PEAS: Package for Elementary Analysis of SNP data

Designed by Shuhua Xu and Li Jin

Code by Shuhua Xu

with contributions from Sanchit Gupta

January 8, 2010

Contact information

Shuhua Xu Ph.D.,
CAS-MPG Partner Institute of Computational Biology, SIBS, CAS
320 YueYang Road, Shanghai 200031, China
Phone: 86-21-54920479
Fax: 86-21-54920451
E-mail: xushua@picb.ac.cn
E-mail: xushua@gmail.com
Website: http://www.picb.ac.cn/~xushua

Contents

1 Introduction

Owing to the advent of high-throughput biological and chemical assays, a wealth of genomic data has been created, of which single nucleotide polymorphism (SNP) data accumulate especially fast. With the release of the Phase III HapMap (The International HapMap Consortium, 2003; 2005; http://www.hapmap.org) data, a resource consisting of over 1.6 million SNPs genotyped in more than 1000 individuals from 11 geographically diverse populations is publicly available. Many similar private or international projects focus on a special of group of genes such as Environmental Genome Project (EGP; http://www.niehs.nih.gov/envgenom) or on regional populations such as PanAsian SNP Project (PASNP; http://pasnpi.biotec.or.th/) generated additional SNP data resources. The modern human genetic studies have been dramatically influenced by the development and release of these data, our insight and knowledge about human genome has been greatly improved due to the analysis of those SNP data.

Many software tools have been developed to extract abundant information from ssuch data. Most of software tools available focus on a certain purpose and perform well on one special aspect. PHASE (Stephens, Smith et al. 2001) is one of the best software tools available for inferring haplotypes from population genotype data; the fastPHASE (Scheet and Stephens 2006) program that developed subsequently performed well in inferring haplotypes in large SNP surveys; Haploview (Barrett, Fry et al. 2005) is widely used software in LD & Haplotype block analysis and tag SNP selection; LDhat (McVean, Myers et al. 2004) is the software tool developed for fine-scale recombination analysis and it performs well in high density SNP data. In addition, a few software tools, which although were developed earlier and not limited in SNP data, are still useful in population genetic analysis of SNP data, for instance, Arlequin (Schneider, Roessli et al. 2000) provides a large set of tools with basic methods in population genetics; STRUCTURE (Pritchard, Stephens et al. 2000; Falush, Stephens et al. 2003) is one of the best software tools available for inferring population structure using genotype data. However, all most all software tools available have been developed for some specific purpose and have private format of input files, whereas both the formatting jobs of input file and manipulation of output files often take people much time, especially for those biologists who do not write program themselves and when the data set is very large. Furthermore, there are still many gaps of analysis for the current available software, such as calculating individual allele sharing distance, population genetic distances, do bootstrapping, calculating LD statistics for large-scale SNP data set and so on. For some basic data manipulations, either the software available currently do not provide or the software do not work very well for large data sets.

Here we developed a software package named PEAS to provide the average user with many basic analysis tools and facilitate people who are involving in analysis of large SNP data set.

2 Overview

All the programs in PEAS are developed to handle very large amount of SNP data with high efficiency. We adopt dynamic memory management, so there is actually no limit of the program for the size of data set, the only limit is the memory of the computer. All the operations of PEAS programs are file(s) to file(s), although PEAS allow the user display results in the GUI which will take huge memory to display on the screen, especially for very large data set. We recommend the user chose not to display data and let program perform background process. In that case, we also provide single separate executables for each PEAS function, so alternatively the user can find all the PEAS component programs separate single executables in the PEAS directory, we will show the details in the following sections.

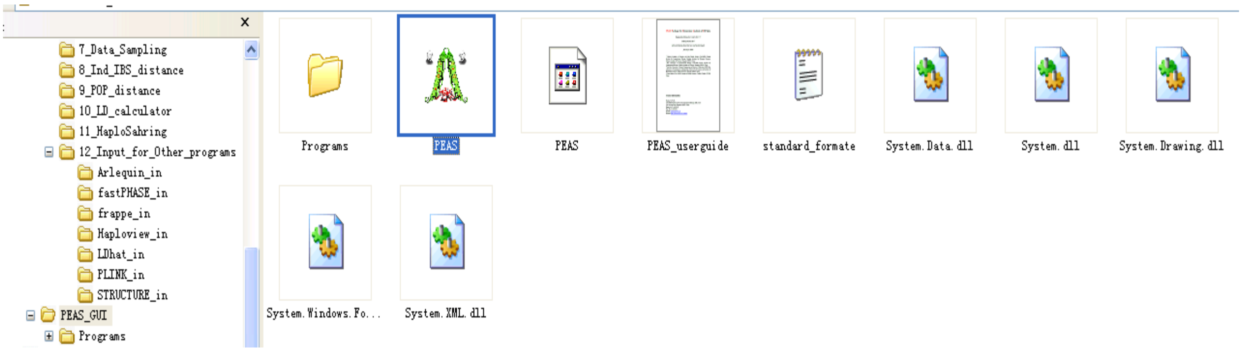
PEAS is versatile in manipulating data. We provide many tools in PEAS for data formatting, which will facilitate the user manipulate data by easy stages before they proceed further analysis. Secondly, we provide tools for some basic manipulations of SNP data. Thirdly, to fill up the gaps of currently available software tools, a few tools focus on population genetic analysis and phylogenetic analysis were implemented.

Finally, a graphical user interface is designed to facilitate the user to rapidly select different PEAS tools and manipulate their data. A screenshot of the GUI of PEAS is shown in Figure 1.

3 Download and installation

PEAS for windows are freely available for academic user from the web page http://www.picb.ac.cn/~xushua/index.files/Software.htm.

1) Download **PEAS_v1.0.zip** to any temporary directory;
2) Extract all files contained in **PEAS_v1.0.zip** in the directory of your choice;
3) Start PEAS by double clicking on the executable icon of PEAS. We distribute executables for PEAS to run under Windows. We also distribute executables for each PEAS program component, which can run under Windows by double clicking the executable, then following the README to enter the name of input file. In most cases, the user need only enter the name of a file list, such as "4pops" without an extension.

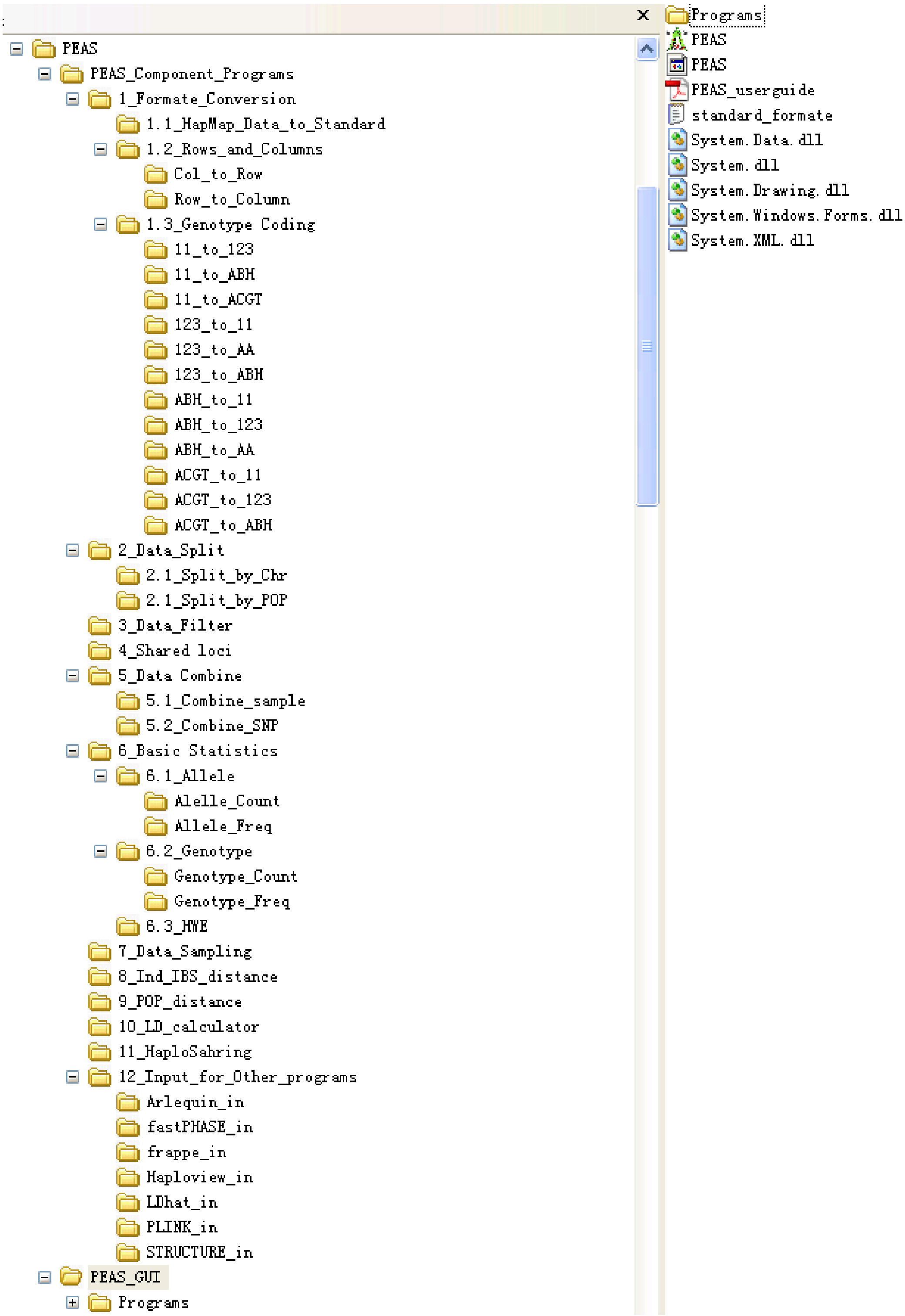


3.1 System requirements

It is recommended that PEAS be run on a machine with Microsoft Windows OS and at least 256M of memory. We adopt dynamic memory management, so there is actually no limit of the program for the size of data set, the only limit is the memory of the computer. We estimate that as large as 1G of memory may be necessary for PEAS to handle very large data set.

3.2 PEAS Component Programs

As we described above, we provide individual component programs for each PEAS function. The user can find all the PEAS component programs separate executables in the PEAS directory, the following snapshot shows the overall structure of the PEAS component programs. Please note that currently not all functions available in GUI, but alternatively the user can perform all the analyses using PEAS component programs.

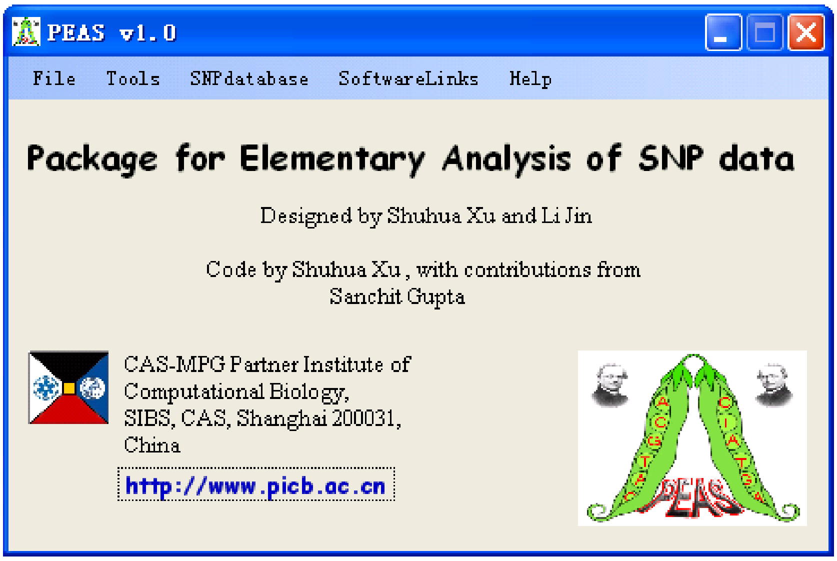


```
⊟ PEAS
   ⊟ PEAS_Component_Programs
      ⊟ 1_Formate_Conversion
            1.1_HapMap_Data_to_Standard
         ⊟ 1.2_Rows_and_Columns
               Col_to_Row
               Row_to_Column
         ⊟ 1.3_Genotype Coding
               11_to_123
               11_to_ABH
               11_to_ACGT
               123_to_11
               123_to_AA
               123_to_ABH
               ABH_to_11
               ABH_to_123
               ABH_to_AA
               ACGT_to_11
               ACGT_to_123
               ACGT_to_ABH
      ⊟ 2_Data_Split
            2.1_Split_by_Chr
            2.1_Split_by_POP
         3_Data_Filter
         4_Shared loci
      ⊟ 5_Data Combine
            5.1_Combine_sample
            5.2_Combine_SNP
      ⊟ 6_Basic Statistics
         ⊟ 6.1_Allele
               Alelle_Count
               Allele_Freq
         ⊟ 6.2_Genotype
               Genotype_Count
               Genotype_Freq
            6.3_HWE
         7_Data_Sampling
         8_Ind_IBS_distance
         9_POP_distance
         10_LD_calculator
         11_HaploSahring
      ⊟ 12_Input_for_Other_programs
            Arlequin_in
            fastPHASE_in
            frappe_in
            Haploview_in
            LDhat_in
            PLINK_in
            STRUCTURE_in
   ⊟ PEAS_GUI
      ⊞ Programs
```

```
Programs
PEAS
PEAS
PEAS_userguide
standard_formate
System.Data.dll
System.dll
System.Drawing.dll
System.Windows.Forms.dll
System.XML.dll
```

3.3 GUI for PEAS

We provide a GUI for PEAS user only to facilitate the user to manage the programs and files of both input and output. All the operations of PEAS programs are file to file, so there is no data loading to GUI to read, just let the program to locate the input files and save the output files in a user defined location.



Package for Elementary Analysis of SNP data

Designed by Shuhua Xu and Li Jin

Code by Shuhua Xu , with contributions from
Sanchit Gupta

CAS-MPG Partner Institute of
Computational Biology,
SIBS, CAS, Shanghai 200031,
China

http://www.picb.ac.cn

Fig. 1. Screenshot of the user graphical interface of PEAS, showing the main page of PEAS and the bottom plot displays the tools list.

The user can find all the analysis tools in the "Tools" menu, as shown in the following figure. The menu is the windows style that many MS windows OS users are familiar with.

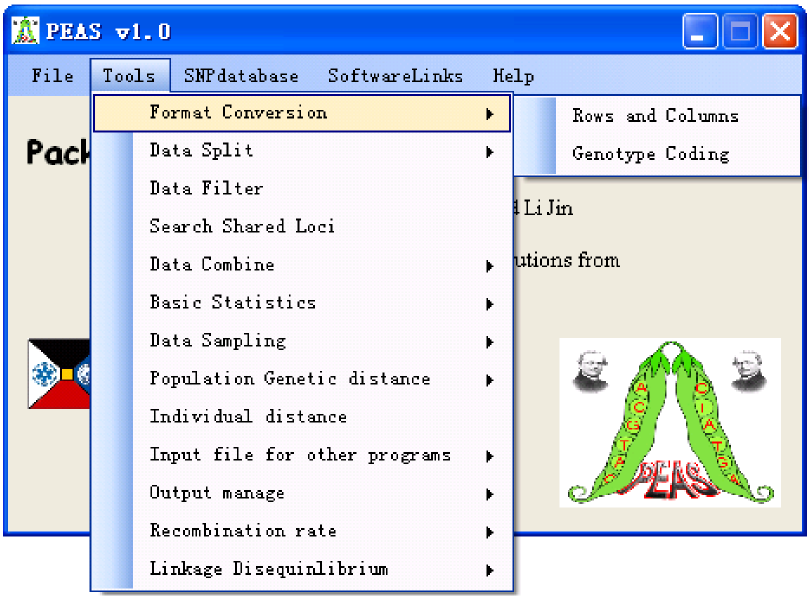


Fig. 2. Screenshot of the user graphical interface of PEAS, showing the tools list.

4 Format for the data file

PEAS recognize many different formats of SNP data, we defined a standard format as the style of HapMap genotype data except the genotypes are coded by single character, with ‘A’ and ‘B’ coding for two homozygotes, ‘H’ coding for heterozygote and ‘U’ coding for missing genotype. Our strategy is transform all the data formats user supply to the standard format of data that PEAS can handle for the subsequent analysis. However, PEAS provide flexible tools so that the user can convert data format from one to another very easily.

Basically, there are two types of data for SNPs, one is the genotype data that are the original data obtained from experiments, and the other is haplotype data that can be obtained from experiment, but are often inferred from genotype data for large SNP surveys. Most of programs in PEAS deal with genotype data, some of them deal with haplotype data.

4.1 Standard format for the genotype data file

This format is the same style as HapMap genotype data, with SNPs in rows and genotypes of sample in columns. But the genotypes are coded by single character, with ‘A’ and ‘B’ coding for two homozygotes, ‘H’ coding for heterozygote and ‘U’ coding for missing genotype. Because for large SNP surveys, there will be much larger number of SNPs than that of individuals, this format will be more readable than the others. The genotype data file is supplied by the user to specify how many individuals there are to be analyzed, how many sites each individual has been typed at, and the genotypes for each individual. The information that the user has to provide includes also ID of SNPs (the first column), which chromosome that each SNP is of (the second column), the physical position of each SNP (the third column), the two possible allele state of each SNP (the fourth column), which DNA strand each SNP was genotyped (the fifth column), followed by genotype data (the rest columns).

One example of standard format of genotype data can be as follows:

SNPID	Chrom	Position	AlleleState	Strand	SampleID1	SampleID2	SampleID3
rs11089130	chr22	14431347	C/G	+	B	A	H
rs915675	chr22	14433659	A/C	+	H	B	A
rs915677	chr22	14433758	A/G	+	B	B	B
rs9604721	chr22	14434713	C/T	+	A	H	A
rs12159982	chr22	14434960	C/T	+	A	A	H
rs4389403	chr22	14435070	A/G	+	H	B	A
rs12628452	chr22	14435171	A/G	+	A	B	**U**
rs7291810	chr22	14435207	C/T	+	A	H	A
rs5746356	chr22	14439734	C/T	+	A	H	H
rs759235	chr22	15647006	A/G	–	H	A	B
rs5748656	chr22	15668200	C/T	+	B	H	B
rs5748657	chr22	15668220	C/T	+	H	A	A
rs2286985	chr22	15668377	G/T	–	A	H	B
rs2072467	chr22	15668988	A/G	–	H	**?**	A
rs2072466	chr22	15669118	A/G	–	A	H	H
rs5746901	chr22	15670010	G/T	+	B	H	H

Note 1: *Genotypes are coded in as A, B, H, here A and B indicate two homozygotes, and H indicate heterozygote. Missing alleles can either be coded as* ***U*** *or as* ***?****.*

Note 2: *All the formats including both the standard format and the following alternative formats are restricted to those sites that have at most 2 alleles segregating. For those sites there are more than 2 alleles present, we select the two most frequent alleles, and treat all the other alleles as missing data.*

Note 3: *There could be extra columns between the strand column (the fifth column) and the genotype columns (the sixth column and the following columns). The used can specify the number of extra columns in the input files.*

4.2 Alternative format for the genotype data file

Besides of the standard format, PEAS recognize 7 alternative formats.

4.2.1 SNPs in rows and individuals in columns

The layouts of the following three formats are the same as the standard format, i.e.

rows store the information of SNPs and columns store the information of individuals. The only difference is the coding of genotype. The user provides SNP ID in the first column, chromosome number in the second column, the physical position of each SNP in the third column, the allele state of each SNP in the fourth column, DNA strand of each SNP in the fifth column and followed by genotype data (the rest columns).

4.2.1.1 Genotype coded by single character

The format is the same as the default except genotypes are coded in as 1, 2, 3, here 1 and 2 indicate two homozygotes, and 3 indicate heterozygote. Missing alleles can either be coded as **0** or as **?**.

