USACIL’S DNA_DataAnalysis v2.1.3
User’s Manual
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This software was developed at the U.S. Army Criminal Investigation Laboratory by Forensic Biologist Tom Overson. The manual was written predominantly by Angela M. Dolph in partial fulfillment of her master’s of science degree. The NEST Project Team and Tim Kalafut contributed to this manual.
1.0 Introduction

1.1 DNA_DataAnalysis was developed by USACIL Forensic Biologist Tom Overson. The program was originally created as a matching program with a few statistical applications, but grew to meet analyst demands.

1.2 The mouse-driven program was written in Visual Basic and runs on Microsoft Excel 2003. If using another version of excel, be aware that this program has only been tested and validated with Excel 2003; therefore, it is highly recommended that DNA_DataAnalysis only be used on Excel 2003. There are no known conflicts of running the program simultaneously with GeneMapper ID or other programs in the Microsoft suite, but problems have been observed while running simultaneously with Genotyper. The program can be run from any drive on a stand-alone PC, from a network, or from an external drive.

1.3 DNA_DataAnalysis is not intended to be an expert system, but is designed to aid analysts in routine DNA analysis in that it enables the analysts to perform several analyses that they would normally want to perform on their GeneMapper ID data. The program allows the user to check ladders, check controls, check for possible stutter, perform matching between evidence samples and references, contamination checking with staff profiles, perform CODIS functions, do mixture interpretation with two to three contributor mixtures, view simulated electropherograms, chart data, perform various biostatistical analyses for single and multiple source samples, print, and save data. All appropriate allele calls and routine inspection of data needs to be completed in GeneMapper ID v3.2 prior to analysis with DNA_DataAnalysis. The program is limited to analysis with the Amf/STR® kits Identifiler™, Profiler Plus™, COfiler™, and YFiler™ and the Promega kit PowerPlex® 16.

2.0 Installation

2.1 System Requirements:
Operating System: Microsoft Windows 2003, specifically Microsoft Excel 2003
Processor Specification: 1-2 GB of RAM
Program is approximately 7500 kB
Can run program from any drive
Security Settings: Medium or low to permit use of specific macros
Changing security settings:
Open Excel 2003 - Tools - Options - Security - Macro Security

2.2 Installation Instructions:
Insert the DNA_DataAnalysis compact disk into the computer CD drive. Installation is simply a copy and paste operation. Copy the DNA_DataAnalysis folder and paste it anywhere on the computer according to user preferences. Once installed, do not remove the folders from the encompassing DNA_DataAnalysis folder if user wants to be able to save and retrieve data. If necessary, change the Table Settings and Drives Folders on the Info page within the program. The Tables Drive is automatically set to the default table location of drive H, which is a generic drive and refers to the program no matter its location. The Tables Folder Path’s default table location is GMID_Tables, which is located inside the DNA_DataAnalysis folder and houses all of the GMID tables. The users can place their output tables into the corresponding sections within this folder or they can create their own output data folder and have the program search at this alternate location.

<table>
<thead>
<tr>
<th>Default Table Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tables Drive</td>
</tr>
<tr>
<td>H</td>
</tr>
<tr>
<td>Tables Folder Path</td>
</tr>
<tr>
<td>GMID_Tables</td>
</tr>
</tbody>
</table>

If Tables Drive = H, then tables will be located in the DNA_DataAnalysis path \GMID_Tables

Examples of changing table locations:

To access a folder C:GMID_Tables
Set the Tables Drive = “C”
Set the Tables Folder Path = “GMID_Tables”

To access a desktop folder named Desktop_GMID_Tables
Set the Tables Drive = “C”
Set the Tables Folder Path like “Documents and Settings\users\Desktop\Desktop_GMID_Tables”
Note: the path may be different on your computer

Folders inside GMID_Tables folder – holds GMID output tables

3.0 Program Navigation

Open different pages, user forms, and perform different functions by left or right-clicking on various sections of the spreadsheets.

3.1 Opening program:
DNA_DataAnalysis program opens in Excel 2003

The Analyst_Data page is automatically opened with the program.

3.2 Opening and closing pages:
Once the program is open, the user can open specific pages depending on analysis.

Right-click in the upper white rows or the top gray line on the spreadsheet to open the “Print / Select pages to view / Select user name” user form

The “Print / Select pages to view / Select user name” user form allows the user to open and close different pages. Open pages appear highlighted in green. Once a gray button is selected, the page automatically opens behind the user form.
To close a page, click on the corresponding highlighted green button. The page is cleared and hidden and the button turns gray. Clicking on the ‘All Pages’ button under the “Clear” heading allows the user to clear all of the pages at once.

The drop-down menu under the “Select User name” heading allows the user to select a user name. Additional user names can be inserted on the Info page.
To close the “Print / Select pages to view / Select user name” user form, select the Close or OK buttons when finished opening or clearing pages.

3.3 Fitting pages to fit screen:
Occasionally, when the pages are opened they will not be formatted to fit entirely on the screen. There are a couple ways to format the pages so that they can be viewed in whole on the screen. The first method is to right click in the gray area in most spreadsheets.

Opening window

The user form “Get data / Compare / Check / Fit / Misc” appears.
Fit the page to the screen by clicking the buttons under the “Fit” heading. The button of the selected fit will highlight green and the fitting action is performed immediately after clicking the button. Click Close to close the user form.

Another method to format the pages on screen is to utilize the excel view menu option and to customize the zoom.

4.0 Operations Flow Chart

A basic flow chart illustrating the most utilized functions and pages.
5.0 GeneMapper ID v3.2

5.1 Before using DNA_DataAnalysis
Normal data examination is conducted within GMID prior to analysis with DNA_DataAnalysis. The sample profiles need to have the proper allele calls with all artifact peaks that have been erroneously called as alleles removed from the profile.

5.2 GMID output table settings
The data table going into DNA_DataAnalysis needs to be formatted and exported from GMID. The output table has specific components and it is recommended to create a generic DNA_DataAnalysis table setting.

Within the Genotypes tab, click on tools and open the “Table Setting Editor…”

Under the Genotypes tab of the Table Setting Editor, check only the boxes shown and change the number of alleles to 35
5.3 Exporting a GMID table

Select the Genotypes Tab and to export the table, click on File and select “Export Table…”
Place the exported table anywhere on the hard drive, network, USB, or in the GMID_Tables folder, which is where the program defaults to when importing a GMID output table.

The output file needs to be in text (.txt) format and the samples should have the following appropriate designations:
   - Ladder = kit specific allelic ladder
   - Pos Con = positive control
     - Can have extra text within the sample name as long as Pos Con is present
     - 9947A is automatically changed to Pos Con
   - Neg Con = negative control
   - Reag Blank = reagent blank
   - QA### = quality control samples
     - Need three number designation (ex: QA1 is QA001)
   - ola = off-ladder allele

6.0 Importing Data Files into DNA_DataAnalysis

6.1 Loading GMID output file
Once the program is open, the Analyst_Data page automatically opens as well. To open the data table into the Analyst_Data or Review_Data pages, right-click the gray section on the spreadsheet to open the “Get data / Compare / Check / Fit / Misc” user form.

To open a GMID data table into the Analyst_Data page, under the “Get Analyst table” heading select the GMID button, which highlights green once selected. Re-clicking the button allows the user to open different types of GMID output files.
Within the Analyst and Review tables buttons:
  I = Identifiler
  P-C = Profiler-COfiler
  Yfiler = Yfiler
  Size Std = Identifiler Size Standard

To import data tables from the various kits, select the appropriate GMID button (Analyst or Review) and click it until the correct designation appears (I, P-C, Yfiler, Size Std). Click OK.

Re-clicking the GMID buttons under the “Get Analyst table” or “Get Review table” changes the button to these designations.

After the appropriate button is selected, the corresponding kit GMID_Tables folder directory is displayed. Find the proper GMID output table and select open. The data table will be formatted to and open in the DNA_DataAnalysis program.
The error “inappropriate text entry” occurs when the formatting of the GMID .txt file is incorrect or if some of the sample names are unrecognized by the program.

Opening a GMID data table into the Review_Data page is the same procedure as opening one in the Analyst_Data page; however, utilizing the GMID button under the “Get Review table” heading.

6.2 Opening a single profile:
To open an already saved, single profile, select the Single Profile button under the “Get a saved file” heading. Click OK.
Note: The profile will open to either the Analyst>Data or Review>Data page depending on which page is open.

6.3 Opening previously saved page data:
To retrieve an already saved page data, re-click the Single Profile button and it will change to Page Data. Click OK.

