Forensic Biology Section

Fusion Casework Analysis and Interpretation with GeneMapper ID-X

1. Scope

1.1. This procedure serves as a general guideline for the interpretation of DNA profiles using Promega's PowerPlex Fusion kit to obtain Short Tandem Repeat (STR) profiles from extracted DNA. This protocol is not an exhaustive list of all interpretation scenarios because the experience and judgment of the DNA Analyst is considered when reporting DNA profiles, and two other qualified analysts review all of the data interpretations and allele calls. If the analyst and the technical reviewers cannot agree on allele calls or data interpretation, the Technical Leader is consulted.

1.2. A Fusion DNA profile is made of 24 reactions across locations (loci) throughout the human genome: the gender marker **Amelogenin** and 23 STR loci (**D3s1358, D1s11656, D2s441, D10s1248, D13s317, Penta E, D16s539, D18s51, D2s1338, CSF1PO, Penta D, TH01, vWA, D21s11, D7s820, D5s818, TPOX, DYS391, D8s1179, D12s391, D19s433, FGA,** and **D22s1045**) are amplified in a single reaction.

1.3. A portion of each amplified sample is run on a capillary electrophoresis 3500 Genetic Analyzer (3500). Allelic ladders are run at the same time to define bins for the alleles at every locus.

1.4. GeneMapper ID-X software (GeneMapper or GMID-X) is used to analyze the data from the 3500. The ILS is used to calculate the base pair size of all the peaks within each sample, the allelic ladders are used to create allele bins, and the peaks in the samples are labeled based on their color and which allelic bin they line up with. GeneMapper then removes the labels from peaks that are in a stutter position and are below the stutter threshold level for that locus.

1.5. The DNA Analyst interprets the data, reviewing and assessing the profile's quality, peak height ratios, potential for mixture, dropout, etc., removing artifacts and confirming true alleles. The final allele calls that are reliable, reproducible, and suitable for comparison are documented on a DNA Profile Summary sheet, including how it compares to other profiles in the case.

2. Specimen:

2.1. Samples amplified with the "Fusion Casework Amp & Detect on 3500" protocol.

3. Instrumentation and Equipment:

- PC Computer
- GeneMapper ID-X software v1.6 or greater (Life Technologies)

4. Quality Assurance:

4.1. Allele calls may be edited, and artifacts may be deleted in the GeneMapper project. All edits and deletions must be documented in GeneMapper. This electronic record will be reviewed during Tech and Admin Reviews.

4.2. Evidence profiles are interpreted independently of the known reference profiles:

4.2.1. First, interpret the data from evidence items, recording the true alleles which are copied or exported to a DNA Profile Summary sheet.

4.2.2. Next, interpret the data from known reference samples, recording the true alleles which are copied or exported to a DNA Profile Summary sheet.

4.2.3. Finally, compare the known reference profiles to the evidence profiles and record conclusions in the case file (e.g., at the bottom of the DNA Profile Summary sheet or in a case note).

4.2.4. Notations about peak height ratios, major/minor, etc. should be entered on the Profile Summary Sheet so the reviewers will be able to understand the analyst's reasoning for interpretation.

5. DNA Profile Terminology:

5.1. Allele Nomenclature

5.1.1. Alleles are designated by the number of tandem repeats they contain. A "14, 16" profile at a given locus would have one allele with 14 repeats and one allele with 16 repeats.

5.1.2. Sometimes a repeat segment lacks a full repeat unit and is called a "microvariant". These microvariants are designated with a numerical suffix. A "9.2" allele would have 9 repeat units plus two additional base pairs.

5.1.3. The peak height of an allele is measured in relative fluorescent units (rfu), a measure of signal intensity. There is a direct correlation between the amount of DNA being amplified and the peak heights within the DNA profile (i.e., peak heights are higher when more DNA is amplified).

5.2. Analytical Threshold

5.2.1. **The analytical threshold is 150 rfu**. This is the minimum peak height at which DNA profiles can be reliably and reproducibly differentiated from background noise.

5.2.2. The analytical threshold is exact and was empirically determined during validation.

5.3. Stochastic Threshold

5.3.1. **The stochastic threshold is 900 rfu**. This threshold is approximate and was determined through validation and practical experience with the DNA profiling system.

5.3.2. Stochastic effect occurs when low levels of DNA are amplified and some alleles are copied more than others in the first few cycles of PCR, resulting in uneven peak heights and dropout.

