Forensic Resource/Reference On Genetics – knowledge base: FROG-kb User's Manual

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1.   Introduction

The purpose of the Forensic Resource/Reference On Genetics – knowledge base (FROG-kb) database is to provide users with online tools for comparing user-provided data with underlying allele frequencies in populations. The database serves as a teaching and research web interface to facilitate the use of single-nucleotide polymorphisms (SNPs) in the forensic field.

FROG-kb is a freely available, web-accessible, and curated resource and reference of allele frequency data for SNPs and other genetic polymorphisms. The data used in FROG-kb calculations derive from the ALlele FREquency Database (ALFRED http://alfred.med.yale.edu), a continually updated database of allele frequency data on SNPs and other genetic polymorphisms.

Current SNP panel categories within FROG-kb include Individual Identification SNPs (IISNPs), Ancestry Inference SNPs (AISNPs), and Phenotype Interference SNPs (PISNPs). IISNPs provide the ability to calculate random match probabilities for user-specified genotypes. AISNPs provide the ability to calculate relative likelihoods of ancestry from different reference populations for user-specified genotypes. PISNPs provide the ability to predict a visible trait for user-specified genotypes for the relevant SNPs.

This User's Manual provides detailed information on these SNP panels and instructions for the navigation and searching of each within FROG-kb. Additional information on FROG-kb and ALFRED can be accessed at each database's website and found in the publications listed in the Reference section (Section 8) of this User's Manual.

2.   Accessing FROG-kb Home Page and Features

FROG-kb can be accessed at **http://frog.med.yale.edu/FrogKB/**. The recommended browsers for accessing FROG-kb are the latest versions of Mozilla Firefox and Google Chrome. Some versions of Internet Explorer are not compatible. If you have any issues please reach out to the database curator using the **Contact Us** page described in **Section 4.3** of this User's Manual.

3.   Home Page and Navigation Tools

Exhibit 1 presents the FROG-kb Home Page, which provides users with information about FROG-kb and a brief description of the types of data sets within the database. The database's Main Menu is shown across the top of the page (see red box). The Main Menu allows easy navigation among menu options and is accessible from any page on the FROG-kb database.

Exhibit 1. **FROG-kb showing the Main Menu (A) options and the Home (B) page**

FROG-kb Forensic R^esource_eference On Genetics knowledge base

| Home | IISNP | AISNP | PISNP | Documentation | Contact Us | Announcements |

Welcome to the New FROG-kb

This new FROG-kb interface has all of the previous functionalities with supplementary reference material and "space" for additional documentation that will be added over the next few weeks. See Announcements for updates. New FROG-kb can be best viewed on latest Mozilla Firefox and Google Chrome. Some versions of Internet Explorer are not compatible. If you come across any issues or have suggestions use the Contact Us function to reach us. "If you see something - say something"

FROG-kb seeks to make allele frequency data for SNPs and other genetic polymorphisms more useful in a forensic setting. The primary objective of FROG-kb is to provide a web interface that, from a forensic perspective, is useful for teaching and research and can serve as a tool facilitating forensic practice. The underlying data are housed in the already extensively used and referenced ALlele FREquency Database (ALFRED). The FROG-kb interface makes the information usable for forensic purposes, including computational tools for comparing user-provided data with underlying allele frequencies in populations. These tools are organized by the methodology followed and the different published SNP/marker panels available.

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description.

As shown in Exhibit 1, the features of the Main Menu are as follows:

- Home
- IISNP
- AISNP
- PISNP
- Documentation
- Contact Us
- Announcements

4.   Navigating FROG-kb

The purpose of this section is to provide the user with an overview of the Main Menu's features, as well as a description of the information that can be found in each.

4.1   SNP Panel Categories: IISNP, AISNP, PISNP

The IISNP, AISNP, and PISNP features on the FROG-kb Main Menu provide access to multiple published SNP panels reviewed and entered by the curator. Clicking any of these three features opens a page that lists the SNP panels under that category.

As shown in *Exhibit 2*, each SNP panel shown in the list includes a reference for the panel. In addition, the right side of each panel includes a link (*Navigate to ALFRED*) that allows the user to access ALFRED for more detail about the panel. Each SNP panel also shows a **Go** button above the panel, which users can click to open the panel.

Exhibit 2. KiddLab -45 Unlinked IISNPs illustrating layout seen in IISNP, AISNP, and PISNP options.

Clicking on the *Navigate to ALFRED* link opens the SNP Set page within ALFRED in a new browser window (*Exhibit 3*). The SNP Set module in ALFRED has multiple options for viewing the presented information, including sorting the SNPs by FST and average heterozygosity.

