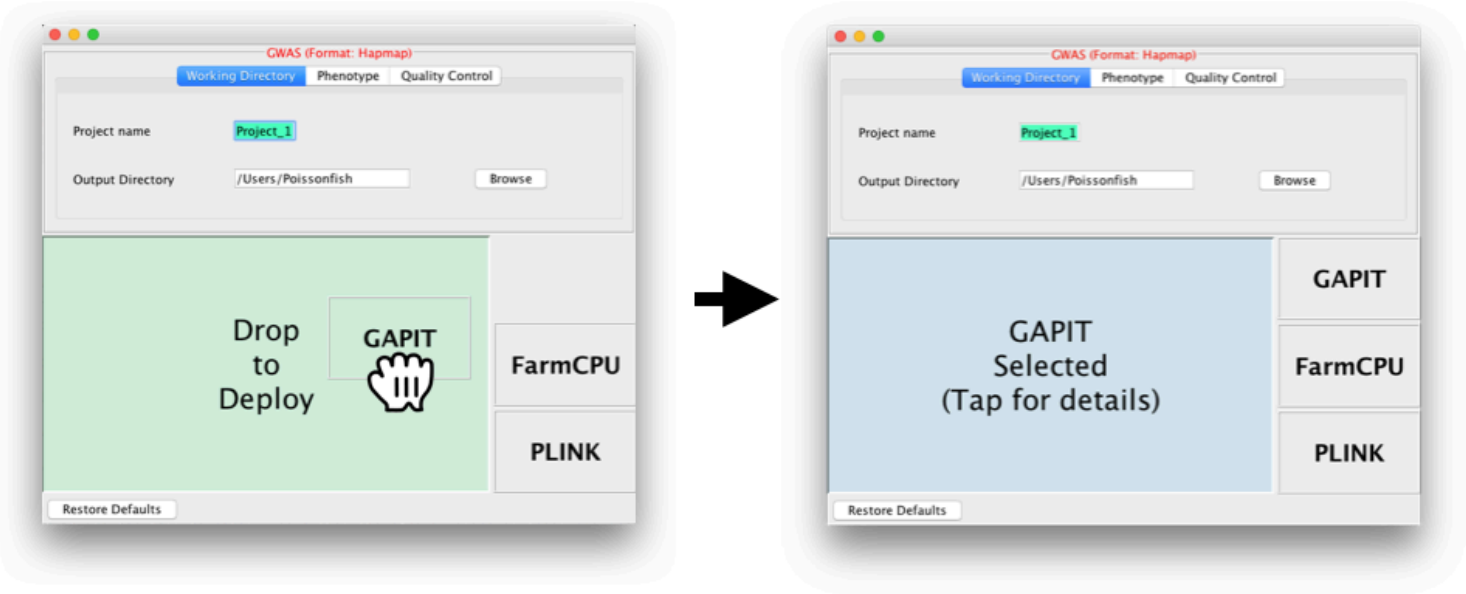
- The panel consist of two sections. The upper one presents a set of input arguments shared by all methods, while users can define method-specific arguments from the lower section.



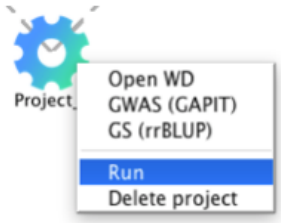
- Available parameters in the upper section :

Tab	Parameters	Definition	Default
Working Directory	Project name	Prefix for output files	Project_x (x is a number starts from 1)
Working Directory	Output Directory	A path where output files will be generated	Home directory
Phenotype	Trait names	Subsetting traits data	All traits are selected
Quality Control	By missing rate	Filtering out markers where certain rate of value is missing	No threshold
Quality Control	By MAF	Filtering out markers based on minor allele frequency	0.05

- To select a method, simply drag a "method block" to the left-side area. And tap on this area for further defining (The information of method-specific arguments can be found in the section 3) .