Note: The profile will open to either the Analyst>Data or Review>Data page depending on which page is open.
The Page_Data folder automatically displays. Locate the appropriate page data file and select open to import the data into the Analyst_Data page.

7.0  Features and Functions

7.1  User Forms:

7.1.1  Print / Select pages to view / Select user name
Depending on the page, this user form is accessed by either right clicking in the top section of a spreadsheet or by right clicking anywhere in a spreadsheet.

For some pages, right click in the top (white) section of a spreadsheet to open the user form.
The pages that will open the Print / Select pages to view / Select user name user form by right clicking in the top of the page are:

- Analyst_Data
- Review_Data
- Temp_Data
- Compare_Data
- Staff_Profiles
- Freq_SS
- Freq_Mix
- Freq_PI
- L_Ratio
- QA_Profiles
- NIST_Profiles

The pages that will open by right clicking anywhere in the body of the spreadsheet are:

- Allele_Freq
- Info

For other pages, right clicking anywhere in the body of the spreadsheet opens the user form.
The pages that do not have a Print / Select pages to view / Select user name user form are:

CMF_Data
CMF
CDF

The basic functions of this user form are to select the pages one wants to print, view, or clear. The user form also allows the user to view the excel menu, view the special Alt and F keys, select a user name, chart, get profiles, and save profiles.
The user form can be displayed differently according to the page because the different pages may not have the same functions. Refer to the specific page sections for discussions on these differences.

7.1.2 Get data / Compare / Check / Fit / Misc

This user form can be displayed by right clicking in the body of the page; however, it is only available on these pages:
- Analyst_Data
- Review_Data
- Compare_Data
- Temp_Data
- Staff_Profiles
- QA_Profiles
- NIST_Profiles
The user form and its functions according to the Analyst_Data page:

- The selected functions are highlighted green. Some of the functions require the user to click OK before they are applied, but some of the functions are applied immediately. Different pages may show fewer buttons because some pages cannot perform all of the functions.

7.1.3 Matching / Send for reference / Send profile / Other
This user form is obtained by right clicking on a Sample ID and can be viewed on the following pages:
- Analyst_Data
- Review_Data
- Temp_Data
- Staff_Profiles
The user form and its functions according to the Analyst_Data page:

7.1.4 Frequency Calculations
This user form appears on the pages that can perform biotistical calculations; however the form differs according to page. It is accessed by right clicking in the body of the page and can be found on the following pages:
- Freq_SS
- Freq_Mix
- Freq_PI
- L-Ratio
Right click in the body of the spreadsheet to open the user form.

The user form and its functions according to the Freq_SS page:

7.1.5 CODIS – CDF
This user form is only found on the CDF page and can be displayed by right clicking in the body of the page.

The CDF page, right clicking within the body of the spreadsheet opens the user form

The user form functions:
7.2 Info page:
The Info page is where users can add or change user names, insert user CODIS names, change user defined threshold values, set header and footer print options, and tell the program where the tables are located.

The user should only change the bold text even though all of it can be edited.
The left-hand side of the page displays the user names and their corresponding CODIS IDs. The names in the list of users should be formatted how the users want their names to be printed in the header or footer. The CODIS IDs are the names that appear in the .cmf CODIS files.

<table>
<thead>
<tr>
<th>Users</th>
<th>User's CODIS IDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen, Benjamin</td>
<td>Allenb</td>
</tr>
<tr>
<td>Bardell, Martha</td>
<td>Bardelmb</td>
</tr>
<tr>
<td>Carter, Sydney</td>
<td>Carters</td>
</tr>
<tr>
<td>Cheung, Frank</td>
<td>Cheungbf</td>
</tr>
<tr>
<td>Coppedale, Noah</td>
<td>Coppedalen</td>
</tr>
<tr>
<td>Barney, Charles</td>
<td>Barneyc</td>
</tr>
<tr>
<td>Batch, Dick</td>
<td>Batchdick</td>
</tr>
<tr>
<td>Defarge, Ernest</td>
<td>Defargee</td>
</tr>
<tr>
<td>Flintworth, Jeremiah</td>
<td>Flintworthq</td>
</tr>
<tr>
<td>Gargessy, Joe</td>
<td>Gargessy</td>
</tr>
<tr>
<td>Handford, Julius</td>
<td>Handfordj</td>
</tr>
<tr>
<td>Honeythunder, Luke</td>
<td>Honeythunder</td>
</tr>
<tr>
<td>Jodler, Grace</td>
<td>Jodlerg</td>
</tr>
<tr>
<td>Jingle, Alfred</td>
<td>Jinglea</td>
</tr>
<tr>
<td>Magnus, Abel</td>
<td>Magnusba</td>
</tr>
<tr>
<td>Merritt, Lucie</td>
<td>MerritteL</td>
</tr>
<tr>
<td>Micawber, Emma</td>
<td>MicawberE</td>
</tr>
<tr>
<td>Murdock, Edward</td>
<td>Murdockee</td>
</tr>
<tr>
<td>Ovesen, Thomas</td>
<td>Ovesent</td>
</tr>
<tr>
<td>Pecksnait, Seth</td>
<td>Pecksnaitt</td>
</tr>
<tr>
<td>Pitch, Tom</td>
<td>Pitcht</td>
</tr>
<tr>
<td>Piirps, Phillip</td>
<td>Piirpsp</td>
</tr>
<tr>
<td>Quall, Daniel</td>
<td>Qualld</td>
</tr>
<tr>
<td>Raffert, George</td>
<td>Rafferto</td>
</tr>
<tr>
<td>Simpson, Harold</td>
<td>Simpsons</td>
</tr>
<tr>
<td>Slidukowsk, Peg</td>
<td>Slidukowskp</td>
</tr>
<tr>
<td>Sponw, Dora</td>
<td>Sponwd</td>
</tr>
<tr>
<td>Steerforth, James</td>
<td>Steerforthj</td>
</tr>
<tr>
<td>Sweeds, Paul</td>
<td>Sweedsps</td>
</tr>
<tr>
<td>Wardle, Emily</td>
<td>Wardle</td>
</tr>
<tr>
<td>Westfield, Agnes</td>
<td>Westfielda</td>
</tr>
</tbody>
</table>

To the right of the user names is the ladder optimization section. Listed are the low and high base pair positions of the alleles within the loci. The default is a 1.5 base pair window in which the sample ladder has to match the low and high alleles of each locus. The program evaluates the ladders based on pattern and the first and last allele positions on a per locus basis. The stutter values for each locus are also listed and can be changed according to laboratory validation results.
Directly below the ladder optimization section is the size standard setting. The default setting instructs the program to look at the sample size standard 246 (250) bp peak and to make sure that it is no more than 0.50 base pairs away from 246.00. This setting is specifically designed for GS 500 LIZ / ROX and is therefore ignored for other size standards. The program does not need the size standard setting to run properly.

<table>
<thead>
<tr>
<th>Size Std</th>
<th>246.00</th>
<th>0.50</th>
</tr>
</thead>
</table>

Size standard setting

Beneath the size standard setting is the GMID table format setting. There is an option here to have it set as USACIL or Generic. Any lab using this program outside of USACIL should use the Generic setting.

<table>
<thead>
<tr>
<th>GMID or OT Table format</th>
<th>USACIL</th>
<th>Generic</th>
</tr>
</thead>
<tbody>
<tr>
<td>If the table format is</td>
<td></td>
<td></td>
</tr>
<tr>
<td>USACIL specific Log Number and Sample ID rearrangement will occur on import of the table into DNA_DataAnalysis; if the Log Number and Sample ID are not in USACIL format, an error will occur</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generic</td>
<td>Use for all non-USACIL</td>
<td></td>
</tr>
</tbody>
</table>

GMID table format setting

The file save location setting is directly below the GMID table format setting. In this section, the user can designate where the saved profiles will be saved. The default setting is within the Saved_Profiles folder. To change this location, simply type in the new folder name.

<table>
<thead>
<tr>
<th>File save location</th>
<th>Saved_Profiles</th>
</tr>
</thead>
</table>

File save location

On the Info page, to the right of the ladder optimization section is the user defined threshold section where the user can regulate the off scale data, low scale data, peak height imbalance, peak height ratio, and minimum peak height thresholds. To change these settings, simply type in the new value in the appropriate cell.

| Off Scale Data | 6990 |
| Low Scale Data | 340  |
| Peak Height Imbalance | 0.5 |
| Peak Height Ratio | 0.5 |
| Minimum Peak Height | 150 |

User defined thresholds that are used in the mixture calculations and sample checks

To the right are the tables drive and tables folder path location settings. The tables drive is automatically set to point to drive H, which is a generic designation and can be used no matter where the program is located. The tables folder path default setting is GMID_Tables, which is where the program defaults when importing a GMID table into the DNA_DataAnalysis program. To change these settings refer to the examples below and simply type in the new locations.
The last item on the Info page is the print header and print footer options. The user can print specific features in specific places on the printed page if they follow the exact syntax shown on the Info page.