SNPID	Chrom	Position	AlleleState	Strand	SampleID1	SampleID2	SampleID3
rs11089130	chr22	14431347	C/G	+	2	1	3
rs915675	chr22	14433659	A/C	+	3	2	1
rs915677	chr22	14433758	A/G	+	2	2	2
rs9604721	chr22	14434713	C/T	+	1	3	1
rs12159982	chr22	14434960	C/T	+	1	1	3
rs4389403	chr22	14435070	A/G	+	3	2	1
rs12628452	chr22	14435171	A/G	+	1	2	**0**
rs7291810	chr22	14435207	C/T	+	1	3	1
rs5746356	chr22	14439734	C/T	+	1	3	3
rs759235	chr22	15647006	A/G	–	3	1	2
rs5748656	chr22	15668200	C/T	+	2	3	2
rs5748657	chr22	15668220	C/T	+	3	1	1
rs2286985	chr22	15668377	G/T	–	1	3	2
rs2072467	chr22	15668988	A/G	–	3	**?**	1
rs2072466	chr22	15669118	A/G	–	1	3	3
rs5746901	chr22	15670010	G/T	+	2	3	3

4.2.1.2 Genotype coded by two characters

Each genotype is indicated by two characters, and the genotypes of each SNP are listed on a single line, locus by locus. Genotypes can be coded in as standard DNA letters, A, C, G, T; missing alleles can either be coded as **N** or as **?**.

SNPID	Chrom	Position	AlleleState	Strand	SampleID1	SampleID2	SampleID3
rs11089130	chr22	14431347	C/G	+	GG	CC	CG
rs915675	chr22	14433659	A/C	+	AC	CC	AA
rs915677	chr22	14433758	A/G	+	GG	GG	GG
rs9604721	chr22	14434713	C/T	+	CC	CT	CC
rs12159982	chr22	14434960	C/T	+	CC	CC	CT
rs4389403	chr22	14435070	A/G	+	AG	GG	AA
rs12628452	chr22	14435171	A/G	+	AA	GG	**NN**
rs7291810	chr22	14435207	C/T	+	CC	CT	CC
rs5746356	chr22	14439734	C/T	+	CC	CT	CT
rs759235	chr22	15647006	A/G	–	AG	AA	GG
rs5748656	chr22	15668200	C/T	+	TT	CT	TT
rs5748657	chr22	15668220	C/T	+	CT	CC	CC
rs2286985	chr22	15668377	G/T	–	GG	GT	TT
rs2072467	chr22	15668988	A/G	–	AG	**??**	AA
rs2072466	chr22	15669118	A/G	–	AA	AG	AG
rs5746901	chr22	15670010	G/T	+	TT	GT	GT

Genotypes can also be coded in as 11, 22, 12, here 11 and 22 indicate two homozygotes, and 12 indicate heterozygote. Missing alleles can either be coded as **0** or as **?**.

SNPID	Chrom	Position	AlleleState	Strand	SampleID1	SampleID2	SampleID3
rs11089130	chr22	14431347	C/G	+	22	11	12
rs915675	chr22	14433659	A/C	+	12	22	11
rs915677	chr22	14433758	A/G	+	22	22	22
rs9604721	chr22	14434713	C/T	+	11	12	11
rs12159982	chr22	14434960	C/T	+	11	11	12
rs4389403	chr22	14435070	A/G	+	12	22	11
rs12628452	chr22	14435171	A/G	+	11	22	**00**
rs7291810	chr22	14435207	C/T	+	11	12	11
rs5746356	chr22	14439734	C/T	+	11	12	12
rs759235	chr22	15647006	A/G	–	12	11	22
rs5748656	chr22	15668200	C/T	+	22	12	22
rs5748657	chr22	15668220	C/T	+	12	11	11
rs2286985	chr22	15668377	G/T	–	11	12	22
rs2072467	chr22	15668988	A/G	–	12	**??**	11
rs2072466	chr22	15669118	A/G	–	11	12	12
rs5746901	chr22	15670010	G/T	+	22	12	12

4.2.2 Individuals in rows and SNPs in columns

In some studies, people prefer to provide another format of genotype data. This format can be taken as a transpose of the layout of the default format, with SNPs in columns and individuals in rows. The genotype data file is supplied by the user to specify how many individuals there are to be analyzed, how many sites each individual has been typed at, and the genotypes for each individual. The information that the user has to provide includes also ID of SNPs (the first row), which chromosome that each SNP is of (the second row), the physical position of each SNP (the third row), the two possible allele state of each SNP (the fourth row), which DNA strand each SNP was genotyped (the fifth row), followed by genotype data (the rest rows).

4.2.2.1 Genotype coded by single character

Genotypes are coded in as A, B, H, here A and B indicate two homozygotes, and H indicate heterozygote. Missing alleles can either be coded as **U** or as **?**.

SNPID	rs11089130	rs915675	rs9604721	rs4389403	rs12628452	rs2072467
Chrom	chr22	chr22	chr22	chr22	chr22	chr22
Position	14431347	14433659	14434713	14435070	14435171	15668988
AlleleState	C/G	A/C	C/T	A/G	A/G	A/G
Strand	+	+	+	+	+	–
SampleID1	B	H	A	H	A	H
SampleID2	A	B	H	B	B	?
SampleID3	H	A	A	A	U	A

Genotypes are coded in as 1, 2, 3, here 1 and 2 indicate two homozygotes, and 3 indicate heterozygote. Missing alleles can either be coded as **0** or as **?**.

SNPID	rs11089130	rs915675	rs9604721	rs4389403	rs12628452	rs2072467
Chrom	chr22	chr22	chr22	chr22	chr22	chr22
Position	14431347	14433659	14434713	14435070	14435171	15668988
AlleleState	C/G	A/C	C/T	A/G	A/G	A/G
Strand	+	+	+	+	+	–
SampleID1	2	3	1	3	1	3
SampleID2	1	2	3	2	2	?
SampleID3	3	1	1	1	0	1

4.2.2.2 Genotype coded by two characters

Each genotype is indicated by two characters, and the genotypes of each SNP are listed on a single line, locus by locus. Genotypes are coded in as standard DNA letters, A, C, G, T; missing alleles can either be coded as **N** or as **?**.

SNPID	rs11089130	rs915675	rs9604721	rs4389403	rs12628452	rs2072467
Chrom	chr22	chr22	chr22	chr22	chr22	chr22
Position	14431347	14433659	14434713	14435070	14435171	15668988
AlleleState	C/G	A/C	C/T	A/G	A/G	A/G
Strand	+	+	+	+	+	–
SampleID1	GG	AC	CC	AG	AA	AG
SampleID2	CC	CC	CT	GG	GG	**??**
SampleID3	CG	AA	CC	AA	**NN**	AA

Genotypes are coded in as 11, 22, 12, here 11 and 22 indicate two homozygotes, and 12 indicate heterozygote. Missing alleles can either be coded as **0** or as **?**.

SNPID	rs11089130	rs915675	rs9604721	rs4389403	rs12628452	rs2072467
Chrom	chr22	chr22	chr22	chr22	chr22	chr22
Position	14431347	14433659	14434713	14435070	14435171	15668988
AlleleState	C/G	A/C	C/T	A/G	A/G	A/G
Strand	+	+	+	+	+	–
SampleID1	22	12	11	12	11	12
SampleID2	11	22	12	22	22	**??**
SampleID3	12	11	11	11	**0**	11

5 Functions in Component Programs

5.1 Basic data format conversion tools.

1.1 A special tool to manipulate HapMap genotype data, which formats HapMap data to PEAS standard format for further analyses.

Program name	Function
HapMap_Data_to_Standard	Convert HapMap data format to PEAS standard format

Procedure to run the program:

[1] Double click the executable file;

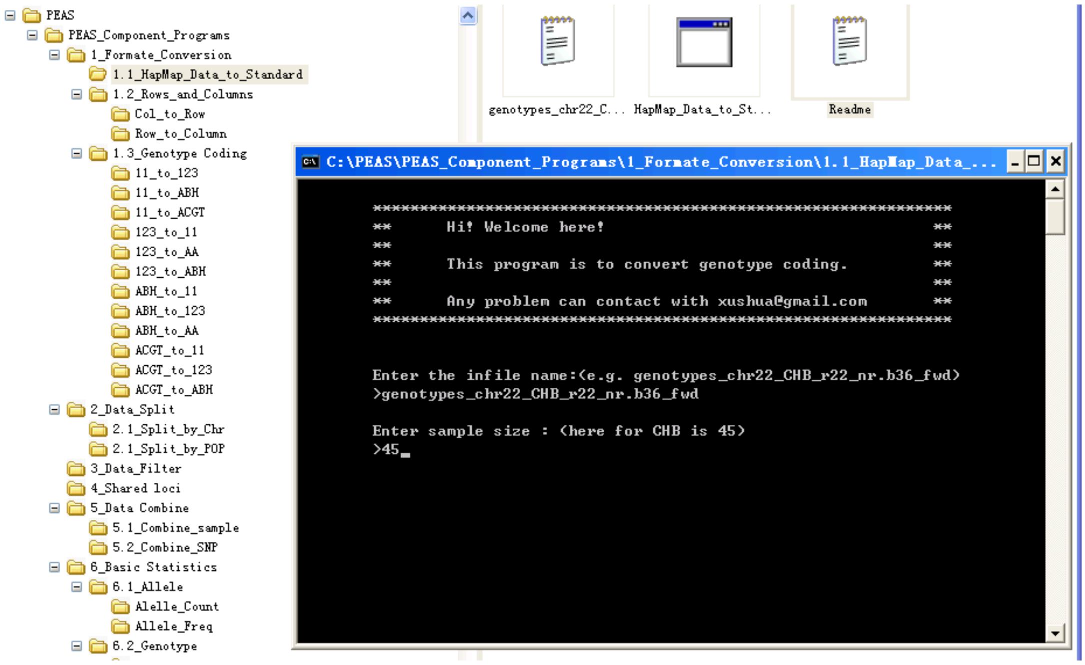
[2] Enter the infile name:(e.g. genotypes_chr22_CHB_r22_nr.b36_fwd);

type genotypes_chr22_CHB_r22_nr.b36_fwd, hit Enter;

[3] Enter sample size : (here for CHB is 45);

type 45, hit Enter;

[4] check the output files.



```
*****************************************************************
**      Hi! Welcome here!                                      **
**                                                             **
**      This program is to convert genotype coding.            **
**                                                             **
**      Any problem can contact with xushua@gmail.com          **
*****************************************************************


Enter the infile name:(e.g. genotypes_chr22_CHB_r22_nr.b36_fwd)
>genotypes_chr22_CHB_r22_nr.b36_fwd

Enter sample size : (here for CHB is 45)
>45_
```

1.2 Two format conversion tools to transpose data between columns and rows.

Program name	Function
colTorow	Convert Linkage-like format to PEAS standard format
rowTocol	Convert PEAS standard format to Linkage-like format

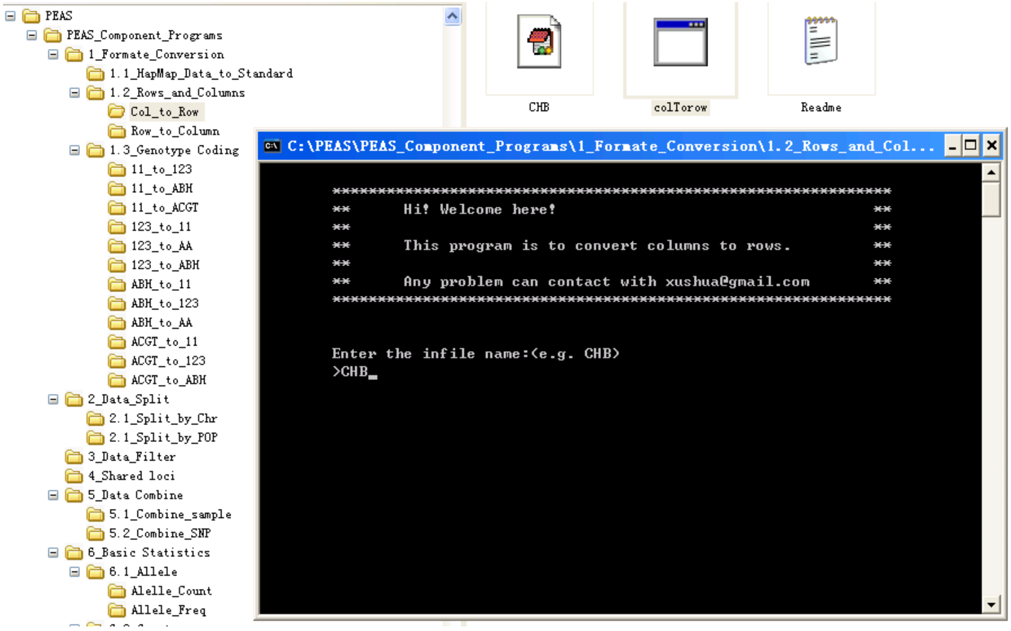
Procedure to run the program:

[1] Double click the executable file;

[2] Enter the infile name:(e.g. CHB);

[3] type CHB, hit Enter;

[4] check the output files.



```
***************************************************************
**      Hi! Welcome here!                                     **
**                                                            **
**      This program is to convert columns to rows.           **
**                                                            **
**      Any problem can contact with xushua@gmail.com         **
***************************************************************


Enter the infile name:(e.g. CHB)
>CHB_
```

1.3 Twelve format conversion tools to re-coding genotype data.

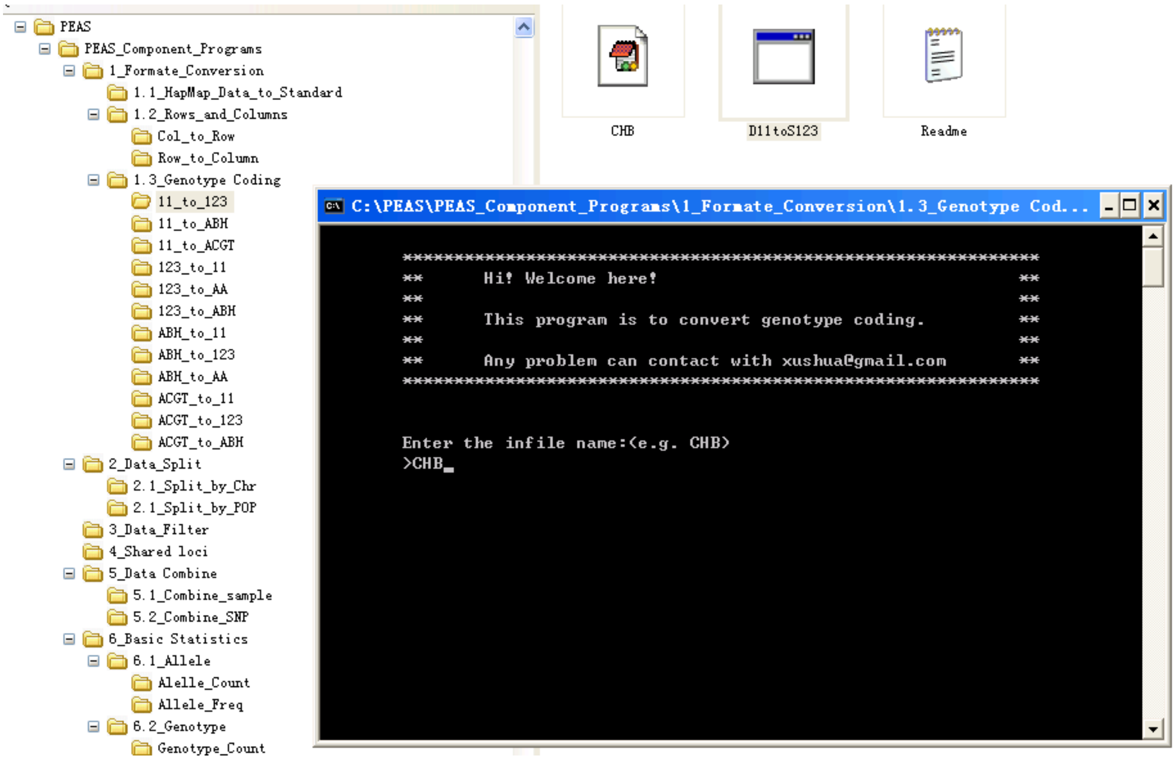
Program name	Function
DAAtoD11	Convert ACGT coding to 11, 12, 22 coding
DAAtoS123	Convert ACGT coding to 1, 2, 3 coding
DAAtoSABH	Convert ACGT coding to A, B, H coding
D11toS123	Convert 11, 12, 22 coding to 1, 2, 3 coding
D11toSABH	Convert 11, 12, 22 coding to A, B, H coding
D11toDAA	Convert 11, 12, 22 coding to ACGT coding
S123toD11	Convert 1, 2, 3 coding to 11, 12, 22 coding
S123toDAA	Convert 1, 2, 3 coding to ACGT coding
S123toSABH	Convert 1, 2, 3 coding to A, B, H coding
SABHtoD11	Convert A, B, H coding to 11, 12, 22 coding
SABHtoS123	Convert A, B, H coding to 1, 2, 3 coding
SABHtoDAA	Convert A, B, H coding to ACGT coding

Procedure to run the program:

[1] Double click the executable file;

[2] Enter the infile name:(e.g. CHB);
type CHB, hit Enter;

[3] check the output files.



```
*************************************************************
**      Hi! Welcome here!                                  **
**                                                         **
**      This program is to convert genotype coding.        **
**                                                         **
**      Any problem can contact with xushua@gmail.com      **
*************************************************************


Enter the infile name:(e.g. CHB)
>CHB_
```

5.2 Data Splitting

A data split tool allows the user split data to multiple sets according to population affinity or chromosomes. For example, the users may like to separate the parents from the kids of YRI samples in most of cases, so that unrelated individuals can be analyzed.

Program name	Function
Split_by_Chr	Split data by chromosomes
Split_by_POP	Split data according to population affinities

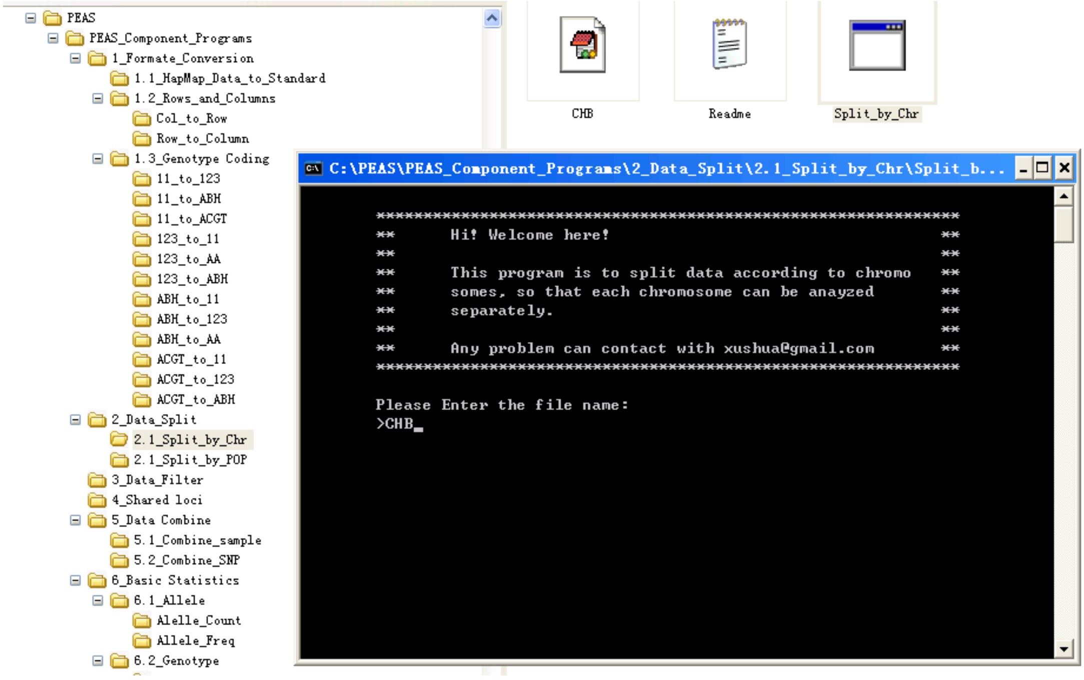
Procedure to run the program:

[1] Double click the executable file;

[2] Enter the infile name:(e.g. CHB);

type CHB, hit Enter;

[3] check the output files.



```
**************************************************************
**      Hi! Welcome here!                                   **
**                                                          **
**      This program is to split data according to chromo   **
**      sones, so that each chromosome can be anayzed       **
**      separately.                                         **
**                                                          **
**      Any problem can contact with xushua@gmail.com       **
**************************************************************

Please Enter the file name:
>CHB_
```

5.3 Data Filtering

A filter tool allows the user filter data by MAF, missing data proportion and HWE states.