Summary Information for Sites From
KiddLab - 45 Unlinked IISNPs

Reference-
Pakstis AJ, Speed WC, Fang R, Hyland FCL, Furtado MR, Kidd JR, Kidd KK. "SNPs for a universal individual identification panel". *Human Genetics* **127**:315-24. (2010) Online citation.

Kidd KK, Kidd JR, Speed WC, Fang R, Furtado MR, Hyland FC, Pakstis AJ. "Expanding data and resources for forensic use of SNPs in individual identification.". *Forensic Sci Int Genet.* **6**:646-52. (2012) Online citation.

Sort by : | Locus | FST | AVG_HET | #Pop |

Sorted by Locus

Locus	Site	dbSNP rs#	Fst	Avg Het	# Pops
ADAMTS2	C____3153696_10	rs338882	0.076	0.459	83
ATP13A4	C____25749280_10	rs6444724	0.051	0.466	85
C8orf73	rs4606077	rs4606077	0.052	0.42	91
CADM1	C____2450075_10	rs10488710	0.02	0.442	50

Exhibit 3. Additional information found on ALFRED database for KiddLab -45 Unlinked IISNPs

Clicking the **Go** button above an SNP panel opens the panel, as well as a row of option buttons for viewing the panel. As shown in ***Exhibit 4***, these option buttons are as follows:

- SNP Set,
- Populations,
- Data Entry,
- Formula, and
- Examples.

Each SNP panel category (IISNP, AISNP, and PISNP) presents the same row of option buttons, except the PISNP panel, which does not include Populations.

KiddLab - 45 Unlinked IISNPs

(First four columns are sortable - click on column header to sort)

Navigate to ALFRED	Navigate to dbSNP	chromosome	chromosome position
SI001900K	rs10092491	8	28,411,072
SI001899B	rs10488710	11	115,207,176
SI001402H	rs1058083	13	100,038,233
SI015392U	rs10773760	12	130,761,696
SI015046Q	rs10776839	9	137,417,308

Exhibit 4. Panel of 45 IISNPs SNP Set information.

The following subsections provide information on each of the option buttons accessible on a SNP panel.

SNP Set

The default view for a SNP panel is a sortable table that presents the **SNP Set** (the first option button shown). As shown previously in Exhibit 4 and to the right, the first column of this table presents an ALFRED UID (e.g., SI001900K) that links to ALFRED for polymorphism information. The second column of the

Navigate to ALFRED	Navigate to dbSNP
SI001900K	rs10092491
SI001899B	rs10488710
SI001402H	rs1058083
SI015392U	rs10773760
SI015046Q	rs10776839

table shows a list of rs numbers (e.g., rs10092491); each numbers is an active link to the dbSNP record for that SNP. Note that within the SNP Set in ALFRED, additional populations may have data for some, but not all, populations, and that those populations are not included in the calculations in FROG-kb.

Populations

Clicking the **Populations** option button provides a list of populations for which comparable calculations can be made (***Exhibit 5***). This is the set of populations for which all SNPs in the set have allele frequency data. Each population name shown in the list is an active link. Clicking on a link opens a new browser window that presents ALFRED information on the population.

Home   IISNP   AISNP   PISNP   Documentation   Contact Us   Announcements

SNP Set   Populations   Data Entry   Formula   Examples

KiddLab - 45 Unlinked IISNPs

(First four columns are sortable - click on column header to sort)

Navigate to ALFRED	Geographic Region	Sample Size (2N)
Adygei	Russia	108
African Americans	Africa	182
Ami	East Asia	80
Atayal	East Asia	84
Biaka	Central Africa	140
Cambodians, Khmer	Southeast Asia	52
Chagga	Eastern Africa	90

Exhibit 5. Panel of 45 IISNPs Population information

Data Entry

Clicking the **Data Entry** option button allows the user to specify an individual, multi-site genotype and to calculate the probability of that genotype in each of the populations. As shown in *Exhibit 6*, two options are provided at the bottom of the Data Entry page for the user to enter genotype data for a particular SNP panel: **File Upload** and **Selection by Radio Button**. *Section 5.1* of this User's Manual provides more information on entering data in FROG-kb.

Exhibit 6. Data Entry for KiddLab – 45 Unlinked IISNPs in FROG-kb.

Formula and Examples

Clicking the **Formula** option button opens a page that presents an explanation on how the probabilities are derived. The **Examples** option button allows users to access a pre-entered data entry page for one individual from a specific population. Both the Example and Formula options are explained further in **Section 6** and **Section 7** of this Users' Manual, respectively.

