# Table of Contents

1. Introduction ................................................................................................................................. 1  
2. Accessing FROG-kb Home Page and Features ......................................................................... 1  
3. Home Page and Navigation Tools ............................................................................................. 2  
4. Navigating FROG-kb ................................................................................................................... 3  
   4.1 SNP Panel Categories: IISNP, AISNP, PISNP ........................................................................ 3  
      SNP Set ........................................................................................................................................ 5  
      Populations ................................................................................................................................. 5  
      Data Entry .................................................................................................................................. 6  
      Formula and Examples .............................................................................................................. 6  
4.2 Documentation ............................................................................................................................ 7  
      Functionalities ........................................................................................................................... 7  
      Pipeline ...................................................................................................................................... 8  
      FAQ ........................................................................................................................................... 8  
      Manual ....................................................................................................................................... 8  
      Resources ................................................................................................................................... 8  
4.3 Contact Us ................................................................................................................................. 9  
4.4 Announcements ......................................................................................................................... 9  
5. Searching ISNP, AISNP, and PISNP Data ...................................................................................... 10  
   5.1 Inputting and Searching Data: Selection by Radio Button .................................................. 10  
      Compute .................................................................................................................................... 11  
      Set All Unselected to Unknown and Compute ......................................................................... 12  
   5.2 Viewing Search Results: Selection by Radio Button ............................................................ 12  
   5.3 Inputting and Searching Data: File Upload ........................................................................... 14  
6. Search Examples ............................................................................................................................ 17  
7. Formulas and Explanations used to Derive Probabilities ............................................................ 18  
   7.1 Derived Probabilities for IISNP and AISNP Panels ............................................................... 18  
   7.2 Derived Probabilities for PISNP Panel ................................................................................... 19  
8. References .................................................................................................................................... 20
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 Home Page](image)

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.

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.
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.

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 table shows a list of rs numbers (e.g., rs10092491); each number 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.

---

**KiddLab - 45 Unlinked IISNPs**

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

<table>
<thead>
<tr>
<th>Navigate to ALFRED</th>
<th>Geographic Region</th>
<th>Sample Size (2N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adygei</td>
<td>Russia</td>
<td>108</td>
</tr>
<tr>
<td>African Americans</td>
<td>Africa</td>
<td>182</td>
</tr>
<tr>
<td>Ami</td>
<td>East Asia</td>
<td>80</td>
</tr>
<tr>
<td>Atavai</td>
<td>East Asia</td>
<td>84</td>
</tr>
<tr>
<td>Bika</td>
<td>Central Africa</td>
<td>140</td>
</tr>
<tr>
<td>Cambodians, Khmer</td>
<td>Southeast Asia</td>
<td>52</td>
</tr>
<tr>
<td>Chagga</td>
<td>Eastern Africa</td>
<td>90</td>
</tr>
</tbody>
</table>

**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.

![Data Entry options]

**Data entry for KiddLab - 45 Unlinked IISNPs in FROG-kb**

There are two options for users to enter genotype data for a particular SNP panel: ‘Selection by Radio Button’ and ‘File Upload’. Selection by Radio Button opens the ability to specify an individual’s multi-site genotype using radio button. The list of SNPs can be sorted by rs-numbers, chromosome, siteUid and chromosomal position for ease of working with the set. For each SNP listed in the data entry table, users can navigate to ALFRED using the ALFRED UID URL link, and navigate to dbSNP using the rs-number URL link. Also displayed in the data entry table is the chromosome and chromosomal position. Radio buttons for the selection of possible genotypes are also displayed in the data entry table. Selecting the Compute button will calculate the probability of that genotype in each of the populations.

File Upload function provides users with an option to enter SNP information and corresponding genotype for a panel in a text area. Example downloadable files for each SNP panel is provided. Users can save these files and modify them by replacing the 'NN'(unknown) genotype to observed genotype in the individual. The file starts with the SNP Set tag. The tag provides information to the internal code on the type of SNP Set. For example, ‘ai34’ for SNPforId 34-plex and ‘ii52’ for SNPforId 52-plex. Copy and paste this file on to the text area provided in the Input Genotype for a Panel function. Select Upload to upload and run the computation.