Program name	Function
filter	Filter data by MAF, missing data proportion and HWE

Procedure to run the program:

[1] Double click the executable file;

[2] Enter the chromosome name (eg. chr1; chr2; ....chrX,chrY):

type: chr22, hit Enter

[3] Enter the population name (eg. CHB):

type: CHB, hit Enter

[4] Enter sample size : (here for CHB is 84)

type:84, hit Enter

[5] Enter extra column : (e.g. 0)

type:0, hit Enter

[6] Do you want missing data filter on (0 for no;1 for yes) :

type:1, hit Enter

[7] Do you want MAF filter on (0 for no;1 for yes) :

type:1, hit Enter

[8] Do you want HWE filter on (0 for no;1 for yes) :

type:1, hit Enter

[9] Enter Threshold for missing data filter (e.g. 0.05):

type:0.05, hit Enter

[10] Enter Threshold for MAF filter (e.g. 0.05):

type:0.05, hit Enter

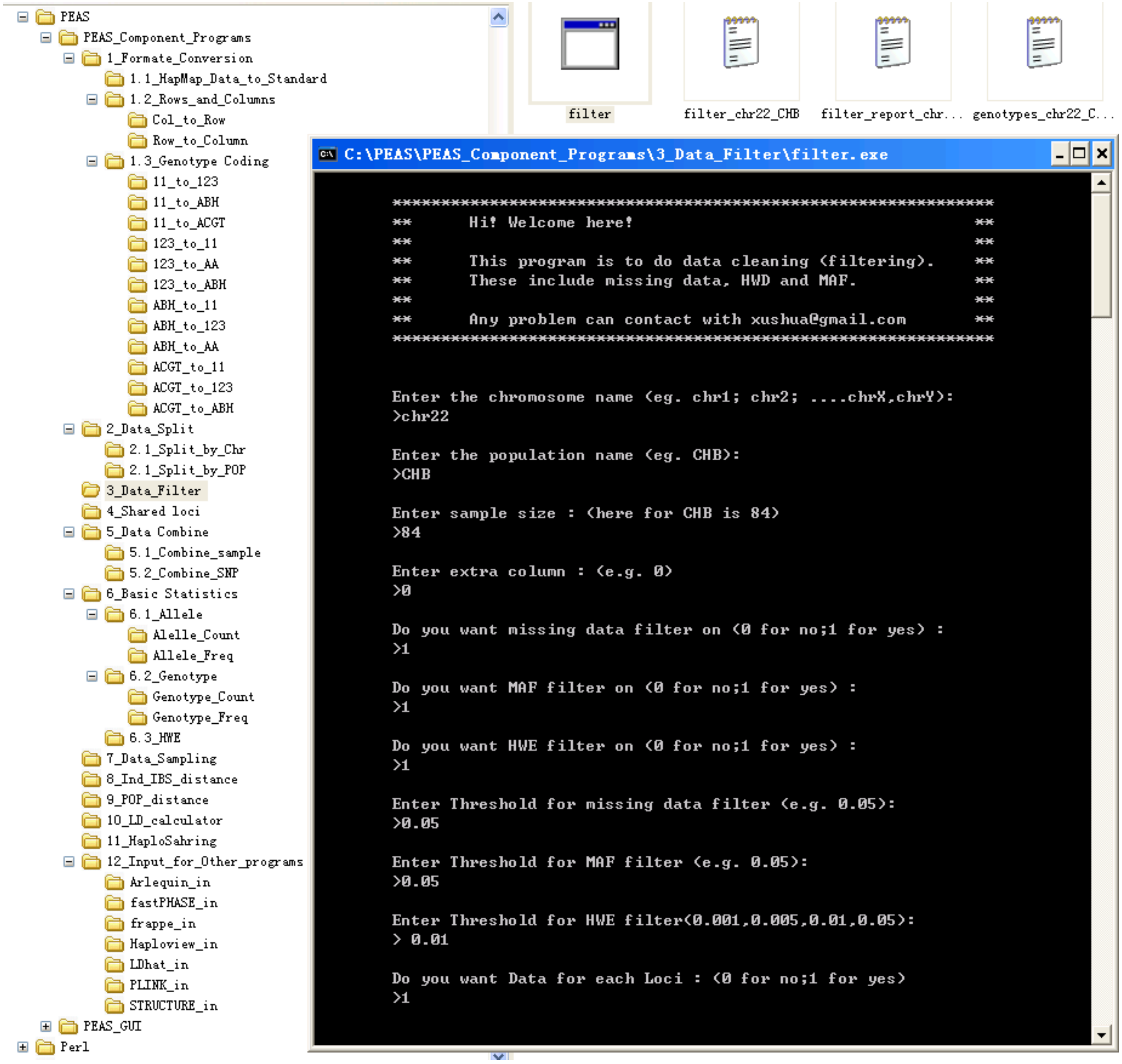
[11] Enter Threshold for HWE filter(0.001,0.005,0.01,0.05):

type: 0.01, hit Enter

[12] Do you want Data for each Loci : (0 for no;1 for yes)

type:1, hit Enter

[13] check the output files.



```
******************************************************************
**      Hi! Welcome here!                                       **
**                                                              **
**      This program is to do data cleaning (filtering).        **
**      These include missing data, HWD and MAF.                **
**                                                              **
**      Any problem can contact with xushua@gmail.com           **
******************************************************************


Enter the chromosome name (eg. chr1; chr2; ....chrX,chrY):
>chr22

Enter the population name (eg. CHB):
>CHB

Enter sample size : (here for CHB is 84)
>84

Enter extra column : (e.g. 0)
>0

Do you want missing data filter on (0 for no;1 for yes) :
>1

Do you want MAF filter on (0 for no;1 for yes) :
>1

Do you want HWE filter on (0 for no;1 for yes) :
>1

Enter Threshold for missing data filter (e.g. 0.05):
>0.05

Enter Threshold for MAF filter (e.g. 0.05):
>0.05

Enter Threshold for HWE filter(0.001,0.005,0.01,0.05):
> 0.01

Do you want Data for each Loci : (0 for no;1 for yes)
>1
```

5.4 Consensus Markers

A consensus data tool allows the users obtain the consensus data for multiple population samples or different resources. The program integrates data according to the information of SNP ID, chromosome, physical position, strand (+/-).

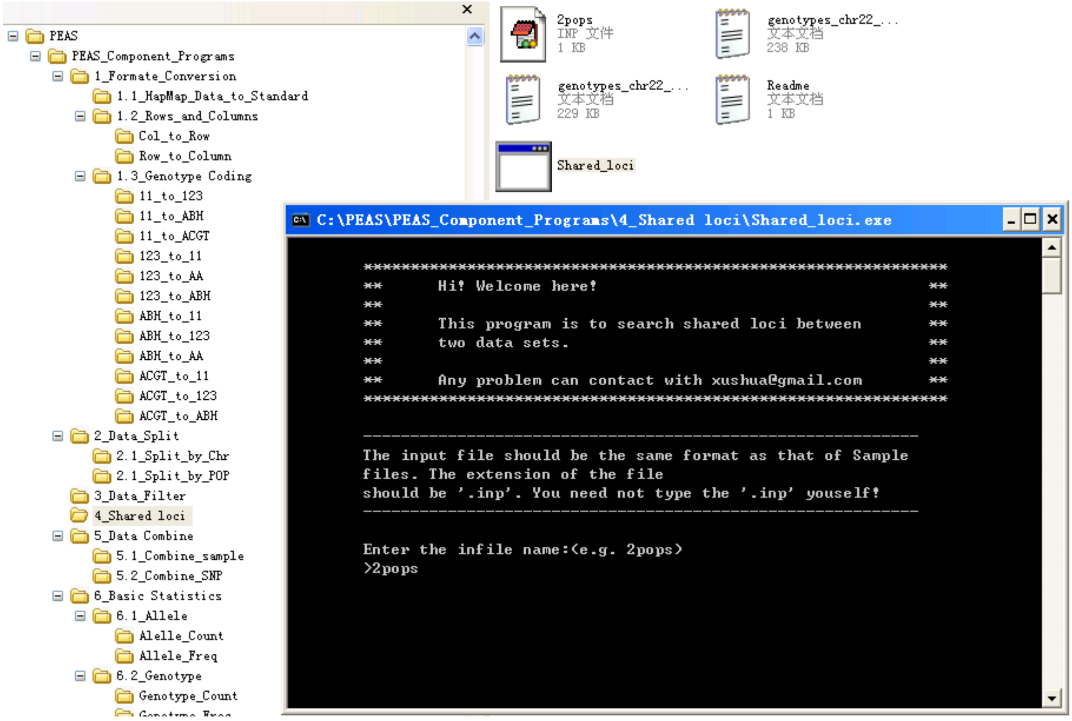
Program name	Function
Shared_loci	To obtain the consensus data for multiple population samples

Procedure to run the program:

[1] Double click the executable file;

[2] Enter the infile name:(e.g. 2pops);
type: 2pops, hit Enter;

[3] check the output files.



C:\PEAS\PEAS_Component_Programs\4_Shared loci\Shared_loci.exe

```
**************************************************************
**      Hi! Welcome here!                                   **
**                                                          **
**      This program is to search shared loci between       **
**      two data sets.                                      **
**                                                          **
**      Any problem can contact with xushua@gmail.com       **
**************************************************************

-----------------------------------------------------------
The input file should be the same format as that of Sample
files. The extension of the file
should be '.inp'. You need not type the '.inp' youself!
-----------------------------------------------------------

Enter the infile name:(e.g. 2pops)
>2pops
```

5.5 Data integration

A data integrate tool allows the user integrate multiple data sets by samples or by chromosomes or by both. For example, people may like to integrate data sets from different population samples when they perform fastPHASE analysis or STRUCTURE analysis.

Program name	Function
Combine_sample	To combine data for multiple population samples
Combine_snp	To combine data for different SNP markers

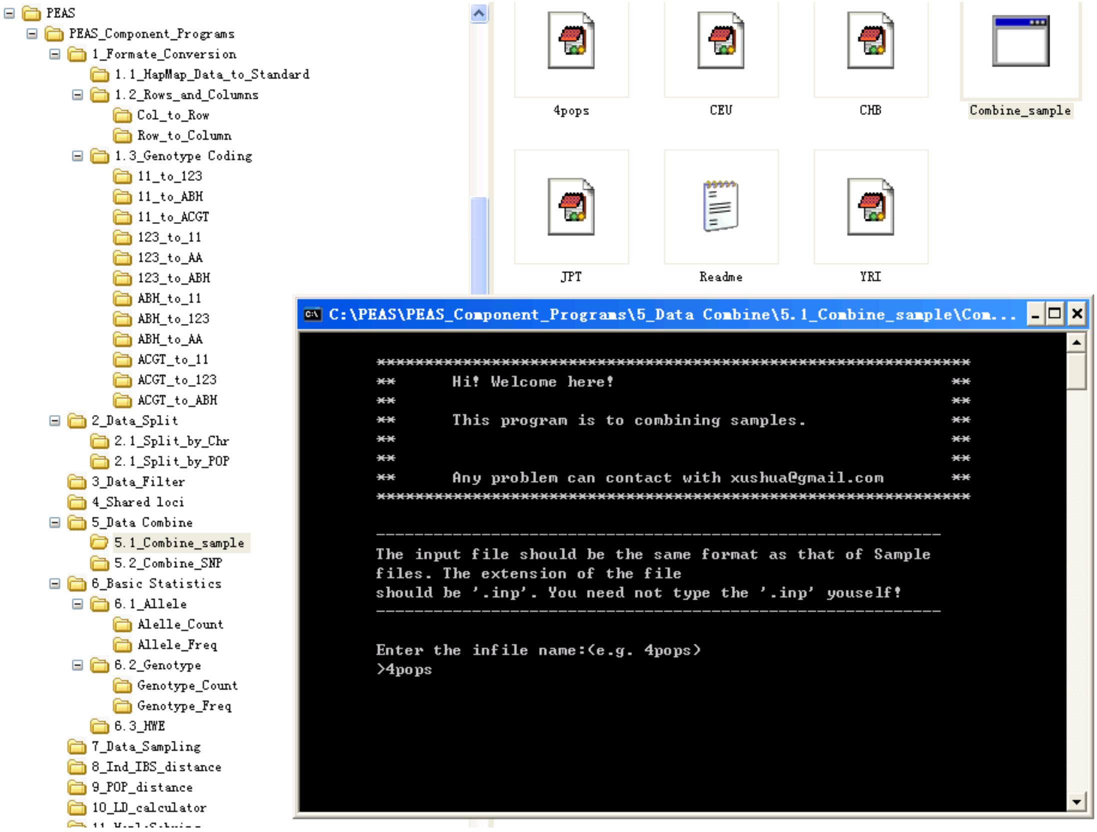
Procedure to run the program:

[1] Double click the executable file;

[2] Enter the infile name:(e.g. 4pops);

type: 4pops, hit Enter;

[3] check the output files.



```
        **************************************************************
        **      Hi! Welcome here!                                   **
        **                                                          **
        **      This program is to combining samples.               **
        **                                                          **
        **                                                          **
        **      Any problem can contact with xushua@gmail.com       **
        **************************************************************

        -----------------------------------------------------------
        The input file should be the same format as that of Sample
        files. The extension of the file
        should be '.inp'. You need not type the '.inp' youself!
        -----------------------------------------------------------

        Enter the infile name:(e.g. 4pops)
        >4pops
```

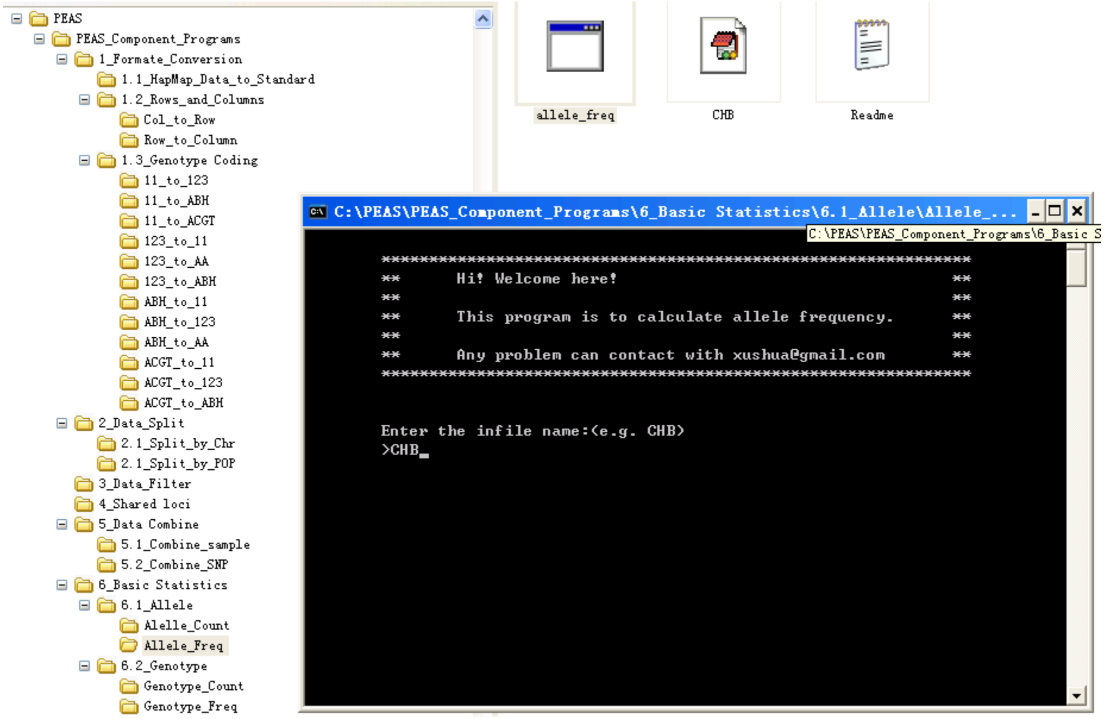
5.6 Basic Statistics

Tools to allow the user calculate allele frequency and genotype frequency, examine the HWE state of each locus.

Program name	Function
allele_count	To conunt number of alleles for each SNP
allele_freq	To calculate allele frequency for each SNP
genotype_count	To conunt number of genotypes for each SNP
genotype_freq	To calculate genotype frequency for each SNP
hwe	To test HWE for each SNP

Procedure to run the program:

[1] Double click the executable file;
[2] Enter the infile name:(e.g. CHB);
type: CHB, hit Enter;
[3] check the output files.



```
**************************************************************
**      Hi! Welcome here!                                   **
**                                                          **
**      This program is to calculate allele frequency.      **
**                                                          **
**      Any problem can contact with xushua@gmail.com       **
**************************************************************


Enter the infile name:(e.g. CHB)
>CHB_
```

5.7 Data Sampling

A sampling tool allows the user sample subsets of data by markers.

Program name	Function
SNP_Sampler	To sample subsets of data by markers, there are several options, sub-datasets can be generated by random sampling (bootstraping), or by setting inter-marker distance, or by setting a paticular heterozygosity, or by setting both inter-marker distance and heterozygosity.

Procedure to run the program:

[1] Double click the executable file;

[2] Enter the infile name:(e.g. 4pops);
type: 4pops, hit Enter;

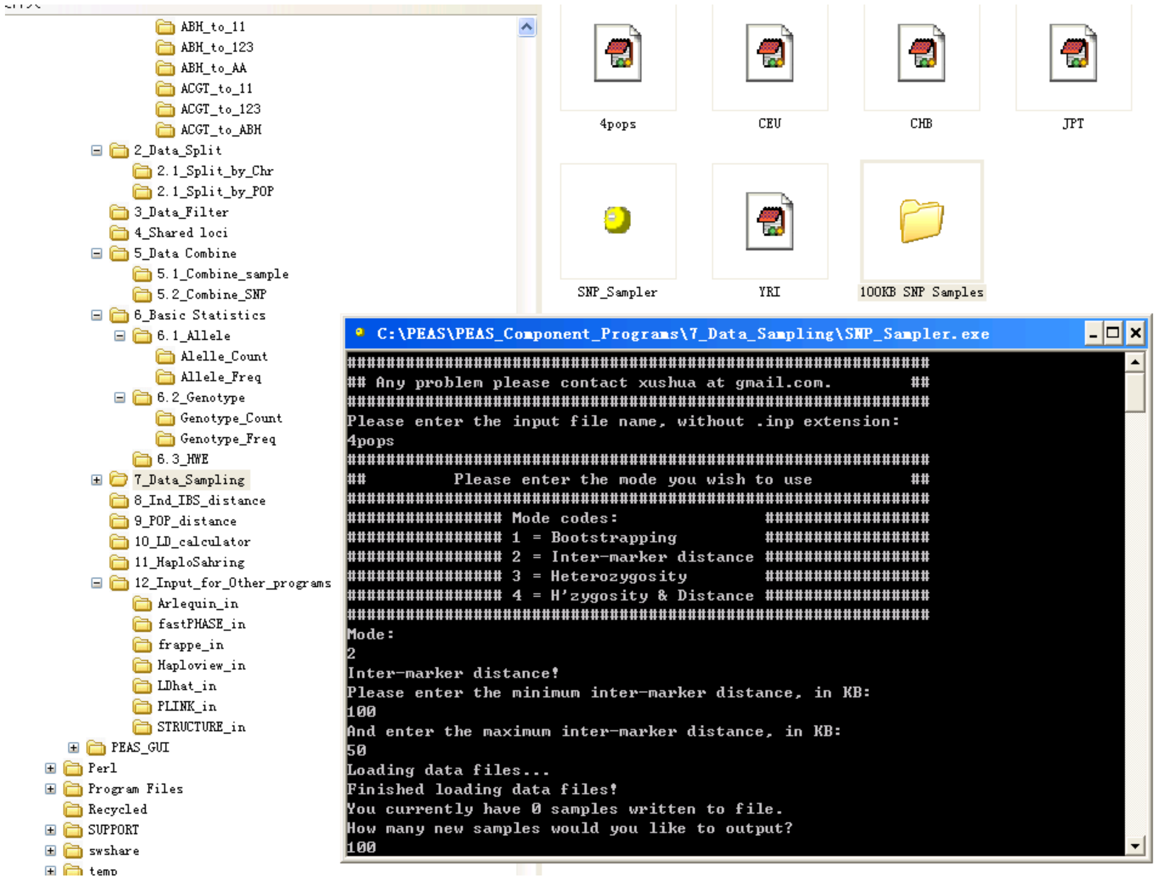
[3] chose mode (1, 2, 3, 4);
type: 2, hit Enter;
Inter-marker distance!