5.3.3. The stochastic threshold is the minimum peak height at which all the alleles in a profile should be consistently detected. Below this threshold, one or more alleles at a locus may be completely missing (i.e., "dropping out").

5.3.4. Below this threshold, interpret apparent homozygous loci and mixtures with caution. Dropout can make a heterozygous locus appear to be homozygous, and alleles could be entirely absent from a low-level mixture which can lead to false exclusions and inaccurate statistical probabilities. Above this threshold, an analyst can be confident all the alleles are present at a locus.

5.3.5. The stochastic threshold is also the minimum peak height at which the general rules of peak height ratios between sister alleles as well as major-minor contributors in mixtures should be consistently observed. Below this threshold, sister alleles at heterozygous loci may be imbalanced and major/minor ratios in mixtures may be inconsistent.

5.3.6. Below this threshold, apparent major-minor contributors in mixtures, and apparent homozygous and heterozygous loci, need to be interpreted with caution. If all the alleles in a locus are below the stochastic threshold, it may be difficult or impossible to distinguish sister alleles from major or minor donors because the peak height ratios may not be reliable.

5.4. True Alleles

5.4.1. A true allele has a peak height greater than or equal to the analytical threshold, has good peak morphology, and typically falls within an allelic bin. Artifacts in a profile need to be identified and labeled as to what type of artifact they are.

5.4.2. Heterozygous peak balance ≥ 60% is typically obtained with a sufficient amount of good quality DNA from a single-source sample. If loci exhibit peak balances < 60%, this may be due to stochastic effect in low level samples, mixture, and/or degradation.

5.4.3. It is possible to obtain imbalanced heterozygous alleles and unusual peak heights even under ideal conditions, presumably due to primer site variations and other mutations.

5.4.4. Mutations in sex chromosome markers (i.e., DYS391 and amelogenin) are more common than in autosomal markers.

5.5. **Off-Ladder Alleles**

5.5.1. Off-ladder (OL) alleles are reproducible peaks that do not fall within an allele bin in the ladder. An OL allele falls above or below the allelic ladder range (due to the number of repeat units exceeding the range in that locus of the ladder), or it falls between the bins of the allelic ladder (due to a repeat unit being smaller than usual).

5.5.2. The size of an OL allele can be interpolated by adding or subtracting the appropriate base pairs from the closest allele, or it can be extrapolated by adding or subtracting the appropriate repeat sizes from the closest allele.

5.5.3. Forensic Unknown samples with OL alleles must be amplified twice (to document reproducibility) before reporting a match at that locus or entering that locus into a database. Otherwise, the locus must be interpreted as inconclusive. To declare a match, the same rare allele must be observed in both the questioned sample and the known sample.

5.5.4. Known Reference samples with OL alleles must be amplified twice if they are going to be reported as matching an evidence item. If a Known Reference sample is excluded as a potential donor to the evidence, the OL allele does NOT need to be verified.

5.5.5. Other reports of microvariant alleles may be found on the STR Fact Sheet listed on the National Institute of Standards and Technology (NIST) Short Tandem Repeat DNA Internet Database web site (https://strbase.nist.gov/).

5.6. **Highly Degraded Samples.**

5.6.1. Typically, the largest loci are the first to demonstrate decreased peak heights or fail to amplify (drop out) as samples become more degraded.

5.6.2. Highly degraded samples must be interpreted with caution: stutter peaks can be higher; degradation products can cause spurious peaks, and there is a higher propensity for unbalanced heterozygous alleles and dropout.

5.7. **Inhibited Samples.**

5.7.1. Inhibited samples are extracts that contain some impurity that halts the polymerase enzyme activity or otherwise impacts the efficiency of the PCR reaction.

5.7.2. Inhibited samples may show unusual stutter peaks as well as unusual balance and dropout patterns within and across loci.

5.7.3. Inhibited samples may mimic the appearance of degraded samples, although the pattern of loci dropping out can be different (i.e., does not correlate to the size of the loci).

5.8. **Stutter Peaks**

5.8.1. During PCR, the polymerase enzyme sometimes adds fewer (and sometimes extra) repeat units to a fraction of the copies it is making due to “enzyme slipping”. These peaks typically appear one repeat unit to the left (or right) of a true allele at a fraction of the true allele’s height.