4.2   Documentation
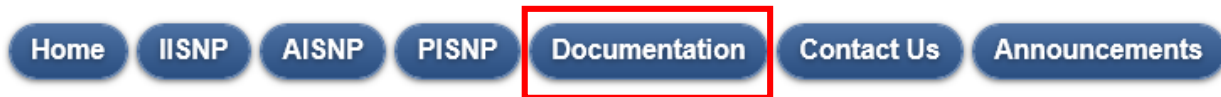
Clicking the **Documentation** feature in the Main Menu opens an additional set of option buttons for users to learn more about FROG-kb capabilities. Similar to the options that appear on the SNP panel pages, the Documentation options appear as orange buttons under the Main Menu.

As shown above, the option buttons that appear under Documentation are as follows:

- Functionalities,
- Pipeline,
- FAQ (Frequently Asked Questions),
- Manual, and
- Resources.

Functionalities

The **Functionalities** option provide an overview for navigating through the SNP panel information, SNP panel example data, SNP panel data entry, and results of the data entry calculations (***Exhibit 7***). The Functionalities serves as an online, secondary location for information provided in this User's Manual.

Functionalities

Below we provide an overview of accessing and searching SNP panels within FROG-kb. This information can also be found in Sections 3, 4, and 5 of the User Manual along with screen shots walking users through the database.

Navigating the SNP Panels

For each SNP panels listed there are several elements. Associated with each of the panel listings is a Detailed Overview of SNPs link into ALFRED precisely for a more detailed overview of the panel. That link opens the SNP Set page within ALFRED into a new browser window.
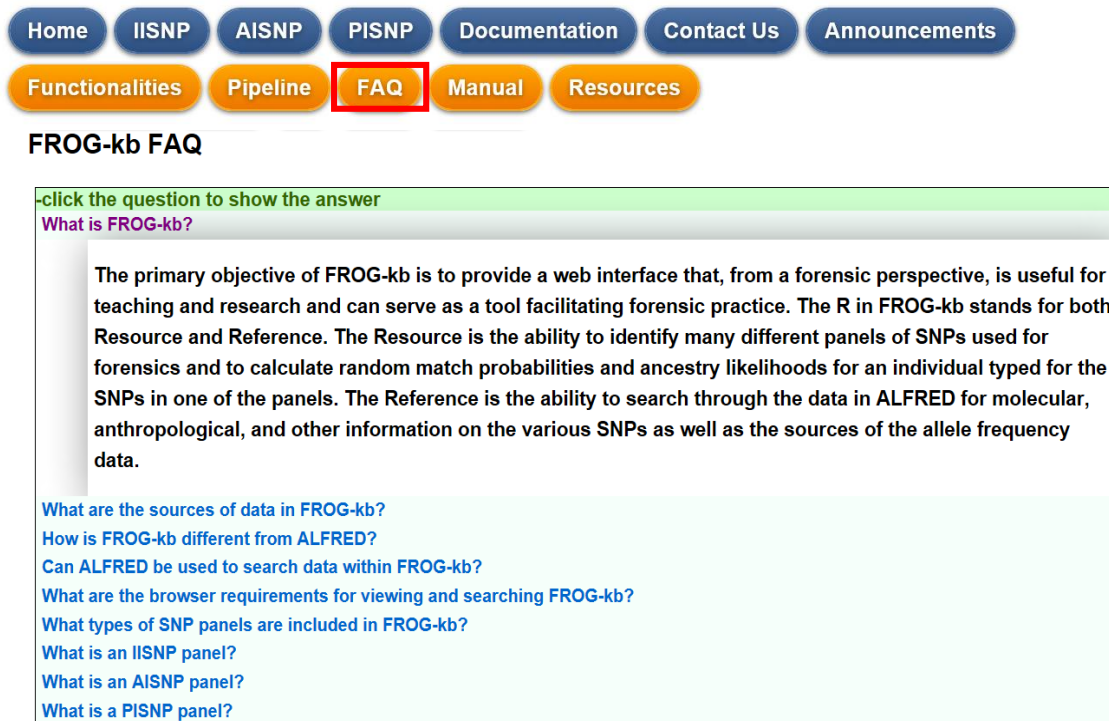
Exhibit 7. Functionalities within the Main Menu option for Documentation provides navigation overview of FROG-kb.

Pipeline

Clicking on the **Pipeline** option opens a graphical representation that illustrates the process for inputting, searching, and retrieving data from FROG-kb.

FAQ

The **FAQ** option presents answers to Frequently Asked Questions about FROG-kb (*Exhibit 8*). Users who have additional questions not found on the FAQ page should follow the process outlined in **Section 4.3** of this User's Manual to submit their questions to the database curator.

Exhibit 8. List of Frequently Asked Questions for FROG-kb.

Manual

Clicking the **Manual** option opens a copy of this User's Manual.