[4] Please enter the minimum inter-marker distance, in KB:
type: 100, hit Enter;

[5] And enter the maximum inter-marker distance, in KB:
type: 50, hit Enter;
Loading data files...
Finished loading data files!
You currently have 0 samples written to file.

[6] How many new samples would you like to output?
type: 100, hit Enter;

[7] check the output files.



```
############################################################
## Any problem please contact xushua at gmail.com.        ##
############################################################
Please enter the input file name, without .inp extension:
4pops
############################################################
##         Please enter the mode you wish to use          ##
############################################################
################ Mode codes:               #################
################ 1 = Bootstrapping         #################
################ 2 = Inter-marker distance #################
################ 3 = Heterozygosity        #################
################ 4 = H'zygosity & Distance #################
############################################################
Mode:
2
Inter-marker distance!
Please enter the minimum inter-marker distance, in KB:
100
And enter the maximum inter-marker distance, in KB:
50
Loading data files...
Finished loading data files!
You currently have 0 samples written to file.
How many new samples would you like to output?
100
```

5.8 Individual Distance

A program allows the user calculate allele sharing distance between each pair of individuals.

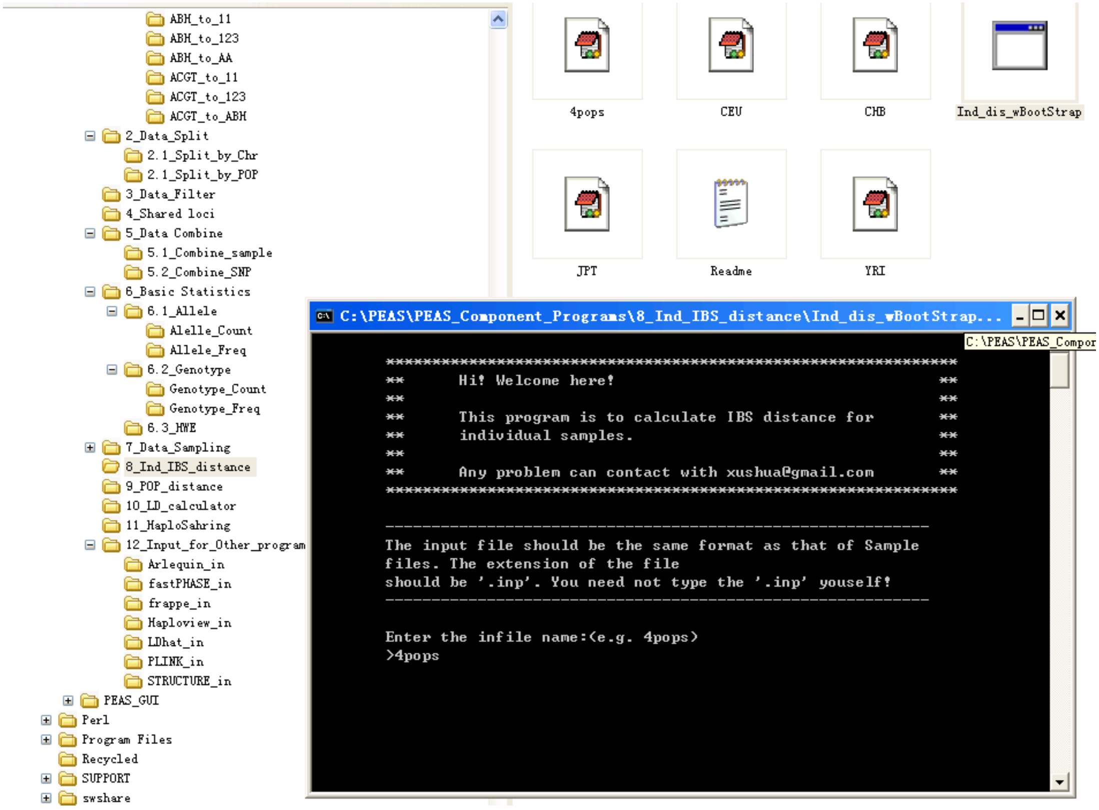
Program name	Function
Ind_dis_wBootStrap	This program will generate multiple distance matrixes by bootstrapping the loci, and provides the output files that can be read by MEGA (Kumar, Tamura et al. 2004) and PHYLIP (Felsenstein 1989) programs for further processing.

Procedure to run the program:

[1] Double click the executable file;

[2] Enter the infile name:(e.g. 4pops);
type: 4pops, hit Enter;

[3] check the output files.



```
C:\PEAS\PEAS_Component_Programs\8_Ind_IBS_distance\Ind_dis_wBootStrap...

**************************************************************
**      Hi! Welcome here!                                   **
**                                                          **
**      This program is to calculate IBS distance for       **
**      individual samples.                                 **
**                                                          **
**      Any problem can contact with xushua@gmail.com       **
**************************************************************

-----------------------------------------------------------
The input file should be the same format as that of Sample
files. The extension of the file
should be '.inp'. You need not type the '.inp' youself!
-----------------------------------------------------------

Enter the infile name:(e.g. 4pops)
>4pops
```

5.9 Population Distance

A program allows the user calculate distances for populations and generates multiple distance matrixes by bootstrapping the loci.

Program name	Function
POP_dis	The population distances that PEAS can provide including Wright's F_{ST}, F_{ST} distance, Nei's standard distance, Nei's DA distance and Cavalli-Sforza's DC distance. The program generate also output files which can be recognized by MEGA and PHYLIP programs for further processing.

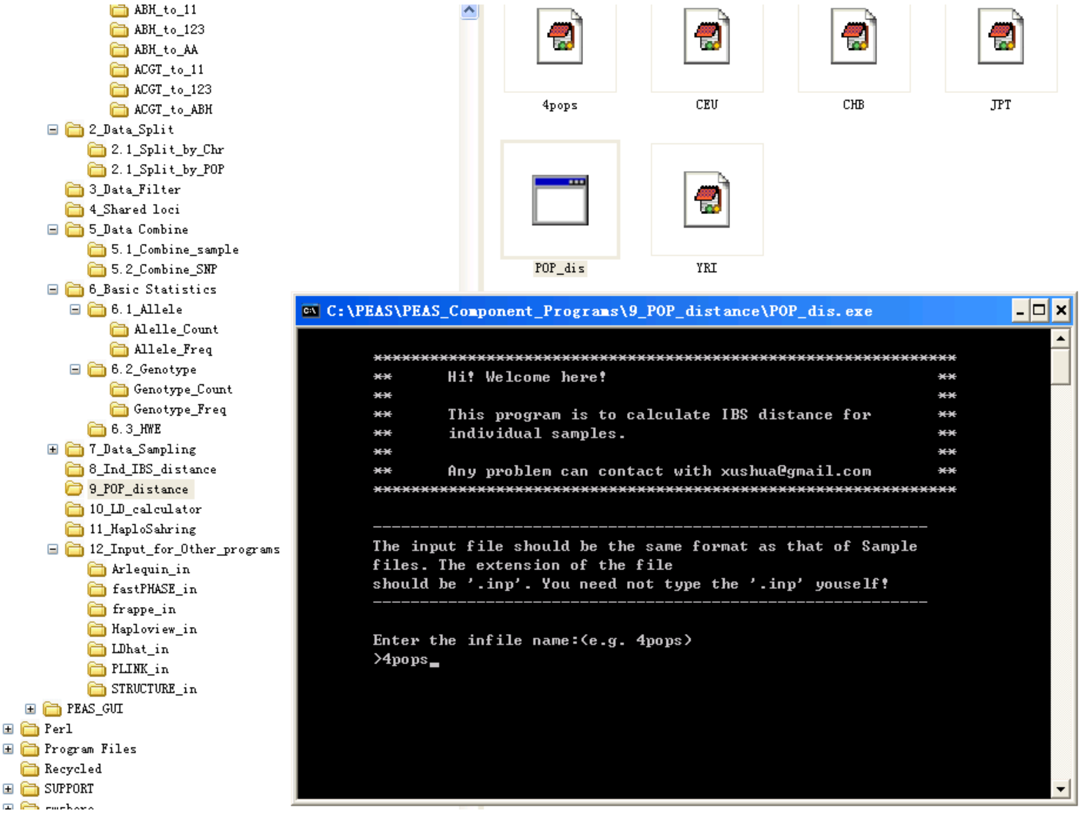
Procedure to run the program:

[1] Double click the executable file;

[2] Enter the infile name:(e.g. 4pops);

type: 4pops, hit Enter;

[3] check the output files.



```
*******************************************************************
**      Hi! Welcome here!                                        **
**                                                               **
**      This program is to calculate IBS distance for            **
**      individual samples.                                      **
**                                                               **
**      Any problem can contact with xushua@gmail.com            **
*******************************************************************

-----------------------------------------------------------------
The input file should be the same format as that of Sample
files. The extension of the file
should be '.inp'. You need not type the '.inp' youself!
-----------------------------------------------------------------

Enter the infile name:(e.g. 4pops)
>4pops_
```

5.10 LD calculator

A program allows the user calculate the two most commonly used LD statistics (r^2 and |*D'*|) and generate LD distribution report files which can be used to plot in MS Excel. This feature is especially useful for very large data set with huge number of SNP sites.

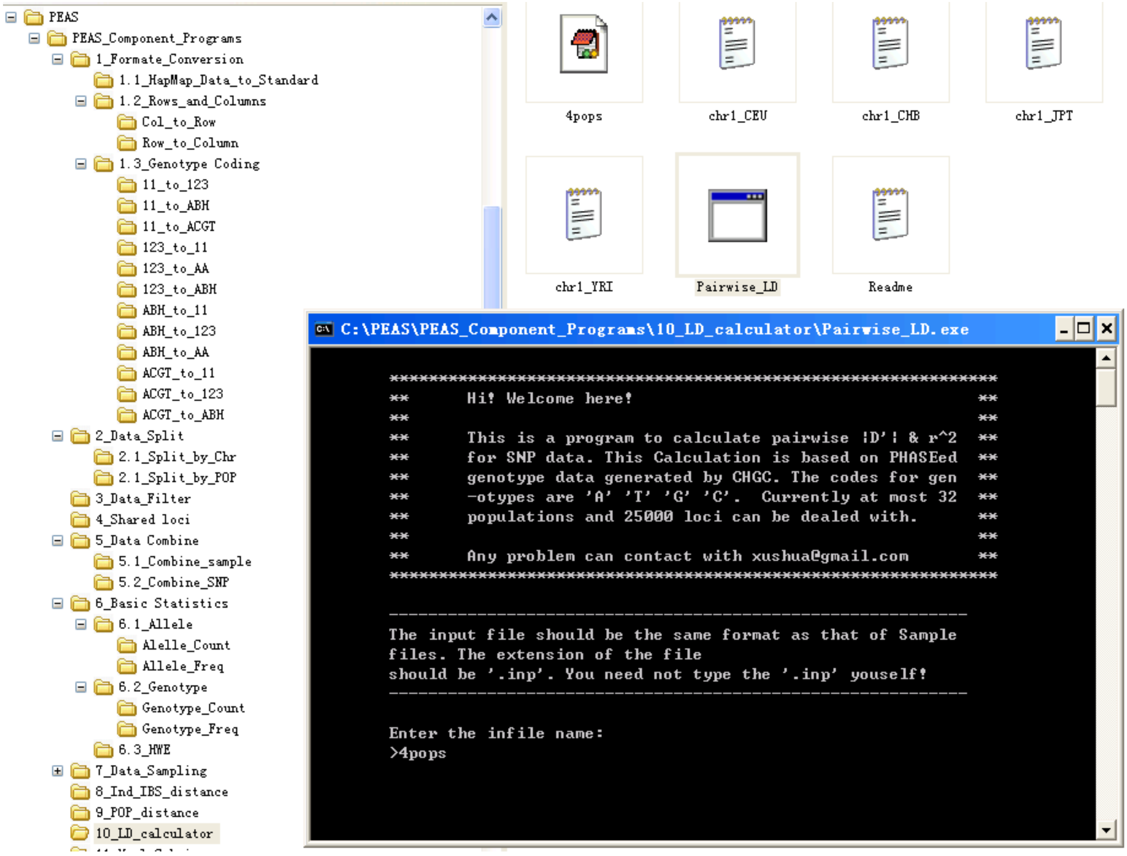
Program name	Function
Pairwise_LD	To calculate the two most commonly used LD statistics (r^2 and |*D'*|). It can handle data from multiple population samples and chromosomes. A summary statistics table will be generated.

Procedure to run the program:

[1] Double click the executable file;

[2] Enter the infile name:(e.g. 4pops);
type: 4pops, hit Enter;

[3] check the output files.



```
**************************************************************
**      Hi! Welcome here!                                   **
**                                                          **
**      This is a program to calculate pairwise |D'| & r^2  **
**      for SNP data. This Calculation is based on PHASEed  **
**      genotype data generated by CHGC. The codes for gen  **
**      -otypes are 'A' 'T' 'G' 'C'.  Currently at most 32  **
**      populations and 25000 loci can be dealed with.      **
**                                                          **
**      Any problem can contact with xushua@gmail.com       **
**************************************************************

-----------------------------------------------------------
The input file should be the same format as that of Sample
files. The extension of the file
should be '.inp'. You need not type the '.inp' youself!
-----------------------------------------------------------

Enter the infile name:
>4pops
```

5.11 Haplotype sharing analysis.

Program name	Function
HaploSharing	To calculate haplotype sharing statistics as we proposed in a recent MBE paper (Xu, Jin et al. 2009).

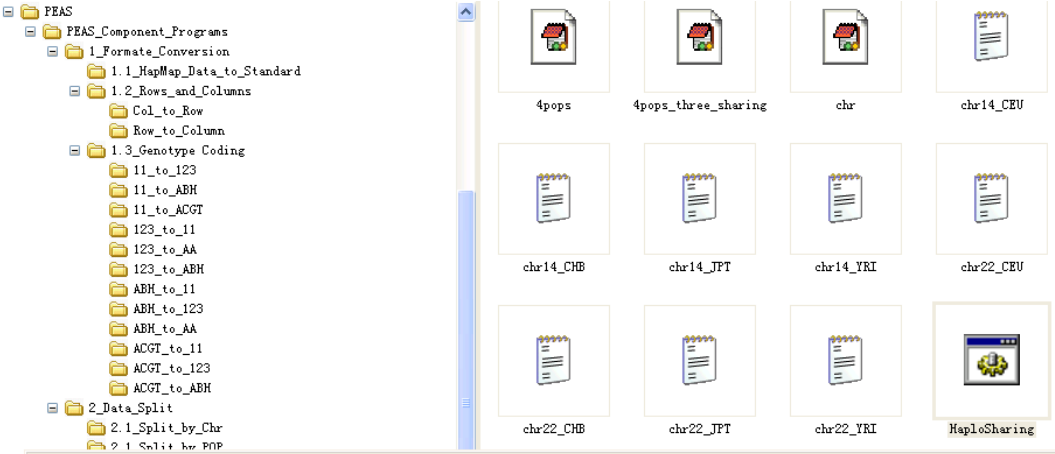
Procedure to run the program:

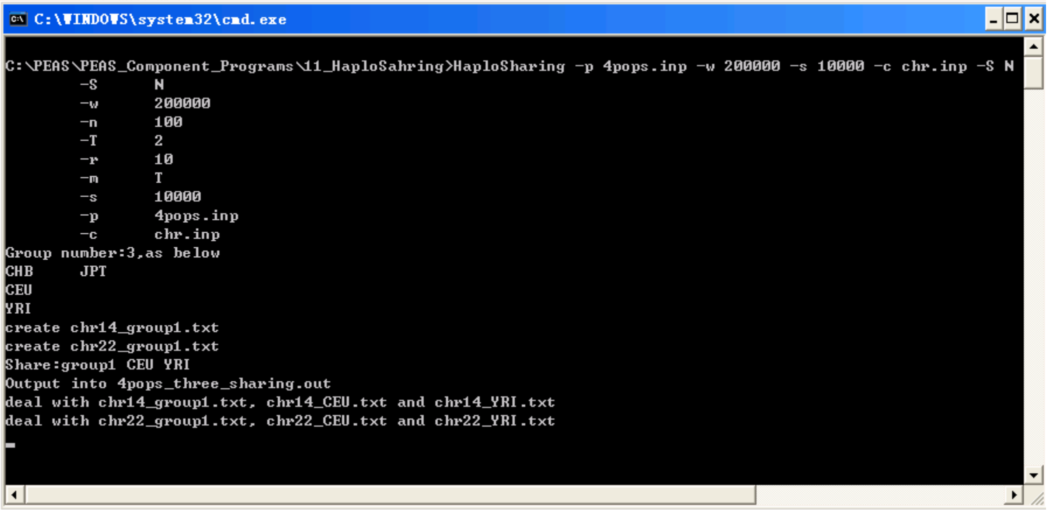
[1] Double click the file "HaploSharing.bat";

[2] check the output files;

[3] you can edit the parameters in HaploSharing.bat.
For example: HaploSharing -p 4pops.inp -w 200000 -s 10000 -c chr.inp -S N -n 100 -r 10 -m T -T 2

[4] check the output files.





```
C:\PEAS\PEAS_Component_Programs\11_HaploSahring>HaploSharing -p 4pops.inp -w 200000 -s 10000 -c chr.inp -S N
        -S      N
        -w      200000
        -n      100
        -T      2
        -r      10
        -m      T
        -s      10000
        -p      4pops.inp
        -c      chr.inp
Group number:3,as below
CHB     JPT
CEU
YRI
create chr14_group1.txt
create chr22_group1.txt
Share:group1 CEU YRI
Output into 4pops_three_sharing.out
deal with chr14_group1.txt, chr14_CEU.txt and chr14_YRI.txt
deal with chr22_group1.txt, chr22_CEU.txt and chr22_YRI.txt
_
```

5.12 Other Software Input

A serious of tools to provide the user input files for many popular softwares which include fastPHASE, PHASE, STRUCTURE, Haploview, Arlequin, LDhat and PLINK.