**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.

**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.


**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.

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.

![KiddLab - 45 Unlinked IISNPs](image)

**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 print the output table and to see the list of SNPs used.

**KiddLab - 45 Unlinked IISNPs**

![KiddLab - 45 Unlinked IISNPs](image)

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

<table>
<thead>
<tr>
<th>Population (Region, Sample Size 2N)</th>
<th>Probability of Genotype in each Population</th>
<th>Likelihood Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jews, Ethiopian (Eastern Africa, 64)</td>
<td>1.892E-18</td>
<td>2.9</td>
</tr>
<tr>
<td>Khanty (Siberia, 100)</td>
<td>6.518E-19</td>
<td></td>
</tr>
<tr>
<td>Pima, Mexico (North America, 106)</td>
<td>3.841E-19</td>
<td>4.93</td>
</tr>
<tr>
<td>Koreans (East Asia, 132)</td>
<td>3.11E-19</td>
<td>6.08</td>
</tr>
<tr>
<td>Han (East Asia, 124)</td>
<td>1.348E-19</td>
<td>14.0</td>
</tr>
<tr>
<td>Han (East Asia, 100)</td>
<td>1.048E-19</td>
<td>18.1</td>
</tr>
<tr>
<td>Ibo (Western Africa, 96)</td>
<td>6.792E-20</td>
<td>27.9</td>
</tr>
<tr>
<td>Chagga (Eastern Africa, 90)</td>
<td>6.429E-20</td>
<td>29.4</td>
</tr>
<tr>
<td>Yakut (Siberia, 102)</td>
<td>5.40E-20</td>
<td>35.0</td>
</tr>
<tr>
<td>Masai (Eastern Africa, 44)</td>
<td>5.399E-20</td>
<td>35.0</td>
</tr>
<tr>
<td>Keralite (South Asia, 60)</td>
<td>5.391E-20</td>
<td>35.1</td>
</tr>
<tr>
<td>Hakka (East Asia, 86)</td>
<td>4.811E-20</td>
<td>39.3</td>
</tr>
</tbody>
</table>

*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.

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.
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.
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 SNPSSet and 'ii' if individual identification SNPSSet.
  - '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 SNPSSet tag (e.g., ai34, ii52, ii45) to identify the SNPSSet 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.
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.

#### Example files for KiddLab - 45 Unlinked IISNPs in FROG-kb

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 compile function from here. The buttons to access these pages are named after the population the individual is from, for example Korean, Hungarian etc. 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 graph for the probability of genotype in each population is displayed. (as described in **Section 5.2** of this User’s Manual).

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

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 ($\pi_1$), blue ($\pi_2$), and otherwise ($\pi_3$) is

$$
\pi_1 = \frac{\exp(\alpha_1 + \sum \beta(\pi_1)x_k)}{1 + \exp(\alpha_1 + \sum \beta(\pi_1)x_k) + \exp(\alpha_2 + \sum \beta(\pi_2)x_k)},
\pi_2 = \frac{\exp(\alpha_2 + \sum \beta(\pi_2)x_k)}{1 + \exp(\alpha_1 + \sum \beta(\pi_1)x_k) + \exp(\alpha_2 + \sum \beta(\pi_2)x_k)}, \text{ and } 
\pi_3 = 1 - \pi_1 - \pi_2.
$$

where $x_k$ is the number of the alleles used in the calculation of the $k^{th}$ 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.

<table>
<thead>
<tr>
<th>rsnumber</th>
<th>ancestral/derived</th>
<th>allele used in calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12913832</td>
<td>A/G</td>
<td>A</td>
</tr>
<tr>
<td>rs1800407</td>
<td>C/T</td>
<td>T</td>
</tr>
<tr>
<td>rs12896399</td>
<td>G/T</td>
<td>G</td>
</tr>
<tr>
<td>rs16891982</td>
<td>C/G</td>
<td>C</td>
</tr>
<tr>
<td>rs1393350</td>
<td>G/A</td>
<td>A</td>
</tr>
<tr>
<td>rs12203592</td>
<td>C/T</td>
<td>T</td>
</tr>
</tbody>
</table>

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