<table>
<thead>
<tr>
<th>Left</th>
<th>Center</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>(case number)</td>
<td>(fuo)</td>
<td>(date)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(page number)</td>
</tr>
</tbody>
</table>

If an entry above contains: the Header or Footer will:

- (case number)
- (user name): show case number user form, print the case number
- (fuo): print the Selected User
- (date): print "For Critical Use Only - Law Enforcement Sensitive"
- (page number): print "Page "

### 7.3 Analyst_Data page:
The Analyst_Data page automatically opens with the program and contains most of the functions the user would want to perform on the data. Once the GMID output table is imported into the Analyst_Data page, the user can open the “Get data / Compare / Check / Fit / Misc” user form by right-clicking on the right side of the spreadsheet under the loci columns, and analyze the data according to the various functions within the user form.

#### 7.3.1 Changing views
Utilizing the buttons under the “Select view” heading within the “Get data / Compare / Check / Fit / Misc” user form, the user can change what is displayed by just viewing the evidence samples, controls, or all. The user can also view the data as alleles, RFUs, or base pairs.

#### 7.3.2 Check controls
The controls are the ladder, positive control, negative control, and any QA samples. To perform a check on the controls, open the “Get data / Compare / Check / Fit / Misc” user form by right clicking within the body of the spreadsheet and select the Controls GMID I button under the “Do checks” heading. Only the control samples will be displayed and a
color key explaining the results is located below the samples. Green indicates that the control is within established parameters and pink indicates that the controls need further evaluation because they are not within the established parameters.

The control checks can be removed by opening the “Get data / Compare / Check / Fit / Misc” user form and selecting the Clear checks button under the “Do checks” heading. This button automatically clears the checks, but if the user wants to see all of the samples again, click on the All button under the “Select view” heading of the same user form. This button is used to clear any of the checks performed on the data.

7.3.3 Evaluate for potential stutter
Evaluating for potential stutter is a two-part process that first involves opening the “Get data / Compare / Check / Fit / Misc” and selecting the Stutter button under the “Do checks” heading. The loci with potential stutter peaks will be highlighted in pink.
To obtain the stutter calculations, right click on one of the cells that have been indicated as having potential stutter. The “Get data / Compare / Check / Fit / Misc” user form opens and the user selects the stutter button again. This opens a Stutter Check window that has the alleles, their corresponding RFUs, and the stutter calculation. Clicking the Close button closes this window. After all loci have been evaluated according to stutter, clear the checks and if any alleles need to be removed from the samples, this needs to be done within GMID.

7.3.4 Check RFUs height and ratio

The thresholds that are used to check for RFUs high, RFUs low, and RFUs ratio are user-defined on the Info page. To perform this check, open the “Get data / Compare / Check / Fit / Misc” user form then select the RFUs high button under the “Do checks” heading. Re-clicking this button changes it from RFUs high to RFUs low to RFUs ratio. Loci that
contain alleles that need to be evaluated based on their RFUs are indicated in pink. The RFUs ratio is only performed on loci with two alleles.

7.3.5 Check for CODIS alleles

One way to obtain the alleles allowed in CODIS is to right click on the colored locus designations on the top of the screen.

This opens an alleles allowed in CODIS information box that lists the allowed alleles per locus and enables the user to view charts of the base pairs associated with the Identifiler and Yfiler Ladders.

Another way to check for CODIS alleles is to open the Matching / Send for reference / Send profile / Other user form and select the CODIS button.
The CODIS functions:

- Matching
  This program has several different matching applications that allow the user to view the data in various ways. The matching functions can be accessed by right clicking on a
sample ID. This action opens the Matching / Send for reference / Send profile / Other user form.

To compare your data to a single reference, under the ‘Select a Reference(s) and do matches’ heading, select the ‘Match to a Reference’ button. This will highlight the reference sample orange, any loci that exactly match the reference sample orange, and any loci that have the reference sample included yellow. The color key is directly below the samples on the spreadsheet for direct reference.

Once a reference is designated, the rest of the Matching / Send for reference / Send profile / Other becomes accessible. The area under the With an existing match (a Ref is at page top) now has useable buttons. There are now four options that can be selected:

a.) Find where Ref is included: Highlights sample profiles which include the reference profile
b.) Find where Ref includes Foreign: Highlights sample profile which contain alleles foreign to the reference profile
c.) Find inclusive in the Reference: Compares the sample profiles to the reference, highlighting the same alleles.
d.) Find foreign to the reference: Compares the sample profiles to the reference, highlight the alleles which are found in the samples, but not in the reference.

To understand the matching results, follow the color designation keys at the bottom of the spreadsheet.
To select multiple samples to be used at references select a sample from the list and right click on its Sample ID bring up the Matching / Send for reference / Send profile / Other user form. Under the ‘Select a Reference(s) and do matches’ heading, select ‘Match to reference 1 of 2 or designate the selected sample as reference 1. To designate a different sample as reference 2 follow the same procedure as above and select the ‘Match to reference 2 of 2’ box. The sample designated as reference 1 will be highlighted in pink, while the sample designated as reference 2 will be highlighted in light blue. Any loci that include both references 1 and 2 will be highlighted yellow.

The Find Foreign Alleles button under the “Select a Reference(s) and do matches” allows the user to find alleles that are foreign to combined references. To combine references,
left click on a Sample ID, hold control, and left click on another Sample ID; repeat until
achieve wanted combination of samples. Then, right click to open the Matching / Send
for reference / Send profile / Other user form and select the Find Foreign Alleles button.
The combined references Sample ID’s are highlighted in orange, the loci that contain
alleles not found in the combined references are highlighted pink, and the loci with
alleles included in the combined references are left white.

Example of Finding Foreign Alleles on Analyst Data page.
The color designation key is located below the samples.

If the user wants to send a sample as a reference to another page, right click on the
sample name listed under the “Sample ID” column. This will bring up the Matching /
Send for reference / Send profile / Other user form. Select the page the analyst would like that profile to be sent to (Analyst Data, Review Data, Temp Data, Staff Profile, QA Profiles or NIST Profiles). The sample name and profile will now appear, highlighted in orange, at the top of the selected page above the listed loci.

7.3.7 Mixture Interpretation Tool
The mixture interpretation tool can be used to aid in deconvolution of two or three person mixtures. The tool can be accessed on several pages by double left clicking on the alleles of a locus within a sample.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>D200079</th>
<th>D21811</th>
<th>D208420</th>
<th>CS11FPC</th>
<th>D32158</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evidence A</td>
<td>13, 14</td>
<td>28, 29, 32, 8, 9, 11</td>
<td>10, 11, 12, 15, 16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evidence B</td>
<td>8, 12, 13, 129, 30, 31, 8, 12</td>
<td>10, 11, 12, 15, 17, 19</td>
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<tr>
<td>Evidence C</td>
<td>8, 12, 13, 129, 30, 31, 8, 12</td>
<td>10, 11, 12, 15, 17, 19</td>
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<td></td>
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</tr>
<tr>
<td>Evidence D</td>
<td>13, 14</td>
<td>28, 29, 32, 8, 9, 11</td>
<td>10, 11, 12, 15, 16</td>
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<td></td>
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<tr>
<td>Evidence E</td>
<td>13, 14</td>
<td>28, 29, 32, 8, 9, 11</td>
<td>10, 11, 12, 15, 16</td>
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<td>Ladder.fsa</td>
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<td></td>
<td></td>
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<tr>
<td>Neg Control</td>
<td>O</td>
<td>2401</td>
<td></td>
<td></td>
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<tr>
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<td>8, 11</td>
<td>10, 12, 16</td>
<td></td>
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<tr>
<td>Suspect 2</td>
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<td>28, 29</td>
<td>8, 11</td>
<td>10, 12, 16</td>
<td></td>
</tr>
<tr>
<td>Suspect 2.5</td>
<td>14</td>
<td>29, 31</td>
<td>8, 11</td>
<td>12, 17, 19</td>
<td></td>
</tr>
<tr>
<td>Victim 1</td>
<td>13, 14</td>
<td>22, 33, 2</td>
<td>8, 9</td>
<td>11</td>
<td>15, 16</td>
</tr>
<tr>
<td>Victim 2</td>
<td>13, 14</td>
<td>20, 32, 2</td>
<td>8, 12</td>
<td>10</td>
<td>15, 17</td>
</tr>
</tbody>
</table>

The basic functions within the mixture interpretation tool are:
To set and apply references, first double left click on an allele cell of a reference profile to open the mixture interpretation tool. Set the reference by clicking on a Ref1…Ref6 button. The Sample ID on the Analyst_Data page will highlight according to the reference number. Up to six references can be set, but only three can be applied simultaneously.
To apply references to the evidence samples, open the mixture interpretation tool by double left clicking on an allele cell of the mixture sample. Under the Apply heading, select the references the user wants to apply and according to the applied references and the user-defined thresholds, the possible genotype combinations will be listed in the gray area at the bottom of the mixture interpretation tool.
The analyst has the option of selecting specific alleles for the different contributors and sending these selections to the Analyst_Data page. To set the user-defined allele designations for the contributors, select the boxes under the P1, P2, etc. areas on the right-hand side of the tool. The P labels are for user reference and can be used for either the major or minor contributors.