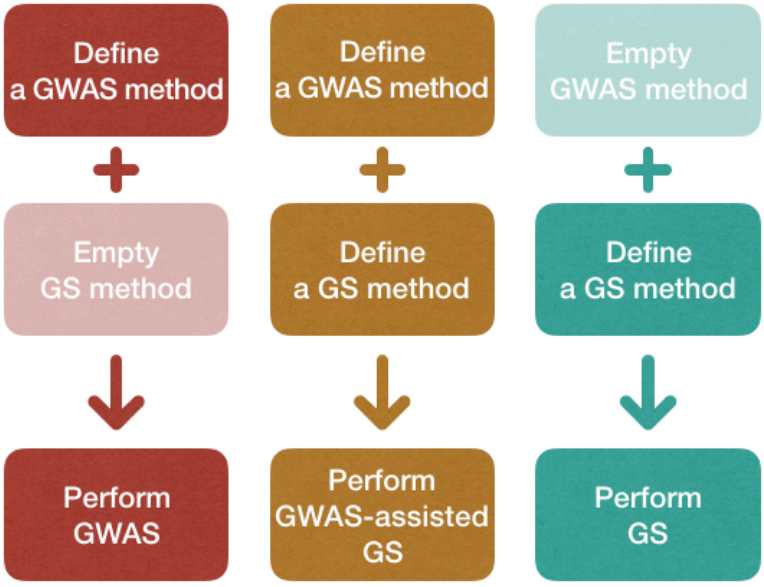


- After defining the analysis, user can start to run the procedure by clicking ‘Run’ at the pop-up menu of the project.

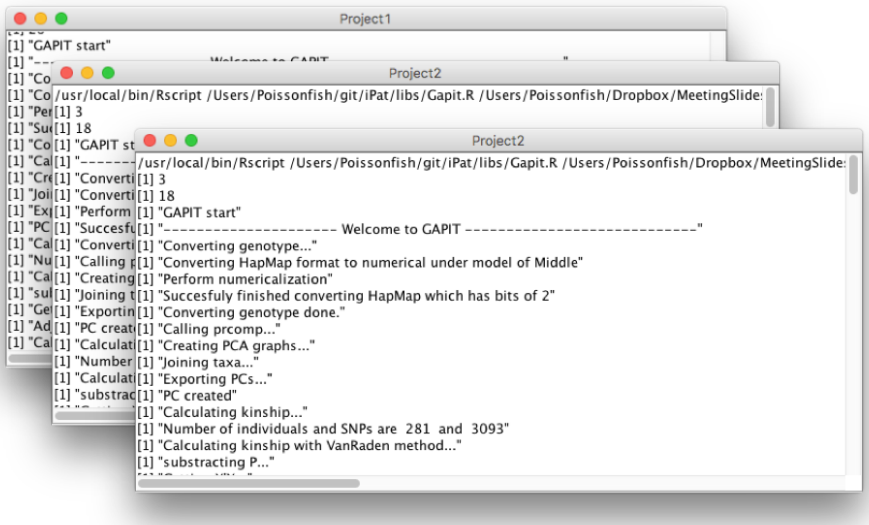


2.6 *Run an analysis*

- In iPat, users are allowed to do genomic studies such as GWAS, GS and GWAS-Assisted GS (Associated SNPs reported by GWAS will be treated as fixed effect in GS). iPat will detect the project configuration and decide which analysis should be implemented afterward.

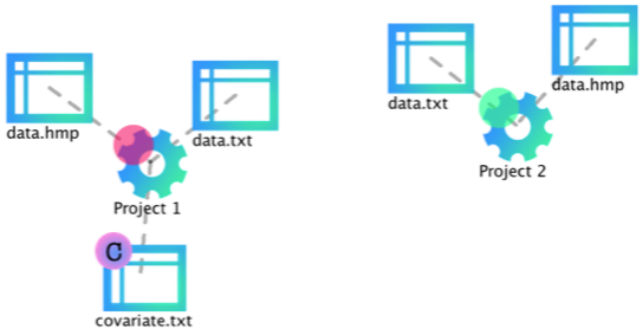


- Each project will generate a console window while running the analysis. User can track the progress of the task from window messages.
- iPat also capable of multitasking. Users can arrange another project even when the previous one have not done yet.

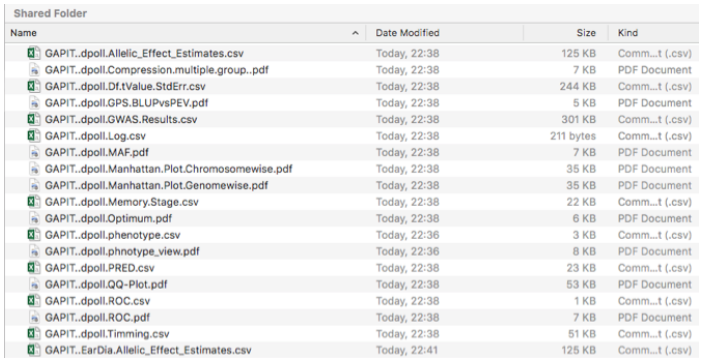


2.7 *Inspect the result*

- When iPat complete a project, the gear icon will show a green dot if the task run successfully without any error occurred. Otherwise it will show a red dot at its top-left to notify users that there're existing at least one error message during the analysis.