5.8.2. GeneMapper will "filter" stutter peaks if they fall below the stutter peak threshold that is defined at that locus. The height of a peak in the stutter position is calculated as a percent of the true allele, and the peak's label is removed if the stutter peak is ≤ the stutter threshold.

5.8.3. Typical stutter peaks are one repeat unit shorter than the true allele (e.g., N–4 for a 4-base pair repeat) and will appear as a small peak to the left of the true allele.

5.8.4. Less commonly, stutter peaks two or more repeat units shorter (e.g., N–8) and forward stutter peaks one repeat unit longer than the true allele (e.g., N+4) appear as small peaks to the left or right of the true allele. When interpreting peaks in these positions, the possibility of stutter as well as mixture should be considered.

5.8.5. If too much DNA is added to the PCR reaction, samples with very high signal (e.g., >30,000 rfu) can exhibit higher than usual stutter peaks. If interpreting a sample with very high peaks is too difficult due to these artifacts, the sample should be re-amplified with less DNA.

5.9. **"N-1" Peaks**

5.9.1. During PCR, the polymerase enzyme should always add an extra adenine nucleotide ("A") to the 3′ end of the PCR product, but if the reaction fails to do this, a small peak one base pair shorter than the true allele may be observed.

5.9.2. N-1 peaks often occur when too much DNA is added to the PCR reaction (typically accompanied by other indications of high signal), but they can occur in some loci at optimum input amounts of DNA (e.g., vWA locus). Peaks in these positions are usually not due to a second donor (because alleles in this position are typically very rare) but the possibility of a mixture should be considered.

5.9.3. The sample may be re-amplified with less DNA to aid interpretation.

5.10. **Pull-up peaks**

5.10.1. If too much DNA is added to the PCR reaction, the fluorescence intensity from the PCR products may saturate the 3500's detector. Samples with very high peaks (e.g., >30,000 rfu) produce too much signal, resulting in an overlap in the emission spectra of the dyes, and can cause a small peak to appear in other colors.

5.10.2. If the peaks have approximately the same bp size and/or scan number as the true peak, the artifact can be interpreted as pull-up.

5.10.3. Re-amplifying with less DNA may aid interpretation.

5.11. **Spikes and Dye Blobs**

5.11.1. Spikes and dye blobs are artifacts in the electrophoretic injection and/or electrophoresis and have peak morphology different from true alleles.

5.11.2. Spikes are much thinner than allele peaks and appear in multiple spectra, with approximately the same bp size or scan number in each color.

5.11.3. Dye blobs are much wider and rounder than allele peaks. They may appear in multiple spectra, but they can be different sizes in each color.

5.12. **Various Other Artifact Peaks**

5.12.1. Long-term storage of amplified samples at 4 °C or higher may produce artifacts at 88–90 bp in the JOE (green) channel. The signal strength of these artifacts will increase from storing the amplification plate at 4 °C for a few days (amplified samples should be stored at –20 °C).

5.12.2. DNA-dependent artifacts (in samples that contain DNA but not in samples that lack DNA) and DNA-independent artifacts (in samples whether they contain DNA or not) may be observed. See artifact table at end of protocol for specifics.

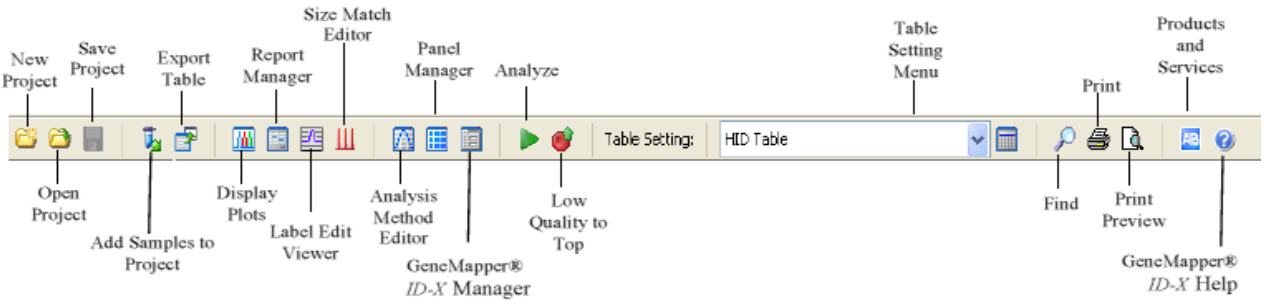
6. STR Analysis

6.1. Importing and Analyzing Data:

6.1.1. Open the GeneMapper software. Select a username and enter the password.

- Each DNA Analyst has their own password-protected username (the software will make them create a new password at the first log in).