Note: When the mixture interpretation tool is first opened, P1, P2 and P3 are available. However, once the ‘2’ or ‘3’ buttons on the top left corner are selected, the P boxes reflect the number of contributors designated for the calculations. For example, selecting the ‘2’ button tells the program that it is a 2-person mixture and only the P1 and P2 boxes are available for user-defined allele designation.
Once the user-defined designations have been selected, the user can send them to the Analyst_Data page by selecting the appropriate button. Sending the user-defined alleles over to the Analyst_Data page is performed separately for each locus.

Note: The button functions can be explained by hovering over them.

![Diagram of user interface]

The left-most button sends the user-defined allele calls to the Analyst_Data page.

Once the user-defined designations are on the Analyst_Data page, the analyst can rename them by simply typing over the existing name according to personal preference. However, if the mixture interpretation tool is closed then re-opened after renaming the user defined profile, and the user continues sending over user-defined values from the same sample, the program puts these designations in a new section; it does not continue from the locus where the user left-off. This allows for the user to have multiple sets of deconvolution results for a given sample. (Every time the user sends alleles to the user defined profiles, the software expects those lines in the table to start with “User defined…” If it doesn’t see that, it inserts (starts over) a line(s) with that name.)

The user can also remove an allele from mixture calculation consideration by double left clicking on the allele designation located in the upper right-hand corner. This action highlights the allele boxes pink to indicate that a change has been made. The removed allele is only removed from the calculations, but is still present in the electropherogram, total data, etc.
Select the ‘Re-set’ button to retrieve the removed allele. The boxes return to white.

The user can also correct for stutter by double left clicking on any RFU value located in the upper right quadrant. This action automatically corrects for maximum stutter, which has a value of 1 in the stutter correction box. The changed RFU values are highlighted in pink. The ‘st’ button sends all of the loci combinations with stutter corrections over to the Mix_Interp page.

The 29 allele was removed from the calculations and the remaining alleles were highlighted pink.
The analyst is able to correct for a different proportion of maximum stutter by utilizing the bottom left and right arrows next to the stutter correction value box. For example, a value of 0.5 corrects for half of maximum stutter. The RFU values change automatically when the arrows are selected.

When finished sample analysis with the mixture interpretation tool, transfer the loci to the mixture interpretation page (Mix_Interp). This can be done locus-by-locus by selecting the light triangle or it can be accomplished by transferring all the loci at once with the dark triangle.

Sending the mixture interpretation results to the Mix_Interp page

7.3.8 Saving
When, exiting the program, be sure to NOT save changes to DNA DataAnalysis; instead, saving the data is done on the Matching / Send for reference / Send profile / Other user form.
To save a single profile that can be brought back into the program, select the Single Profile button. This opens a dialogue box that allows the user to type in a file name and to save it to the Saved Profiles folder.

To save the Page Data that can be brought back into the program, select the Page Data button. This opens the same dialogue box as before, but saves the whole page of data rather than just a single profile.

The user also has the option of saving a page view by selecting the Page View button; however, this cannot be brought back into the program. The same dialogue box is opened and the user can type in a file name.

Using the Print / Select pages to view / Select user name user form, the user also has the options of creating special folders. Open the user form and select the ‘Select new location’ button. This clears the folder name and allows the user to type in a new one. After typing in a new folder name, re-click ‘Select new location’ and close the user form.
To save within the new folder, right-click on a sample name to open the Matching / Send for reference / Send profile / Other user form and select the appropriate button under the ‘Set new location’ button.

Type in a new folder name and re-click the ‘Set new location’ button.

To add in a special folder for saving, click the ‘Set new location’ button within the Print / Select pages to view / Select user name user form.

To save within the new folder, right-click on a sample name to open the Matching / Send for reference / Send profile / Other user form and select the appropriate button under the
“Save” heading. This opens the “Enter a file name” dialogue box and the default folder and the special folder buttons will be available.

Both the default folder and newly created folder are available for saving.

The user can create an unlimited number of specific folders, but to be able to save in the folder, one has to re-type the folder name onto the Print / Select pages to view / Select user name user form with the ‘Select new location’ button. An error will appear, but ignore it and the wanted folder will be available for saving.

The error that appears on the Print / Select pages to view / Select user name user form when trying to type in an already existing folder name. Simply ignore this error when trying to save into the folder.

7.4 Review_Data page:
Refer to section 6.1 when importing a GMID output table into the Review_Data page.
The Review_Data page is utilized to compare the same samples that have been analyzed by different analysts. In order to be compared, the review data and analyst data need to have the same Sample IDs and the samples need to be in the same order. After importing the Analyst and Review data compare the results by opening the Get data / Compare / Check / Fit / Misc user form. To access this user form, right click within the body of the spreadsheet under the loci designations.

To compare the analyst and review data, select the Analyst to Review button under the Compare tables heading within the user form. This action opens another page, Compare_Data page, to display the results.
The Review_Data page can be utilized to perform any function that was discussed under the Analyst_Data page, section 7.3.

7.5 Compare_Data page:
The Compare_Data page automatically opens after the user selects the Analyst to Review button under the Compare tables heading within the Get data / Compare / Check / Fit / Misc user form. This page displays a line-by-line comparison of the samples. A green designation indicates that the analyst data matches the review data; a pink designation indicates that the analyst data does not match the review data. If differences arise, both the analyst and the reviewer should discuss why there were differences and make any necessary changes to the data in GeneMapper ID.

The Compare_Data page can be used to perform some analysis, but does not have as many functions as the Analyst_Data page or Review_Data page.

The Get data / Compare / Check / Fit / Misc user form according to the Compare_Data page
The Compare_Data page cannot perform any of the functions on the Matching / Send for reference / Send profile / Other user form because the page does not have this user form.

The Compare_Data page does have the mixture interpretation tool.

7.6 Temp_Data page:

The Temp_Data page is used to compare specific profiles from the same or different data sets. To send a sample to the Temp_Data page, open the Matching / Send for reference / Send profile / Other user form from the Analyst_Data page, Review_Data page, or any of the profiles pages and select the Temp Data button under the Send selection to heading.
The Temp_Data page cannot perform all of the functions discussed for the Analyst_Data page. Its Get data / Compare / Check / Fit / Misc user form has only some of the function buttons.

The Matching / Send for reference / Send profile / Other user form has all of the function buttons except the Send all data to Total Data and CODIS buttons.

To send a sample to the Temp_Data page, select the Temp Data button within the Matching / Send for reference / Send profile / Other user form that is available on certain pages.
The Mix_Interp page is automatically opened once the mixture interpretation tool is accessed, which is only available on certain pages. To transfer data into the Mix_Interp page, utilize the light and dark triangle buttons within the mixture interpretation tool. If the user wants to chart the data, make sure that all of the appropriate references are applied; two for a two-person mixture and three for a three-person mixture.

When sending loci to the summary Mix_Interp page one at a time, the user can change the PHr (peak height ratio) parameters, correct for stutter, kick out an allele, etc. and it will be more of a “custom” summary page. Alternatively, the user can send all the loci at once, and then if the analyst wants to re-send a single locus with a correction applied, the locus will be appended to the end of the list on the Mix_Interp page, but the graph will use the second sending of the locus when charting.

Once the data is transferred to the mixture interpretation page, it is organized according to loci with the Sample ID placed above the different loci. The most probable genotype for each locus is highlighted in green. The only user form accessible on the Mix-Interp page is the Print / Select pages to view / Select user name user form.
The Condense View button on the Print / Select pages to view / Select user name condenses the list down to just the possible genotypes highlighted in green.