Resources

The **Resources** option button provides users with links (STR- and SNP-based) and references to potentially relevant databases and sites. This page also lists poster presentations pertaining to FROG-kb that have been presented at annual conferences and that are freely accessible to users.

4.3 Contact Us

The **Contact Us** feature in the FROG-kb Main Menu serves as a resource for users to provide feedback and questions to the database curator. Users can also inquire about submitting data and providing additional information that will assist the community. As shown in ***Exhibit 9***, the Contact Us page presents a form where users can provide their email address and a subject and message. Clicking the **Submit** button at the bottom of the page sends the message to the database curator.

Exhibit 9. Contact Us submission form

4.4 Announcements

The **Announcements** feature on the Main Menu provides users with updates pertaining to FROG-kb (***Exhibit 10***). These updates may include additional functionalities or upgrades to the web interface, additional panels, and expansion of existing panels in FROG-kb.

FROG-kb Announcements

2016

New FROG-kb: We have revised the FROG-kb web interface with the intent of making it more user friendly and providing new features. Following are some of the upgrades:

- Sortable SNP and Population tables.
- Users can now navigate to previous pages by clicking the 'Back' button on the browser.

New AISNP Panel: A new AISNP panel was added to FROG-kb - Overlap set of AISNPs - based on analyses in a manuscript submitted for publication.

Expanded Daniele Podini's list of 32 AISNPs: Since only 25 out of 32 SNPs in the panel had data on all the reference populations listed previously only those 25 were included in the calculations of likelihood. We have added four more SNPs which will bring the count to 29. The remaining three SNPs have data on fewer and diverse populations.

Additional reference populations for panels:
AISNP panel:
The 1000 Genomes populations are being added as reference populations to the SNP Sets in ALFRED and to the calculations for most AISNP panels in FROG-kb. The following panels have additional reference populations.

- Kayser's set of 24 Ancestry Informative Markers
- Daniele Podini's list of 32 AISNPs
- Nievergelt's Set of 41AIMs
- Kidd Lab set of 55 AISNPs

Exhibit 10. FROG-kb Announcements Page

5.   Searching ISNP, AISNP, and PISNP Data

Section 4.1 of this User's Manual, *SNP Panel Categories: IISNP, PISNP, AISNP*, provides an overview for navigating the SNP panels and the functionalities within each. The following sections expand on that information by walking users through the process for inputting, searching, and retrieving data in FROG-kb.

Exhibit 11 shows the Data Entry default page for the IISNP panel. The page presents two options for users to enter genotype data for an SNP panel: **File Upload** and **Selection by Radio Button**.

Exhibit 11. Data Entry Page for IISNP Data Entry.

5.1   Inputting and Searching Data: Selection by Radio Button

Exhibit 12 shows the data entry form for the **Selection by Radio Button** option. This form allows the user to specify an individual's multi-site genotype. By default, the list of SNPs is

sorted by rs numbers; however, users can also sort the list by any of the first four columns by clicking on the preferred header.

As shown in the first two columns of the table, for each SNP on the list, the ALFRED UID and dbSNP rs numbers are displayed as active links to the respective databases. The third column presents the chromosome (chr), and the fourth column provides the chromosome position. As shown in the red box, the next three columns present radio buttons for categorizing the possible genotypes. Users can enter the genotype by simply clicking on the radio button for the genotype. If the genotype is unknown, the last column in the data entry table allows the user to select the Unknown (NN) option.

The two action buttons of this page are **Compute** and **Set all unselected to known and compute**. These action buttons are discussed in the following subsections.

Exhibit 12. KiddLab – 45 Unlinked IISNPs Data Entry table.

Compute

Once all the information has been entered, the user can select **Compute** at the bottom of the Data Entry table to initiate the calculation and display the results. If any SNPs do not have a radio button entry (Genotype or Unknown), the system will flag the missing entry and a warning box

will appear indicating that the user must add the missing information. Once a radio button has been selected for each SNP, the user must again click Compute to run the calculation.

Set All Unselected to Unknown and Compute

FROG-kb also provides user with a **Set all unselected to unknown and compute** option, which is shown at the bottom of the Data Entry table next to the Compute button. If preferred, a user can click this option instead of Compute, and the system with automatically capture any genotypes for which a radio button was not selected and mark these as Unknown. Note that using this feature assumes that the user has already entered in all known genotypes.

Both **Compute** and **Set all unselected to unknown** initiate the FROG-kb calculation and display the results when all SNPs have an entry.

5.2   Viewing Search Results: Selection by Radio Button

As shown in *Exhibit 13*, the results of a calculation are displayed as a table with three columns that outline the following: (1) the name of the population sampled with its geographic region and the sample size, (2) the probability of the entered genotype in that population, and (3) the likelihood ratio of the most probable population to the specified population.