GeneMapper® *ID-X* Project Window Toolbar



6.1.2. Choose Edit → Add Samples to Project (or type control-K or click), double-click on "This PC" and navigate to H:\Crimelab\DNA\3500 runs.

- Choose a user's data folder, click on the relevant run folder, click "Add to List", and then "Add" or "Add & Analyze".
- If not already set, specify the Sample Type (Sample, Allelic Ladder, Positive Control, Negative Control). *The Sample Type can be set in Data Collection on the 3500 Genetic Analyzer.*
- If not already set, specify the Analysis Method (Fusion_3500 or Y23_3500), Panel (PowerPlex_Fusion_Panels_IDX_v2.0 or PowerPlexY23_IDX_v2.0), and Size Standard (WEN_ILS_500_IDX). *The Analysis Method, Panel, and Size Standard can be set to default values (File → Project Options → Add Samples) and changed later if analyzing Y23.*

6.1.3. Choose Analysis → Analyze (or type control-R or click). Can also highlight specific samples and choose Analysis → Analyze Selected Samples (to force reanalysis of specific samples) or choose Analysis → Analyze All (to force reanalysis of all samples in the project).

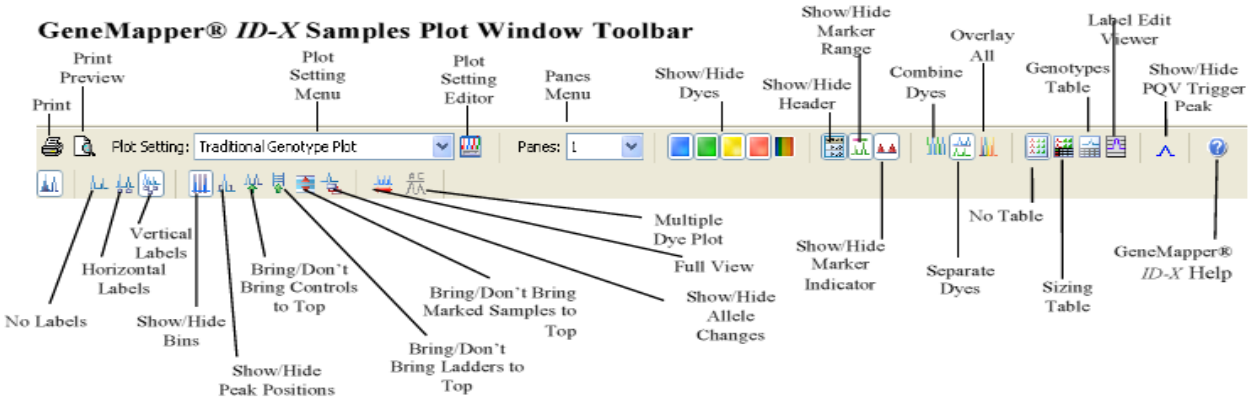
6.1.4. When analysis of the project is complete, the project displays Quality Metrics in the righthand columns. For example, SFNF (sample file not found), SOS (Sample Off Scale), SQ (Sizing Quality), SSPK (Sample Spike), MIX (Mixture), OMR (Outside Marker Range), and CGQ (Composite Genotype Quality).

6.2. Viewing Electropherograms:

6.2.1. Click or control-click the sample(s) of interest (or control-A to select all the samples), and then View → Display Plots (or type control-L or click) to view the electropherograms.

- The electropherogram view can also be changed by choosing from the drop-down list next to Plot Setting (e.g., check WEN-ILS, Fusion, Y23).
- The display area can be zoomed-in clicking-and-dragging in the base pair size area just above the electropherogram. To zoom-out, double-click in locus name or the base pair size area.

- Choosing the number of panes, the blue/green/yellow/red/orange dyes, whether tables are displayed or not, displaying the ladder at the top of the window, etc. are all controlled with buttons across the top of the electropherogram window.
- To save a view, go to Tools → Plot Settings (or type control-T or click) while viewing the electropherograms and choose Save.