To chart the mixture interpretation data, right click anywhere on the spreadsheet to open the Print / Select pages to view / Select user name user form and select the Make Chart button. The data will only chart if there is one genotype possibility for most of the loci and if all of the necessary references were applied in the mixture interpretation tool before sending the results over to the Mix-Interp page. For example, if charting a two person mixture, both references need to be applied in the mixture interpretation tool before sending the genotypes over to the Mix-Interp page.
A chart appears that plots the calculated proportions for the contributors. If a locus is not plotted then either no genotype possibilities exist or more than one genotype possibility has been calculated as plausible. The contributor, in this case the suspect, with the largest proportion is always plotted on the bottom (red) and the contributor, in this case the victim, with the smallest proportion is always plotted on the top (red) with both of the proportions adding up to 100 percent.

Within the Print / Select pages to view / Select user name user form, select the Make Chart button to chart the mixture interpretation results with the proportions from the two contributors.
A chart for a three-component mixture looks similar to the two-component mixture chart, but has an added proportion; however, all three proportions still add to 100 percent.

The charts can also be re-arranged according to locus size order. This is done by accessing the Print / Select pages to view / Select user name user form by right clicking on the spreadsheet and selecting the Locus Order button. The results stay the same, but the loci within the chart are simply re-arranged.
The chart can also be changed from a stacked view to a side-by-side view by double left clicking outside of the graph boundary.

![Side-by-side view of chart](image)

### 7.8 Total_Data page:
The Total_Data page simply formats the data from other pages into tables that separate the data according to Sample ID, locus, alleles, RFUs, and base pairs. To import data into the Total_Data page, open the Matching / Send for Reference / Send profile / Other user form by right clicking on a Sample ID and selecting the Total Data button under the Send all data to heading.
An example view of data imported into the Total_Data page:

<table>
<thead>
<tr>
<th>Evidence ID</th>
<th>Evidence A, B, C</th>
<th>Alleles</th>
<th>RFUs</th>
<th>Base Pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2S3179</td>
<td>13, 14, 15</td>
<td>14472, 14935</td>
<td>355, 849</td>
<td></td>
</tr>
<tr>
<td>D2S3141</td>
<td>16, 17, 18</td>
<td>20124, 20469, 21918</td>
<td>41, 345</td>
<td></td>
</tr>
<tr>
<td>D9S290</td>
<td>19, 20, 21</td>
<td>26467, 26640, 27576</td>
<td>126, 137, 1069</td>
<td></td>
</tr>
<tr>
<td>D9S290c</td>
<td>22, 23, 24</td>
<td>32166, 32785, 33835</td>
<td>123, 378, 869</td>
<td></td>
</tr>
<tr>
<td>D9S290d</td>
<td>25, 26, 27</td>
<td>32447, 32805, 33835</td>
<td>415, 401</td>
<td></td>
</tr>
<tr>
<td>D1S610</td>
<td>12, 14, 16</td>
<td>17978, 17995, 30911</td>
<td>327, 397, 1906</td>
<td></td>
</tr>
<tr>
<td>D1S611</td>
<td>17, 19, 20</td>
<td>23279, 23678, 24078</td>
<td>318, 377, 1327</td>
<td></td>
</tr>
<tr>
<td>D1S612</td>
<td>21, 22, 23</td>
<td>52853, 52958, 53059</td>
<td>240, 217</td>
<td></td>
</tr>
<tr>
<td>D1S613</td>
<td>24, 25, 26</td>
<td>31144, 32013, 3440</td>
<td>352, 186, 1406</td>
<td></td>
</tr>
<tr>
<td>D1S614</td>
<td>27, 28, 29</td>
<td>31439, 31913, 32235</td>
<td>388, 325, 1277</td>
<td></td>
</tr>
<tr>
<td>D1S615</td>
<td>30, 31, 32</td>
<td>17353, 17989, 30059</td>
<td>567, 123</td>
<td></td>
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<tr>
<td>D1S616</td>
<td>33, 34, 35</td>
<td>20053, 21202, 28469</td>
<td>200, 167</td>
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</tr>
<tr>
<td>D1S617</td>
<td>36, 37, 38</td>
<td>28010, 30818, 31906, 31907</td>
<td>385, 116, 159, 1879</td>
<td></td>
</tr>
<tr>
<td>D1S618</td>
<td>39, 40, 41</td>
<td>1006, 1127, 6166</td>
<td>1145, 1066</td>
<td></td>
</tr>
<tr>
<td>D2S3179</td>
<td>13, 14, 15</td>
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<td>1096, 1238</td>
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<tr>
<td>D9S290d</td>
<td>25, 26, 27</td>
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<td>D1S610</td>
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<td>21, 22, 23</td>
<td>52853, 52958, 53059</td>
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<td></td>
</tr>
<tr>
<td>D1S613</td>
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<td>31144, 32013, 3440</td>
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<td></td>
</tr>
<tr>
<td>D1S614</td>
<td>27, 28, 29</td>
<td>31439, 31913, 32235</td>
<td>388, 325, 1277</td>
<td></td>
</tr>
<tr>
<td>D1S615</td>
<td>30, 31, 32</td>
<td>17353, 17989, 30059</td>
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<td></td>
</tr>
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<td>D1S616</td>
<td>33, 34, 35</td>
<td>20053, 21202, 28469</td>
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<td></td>
</tr>
<tr>
<td>D1S617</td>
<td>36, 37, 38</td>
<td>28010, 30818, 31906, 31907</td>
<td>385, 116, 159, 1879</td>
<td></td>
</tr>
<tr>
<td>D1S618</td>
<td>39, 40, 41</td>
<td>1006, 1127, 6166</td>
<td>1145, 1066</td>
<td></td>
</tr>
<tr>
<td>D2S3179</td>
<td>13, 14, 15</td>
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<td>1096, 1238</td>
<td></td>
</tr>
<tr>
<td>D2S3141</td>
<td>16, 17, 18</td>
<td>20124, 20469, 21918</td>
<td>41, 345</td>
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<tr>
<td>D9S290</td>
<td>19, 20, 21</td>
<td>26467, 26640, 27576</td>
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<td>D9S290c</td>
<td>22, 23, 24</td>
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<td>D9S290d</td>
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<tr>
<td>D1S610</td>
<td>12, 14, 16</td>
<td>17978, 17995, 30911</td>
<td>327, 397, 1906</td>
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<tr>
<td>D1S611</td>
<td>17, 19, 20</td>
<td>23279, 23678, 24078</td>
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<td>21, 22, 23</td>
<td>52853, 52958, 53059</td>
<td>240, 217</td>
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</tr>
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<td>D1S613</td>
<td>24, 25, 26</td>
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<td>33, 34, 35</td>
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<td></td>
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<td>D1S617</td>
<td>36, 37, 38</td>
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<td>D1S618</td>
<td>39, 40, 41</td>
<td>1006, 1127, 6166</td>
<td>1145, 1066</td>
<td></td>
</tr>
</tbody>
</table>

The mixture interpretation tool can be accessed by double left clicking on an alleles cell. However, references already need to be set in a previous page in order for them to be applied while using the mixture interpretation tool within the Total_Data page.
The only other user form that can be utilized within the Total_Data page is the Print / Select pages to view / Select user name user form and it is missing the profiles and chart buttons. The user form is displayed by right clicking anywhere within the spreadsheet except for in an allele cell, which opens the Matching / Send for reference / Send profile / Other user form.

7.9 Staff_Profiles page:
The Staff_Profiles page contains all of the profiles from people working at the laboratory. It is highly useful when an unknown contamination occurs and the user wants to check the contaminated evidence with the staff profiles. To check the contaminated evidence against the staff profiles, first send the evidence to the Staff_Profiles page. This is accomplished by right clicking on the Sample ID of the evidence to open the Matching / Send for reference / Send profile / Other user form.

Right click on the contaminated evidence’s Sample ID to open the user form. Select the Staff Profiles button.

Select the Send selection as reference to Staff Profiles button, which automatically opens the Staff_Profiles page and places the profile of the contaminated evidence, highlighted in orange, on the top of the page.
To perform the matching with the reference, right click on one of the staff profiles sample designations under the “SD” column, which opens the Matching / Send for reference / Send profile / Other user form, and select the Find incl in the Ref button under the With an existing match heading. This function goes through the staff profiles and finds all of the alleles that are included in the reference profile.