The text above the table (see red box in exhibit) indicates the number of SNPs used in the calculation, and the **Print Table Format** and **View SNPs Used** buttons allow the user to to print the output table and to see the list of SNPs used.

KiddLab - 45 Unlinked IISNPs

Based on 37 SNPs

Print Table Format   View SNPs Used

● Indicates the values are within an order of magnitude of the highest likelihood.

Population (Region,Sample Size 2N)	Probability of Genotype in each Population	Likelihood Ratio
Jews_ Ethiopian(Eastern Africa,64)	● 1.892E-18	
Khanty(Siberia,100)	● 6.518E-19	2.9
Pima_ Mexico(North America,106)	● 3.841E-19	4.93
Koreans(East Asia,132)	● 3.11E-19	6.08
Han(East Asia,124)	1.348E-19	14.0
Han(East Asia,100)	1.048E-19	18.1
Ibo(Western Africa,96)	6.792E-20	27.9
Chagga(Eastern Africa,90)	6.429E-20	29.4
Yakut(Siberia,102)	5.406E-20	35.0
Masai(Eastern Africa,44)	5.399E-20	35.0
Keralite(South Asia,60)	5.391E-20	35.1
Hakka(East Asia,86)	4.811E-20	39.3

Exhibit 13. Results from KiddLab-45 Unlinked IISNPs data entry.

The probability of the entered genotype is equivalent to the probability of a random match, assuming no deviation from Hardy-Weinberg ratios in the population. The populations are ordered by the probabilities of the entered genotype from highest to lowest; therefore, the likelihood ratios range from 1 to some larger number for the least likely population, representing how many times more likely the entered genotype is in the highest-ranking populations compared to the specific population. As a rough measure of significance, a flag indicates those populations with probability values that are within an order of magnitude of the best and are therefore considered not significantly less likely to be the best.

Separately, the log of the probabilities (*Exhibit 14*) of the entered genotype is plotted from highest to lowest as a mouse-over graph that specifies the population and value at the point indicated.
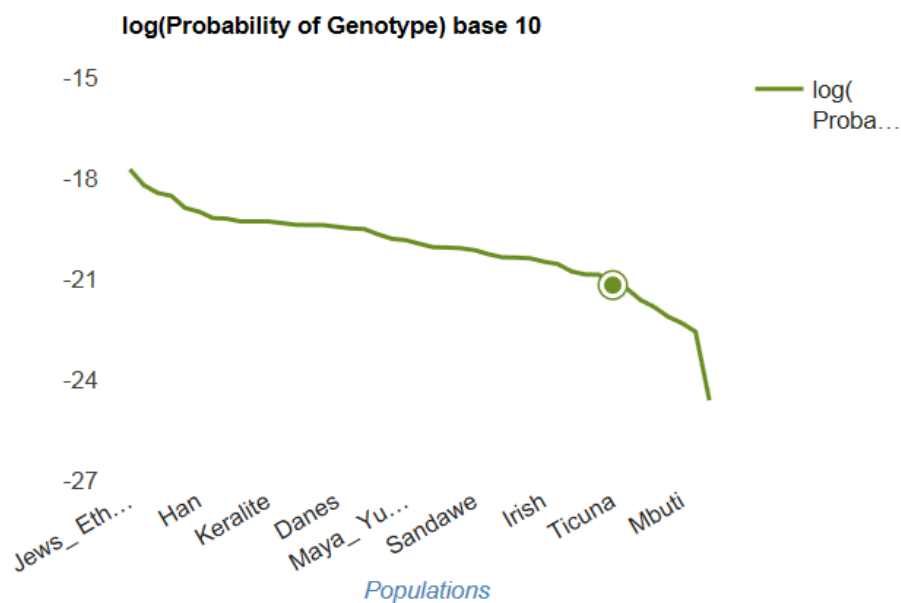
Exhibit 14. Log of probabilities for data entered in KiddLab-45 Unlinked IISNPs.

The IISNP panels have less information on actual ancestry than the AISNP panels. It is up to the user to decide whether the relevant breadth of global coverage is sufficient to use the highest probability for the entered genotype as a forensically relevant maximum probability for a random match.

No AISNP panel has data on all of the roughly 7,000 different human populations. The user must determine whether sufficient biogeographic regions of the world are represented as possible origins of the sample. For example, for the SNPforID 34-plex AISNP panel, there is no Korean population typed for all SNPs that can be used for the calculations. In the input examples, the Korean individual has data on only 24 of the SNPs. In the results table, the top eight populations

are not significantly different, and these eight and the next four populations all come from East Asia. Therefore, while a user cannot use these data to specify the population, one can safely conclude that the sample was an individual from an East Asian population.