6.2.2. The Controls should be inspected:

- ladders should be inspected for proper allele bins.
- Reagent Blanks and Negative Controls should be examined for any interpretable profile.
- Positive Controls should be examined for the expected DNA profile (see Expected Control Values section). The 2800M Control DNA is extracted from a cell line and can have poor balance within loci as well as between loci.
- The migration of the ILS should be checked for appropriate labeling of peaks and/or peak morphology due to migration issues. If these peaks fail to size properly, the sample may need to be deleted from the project.

6.2.3. The Samples should be inspected:

- Examine sample profiles to assess peaks and delete any artifacts (include reason for edits).
- The migration of the ILS should be checked for appropriate labeling of peaks and/or peak morphology due to migration issues. If these peaks fail to size properly, the sample may need to be deleted from the project.

6.3. **Documenting DNA Profiles:**

6.3.1. DNA profiles can be edited in GeneMapper (e.g., deleting artifacts and high stutter). The saved project will be reviewed at Technical and Administrative reviews.

6.3.2. Notations about deleting artifacts (e.g., pull up, spikes, stutter) should be done in GeneMapper. Left click to select the peak then right-click, choose 'Rename Allele Label' and select relevant reason for renaming is documented for review. The additional 'Reason' box can be used to record % stutter, etc.

6.3.3. Notations about peak height ratios, major/minor, etc. should be entered on the Profile Summary Sheet so the reviewers will be able to understand the analyst's reasoning for interpretation.

6.3.4. To avoid transcription errors, the interpreted casework DNA profiles should be copied from GeneMapper and recorded on DNA Profile Summary sheets.

6.3.5. After analyzing, editing, and saving the project, click on the **Genotypes Tab**.

6.3.6. Highlight the rows and columns to be copied (or type Control-A to highlight everything).

6.3.7. Type **Control-Shift-C** to copy the cells and the column headers.

6.3.8. Launch Excel and type **Control-V** to paste the values into the spreadsheet.

6.3.9. Each row will have the sample name and marker, and the alleles and peak heights for that locus.

6.3.10. It is easier to format the data in Excel (e.g., deleting columns that have no alleles, extraneous columns such as the Run Name, deleting extraneous rows of ladders and blanks, and bolding allele calls and headers).

6.3.11. Click any cell containing data on in the spreadsheet and type control-A and then control-C. This will highlight all the cells that have data in them and then copy them.

6.3.12. Go to the H:\Crimelab\DNA\DOCS\FORMS folder, open the Word template for Fusion, and type control-V to paste the data copied from Excel into the Word document.

6.3.13. Go back to the top of the first page in the Word doc (Control-Home) and click in the first row of the table and click on the REPEAT HEADER ROWS button  (copies the first row to top of every page).

6.3.14. Type control-A to select the entire table and then click the ALL-BORDERS button  to add lines around all the cells in the table.

6.3.15. Hover the mouse over the vertical lines between columns and double-click on the vertical line or drag the vertical line to the right (this makes the column wider, so all the cells have enough room for the data).

6.3.16. Click in a cell in the column furthest to the right and then click the "insert column to the right" button  .

6.3.17. Hover the mouse over the far-right border of the table and drag the vertical line to the right (this makes the column wider so there will be room to write notes about each locus).

6.3.18. Choose File → Save As (or click the SAVE AS button ) to save the DNA Profile Summary pages. Print all the pages and insert them into their respective case folder.

6.3.19. The Pos, Neg, and RB controls can be copied or re-printed and placed in all the relevant case folders, or a note can be written in each case file stating the controls are appropriate.

6.3.20. In a profile summary sheet, balanced alleles (e.g., ≥60%) should be separated by a comma, alleles that appear to be minor (e.g., ≤ 30%) should be separated by parentheses, and alleles that are between balanced and minor (e.g., between 30% and 60%) should be separated by less-than or greater-than signs.

6.4. Printing DNA Profile Sheet with Multiple Samples

6.4.1. A profile summary sheet with **multiple**-samples-per-page can be useful for sample-to-sample comparisons, but the one-sample-per-page DNA profile summary sheets still need to be created to document the thought process behind the interpretation of each sample.

6.4.2. Highlight the samples to include in the Table (can exclude Ladders, Positive and Negative Controls).

6.4.3. Click the Report Manager button  .

6.4.4. Click Edit → View Table by Marker

6.4.5. Click File → Print, Click Print.

6.4.6. The samples are arranged in alphabetical order by their Sample Name. It is optional to save the report after printing is complete.