There is a color designation key at the bottom of the spreadsheet. A yellow designation indicates that the profile is included in the reference, an orange designation indicates that the profile exactly matches the reference, and a white designations indicates that the profile is not included in the reference. After the matching is performed, the reference Sample ID at the top of the page changes from orange to yellow, and the name changes to Combined References – Inclusions in the Reference. To obtain the staff profile that most closely matches the contaminated evidence, double left click on count located at the far right of the spreadsheet. This action lists the staff profiles in descending order of matches to the reference profile. In this example, staff profile SD082 has the highest match with a count of 13 loci exactly matching or included in the reference profile. To perform further analysis on the possible staff contaminated evidence, transfer all applicable profiles to the Temp_Data page and perform more extensive matching and analysis.

To clear the match on the Staff_Profiles page, re-open the Matching / Send for reference / Send profile / Other user form and select the Clear Match button.

The Staff_Profiles page has the user forms: Print / Select pages to view / Select user name, Get data / Compare / Check / Fit / Misc, and Matching / Send for reference / Send
profile / Other. However, most of them do not appear in their entirety and have all of the active functions, as they would on other pages.

The Print / Select pages to view / Select user name user form as it appears on the Staff_Profiles page

The Get Staff button on the Print / Select pages to view / Select user name user form allows the user to retrieve a staff profile and bring it into the Staff_Profiles page. The Save Staff button enables the user to save the new Staff_Profiles page.

The Get data / Compare / Check / Fit / Misc user form as it appears on the Staff_Profiles page
To open the Print / Select pages to view / Select user name user form, right click at the top of the spreadsheet where the reference profile is located. To open the Get data / Compare / Check / Fit / Misc user form, right click within the body of the spreadsheet, except under the SD column or the count column. Right clicking under the count column, opens a Select System box that allows the user to pick between the Identifiler and Yfiler STR kits. Finally, to open the Matching / Send for reference / Send profile / Other user form, right click on a sample ID under the SD column.

7.10 QA_Profiles page:
The QA_Profiles page works like the Staff_Profiles page except that it contains quality assurance profiles instead of staff profiles. To check a sample against the QA profiles, follow the same steps as checking contaminated evidence against the staff profiles. Transfer the sample to the QA Profiles page, perform the matching, and double click on count.
The QA_Profiles page has all of the same user forms as the Staff_Profiles pages with the same active buttons. The user forms are accessed in the same manner as on the Staff_Profiles page.

### 7.11 NIST_Profiles page:
The NIST_Profiles page operates in the same manner as the Staff_Profiles and QA_Profiles pages except that it contains the NIST Standard Reference Materials profiles. It has all of the same user forms, which are accessed in the same manner as the other profiles pages.

### 7.12 Allele_Freq page:
The Allele_Frequency page lists the allele frequencies for Caucasian, African American, and Hispanic ethnicities. These frequencies are used for the biostatistical calculations performed on the various statistics pages. Care should be taken when opening this page because a simple typing in the cells replaces the existing text; therefore, it is readily easy to change the alleles and their frequencies, which may result in incorrect statistical representation of the data.

### 8.0 CODIS Features and Functions

#### 8.1 CDF Page
The CDF page is the CODIS Disposition Form, where the user sends the Evidence, Suspect, Victim, and Elimination profiles.
To send a profile to the CDF page, right click on its Sample ID to open the Matching / Send for reference / Send profile / Other user form and select the CODIS button.

View of the CDF page; the profile was send as a suspect profile

<table>
<thead>
<tr>
<th>Evidence</th>
<th>Victim</th>
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</thead>
<tbody>
<tr>
<td>D800189</td>
<td>D30019</td>
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<tr>
<td>D300189</td>
<td>D30019</td>
</tr>
<tr>
<td>D770020</td>
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<tr>
<td>C27F000</td>
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<tr>
<td>D300550</td>
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<td>T3001</td>
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<td>T5001</td>
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<tr>
<td>LVA</td>
<td>LVA</td>
</tr>
<tr>
<td>TFOX</td>
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<tr>
<td>D300550</td>
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<tr>
<td>TFOX</td>
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<table>
<thead>
<tr>
<th>Suspect</th>
<th>Film</th>
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<tr>
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<table>
<thead>
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<th>Suspect Info</th>
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<tr>
<td>19</td>
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<tr>
<td>5A</td>
<td>5A</td>
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<td>23</td>
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<td>20</td>
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<table>
<thead>
<tr>
<th>Signature</th>
<th>Date</th>
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</thead>
<tbody>
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<td>D300189</td>
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<td>D300189</td>
<td>D300189</td>
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</tbody>
</table>
Selecting the CODIS button allows the user to view the CODIS functions.

To send a sample to the CDF page, select the appropriate button under the Send selection to CDF heading.
8.2 CODIS Functions
The CODIS user form can be seen in the previous section.

8.2.1 CODIS Checks
The CODIS checks are similar to the controls checks, except they are for a database lab. The Check for > 2 button highlights profiles with more than two alleles. The Check for X button highlights female samples. The Check PHR button checks for peak height ratios under a user defined phr thresholds. The Check OSD checks for off scale data. The Check multi checks to see if there are different allele calls for multiple injection of the same CODIS ID. The Clear Checks button clears all highlighting from the checks performed.

8.2.2 CODIS CMF
The Validate for CMF button red highlights samples ineligible for CODIS entry. The CMF de/select can select or deselect (remove/add the red highlighting of) samples highlighted by the Validate for CMF button. The Make a CMF button makes the CMF file either as a floppy disk (A: drive) or on a designated H: drive. The CMF file created is in the 1.0 format; no .html files are created.

8.2.3 CODIS Controls Report
The Open Controls Check button opens another workbook, the control check workbook, that allows a laboratory to retain data on all of its positive controls, negative controls, reagent blanks, etc. Right click on the body of the spreadsheet to open its specific user form. The Send Controls button checks and sends the controls to the control check workbook. The Send ReAn orig and Send ReAn rept buttons send the selected Sample ID profile to the control check workbook. When a sample is reanalyzed, same Sample ID required, it is also sent to the control check workbook where it can be compared to the original.

9.0 Statistics
9.1 Freq_SS
The Freq_SS page is used to perform statistics on single source samples. To send a single source sample to the Freq_SS page, right click on its Sample ID to open the Matching / Send for reference / Send profile / Other user form and select the Freq SS button under the Send selection for statistics to heading. The Freq_SS page automatically opens.
Once the sample has been transferred to the Freq_SS page, the appropriate loci and their corresponding alleles are imported.

To send a single source sample to the Freq_SS page, right click on the Sample ID to open the Matching / Send for reference / Send profile / Other user form and select the Freq SS button.
Double left click on the Locus Profile heading to add the allele frequencies and perform the calculations.

The loci and the sample alleles are automatically imported into the Freq_SS page.
The calculation results are displayed at the bottom of the spreadsheet in both scientific notation and word format.

Right clicking within the body of the Freq. SS spreadsheet opens the Frequency Calculations, refer to section 7.1.4 for a discussion on this user form.
9.2 Freq_Mix
The Freq_Mix page is used to perform frequency calculations on a two-person mixture sample. The calculations can either be performed with or without references applied. If applying references, set the appropriate references before sending the mixture sample to the Freq_Mix page. To set the references, double left click on an allele cell of one of the references, which opens the 2 or 3 Contributor Mixture Interpretation tool. Set the reference by selecting one Ref button under the Set heading. The Sample ID of the reference should highlight the same color as the Ref button. Repeat this process for setting up to six references.

After the references have been set, send the mixture sample to the Freq_Mix page by right clicking on its Sample ID and opening the Matching / Send for reference / Send profile / Other. Select the Freq Mix button under the Send selection for statistics to heading. The Freq_Mix page automatically opens and the loci and alleles from the sample are imported.
Add the corresponding allele frequencies to the page by double left clicking on the Locus Profile heading. The alleles and their frequencies will add under the Allele 1 – Allele 4 headings.

The loci and alleles from the mixture sample are automatically imported into the Freq_Mix page.
To obtain the genotypes under the Type 1 – Type 5 headings, the user has to go through locus-by-locus to find the required allele(s). Type 5 is always designated Allele, Any to account for any drop-out in the sample and can be removed if drop-out is not suspected. The figure below illustrates how to find the required allele(s) for a locus.
Once the required allele(s) has been identified, close the mixture interpretation tool and double left click on the required allele under the Allele heading. This action imports all of the possible genotype combinations with that allele under the Type headings.

To remove any of the unwanted genotypes, double left click on their allele designations.