5.3   Inputting and Searching Data: File Upload

The second option to input data is the File Upload function shown previously in Exhibit 12. The File Upload function provides users with an option to enter SNP information and a corresponding genotype for a panel in a text-area. *Exhibit 15* shows the default File Upload page. Note that in addition to the input-specific buttons shown at the bottom of this page (**File Function** and **Input Genotype for Panel**), the page provides an **About** option that serves as an online reference for users on inputting genotype data using the File Upload function. The information provided on the **About** page is the same as the instructions outlined in this User's Manual.

- **File Format -** lists all the SNPSets and sample genotype files for each SNPSet that can be downloaded. After you click on the link for the file, you may chose to browse or save the file. The tsv file can be browsed in Notepad, WordPad or TextPad. You could save the file as well for later use. Copy and paste the complete file in the text area provided in the 'Input Genotype for a Panel' function. Replace 'NN' adjacent to each SNP with the corresponding genotype observed in the individual. Click 'Upload' to upload and run the computation. Users are encouraged to download and examine the file of their interest; especially the following details.

- *First line on the file -* should start with 'ai' if an ancestry inference SNPSet and 'ii' if individual identification SNPSet. 'ai' or 'ii' should be followed by the number of SNPs in the set.(eg. 'ai34' for SNPforID 34-plex and 'ii52' for SNPforID 52-plex). The internal code uses this SNPSet tag (ai34, ii52, ii45 etc.)to identify the SNPSet the user is inserting genotype for.

  DNA Profile UId - should be provided on the first line following the panel identification text. Leave a forward slash '/' after the panel identification text. After the forward slash provide the unique Id you would like to identify the input DNA profile with. **(eg. ai55 Pilot Panel of 55 AISNPs/DNAProfile_UId).**

  Second line on the file - contains the column heads. The most important columns are 'ALFRED_UID' and 'genotype'. The other columns (dbSNP_rsnumber, chrom, chrom_pos, Alleles)are provided for the user to identify the SNPs.

  Rest of the file - contains the data. All six columns should be present; the code will extract only the first and sixth columns.

  The genotypes (genotype) should correspond to the alleles (Alleles) given in the file. The program will ignore the SNP otherwise. Enter 'NN' if genotype is unknown or omit the SNP completely from the file. The columns should be tab-delimited.

  Input Genotype for a Panel - provides a text area for user to copy and paste an individual's genotype. Follow the instructions given above while creating the file. Click 'Upload' after pasting the genotype information. We expect users will give us feedback after using this function.

Exhibit 15. Explanation on using the File Upload option for Data Entry

Clicking on the **File Format** option at the bottom of the File Upload page lists all the SNP Sets and the sample genotype files that can be downloaded for each set, as shown in *Exhibit 16*.

Format of input file and example files

File Format | Input Genotype for a Panel | About

IISNPs

Panel	File	Example File
KiddLab - 45 Unlinked IISNPs	ii45 45 Unlinked IISNPs	45_iisnps_Ex.tsv
SNPforID 52-plex	ii52 SNPforID 52-plex	52_plex_iisnps_Ex.tsv
Qiagen Investigator DIPplex Kit	ii30 Qiagen Investigator DIPplex Kit	

AISNPs

Panel	File	Example File
Seldin's list of 128 AISNPs	ai128 Seldin's list of 128 AISNPs	128_aisnps_Ex.tsv
SNPforID 34-plex	ai34 SNPforID 34-plex	34_plex_aisnps_Ex.tsv
KiddLab - Set of 55 AISNPs	ai55 Set of 55 AISNPs	55_aisnps_Ex.tsv
Kayser's set of 24 Ancestry Informative Markers	ai24 Kayser's set of 24 AISNPs	24_aisnps_Ex.tsv
Daniele Podini's list of 32 AISNPs	ai32 Podini's list of 32 AISNPs	32_aisnps_Ex.tsv
Eurasiaplex 23 SNP Panel	ai23 Eurasiaplex 23 SNP Panel	23_Eurasia_aisnps_Ex.tsv
Nievergelt's Set of 41AIMs	ai41 Nievergelt's Set of 41AIMs	41_aisnps_Ex.tsv