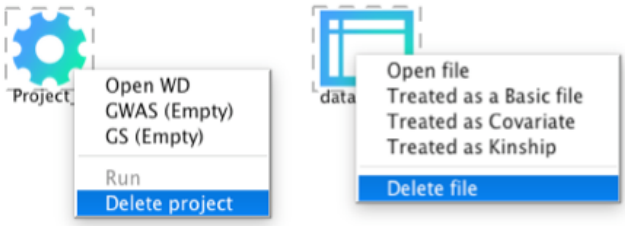


- Users can inspect the results by double clicking on the gear icon, which will direct users to the folder where output files generated.



2.8 *Remove files from iPat*

- Users are allowed to remove projects or files from iPat in the pop-up menu through right clicking on them, or simply press `Backspace` (press `delete` on Mac) after selecting them.



3. GWAS and GS

Tools implemented in iPat allow users to do genome-wide associate study (GWAS) and genomic selection (GS). Curretly GWAS can be performed by GAPIT, FarmCPU and PLINK, and GS can be done by GAPIT, rrBLUP and BGLR in iPat. Tables below are the input arguments available in iPat:

3.1 GAPIT

Tab	Parameters	Definitions	Default
Covariates	Covaraite names	Subsetting covaraites data	All covariates are selected
GAPIT input	Model	Which linear model to be used in GWAS	GLM
GAPIT input	kinship.cluster	Clustering algorithm to group individuals based on their kinship	average
GAPIT input	kinship.group	Method to derive kinship among groups	Mean
Advance	SNP.fraction	Fraction of SNPs Sampled to Estimate Kinship and PCs	1
Advance	File.fragment	The Fragment Size to Read Each Time within a File	512
Advance	Model selection	Conduct Bayesian information criterion (BIC)-based model selection to find the optimal number of PCs for inclusion in the GWAS models	FALSE

3.2 FarmCPU

Category	Parameters	Definitions	Default
Covariates	Covaraite names	Subsetting covaraites data	All covariates are selected
FarmCPU inpute	method.bin	It uses fixed or optimized of possible QTN window size and number of possible QTNs selected into FarmCPU model.	static
FarmCPU inpute	maxLoop	Maximum number of iterations allowed	10

3.3 PLINK

Category	Parameters	Definitions	Default
Covariates	Covaraite names	Subsetting covaraites data	All covariates are selected
PLINK input	C.I.	The desired coverage for a confidence interval	0.95

3.4 rrBLUP

Category	Parameters	Definitions	Default
Covariates	Covaraite names	Subsetting covaraites data	All covariates are selected
rrBLUP input	Shrinkage estimation	Shrinkage estimation can improve the accuracy of genome-wide marker-assisted selection, particularly at low marker density (Endelman and Jannink 2012)	
rrBLUP input	impute.method	Imputation algorithm for missing values in markers data	mean

3.5 BGLR

Category	Parameter	Definitions	Default
Subset	Subset of traits data	Users can select all or partial of traits to be analyzed	All traits
BGLR	Regression model for predictor (Markers)	The regression type for the markers data	BRR
BGLR	response_type	Data type of the response (y)	gaussian
BGLR	nIter	The number of iterations of the sampler	1200
BGLR	burnIn	The number of samples discarded	200
BGLR	thin	The number of thinning	5

4 Support

- If there is any difficulty on iPat, please leave your question in the page of issue report.
- Or you can directly send an email to the author James Chen

5 Citation

- Bradbury,P.J. et al. (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics, 23, 2633–2635.
- Endelman,J. (2011) Ridge regression and other kernels for genomic selection in the R package rrBLUP. Plant Genome, 4, 250–255.
- Kang,H.M. et al. (2008) Efficient control of population structure in model organism association mapping. Genetics, 178, 1709–1723.
- Liu,X. et al. (2016) Iterative Usage of Fixed and Random Effect Models for Powerful and Efficient Genome-Wide Association Studies. PLoS Genet., 12, e1005767.
- Purcell,S. et al. (2007) PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. Am J Hum Genet, 81, 559–575.
- Tang,Y. et al. (2016) GAPIT Version 2: An Enhanced Integrated Tool for Genomic Association and Prediction. Plant J., 9.