The wanted genotypes depend on the calculated possibilities determined by the analyst after utilizing the mixture interpretation tool. In this example, the analyst concluded that
the only possible example for the suspect is a 14,14; therefore, the Freq_Mix page for this locus depicts this calculation.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Locus Profile</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Allele 3</th>
<th>Allele 4</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
<th>Type 4</th>
<th>Type 5</th>
<th>Sum of</th>
</tr>
</thead>
<tbody>
<tr>
<td>13,14</td>
<td></td>
<td>14</td>
<td>14</td>
<td></td>
<td></td>
<td>24</td>
<td>24</td>
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<tr>
<td>0.2099</td>
<td>0.2013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0422</td>
<td></td>
<td></td>
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<td>0.2222</td>
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<td>0.3251</td>
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<td>0.0625</td>
<td></td>
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</tbody>
</table>

Depending on the results from the mixture interpretation tool, the user has the option of selecting more than one required allele. To select more than one required allele, simply click on the alleles under the Allele headings until the user obtains the wanted genotypes.

After all of the genotypes for all of the loci have been selected, double left click on the Sum of heading to calculate the results. The results are in both scientific notation and words at the bottom of the spreadsheet.

9.3 Freq_PI
The Freq_PI page calculates both probability of inclusion and probability of exclusion for single source and mixture samples. To send a sample over to the Freq_PI page, right click on its Sample ID to open the Matching / Send for reference / Send profile / Other user form. Select the Freq PI button under the Send selection for statistics to heading. The Freq_PI page automatically opens with the loci and their corresponding alleles already imported.
To calculate the PI and PE, double left click on the Locus Profile heading. The allele frequencies are imported and the results are in scientific notation at words at the bottom of the spreadsheet.

The loci and their corresponding alleles are automatically imported onto the Freq_PI page once the sample is sent to the page for statistics.
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The mixture interpretation tool can be accessed on the Freq_PI page by double left clicking on the alleles under the locus profile heading. Right clicking within the body of the spreadsheet opens the Frequency Calculations user form and right clicking at the top of the spreadsheet opens the Print / Select pages to view / Select user name user form opens.

9.4 L_Ratio

The L_Ratio page is used to calculate likelihood ratios for mixture samples. Before sending a mixture sample to the L_Ratio page, set the appropriate references. Refer to section 9.2 on how to set the references. Send the mixture sample to the L_Ratio page by double left clicking the Locus Profile heading inserts the allele frequencies and the results are displayed at the bottom of the page.
right clicking on its Sample ID. This opens the Matching / Send for reference / Send profile / Other user form and select the Send selection for statistics to L Ratio button. The L_Ratio page automatically opens with the appropriate loci and alleles inserted.

Insert the allele frequencies by double left clicking on the Locus Profile heading. After the allele frequencies are imported, to calculate a likelihood ratio, the user moves through the data locus-by-locus, in a similar fashion as the Freq_Mix page. First, double left click on a locus the user wants to view. This turns the locus designation green and inserts the alleles of that locus in two rows of pink; one row always is at the top of the spreadsheet (defense) and the second row (prosecution) always is across from the corresponding locus.
Open the mixture interpretation tool by double left clicking on the alleles under the Locus Profile heading. Apply the appropriate references and decipher which alleles belong to the suspect, or unknown, contributor. In this example, the victim’s profile was applied and the suspect is calculated to be a 8,12.

Once the suspect alleles are identified, close the mixture interpretation tool and locate the suspect alleles on the defense row of alleles (bottom pink row). Double left click on the suspect alleles to turn them from pink to green. The green color indicates that the alleles are from an unknown source and the pink color indicates that the alleles are from a known source. Therefore, for the defense hypothesis, the suspect alleles should be changed to green to distinguish them as unknown alleles and all of the alleles should remain pink for the prosecution’s hypothesis.

Double left clicking on the suspect’s alleles changes them from pink (known) to green (unknown)
To calculate a likelihood ratio, right click under the Px heading. On the first calculation, a box appears that allows the user to set the C1 and C2 values. C1 is the number of unknown contributors for the prosecution, and in this example is zero. C2 is the number of unknown contributors for the defense, and in this example is one. Once the values are set, they can not be changed for the other loci. Click calculate.

Clicking Calculate builds a likelihood ratio and performs the calculations for C1 and C2 according to the ethnicities Caucasian, African American, and Hispanic.

Note: Once the likelihood ratio for the first locus has been calculated, the user can calculate for the rest of the loci, after choosing the appropriate defense unknown alleles, by double left clicking the cell that ends up with the conditional (Px) statement.

Repeat the procedure of selecting a locus, finding the suspect alleles, and performing a likelihood ratio for the rest of the loci in the sample. Illustrated is the next locus in this example. The D21S11 designation is highlighted green, as are the suspect 29 and 31 alleles. Note that the defense row of alleles is still located in the same position, but the prosecution alleles have moved to be across from their locus designation.

The LR calculations for one locus

The L_Ratio page performs the overall calculations as each loci is calculated and the results are expressed in both scientific notation and words at the bottom of the spreadsheet.

The Frequency Calculations user form can be accessed by right clicking on the left hand side of the spreadsheet; the C1 and C2 box opens when right clicking under Px. The Print / Select pages to view / Select user name user form can be accessed by right clicking on the top of the spreadsheet, above the headings.
10.0 Version Changes

10.1 Version 2.1.2

- Problem: on the Analyst_Data page (or other match pages), sometimes at locus Amelogenin the Find Foreign Alleles process terminated. VBA Modification-Solution: Find Foreign Alleles processes to the list end.

- Problem: when using the Mixture Interpretation user form, re-calculate for stutter was only for maximum stutter. VBA Modification-Solution: re-calculate for stutter is now adjustable in steps 0.1 to 1.0 (10 to 100% of maximum stutter).

- Problem: when using the Mixture Interpretation user form, generic categories AB, AC & AD; AB, AC & BD; AB, AC & BC did not account for proportions under minimum peak height. VBA Modification-Solution: calculations account for proportions under minimum peak height.

- Problem: Staff_Profiles, QA_Profiles, and NIST_Profiles data for import are in text file format. VBA Modification-Solution: format was changed to Page View (user friendly Excel workbook format).

- Problem: peak height ratios on the Mix_Interp page contain too many significant figures, and normal rounding rules. VBA Modification-Solution: changed to 3 figures, with no rounding.

- Problem: Freq_SS calculations produced an error if the database is size set = 0. VBA Modification-Solution: if the database size re-sets = 1.

- Problem: on the Analyst_Data page, the check for > 2 alleles only checked for 3 or 4 alleles. VBA Modification-Solution: all > 2 alleles are checked.

- Problem: not all data on Compare_Data page was left aligned. VBA Modification-Solution: all data is left aligned.

- Problem: the inclusion count on Staff_Profiles (QA_Profiles, NIST_Profiles) did not include inclusion matches in the sum of matching loci. VBA Modification-Solution: the sum of matching loci includes inclusions and exact matches.

10.2 Version 2.1.3

- Problem: when using the Mixture Interpretation user form, if Ref1 is Set and Applied, and then if Ref1 is clicked to clear the reference, the user form loses function (similar for other Refs): code is interrupted, an Excel error message appears, and the user needs to close and re-open DNA_DataAnalysis to regain function with the Mixture Interpretation user form. VBA Modification-Solution: Ref1 Set and Apply are both re-set to grey (or other Refs), and associated Alleles and Description textboxes are hidden, so that the user form does not lose function.
• Problem: when using the Mixture Interpretation user form, if the “2” button (calc for 2 contributors) is selected, and then more than two references are applied, the view of combinations may incorrectly indicate that more than two references are included in the profile. VBA Modification-Solution: the combinations area shows a message stating that too many references are selected.

• Problem: when using the Mixture Interpretation user form, if the “3” button (calc for 3 contributors) is selected, and then more than three references are applied, the view of combinations may incorrectly indicate that more than three references are included in the profile. VBA Modification-Solution: the combinations area shows a message stating that too many references are selected.

• Problem: when giving large-screen presentations, it is difficult for the viewer to distinguish between on-screen colors (match, etc). VBA Modification-Solution: feature added so that the user may apply custom colors for matching, etc (Info page display and selection of colors).

• Problem: when using the Mixture Interpretation user form, LSD calculations were not displaying if Refs were selected. VBA Modification-Solution: LSD calculations display if Refs are selected.


• Problem: on the Analyst_Data page (or other match pages), not all matches to Match to a Reference are processing to the list end. VBA Modification-Solution: Match to a Reference processes to the list end.

• Problem: on the Analyst_Data page (or other match pages), match color key info extended too far to the right. VBA Modification-Solution: the color key info does not extend to the right.