Exhibit 16. List of all SNP Sets and sample genotypes for download

After clicking on the link for the preferred file (see red box in Exhibit 16), users can choose to browse or save the file. The .tsv file can be browsed in Notepad, WordPad, or TextPad. *Exhibit 17* shows a .tsv file for the 45 IISNPs panel opened in Notepad.

```
45_iisnps_download.tsv - Notepad

File  Edit  Format  View  Help
ii45 Panel of 45 IISNPs
ALFRED_UID       dbSNP_rsnumber    chrom    chrom_pos          alleles  genotype
SI001900K        rs10092491        8        "28,466,991"       C/T      NN
SI001899B        rs10488710        11       "114,712,386"      C/G      NN
SI001402H        rs1058083         13       "98,836,234"       A/G      NN
SI015392U        rs10773760        12       "129,327,649"      A/G      NN
SI015046Q        rs10776839        9        "136,557,129"      G/T      NN
SI001909T        rs1109037         2        "10,003,173"       A/G      NN
SI015382T        rs1294331         1        "231,515,036"      C/T      NN
SI001396T        rs12997453        2        "182,121,504"      A/G      NN
SI001390N        rs13182883        5        "136,661,237"      A/G      NN
SI001397U        rs13218440        6        "12,167,940"       A/G      NN
SI001915Q        rs1336071         6        "94,593,976"       C/T      NN
SI015123M        rs1498553         11       "5,665,604"        C/T      NN
SI001914P        rs1523537         20       "50,729,569"       C/T      NN
SI015134O        rs159606          5        "17,427,898"       A/G      NN
SI015124N        rs1736442         18       "53,376,775"       C/T      NN
SI001913O        rs1821380         15       "37,100,694"       C/G      NN
SI001403I        rs214955          6        "152,739,399"      C/T      NN
SI015402M        rs221956          21       "42,480,066"       C/T      NN
SI015128R        rs2269355         12       "6,816,175"        C/G      NN
SI015395X        rs2342747         16       "5,808,701"        A/G      NN
SI015385W        rs2399332         3        "111,783,816"      G/T      NN
SI001391O        rs279844          4        "46,024,412"       A/T      NN
SI015053O        rs2920816         12       "39,149,319"       A/G      NN
SI001906Q        rs321198          7        "136,680,378"      C/T      NN
SI001401G        rs338882          5        "178,623,331"      A/G      NN
SI001904O        rs3780962         10       "17,233,352"       A/G      NN
SI015042M        rs430046          16       "76,574,552"       C/T      NN
SI015054P        rs4364205         3        "32,392,648"       G/T      NN
SI001912N        rs445251          20       "15,072,933"       C/G      NN
SI015393V        rs4530059         14       "103,840,194"      A/G      NN
SI015387Y        rs4606077         8        "144,727,897"      C/T      NN
```

Exhibit 17. 45 IISNPs .tsv file opened in Notepad

Users are encouraged to download and examine the files of their interest in a .tsv file, especially the following details:

- The first line on the file should start with 'ai' if an ancestry inference SNPSet and 'ii' if individual identification SNPSet.
    - 'ai' or 'ii' should be followed by the number of SNPs in the set.(e.g., 'ai34' for SNPforID 34-plex and 'ii52' for SNPforID 52-plex).
    - The internal code uses this SNPSet tag (e.g., ai34, ii52, ii45) to identify the SNPSet for which the user is inserting the genotype.
- The second line on the file contains the column headers. The most important columns are 'ALFRED_UID' and 'genotype'. The other columns (dbSNP_rsnumber, chrom, chrom_pos, Alleles) are provided for the user to identify the SNPs.
- The rest of the file contains the data. All six columns should be present; the code will extract only the first and sixth columns.
- The genotypes (genotype) should correspond to the alleles (Alleles) given in the file. The program will ignore the SNP otherwise. Enter 'NN' if genotype is unknown, or omit the SNP completely from the file. The columns should be tab-delimited.

Once users have examined the text within a .tsv file, users should copy the complete file in the text area and paste it into the provided **Input Genotype for a Panel** function (*Exhibit 18*). Users should replace NN adjacent to each SNP with the corresponding genotype observed in the individual.

Exhibit 18. 17 SNP set data pasted into the Input Genotype for a Panel function

To upload and run the computation, the user should click **Upload** at the bottom of the page. The results from the data entry using the File Upload are provided in the same format as described in **Section 5.2**, *Viewing Search Results: Selection by Radio Button*.

6.   Search Examples

Users can access a pre-entered data entry page for one individual from a specific population. These pages are dynamic, and users can run the **Compute** function from these pages. The buttons that access these pages are named after different populations (e.g., Korean, Hungarian) *Exhibit 19* shows an example of a Korean population for the KiddLab – 45 Unlinked IISNPs in Frog-kb.