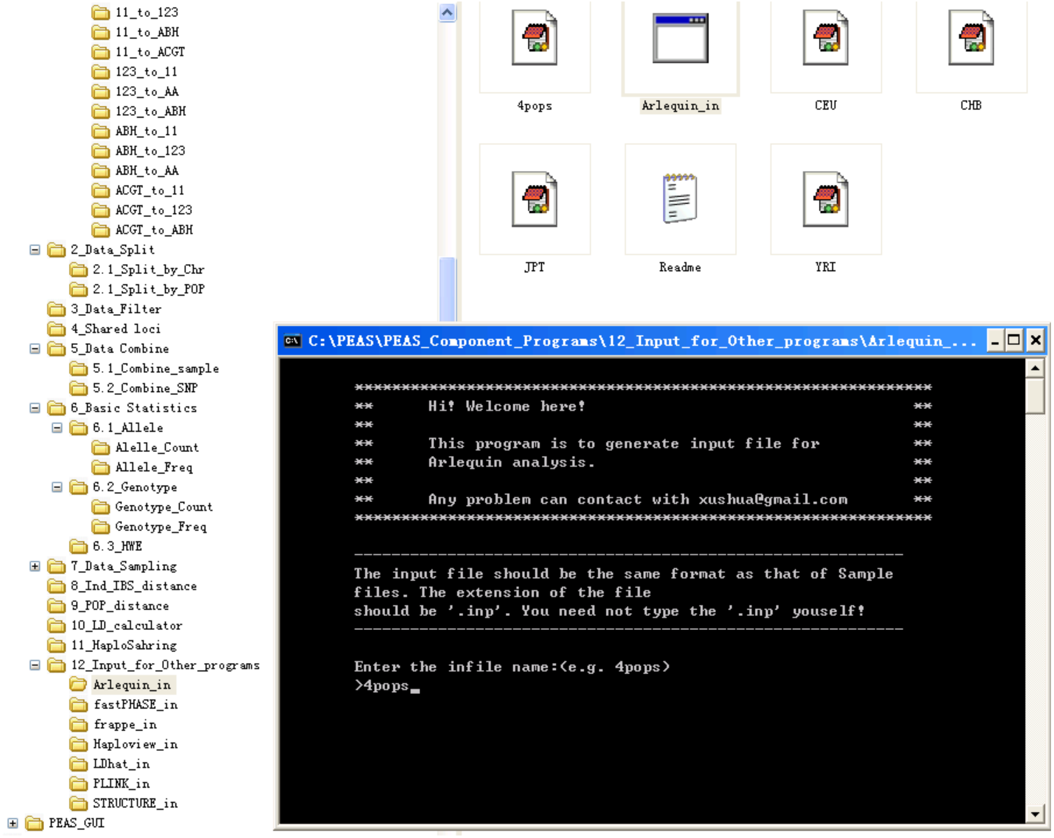
Program name	Function
Arlequin_in	To generate input file for Arleauin analysis
fastPHASE_in	To generate input file for fastPHASE analysis
frappe_in	To generate input file for *frappe* analysis
Haploview_in	To generate input file for Haploview analysis
LDhat_in	To generate input file for LDHat analysis
PLINK_in	To generate input file for PLINK analysis
STRUCTURE_in	To generate input file for STRUCTURE analysis

Procedure to run the program:

[1] Double click the executable file;

[2] Enter the infile name:(e.g. 4pops);
type: 4pops, hit Enter;

[3] check the output files.



```
************************************************************
**      Hi! Welcome here!                                 **
**                                                        **
**      This program is to generate input file for        **
**      Arlequin analysis.                                **
**                                                        **
**      Any problem can contact with xushua@gmail.com     **
************************************************************


----------------------------------------------------------
The input file should be the same format as that of Sample
files. The extension of the file
should be '.inp'. You need not type the '.inp' youself!
----------------------------------------------------------

Enter the infile name:(e.g. 4pops)
>4pops_
```

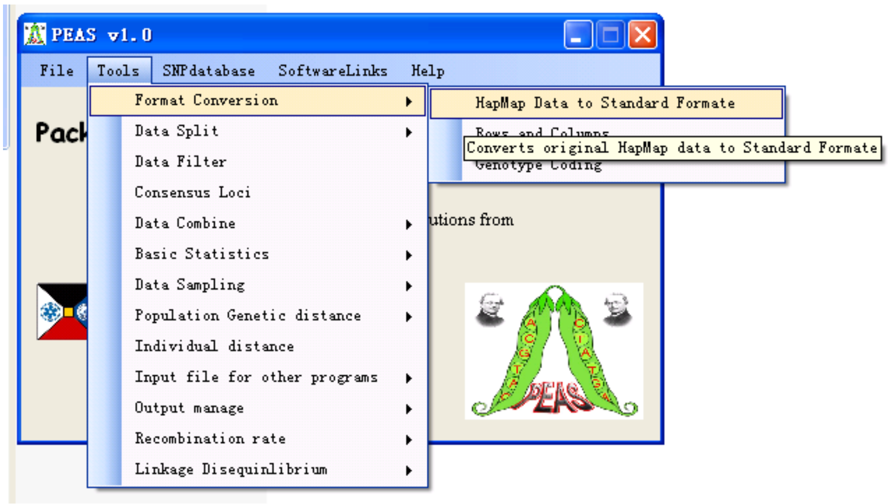
6 Functions in GUI

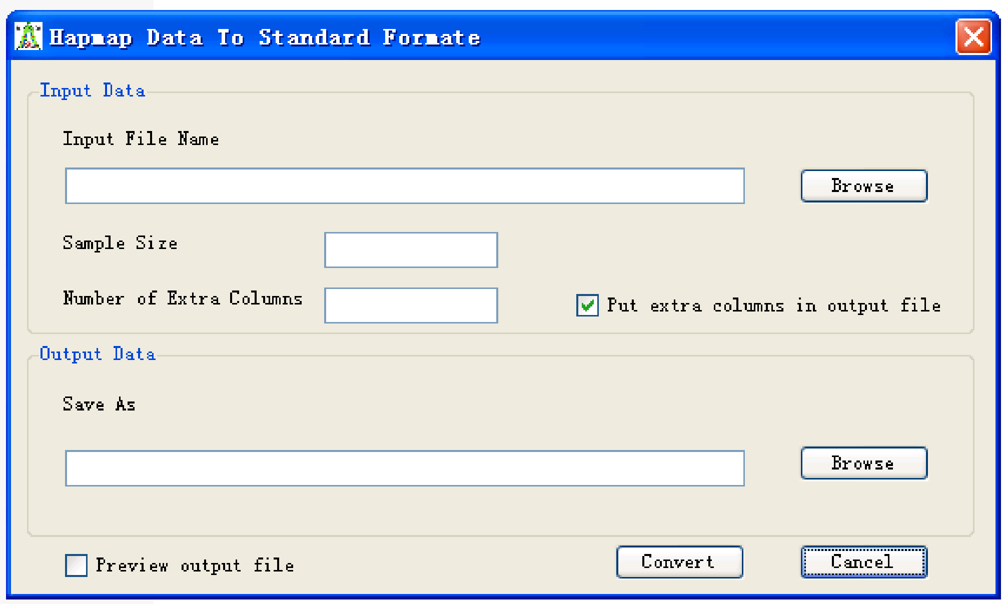
6.1 Basic data format conversion tools.

PEAS are able to transform all the 8 formats mentioned above from one the user supplied to all the other seven ones. However, to use PEAS to do further analysis, we recommend user convert their data into the standard format (with ABHU coding for all possible genotypes for each SNP), because this format is easy to be handled by all the program components in PEAS package.

6.1.1 HapMap data to Standard format

We provide a special tool for converting HapMap data format to our standard format, as shown in the following snapshots.





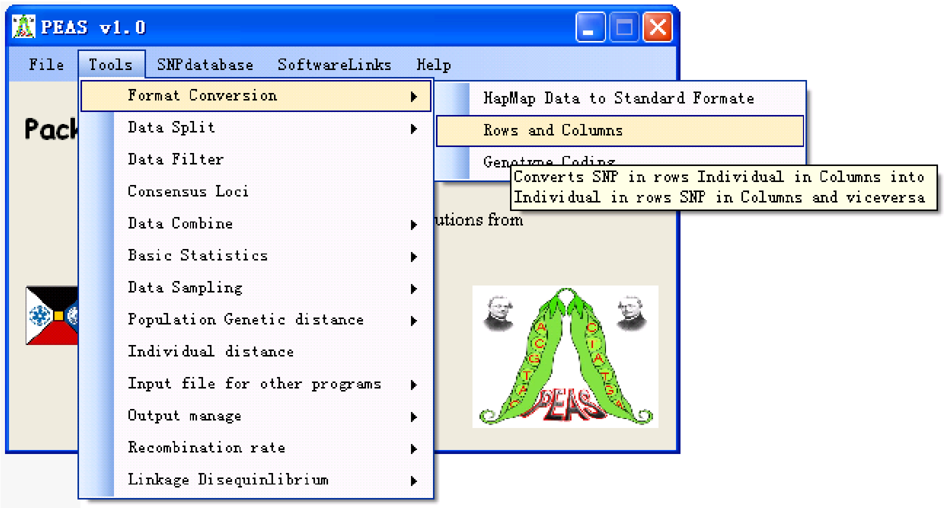
☑ Put extra columns in output file

☐ Preview output file

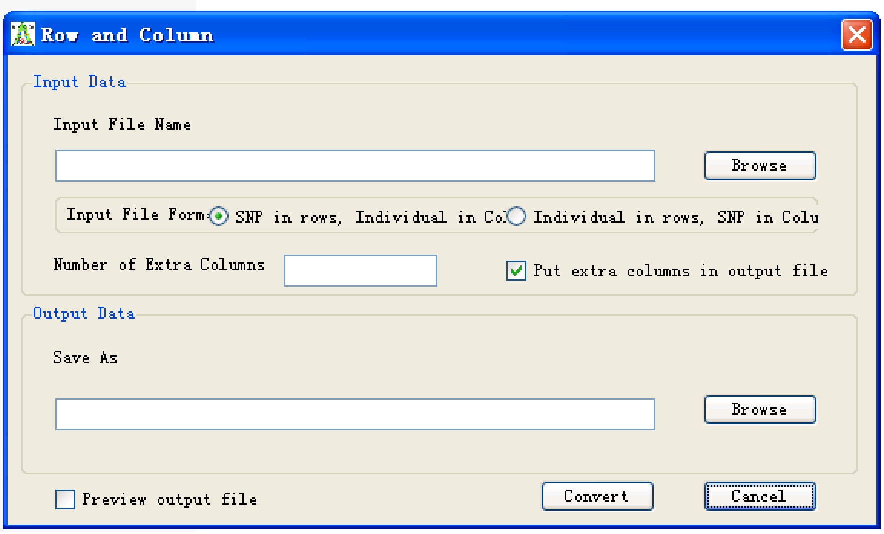
6.1.2 Two format conversion tools to transpose data between columns and rows.

Since in most occasions the number of SNPs is much larger than that of individual samples, the common format of SNP data is markers in rows and individuals in colums, such as those in HapMap database. There are also many software using pedgree-like format as input, such as PLINK, EIGENSOFT etc.

We provided a tool to convert data between rows and columns, as shown in the following snapshots.



Converts SNP in rows Individual in Columns into Individual in rows SNP in Columns and viceversa



Row and Column

Input Data

Input File Name

Browse

Input File Form ● SNP in rows, Individual in Co ○ Individual in rows, SNP in Colu

Number of Extra Columns

☑ Put extra columns in output file

Output Data

Save As

Browse

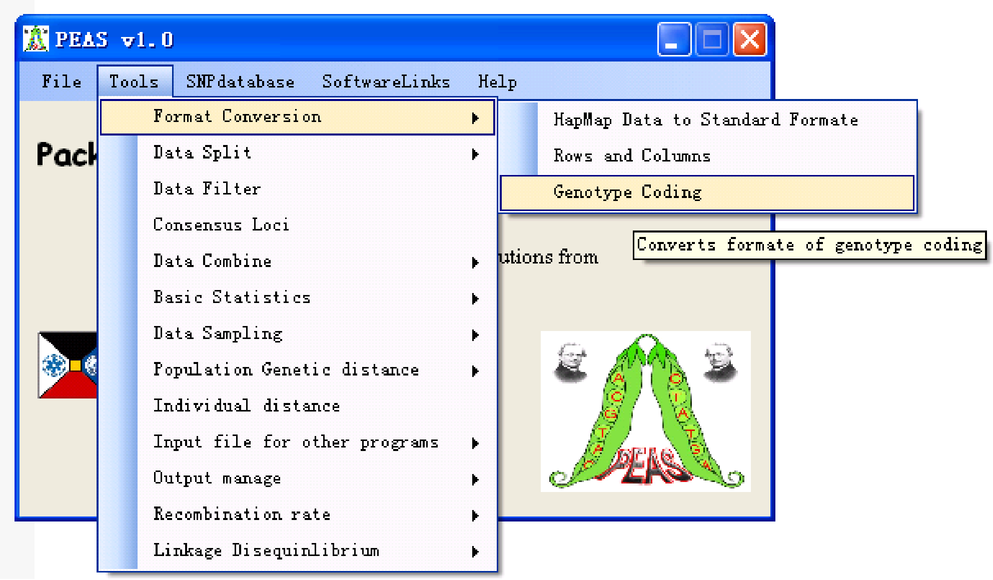
☐ Preview output file

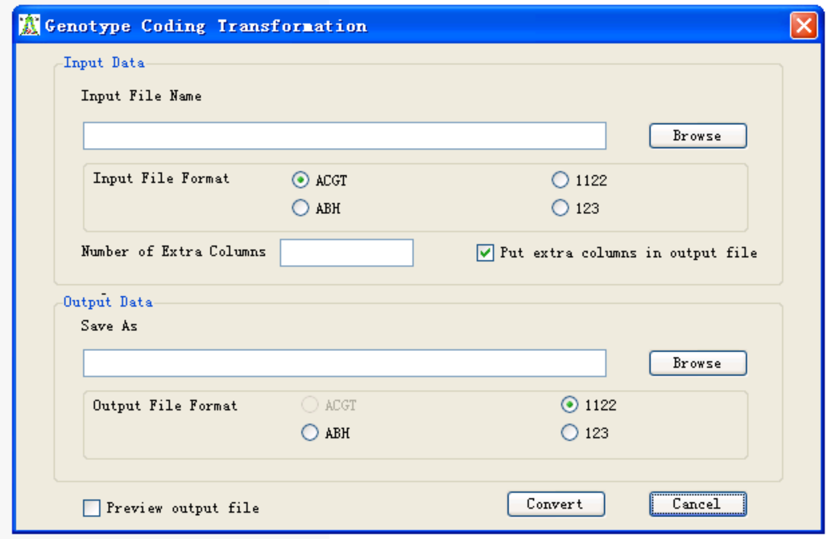
Convert

Cancel

6.1.3 Genotype coding transformation

Genotypes of a SNP can be coded as either characters or numbers. As data from different sources could have different coding schemes, we provide a tool to do conversion between these different genetype codings. These include the following possible conversions: ACGT to 1122; ACGT to ABH; ACGT to 123; 1122 to ACGT; 1122 to ABH; 1122 to 123; ABH to ACGT; ABH to 1122; ABH to 123.





Input File Format ● ACGT ○ 1122 ○ ABH ○ 123

Number of Extra Columns ☑ Put extra columns in output file

Output File Format ○ ACGT ● 1122 ○ ABH ○ 123

□ Preview output file

6.2 Data split

6.2.1 Split data by samples

In some cases, data of all the samples may stored in one single file, if the user want to separate certain group samples, such as separate kids from parents, as YRI and CEU in HapMap data, or separate samples of one population from the other populations, as

CHB+JPT data file in HapMap data. PEAS reads one file with sample information that the user defined, then separate data by sample groups that the user defined.

The structure for sample information file can be represented as follows:

```
NumberOfIndividuals
NumberOfExtraColumns
NumberOfGroups
GroupIndicator    GroupName
GroupIndicator    GroupName
…
GroupIndicator    GroupName

SampleNumber      GroupIndicator
SampleNumber      GroupIndicator
…
SampleNumber      GroupIndicator
```

Where the quantities above are as follows:

1. **NumberOfIndividuals** An integer specifying the number of individuals who have been genotyped. It is often the total sample size in the original data file.
2. **NumberOfExtraColumns** An integer specifying the number of extra columns which is relative to the standard format of genotype file. It is actually the number of columns, if any, between the strand information (the fifth column) and genotype data (the sixth column without extra columns).
3. **NumberOfGroups** An integer specifying the number of groups that all the samples will be divided into.
4. **GroupIndicator** An integer will be taken as indicator of group, the number of **GroupIndicator**s must be the same as the number of groups, i.e. the total number of **GroupIndicator**s used must be **NumberOfGroups**.
5. **GroupName** A string indicating the name of group, this is also used to name the files that store the data of this group samples.

6. **SampleNumber** An integer indicating the samples in the order of that in original data file, i.e. the first sample specified by 1, the second sample by 2, and so on., followed by **GroupIndicator** defined previously indicating which group this sample is of.

An example file to show the sample information of CEU in HapMap project is as follows:

```
90
6
2
0 parent
1 kid

1    0
2    1
3    0
4    0
5    0
6    1
7    0
8    1
9    0
10   1
11   0
12   0
13   0
14   1
15   0
16   1
17   1
18   1
19   1
20   1
21   1
22   1
23   1
24   1
25   1
26   1
27   1
```

28	1
29	1
30	1
31	1
32	0
33	0
34	0
35	0
36	0
37	0
38	0
39	0
40	0
41	0
42	0
43	0
44	0
45	0
46	0
47	0
48	0
49	0
50	0
51	0
52	0
53	0
54	0
55	0
56	0
57	0
58	0
59	0
60	0
61	0
62	0
63	0
64	1
65	0
66	0
67	1
68	0
69	0
70	1
71	1
72	0

```
73    0
74    0
75    0
76    1
77    1
78    0
79    0
80    0
81    0
82    1
83    1
84    0
85    0
86    0
87    0
88    1
89    0
90    0
```

In this example file, the first number says there are 90 CEU individuals. The second number says there are 6 extra columns relative to the standard format of genotype file. The third number says the 90 individuals will be separated as 2 groups. The fourth line indicates 0 will be used as indicator of parent group. The fifth line indicates 1 will be used as indicator of kid group. The followed lines indicate how the 90 individuals should be grouped, for example, the first individual (1) is of parent group (indicator 0), the second individual (2) is of kid group (indicator 1), and the last individual (90) is of parent group (indicator 0).

If the user run **SampleSplit** program, the original file will be separated as two files, one file named "*_parent" store the genotype data of 60 parents with the same format as the original data file, the other file named "*_kid" store the genotype data of 30 kids with the same format as the original data file.

There is an option let the user chose to output the extra columns or not.