• Problem: on the Analyst_Data page (or other controls check pages), not all controls checks are processing to the list end. VBA Modification-Solution: controls checks process to the list end.

• Problem: Set system dye colors on the “alleles allowed in CODIS” user form did not updating to all appropriate pages. VBA Modification-Solution: the function was removed from this user form (see next item).

• Problem: locus names and dye colors were not updating to all appropriate pages (when user changes to Identifiler, Yfiler, etc). VBA Modification-Solution: locus names with dye colors can be set from the “Print / Select pages to view / Set user name” user form; locus names are updated on Analyst_Data, Review_Data, Compare_Data, Temp_Data, Freq_SS, Freq_Mix, Freq_PI, L_Ratio and CDF pages. Locus names with dye colors are
set on table import: locus names are updated on Analyst_Data, Review_Data, Compare_Data, Temp_Data, Freq_SS, Freq_Mix, Freq_PI, L_Ratio and CDF pages.

- Problem: Powerplex16 tables can not be imported. VBA Modification-Solution: Powerplex16 table import has been added.

- Problem: SRM profiles are inappropriately designated as NIST profiles. VBA Modification-Solution: references to NIST (NIST_Profiles page name, NIST (Profiles) on buttons, NIST_Profiles folder) have been changed to SRM (Profiles).

  - Note: DNA_DataAnalysis: (1) is flexible with regard to adding new systems with up to 16 loci; (2) is flexible with regard to changing the locus order and dye colors for any given system; (3) is flexible with regard to statistical calculations (as long as the appropriate database information has been added); (4) matching on Analyst_Data, Review_Data, Compare_Data, and Temp_Data is done without regard to locus column headings; (5) Staff_Profiles and QA_Profiles can import Identifiler or Yfiler tables (or other systems – once prepared) for profile matching.

- Problem: when using the Mixture Interpretation user form, if the “2” button (calc for 2 contributors) is selected, when the [3] button should be selected, an Excel error message appears when the user tries to remove an allele from the calculations. VBA Modification-Solution: the process stops; no error message is produced.

- Problem: when using the Mixture Interpretation user form, the “not excluded” message requires further clarification. VBA Modification-Solution: the message now reads “not excluded; meets mPH = (value) and PHr = (value) thresholds”.

- Problem: on the L_Ratio page, the user click-select positions for the C1 (prosecution) and C2 (defense) hypotheses are not intuitive (C1 should be over C2). VBA Modification-Solution: the C1 and C2 user click-selections positions are switched.

- Problem: some controls run from the Get data / Compare / Check / Fit / Misc user form don’t display color-key messages. VBA Modification-Solution: The Stutter check displays “Evaluate for potential stutter”; CODIS alleles check displays “Profile contains text – or alleles not accepted by CODIS”; RFUs high check displays “Evaluate for RFUs over (6000)”; RFUs low displays “Evaluate for RFUs under (300)”; RFUs ratio displays “Evaluate for peak height ratios under (0.5)”.

- Problem: when using the Mixture Interpretation user form, if a reference contains “ola”, “<” or “>”, the combinations displayed may not be accurate (the mixture tool requires that all alleles have a specific numeric value). VBA Modification-Solution: if a reference is applied, and the reference contains “ola”, “<” or “>”, the message “Check reference alleles; replace ola, <, or > with a numeric value” displays.

11.0 Appendix
11.1 Frequency Calculations used in DNA_DataAnalysis
Freq_SS (single source)

Unrelated Locus (with allele frequencies p, q)

homozygotes: \( p^2 + p(1 - p)\Theta, \quad \Theta = 0.01 \) (default) or 0.03
heterozygotes: \( 2pq \)

Full siblings Locus (with allele frequencies p, q)

homozygotes: \( (1 + 2p + p^2)/4 \)
heterozygotes: \( (1 + p + q + 2pq)/4 \)

Parents and Offspring Locus (with allele frequencies p, q)

homozygotes: \( p^2 + 4p(1 - p)/4 \)
heterozygotes: \( 2pq + 2(p + q - 4pq)/4 \)

Half-Siblings, Uncles and Nephews Locus (with allele frequencies p, q)

homozygotes: \( p^2 + 4p(1 - p)/8 \)
heterozygotes: \( 2pq + 2(p + q - 4pq)/8 \)

Freq_SS (single source)

First Cousins Locus (with allele frequencies p, q)

homozygotes: \( p^2 + 4p(1 - p)/16 \)
heterozygotes: \( 2pq + 2(p + q - 4pq)/16 \)

Overall

\((\text{Locus 1})(\text{Locus 2})\ldots(\text{Locus n})\)

Freq_Mix (mixtures, multiple possibilities may exist at a locus)

Locus (with allele frequencies p, q)
the sum of all applicable homozygotes and heterozygotes

homozygotes: \( p^2 + p(1-p)\Theta \), \( \Theta = 0.01 \) (default) or 0.03

heterozygotes: \( 2pq \)

or for Any single allele + any allele

\[ \text{Any} = p^2 + p(1-p) \Theta + 2p(1-p) \]

\( p^2 + p(1-p) \Theta \) for the homozygote possibility

\( 2p(1-p) \) for all heterozygote possibilities

**FreqMix** (mixtures, multiple possibilities may exist at a locus)

**Overall**

(Locus 1)(Locus 2)...(Locus n)

**Freq_PI** (PE probability of inclusion, PE probability of exclusion)

**Locus** (with allele frequencies a, b … n)

\[ P = \text{sum}(a + b + \ldots + n) \]

\[ Q = 1 - P \]

\[ \text{PE} = Q^2 + 2PQ \]

\[ \text{PI} = 1 - \text{PE} \]

**Overall**

(Locus 1)(Locus 2)...(Locus n)

**L_Ratio** (Likelihood Ratio)

**Profiles with one allele a**

Allele a from x unknown contributors

\[ P_x(a|a) = p_a^{2x} \]

If knowns contribute a to the profile
$P_x(\phi|a) = p_a^{2x}$

Profiles with two alleles $a$, $b$

Allele $a$ from $x$ unknown contributors ($b$ is from a known contributor)

$P_x(a|ab) = (p_a + p_b)^{2x} - p_b^{2x}$

For no known contributors

$P_x(ab|ab) = (p_a + p_b)^{2x} - p_a^{2x} - p_b^{2x}$

If knowns contribute $a$, $b$ to the profile

$L_Ratio$ (Likelihood Ratio)

Profiles with three alleles $a$, $b$, $c$

Allele $a$ from $x$ unknown contributors ($b$, $c$ are from a known contributor)

$P_x(a|abc) = (p_a + p_b + p_c)^{2x}$

Alleles $a$, $b$ from $x$ unknown contributors ($c$ is from a known contributor)

$P_x(ab|abc) = (p_a + p_b + p_c)^{2x} - (p_a + p_b)^{2x} - (p_a + p_c)^{2x} + p_c^{2x}$

Alleles $a$, $b$, $c$ from $x$ unknown contributors

$P_x(abc|abc) = (p_a + p_b + p_c)^{2x} - (p_a + p_b)^{2x} - (p_b + p_c)^{2x} - (p_a + p_c)^{2x}
+ p_a^{2x} + p_b^{2x} + p_c^{2x}$

If knowns contribute $a$, $b$, $c$ to the profile

$L_Ratio$ (Likelihood Ratio)

Profiles with four alleles $a$, $b$, $c$, $d$

Allele $a$ from $x$ unknown contributors ($b$, $c$, $d$ are from known contributors)
\[ P_x(a|abcd) = (p_a + p_b + p_c + p_d)^{2x} - (p_b + p_c + p_d)^{2x} \]

Alleles a, b from x unknown contributors (c, d are from a known contributor)

\[ p_d^{2x} + (p_c + p_d)^{2x} \]

Alleles a, b, c from x unknown contributors (d is from a known contributor)

\[ P_x(ab|abcd) = (p_a + p_b + p_c + p_d)^{2x} - (p_b + p_c + p_d)^{2x} - (p_a + p_c + p_d)^{2x} + (p_c + p_d)^{2x} \]

Alleles a, b, c, d from x unknown contributors

\[ P_x(abc|abcd) = (p_a + p_b + p_c + p_d)^{2x} - (p_b + p_c + p_d)^{2x} - (p_a + p_c + p_d)^{2x} - (p_a + p_c + p_d)^{2x} + (p_c + p_d)^{2x} + (p_b + p_d)^{2x} + (p_c + p_d)^{2x} - p_d^{2x}, \; x > 1 \]

If knowns contribute a, b, c, d to the profile

\[ P_x(\phi|abcd) = (p_a + p_b + p_c + p_d)^{2x} \]