Exhibit 19. Korean population example for KiddLab – 45 Unlinked IISNPs in Frog-kb

Selecting the Korean population in Exhibit 19 provides a data entry table similar to Exhibit 12 but it is pre-entered for the genotypes of the Korean population. At the bottom of the pre-entered table, select **Compute** to view the results for the example population. After selecting Compute, a table of the results and a graph outlining the probability of the genotype in each population is displayed (as described in **Section 5.2** of this User's Manual).
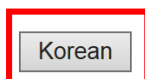
Search examples can be found in each of the SNP panels: IISNP, AISNP, and PISNP. These are good place to start if users have questions on inputting and viewing the resulting data.

7.   Formulas and Explanations used to Derive Probabilities

7.1   Derived Probabilities for IISNP and AISNP Panels

Calculations for IISNP and AISNP panels assume Hardy-Weinberg proportions of the genotypes based on the allele frequencies available for each SNP in each population. This probability of each genotype is stored in FROG-kb after being pre-calculated from the allele frequencies in ALFRED.

Thus, the probability of each genotype at one locus is given as

A.) P_r(homozygous allele 1) = (frequency allele 1) ^{2} = p^2
B.) P_r(heterozygous) = 2*(frequency allele 1)*(frequency allele 2) = 2pq
C.) P_r(homozygous allele 2) = (frequency allele 2) ^{2} = q^2

where "p" is the frequency of allele 1 and "q" is the frequency of allele 2 in the population.

The probability of a specific multi-locus genotype in a specific population is then simply the product across all loci of the locus-specific genotype probabilities, known as the product rule in forensics. The program implements this basic rule of probability by using the genotype probabilities corresponding to the genotypes entered by the user. The calculation is repeated for each population, and the population-specific probabilities are printed and graphed with the populations. The probabilities are ordered from highest to lowest. If there are no input data for one locus, that locus is skipped in the calculations for all populations.

For the IISNP panels, these are the population-specific match probabilities. For the AISNP panels, these are the probabilities of the input, multi-locus genotype arising in the different populations and can be considered the relative likelihoods of the populations being the origin of the multi-locus profile.

Special consideration is needed for those situations in which one allele is not observed in a population. The simple estimate of the allele frequency is zero, and two genotypes would strictly be estimated to be zero (i.e., absent in the population). Given the sample sizes involved, an allele at a frequency of less than 1% might never be seen, and one can never prove the absence of an allele. Entering a zero into the multiplication would clearly lead to an incorrect conclusion that the genotype could not exist in that population. A very small allele frequency needs to be assumed to be conservative. Taking sample size into account, one can assume that the next individual sampled would be the first heterozygote for the previously unseen allele. Then the allele frequency estimate would be 1/(2n+2), where n is the original sample size in which the

allele was not seen. That assumption has been made in the pre-calculation of the genotype probabilities stored in the FROG-kb database.

7.2   Derived Probabilities for PISNP Panel

The eye color of each individual can be probabilistically predicted based on his or her genotypes and the derived alpha and beta. The probabilities of each individual being brown (π_1), blue (π_2), and otherwise (π_3) is

$$\pi_1 = \frac{\exp(\alpha_1 + \sum \beta(\pi_1)_k x_k)}{1 + \exp(\alpha_1 + \sum \beta(\pi_1)_k x_k) + \exp(\alpha_2 + \sum \beta(\pi_2)_k x_k)}$$

$$\pi_2 = \frac{\exp(\alpha_2 + \sum \beta(\pi_2)_k x_k)}{1 + \exp(\alpha_1 + \sum \beta(\pi_1)_k x_k) + \exp(\alpha_2 + \sum \beta(\pi_2)_k x_k)}, \text{ and}$$

$$\pi_3 = 1 - \pi_1 - \pi_2.$$

where x_k is the number of the alleles used in the calculation of the kth SNP.

The model parameters alpha and beta were derived based on 3,804 Dutch individuals in the model-building set of a previous study (Lui et al., 2009). These probabilities can be calculated using the macro provided in the link ***here*** (Walsh et al., 2011). ***Exhibit 20*** shows the allele information relative to the forward strand and is used in the probability calculations provided in the macro.

rsnumber	ancestral/derived	allele used in calculation
rs12913832	A/G	A
rs1800407	C/T	T
rs12896399	G/T	G
rs16891982	C/G	C
rs1393350	G/A	A
rs12203592	C/T	T

Exhibit 20. Alleles relative to forward strand used in probability calculations

8. References

Liu F, van Duijn K, Vingerling JR, Hofman A, Uitterlinden AG, Janssens AC, Kayser M. Eye color and the prediction of complex phenotypes from genotypes. *Curr Biol. 19*(5):192-193. (2009)

Walsh S, Liu F, Ballantyne KN, van Oven M, Lao O, Kayser M. IrisPlex: a sensitive DNA tool for accurate prediction of blue and brown eye colour in the absence of ancestry information. Forensic *Sci Int Genet 5*:170-80. (2011)