Another example is separate CHB and JPT samples in HapMap project.

```
90
```

```
6
2
0 CHB
1 JPT

1	0
2	0
3	0
4	0
5	0
6	0
7	0
8	0
9	0
10	0
11	0
12	0
13	0
14	0
15	0
16	0
17	0
18	0
19	0
20	0
21	0
22	0
23	0
24	0
25	0
26	0
27	0
28	0
29	0
30	0
31	0
32	0
33	0
34	0
35	0
36	0
37	0
38	0
39	0
40	0
```

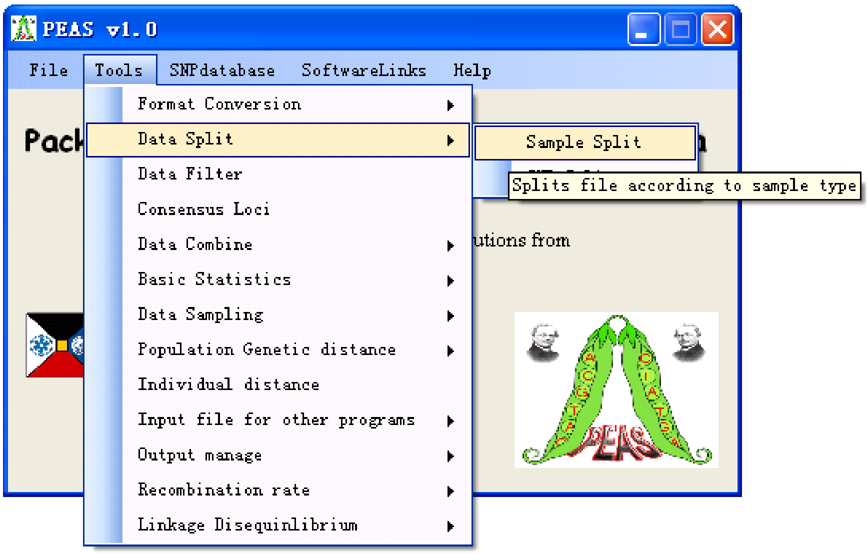
41	0
42	0
43	0
44	0
45	0
46	1
47	1
48	1
49	1
50	1
51	1
52	1
53	1
54	1
55	1
56	1
57	1
58	1
59	1
60	1
61	1
62	1
63	1
64	1
65	1
66	1
67	1
68	1
69	1
70	1
71	1
72	1
73	1
74	1
75	1
76	1
77	1
78	1
79	1
80	1
81	1
82	1
83	1
84	1
85	1

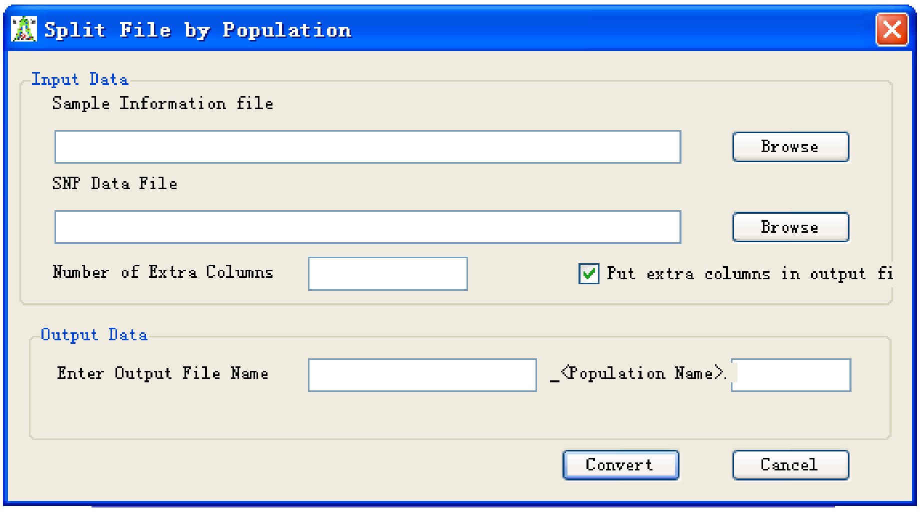
```
86    1
87    1
88    1
89    1
90    1
```

In this example file, the first number says there are 90 individuals. The second number says there are 6 extra columns relative to the standard format of genotype file. The third number says the 90 individuals will be separated as 2 groups. The fourth line indicates 0 will be used as indicator of CHB group. The fifth line indicates 1 will be used as indicator of JPT group. The followed lines indicate how the 90 individuals should be grouped, for example, the first 45 individual (1 to 45) is of CHB group (indicator 0), and the rest 45 individual (46 to 90) is of JPT group (indicator 1).

If the user run **SampleSplit** program, the original file will be separated as two files, one file named "*_CHB" store the genotype data of 45 CHB individuals with the same format as the original data file, the other file named "*_JPT" store the genotype data of 45 JPT individuals with the same format as the original data file.

Note 5: PEAS can split the samples to any number of groups, as defined by the user in the information file, but to make this split to be meaningful, the number of groups should be no more than the total number of individuals, i.e. the maximal number of groups should be smaller than the total sample size.

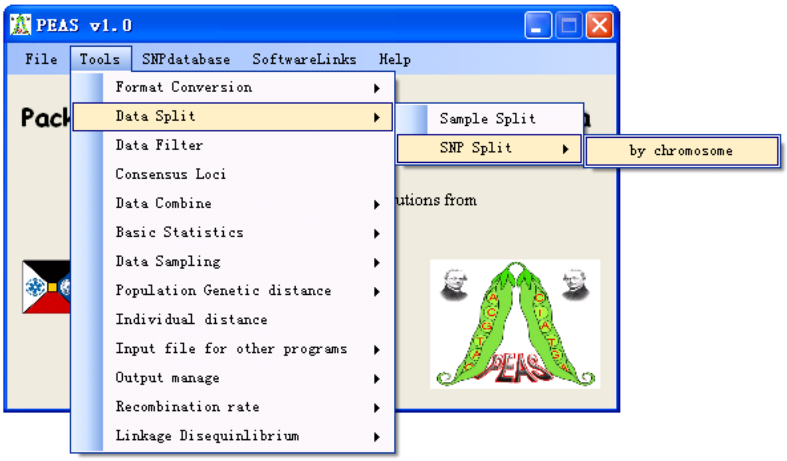


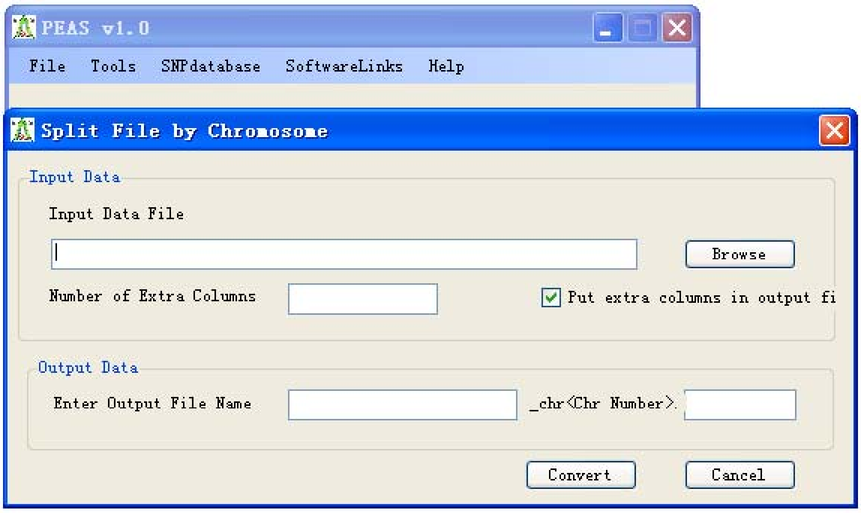


☑ Put extra columns in output fi

6.2.2 Split data by markers

PEAS can also split data by chromosome information, so that data of each single chromosome can be analyzed separately.

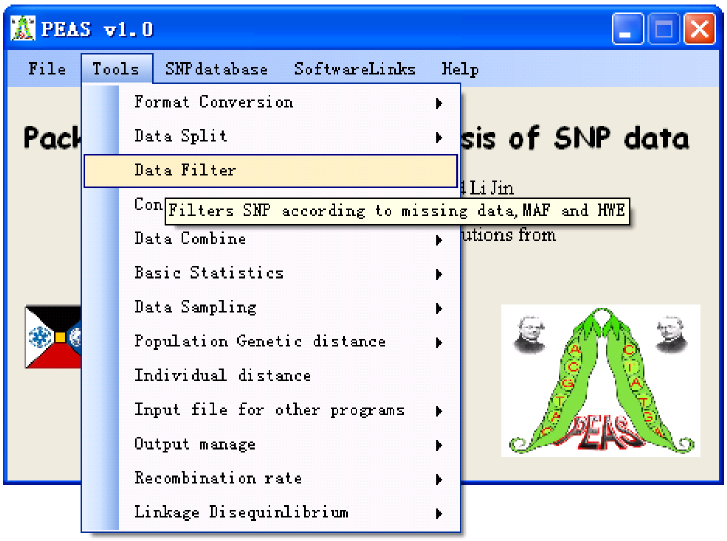


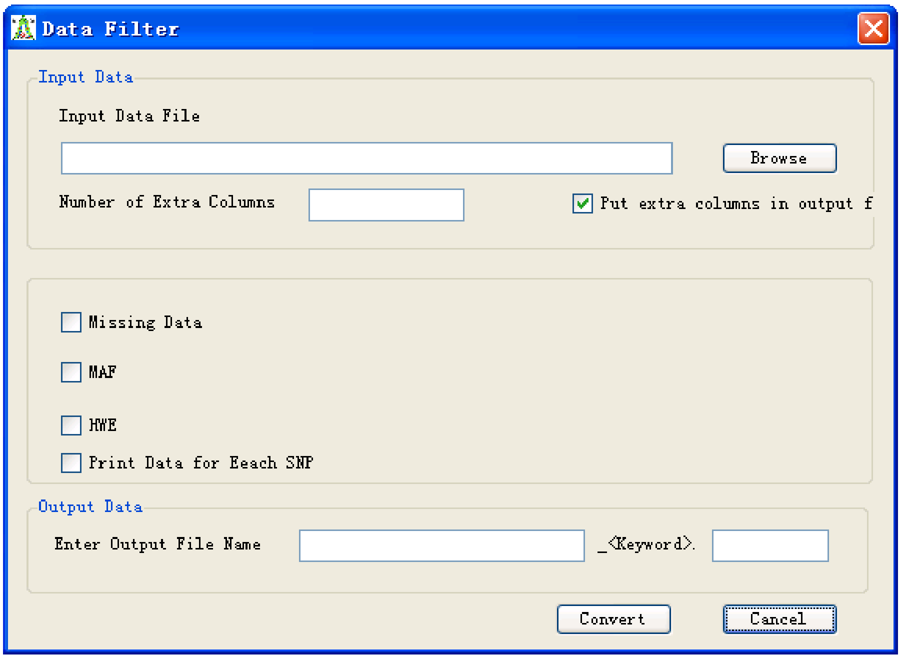


☑ Put extra columns in output fi

6.3 Data filtering

PEAS has three basic data filters, missing data filter, HWD filter and MAF filter, which are implemented in the program **DataFilter**. PEAS allow user set thresholds for missing data control, HWE test p-values and MAF lower bound. PEAS generate a report file which lists missing data proportion, HWE p-value and MAF for each site, and also the distribution of missing data, MAF, which the user can specify the intervals for each distribution.





6.4 Search shared loci

For many purposes of data analysis, it is necessary to use the shared loci among multiple populations. PEAS provides a program **SharedLociSearcher** to search the shared loci among populations. The user should provide a file specify some basic information of the populations, the structure of the file is as follows:

NumberOfPopulations

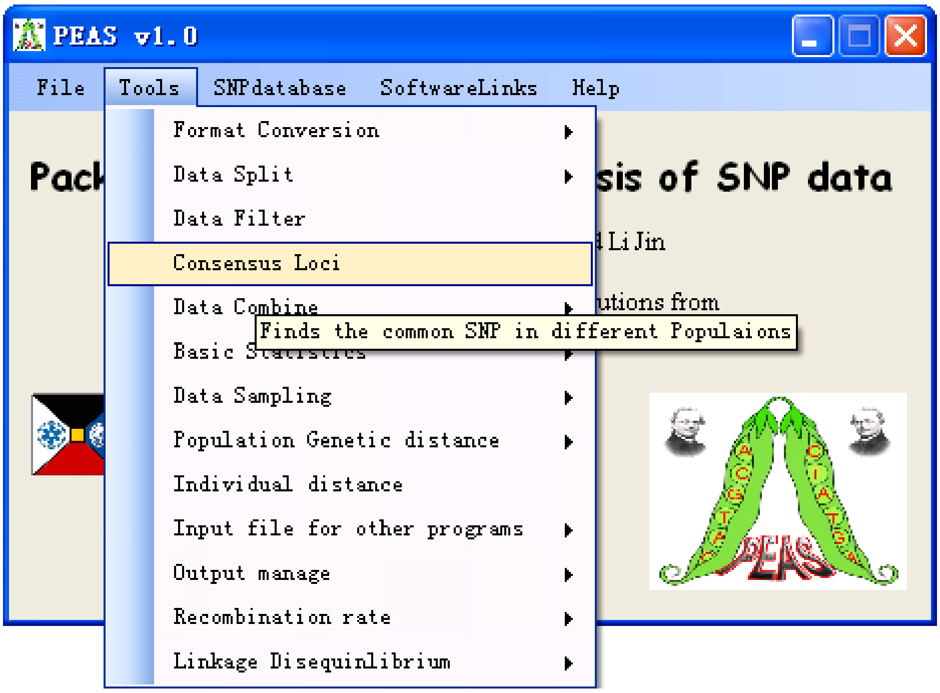
PopulationName ExtraColumns SampleSize

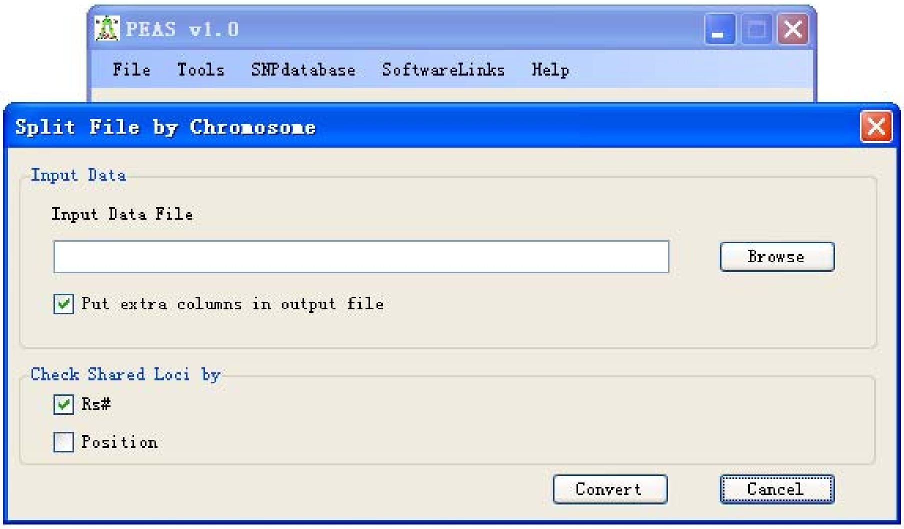
…

PopulationName ExtraColumns SampleSize

For example, the following file says there are 2 populations need to be searched shared loci, CEU is the name of the first population, the data file has 0 (no) extra column, the sample size of CEU is 90, YRI is the name of the second population, the data file has 0 (no) extra column, the sample size of YRI is 90.

```
2
CEU      0    90
YRI      0    90
```





Split File by Chromosome

Input Data

Input Data File

☑ Put extra columns in output file

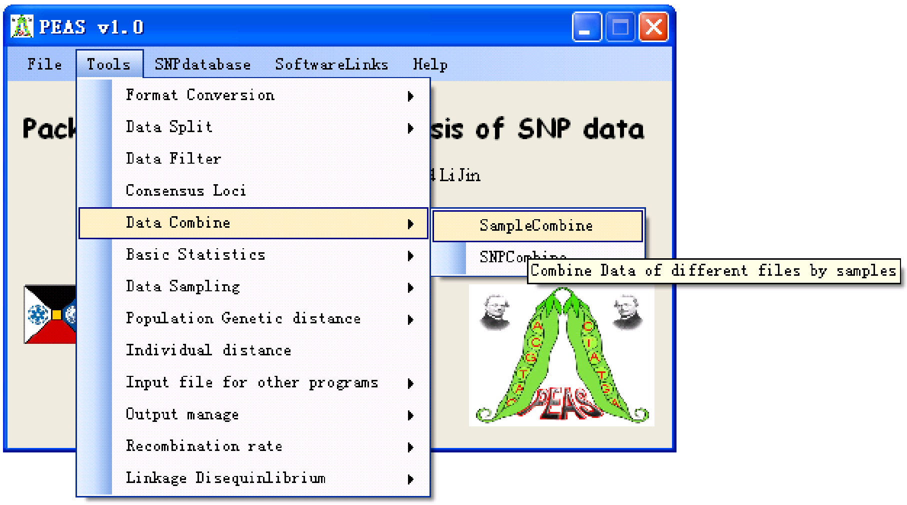
Check Shared Loci by

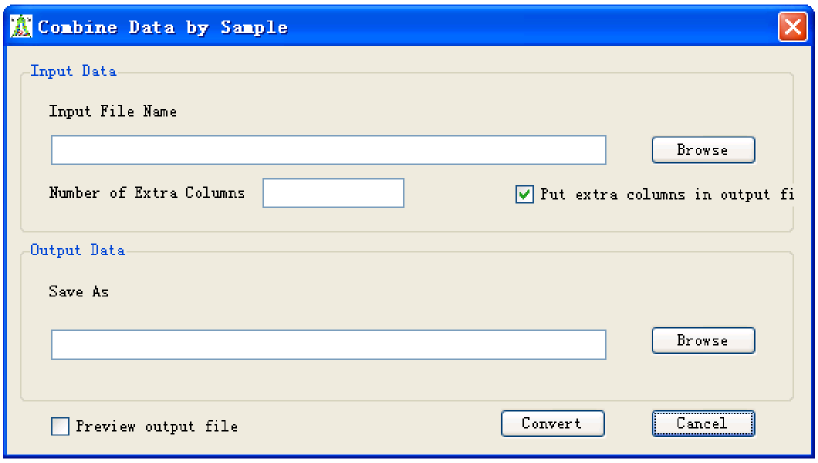
☑ Rs#

□ Position

6.5 Data combination

As the princinple of data split, PEAS can also combine data from different individuals, or from different SNP markers.




Combine Data by Sample

Input Data

Input File Name

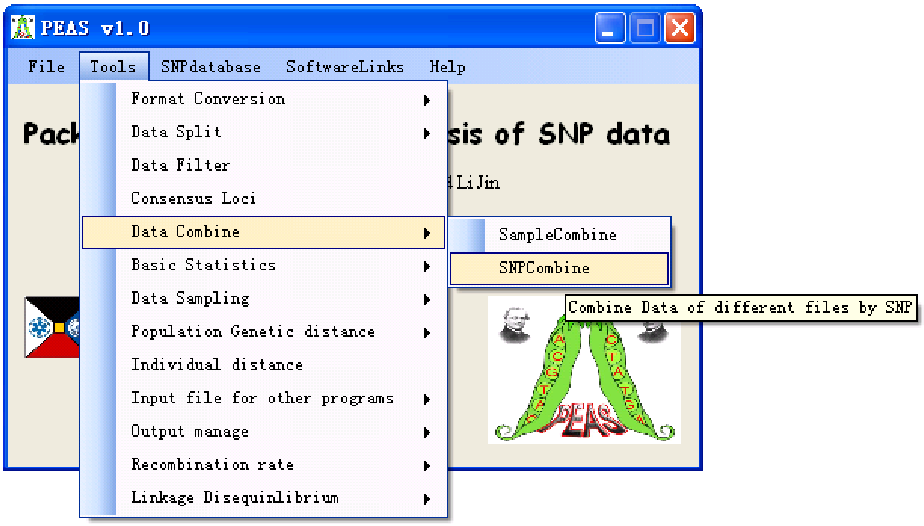
Number of Extra Columns

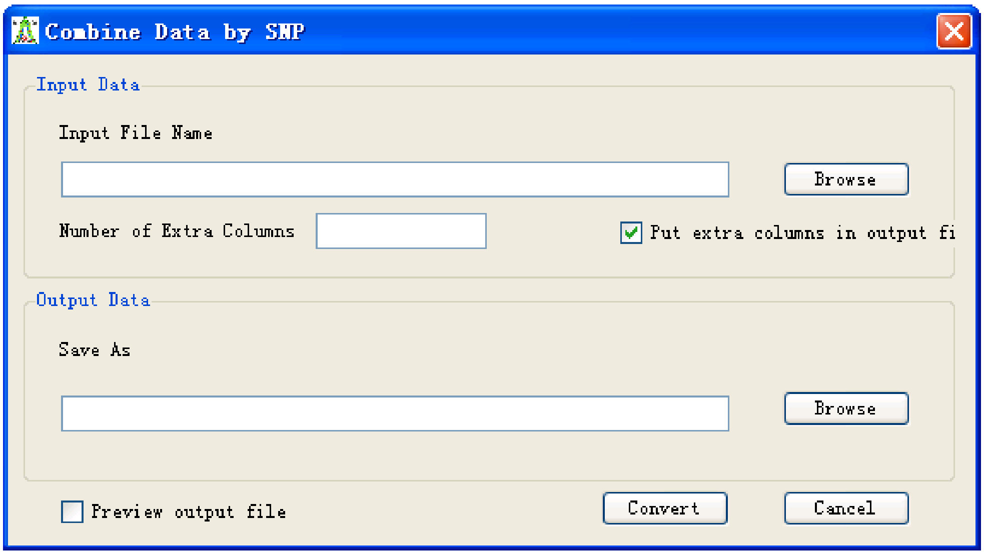
☑ Put extra columns in output fi

Output Data

Save As

□ Preview output file





☑ Put extra columns in output fi

□ Preview output file

6.6 Basic statistics

6.6.1 Allele count

Input: standard format

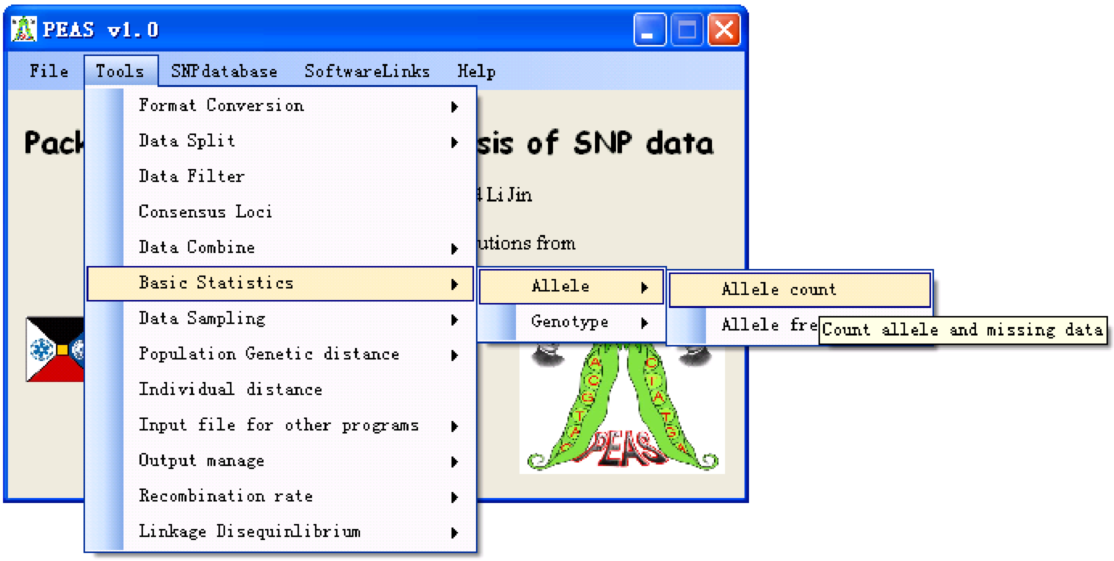
Output:

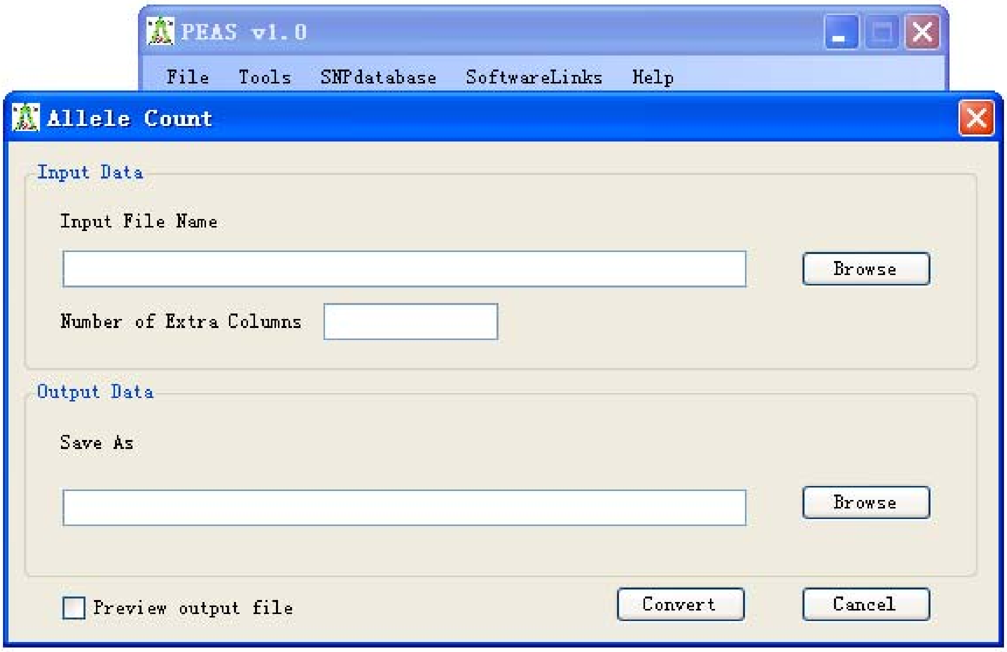
loci #

rs# (compulsional) chr# (optional) position (optional) strand (optional)

Allele (optional) first allle # the other allele# sample size missing

Allele count

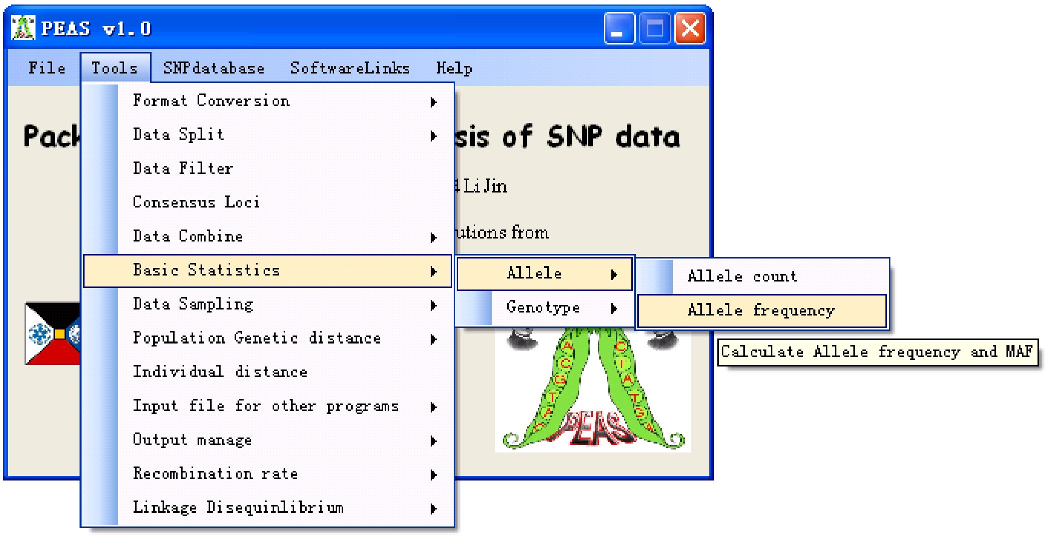


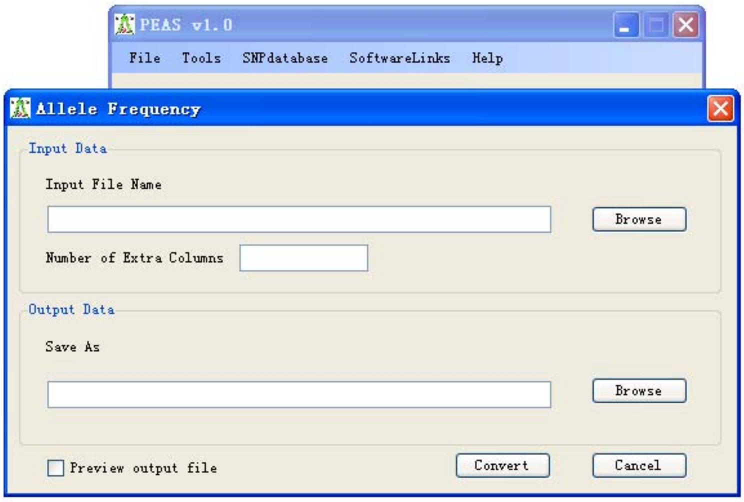


6.6.2 Allele frequency

rs# (compulsional) chr# (optional) position (optional) strand (optional)
Allele (optional) first alle freq the other allele freq minor allele freq (MAF)
sample size missing (0.01)

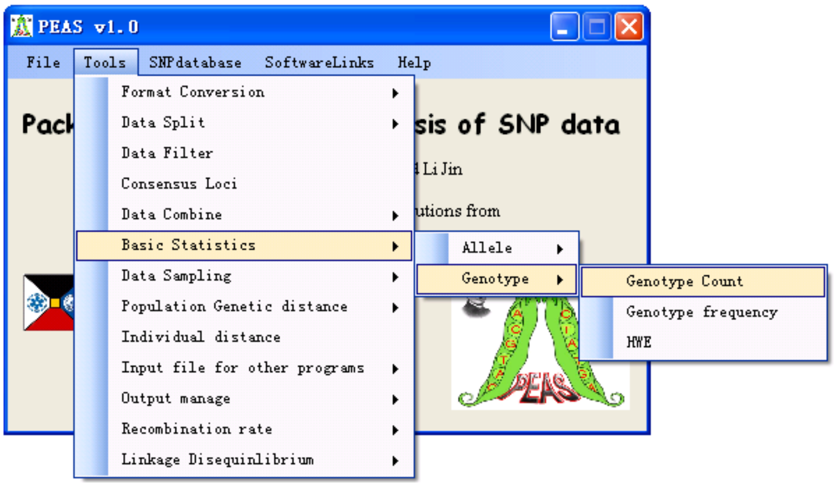
Allele frequency





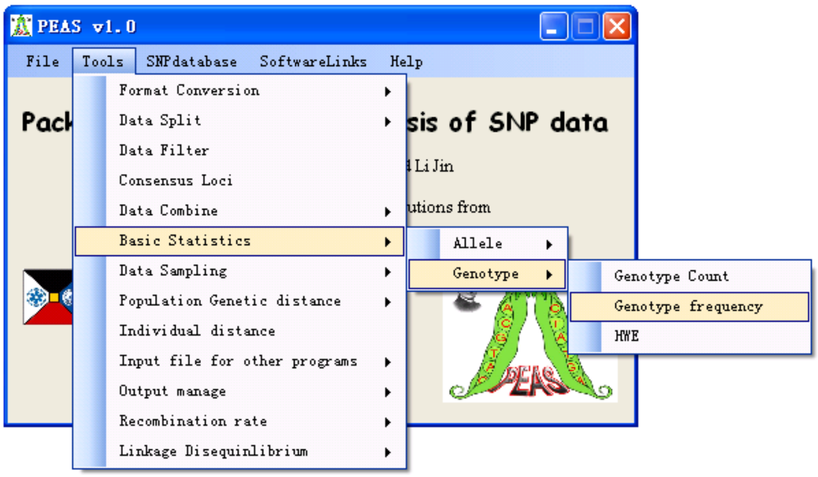
6.6.3 Genotype count

rs# (compulsional) chr# (optional) position (optional) strand (optional) Allele (optional) A#, B#, H#, U#



6.6.4 Genotype frequency

rs# (compulsional) chr# (optional) position (optional) strand (optional) Allele (optional) A_freq, B_freq, H_freq, U_freq

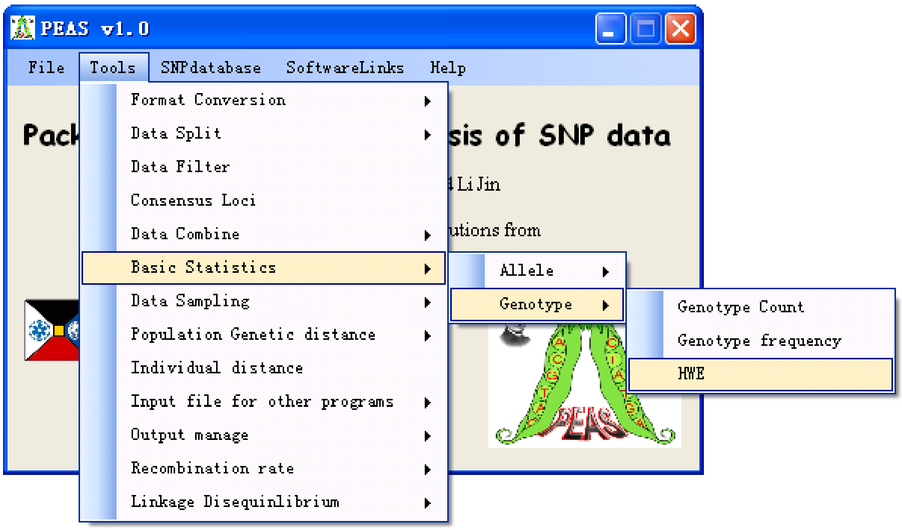


6.6.5 Hardy-Weinberg equilibrium (p-value threshold, 0.05)

rs# (compulsional) chr# (optional) position (optional) strand (optional) Allele (optional) Observed Heterozygosity Expected Heterozygosity X2 p-value E/D

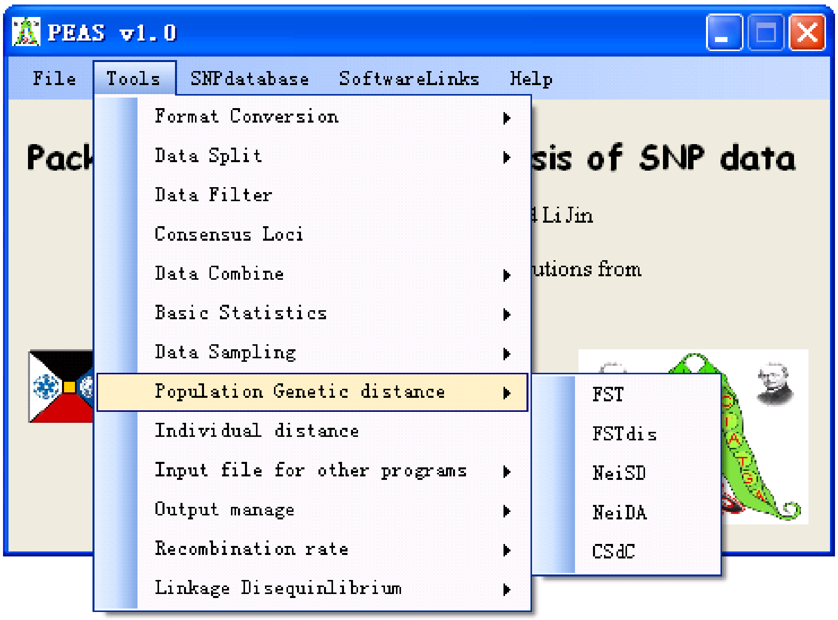
In some studies, people prefer to provide another format of genotype data. This

For many purposes, allele frequencies provide enough information. PEAS provides a program **AlleleFreq** caluculate allele frequency for multiple populations and generates some basic information such as MAF and missing data proportion for each SNP in each population.



6.7 Population genetic distances

Many people are interested in the genetic differentiation of populations, some genetic distances are used to describe the genetic difference between populations, such as F_{ST}, Nei's standard distance, Nei's D_A distance, F^*_{ST} distance and Cavalli-Sforza's d_C distance. PEAS provides a program **POPdis** to calculate those commonly used distances. PEAS generates distance matrix as MEGA format so that the results can be directly used to reconstruct phylogenetic trees. PEAS also does bootstrapping and generate Phylip format files and the results can be read and analyzed by Neighbor program and Consence program in Phylip package.



The formula used to calculate the distances are as follows:

6.7.1 Estimates of F_{ST}

Unbiased estimates of F_{ST} were calculated as described by Weir and Hill 2002(Weir and Hill 2002). Suppose we have i subpopulations (where i = 1,…, r), we denote sample allele frequency as $\tilde{p}_i$, and denote the average frequency over samples as $\bar{p}_i$. The jth allele in the ith sample is denoted by x_{ij}. If there are n_i alleles sampled from the ith of r populations:

$$\widetilde{p}_i = \frac{1}{n_i}\sum_{j=1}^{n_i} x_{ij}$$

$$\overline{p} = \frac{1}{\sum_i n_i}\sum_{i=1}^{r} n_i \widetilde{p}_i$$

The observed mean square errors for loci within populations are denoted by MSG:

$$MSG = \frac{1}{\sum_{i=1}^{r}(n_i - 1)}\sum_{i}^{r} n_i \widetilde{p}_i (1 - \widetilde{p}_i)$$

The observed mean square errors for between populations are denoted by MSP:

$$MSP = \frac{1}{r-1}\sum_{i}^{r} n_i (\widetilde{p}_i - \overline{p}\ )^2$$

Then F_{ST} can be estimated as follows:

$$F_{ST} = \frac{MSP - MSG}{MSP + (n_c - 1)MSG}$$

Where n_c is the average sample size across samples that also incorporates and corrects for the variance in sample size over subpopulations:

$$n_c = \frac{1}{r-1}\left(\sum_{i=1}^{s} n_i - \frac{\sum_{i=1}^{r} n_i^2}{\sum_{i=1}^{r} n_i}\right)$$

Because negative values of F_{ST} do not have biological interpretation, we set negative values of F_{ST}=0.0.

6.7.2 F*$_{ST}$ Distance

According to Latter(Latter 1972),

$$F_{ST}^{*} = \frac{\frac{1}{2}\left(\hat{J}_X + \hat{J}_Y\right) - \hat{J}_{XY}}{1 - \hat{J}_{XY}}$$

where $\hat{J}_X$, $\hat{J}_Y$ and $\hat{J}_{XY}$ are the unbiased estimates of average of $\sum x_i^2$, $\sum y_i^2$ and $\sum x_i y_i$ for all loci respectively. For a single locus, the unbiased estimates of $\sum x_i^2$, $\sum y_i^2$ and $\sum x_i y_i$ are:

$$\hat{j}_X = \frac{2m_X \sum \hat{X}_i^2 - 1}{2m_X - 1}$$

$$\hat{j}_Y = \frac{2m_Y \sum \hat{Y}_i^2 - 1}{2m_Y - 1}$$

$$\hat{j}_{XY} = \sum \hat{X}_i \hat{Y}_i$$

where m$_X$ and m$_Y$ are the number of diploids sampled from population X and Y respectively, $\hat{x}_i$ and $\hat{y}_i$ are allele frequencies in samples(Nei 1987). Therefore, $\hat{J}_X$, $\hat{J}_Y$ and $\hat{J}_{XY}$ are the averages of $\hat{j}_X$, $\hat{j}_Y$ and $\hat{j}_{XY}$ in all loci.

6.7.3 Nei's Standard Distance

According to Nei(Nei 1972),

$$D = -\ln(I)$$

where

$$I = \frac{\hat{J}_{XY}}{\sqrt{\hat{J}_X \hat{J}_Y}}$$

where $\hat{J}_X$, $\hat{J}_Y$ and $\hat{J}_{XY}$ are the unbiased estimates of average of $\sum x_i^2$, $\sum y_i^2$ and $\sum x_i y_i$ for all loci respectively. For a single locus, the unbiased estimates of $\sum x_i^2$, $\sum y_i^2$ and $\sum x_i y_i$ are:

$$\hat{j}_X = \frac{2m_X \sum \hat{X}_i^2 - 1}{2m_X - 1}$$

$$\hat{j}_Y = \frac{2m_Y \sum \hat{Y}_i^2 - 1}{2m_Y - 1}$$

$$\hat{j}_{XY} = \sum \hat{X}_i \hat{Y}_i$$

where m$_X$ and m$_Y$ are the number of diploids sampled from population X and Y respectively, $\hat{x}_i$ and $\hat{y}_i$ are allele frequencies in samples(Nei 1987). Therefore, $\hat{J}_X$, $\hat{J}_Y$ and $\hat{J}_{XY}$ are the averages of $\hat{j}_X$, $\hat{j}_Y$ and $\hat{j}_{XY}$ in all loci.

6.7.4 Cavalli-Sforza's d_C

According to Cavalli-Sforza and Edwards(Cavalli-Sforza and Edwards 1967),

$$d_C = \frac{2\sqrt{2\left(1-\sum_{i=1}^{q}\sqrt{x_i y_i}\right)}}{\pi}$$

where q is the number of alleles in kth locus.

6.7.5 Nei's D_A

According to Nei(Nei, Tajima et al. 1983) ,

$$D_A = \frac{\sum_{k=1}^{L}\left(1-\sum_{k=1}^{q_k}\sqrt{x_{ik} y_{ik}}\right)}{L}$$

where q_k is the number of alleles in kth locus, x_{ik} and y_{ik} are frequencies of allele i of locus k in population x and y respectively, L is the number of loci detected.

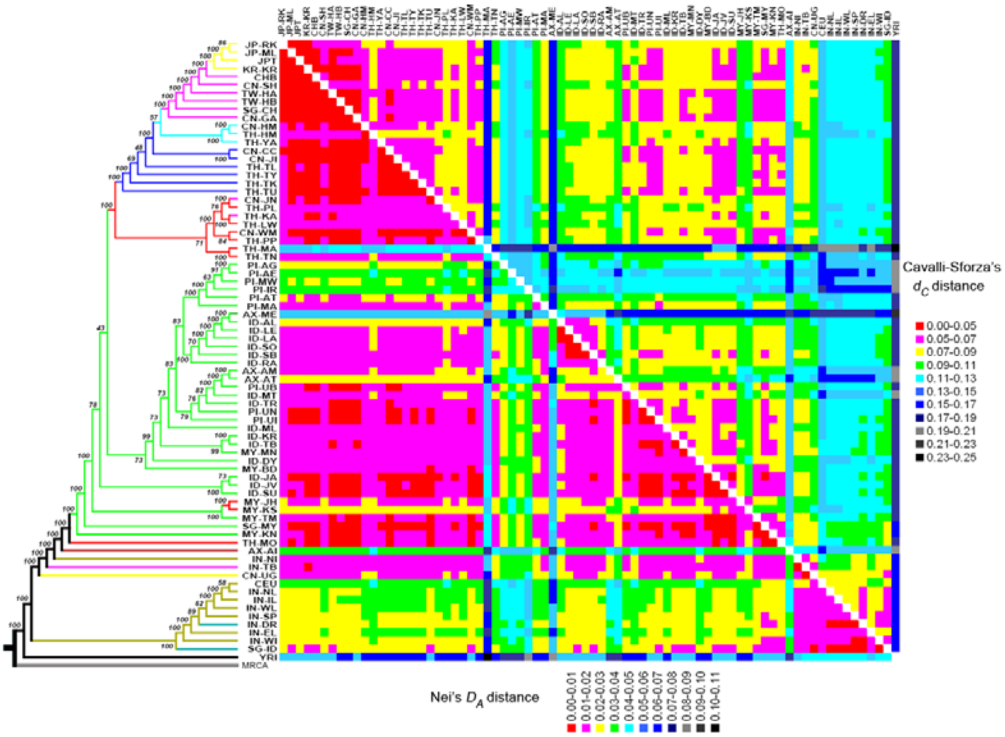


Fig. 4 Population genetic distance matrix and population tree reconstructed from the distance matrix.

6.7.6 Output distance matrix for MEGA and PHYLIP analysis

All the distance matrices calculated by PEAS are converted to MEGA and PHYLIP input format, so that phylogenetic analysis could be done easily.

6.7.7 Bootstrapping for distance calculation

As for trees, the best check of the validity of the conclusions is their independence from the markers employed: that is, their reproducibility with different sets of markers.

6.8 Individual genetic distances

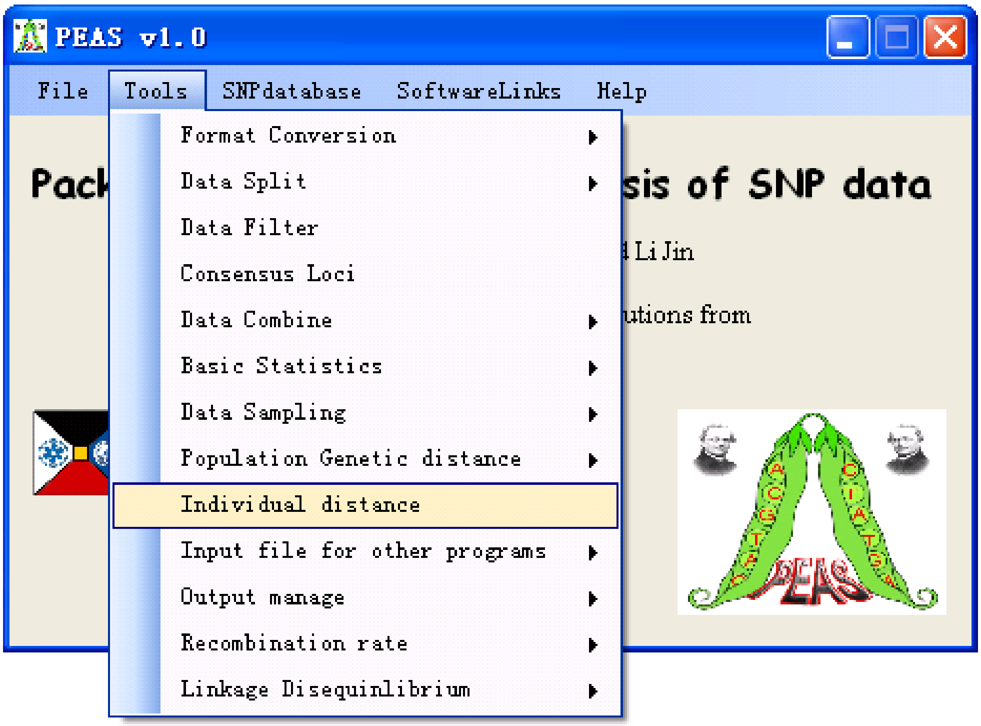
PEAS also provide a program **Indis** to calculate the genetic distance between individuals.

6.8.1 Allele sharing distance

We used an allele sharing distance(Mountain and Cavalli-Sforza 1997) as the genetic distance between individuals and reconstructed an individual tree to explore their relationship using Neighbor-Joining algorithm(Saitou and Nei 1987). Consider one biallelic SNP; there are three possible genotypes, AA, Aa, and aa. The genetic distances between individuals were calculated as follow:

$$D_{ij} = \frac{1}{l}\sum_{k=1}^{l} d_{kij} \ ,$$

where *l* is the number of loci for which both individuals have been tested, and d_{kij}=0 if individual *i* and individual *j* have identical genotypes at locus k (e.g., AA:AA or Aa:Aa or aa:aa), d_{kij}=0.5 if individual *i* and individual *j* have one one allele identical one allele different (e.g., AA:Aa or aa:Aa), and d_{kij}=1.0 if individual *i* and individual *j* have no alleles in common (e.g., AA:aa).



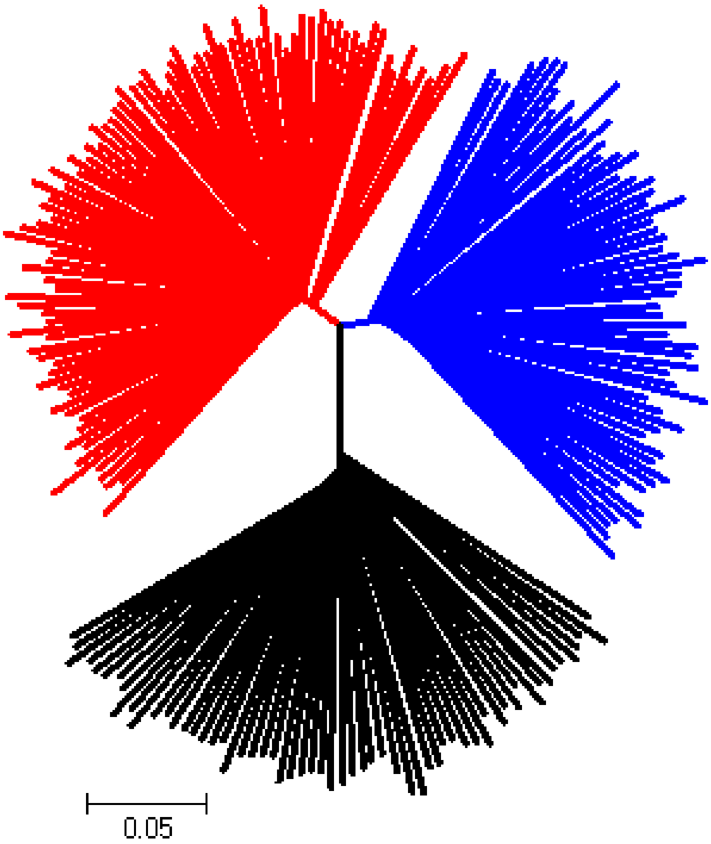
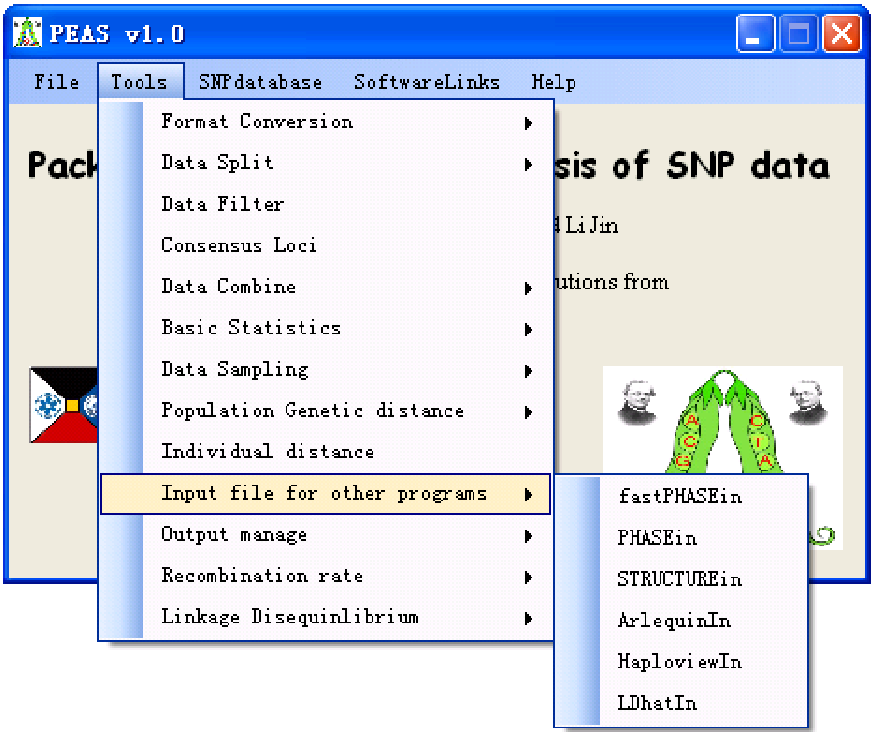


Fig. 5 Individual tree of 270 HapMap samples, red branches indicate CHB and JPT samples, blue branches indicate CEU samples and black branches indicate YRI samples.

6.9 Input files for other programs

As described in PEAS component programs, PEAS can generate input file for the following popular computer programs which have been used frequently in studies on human population genetics.



6.9.1 Format input files for PHASE

PEAS provides a program **PHASEin** to format the genotype data to the input file for PHASE (Stephens, Smith et al. 2001) program.

6.9.2 Format input files for fastPHASE

PEAS also provides a program **fastPHASEin** to format the genotype data to the input file for fastPHASE (Scheet and Stephens 2006) program.

6.9.3 Format input files for STRUCTURE

PEAS also provides a program **STRUCTUREin** to format the genotype data to the input file for STRUCTURE (Falush, Stephens et al. 2003) program.

6.9.4 Format input files for Arlequin

PEAS also provides a program **ArlequinIn** to format the genotype data to the input file for Arlequin (Excoffier and Schneider 2005) program.

6.9.5 Format input files for Haploview

PEAS also provides a program **HaploviewIn** to format the genotype data to the input file for Haploview (Barrett, Fry et al. 2005) program.

6.9.6 Format input files for LDhat

PEAS also provides a program **LDhatIn** to format the genotype data to the input file for LDhat (McVean, Myers et al. 2004) program.

6.10 Linkage Disequilibrium

6.10.1 Measures of Linkage Disequilibrium

Hedrick (Hedrick 1987) has reviewed the numerous measures of linkage disequilibrium. In his review, Hedrick demonstrates the conditions under which the measures, or at least a subset thereof, are highly correlated. Devilin and Risch (Devlin and Risch 1995) compared linkage disequilibrium for fine-scale mapping.

Consider two loci, A and B, each locus having two alleles: A_1, A_2 of locus A, B_1, B_2 of locus B respectively. The layout and notation of the 2 × 2 table from a sample from the population are given in **Table 1**. In **Table 1**, p_{11}, p_{12}, p_{21}, p_{22} denote the observed frequencies of haplotype A_1B_1, A_1B_2, A_2B_1, A_2B_2, p_{1+}, p_{2+}, p_{+1}, p_{+2} denote the frequencies of allele A_1, A_2, B_1, B_2.

Table 1 Layout and Notation for Sample Haplotype Frequencies in a 2 × 2 Table

marker	B_1	B_2	
A_1	p_{11}	p_{12}	p_{1+}
A_2	p_{21}	p_{22}	p_{2+}
	p_{+1}	p_{+2}	1

Naturally the *p*'s are only sample estimates of some underlying unknown parameters, denoted by *p*'s. We use *p*'s in the definitions that follow, with the understanding that these unknown quantities are estimated from the observed sample quantities.

The basic component of many measures of disequilibrium is the difference between the observed and the expected (under independence) number of haplotypes bearing the A1 and the B1 allele or its equivalent expressions:

$$D \begin{aligned} &= \pi_{11} - \pi_{1+}\pi_{+1} \\ &= \pi_{22} - \pi_{2+}\pi_{+2} \\ &= -\pi_{12} + \pi_{1+}\pi_{+2} \\ &= -\pi_{21} + \pi_{2+}\pi_{+1} \\ &= \pi_{11}\pi_{22} - \pi_{12}\pi_{21} \end{aligned} \text{, the corresponding estimation is } D \begin{aligned} &= p_{11} - p_{1+}p_{+1} \\ &= p_{22} - p_{2+}p_{+2} \\ &= -p_{12} + p_{1+}p_{+2} \\ &= -p_{21} + p_{2+}p_{+1} \\ &= p_{11}p_{22} - p_{12}p_{21} \end{aligned} .$$

Although the measure D captures the intuitive concept of LD, its numerical value is of little use for measuring the strength of and comparing levels of LD. This is due to the dependence of D on allele frequencies. As a result, several alternative measures based on D have been devised (Devlin and Risch 1995).

According to Hill and Weir (Hill and Weir 1994), the most frequently used measure of disequilibrium is the square of standardized measure

$$\Delta = \frac{\pi_{11}\pi_{22} - \pi_{12}\pi_{21}}{\sqrt{\pi_{1+}\pi_{2+}\pi_{+1}\pi_{+2}}},$$

or Δ^2. Δ is commonly squared to remove the arbitrary sign introduced when the marker alleles are labeled.

Another common measure, introduced by Lewontin (Lewontin 1964), is defined as

$$D' = \begin{cases} \dfrac{\pi_{11}\pi_{22} - \pi_{12}\pi_{21}}{\min(\pi_{1+}\pi_{+2}, \pi_{+1}\pi_{2+})} & D > 0 \\ \dfrac{\pi_{11}\pi_{22} - \pi_{12}\pi_{21}}{\min(\pi_{1+}\pi_{+1}, \pi_{+2}\pi_{2+})} & D < 0 \end{cases} \text{, where}$$

$$D = \pi_{11} - \pi_{1+}\pi_{+1} = \pi_{22} - \pi_{2+}\pi_{+2} = -\pi_{12} - \pi_{1+}\pi_{+2} = -\pi_{21} - \pi_{2+}\pi_{+1} = \pi_{11}\pi_{22} - \pi_{12}\pi_{21} .$$

7 How to cite this program

Shuhua Xu, Sanchit Guputa and Li Jin. 2010. PEAS: A Package for Elementary Analysis of SNP Data. Chinese Academy of Sciences and Max Planck Society (CAS-MPG) Partner Institute for Computational Biology, Shanghai Institutes for

Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China.

8 References

Barrett, J. C., B. Fry, et al. (2005). "Haploview: analysis and visualization of LD and haplotype maps." Bioinformatics **21**(2): 263-5.

Cavalli-Sforza, L. L. and A. W. Edwards (1967). "Phylogenetic analysis. Models and estimation procedures." Am J Hum Genet **19**(3): Suppl 19:233+.

Devlin, B. and N. Risch (1995). "A comparison of linkage disequilibrium measures for fine-scale mapping." Genomics **29**(2): 311-22.

Excoffier, L. and S. Schneider (2005). "Arlequin ver. 3.0: An integrated software package for population genetics data analysis." Evolutionary Bioinformatics Online **1**: 47-50.

Falush, D., M. Stephens, et al. (2003). "Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies." Genetics **164**(4): 1567-87.

Felsenstein, J. (1989). "PHYLIP--Phylogeny Inference Package (Version 3.2)." Cladistics **5**: 164-166.

Hedrick, P. W. (1987). "Genetic bottlenecks." Science **237**(4818): 963.

Hill, W. G. and B. S. Weir (1994). "Maximum-likelihood estimation of gene location by linkage disequilibrium." Am J Hum Genet **54**(4): 705-14.

Kumar, S., K. Tamura, et al. (2004). "MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment." Brief Bioinform **5**(2): 150-63.

Latter, B. D. (1972). "Selection in finite populations with multiple alleles. 3. Genetic divergence with centripetal selection and mutation." Genetics **70**(3): 475-90.

Lewontin, R. C. (1964). "The Interaction of Selection and Linkage. Ii. Optimum Models." Genetics **50**: 757-82.

McVean, G. A., S. R. Myers, et al. (2004). "The fine-scale structure of recombination rate variation in the human genome." Science **304**(5670): 581-4.

Mountain, J. L. and L. L. Cavalli-Sforza (1997). "Multilocus genotypes, a tree of individuals, and human evolutionary history." Am J Hum Genet **61**(3): 705-18.

Nei, M. (1972). "Genetic distance between populations." Am. Nat. **106**: 283-292.

Nei, M. (1987). Molecular evolutionary genetics. New York, Columbia University Press.

Nei, M., F. Tajima, et al. (1983). "Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data." J Mol Evol **19**(2): 153-70.

Pritchard, J. K., M. Stephens, et al. (2000). "Inference of population structure using multilocus genotype data." Genetics **155**(2): 945-59.

Saitou, N. and M. Nei (1987). "The neighbor-joining method: a new method for reconstructing phylogenetic trees." Mol Biol Evol **4**(4): 406-25.

Scheet, P. and M. Stephens (2006). "A fast and flexible statistical model for large-scale population genotype data: applications to inferring missing genotypes and haplotypic phase." Am J Hum Genet **78**(4): 629-44.

Schneider, S., D. Roessli, et al. (2000). "Arlequin: A software for population genetics data analysis. Ver

2.000." Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

Stephens, M., N. J. Smith, et al. (2001). "A new statistical method for haplotype reconstruction from population data." Am J Hum Genet **68**(4): 978-89.

TheInternationalHapMapConsortium (2003). "The International HapMap Project." Nature **426**(6968): 789-96.

TheInternationalHapMapConsortium (2005). "A haplotype map of the human genome." Nature **437**(7063): 1299-320.

Weir, B. S. and W. G. Hill (2002). "Estimating F-statistics." Annu Rev Genet **36**: 721-50.

Xu, S., W. Jin, et al. (2009). "Haplotype-sharing analysis showing Uyghurs are unlikely genetic donors." Mol Biol Evol **26**(10): 2197-206.