6.5. **Reopening or Reviewing Projects:**

6.5.1. Log into the GeneMapper software.

6.5.2. Choose File → Open Project (or use control-O or click). Projects previously saved in GMID-X will appear in the GMID-X Database (with newest projects at the top of the list).

6.5.3. Holding Shift and clicking on a column header will sort the list by that column, or click on a column header, type part of the name being searched, and click on Search.

6.5.4. Scroll down the list of projects and double-click on a project to open it.

6.5.5. Click, click-and-drag, or control-click on sample to select samples (or control-A to select all the samples), and then View → Display Plots (or control-L or click) to view electropherograms.

6.5.6. When viewing electropherograms, choose Plots →Tables →Label Edit Viewer (or click) to see a list of edits made to allele labels in a window at the bottom of the screen. Clicking on each row in the list shows the corresponding locus (with the edited allele highlighted or colored in) in the upper window. Viewing may be easier if "1" is chosen in the 'number of panes' pulldown list.

6.6. **Printing DNA Electropherograms (optional):**

6.6.1. Electropherograms will print as displayed on the screen. Additional views can be created and saved specifically for printing electropherograms.

6.6.2. Zoom in or out to the region of interest, click Print Preview to verify the number of panes that will fit on each page, and then click Print.

6.7. **Exporting Data and Projects**

6.7.1. The electronic data files from the 3500 Genetic Analyzer (Sample files or .hid files) are in their individual run folders on the H-Drive. **To create duplicates of the electronic data** (e.g., for a discovery request), navigate to the 3500 Runs folder on H:\Crimelab\DNA\3500 runs, make a copy of the run folder on a separate computer, and delete any .hid files that do NOT pertain to the discovery request in the copied run folder. Add the GeneMapper project to the folder (see below).

6.7.2. The analyzed GMID-X projects (.ser files) are located within the GeneMapper database. **To create a duplicate of a GMID-X project** (e.g., for a discovery request), log into GeneMapper, click on the GeneMapper Manager button, highlight the project's name under the Projects tab, click the Export button, navigate to the copied run folder created earlier (see above), and click Save.

7. Recognition of Single-Source Profiles

7.1. A single-source DNA profile appears to originate from only one individual based on an interpretation of all the loci within a profile.

7.2. The number of alleles at each locus is the most obvious indicator of the minimum number of contributors. A single-source profile should have no more than two alleles at the 22 autosomal loci (except for rare tri-allelic loci) and typically no more than one allele at DYS391 in male profiles. All the alleles within a locus should be above the dropout stochastic threshold for this rule to be reliable.

7.3. The peak height ratio at each locus is also an important indicator of the minimum number of contributors. Sister alleles at heterozygous loci should be balanced, and all the alleles within a locus should be above the peak height ratio stochastic threshold for this rule to be reliable.

8. Interpretation of Single-Source Profiles

8.1. Examining the electropherograms and the DNA Profile Summary sheet, apply the rules of expected peak height ratios and stochastic thresholds.

8.2. All the alleles meeting the criteria for a 'true' allele should be interpreted and recorded on the DNA Profile Summary sheet, but any locus that is believed to be missing an allele (due to dropout) may need to be interpreted as 'inconclusive' (although any alleles above the analytical threshold may be recorded with some notation that the locus is inconclusive).

8.3. A **complete profile** is a single-source or deduced profile that contains reportable results for all 22 autosomal loci, and no dropout has occurred (i.e., all the autosomal loci are suitable for comparison). A profile can have sister allele imbalance and still be considered "complete".

8.4. A **partial profile** is a single-source or deduced profile that is missing an allele or alleles at any of the autosomal loci. Loci considered "inconclusive" are not used for comparison purposes.

8.5. A **composite profile** is a profile generated by combining multiple injections and/or multiple amplifications of the same DNA extract. Separate extracts from different locations on an evidentiary item cannot be combined into a composite profile unless there is a reasonable expectation of the samples originating from a common source (e.g., duplicate vaginal swabs, multiple samples of the same bone/muscle/tissue, contiguous cuttings from a small stain, or a known reference sample).

8.6. The sex-linked Amelogenin locus can provide information as to whether male or female DNA is present. If amelogenin does not amplify, exercise caution in calling any other loci since it may be non-human DNA, but if there is high-quality data for several other loci, the profile may be reported with "indeterminate sex/gender".

8.7. The Y-chromosome locus DYS391 is a male-specific marker that can confirm the presence of male DNA (especially when the amelogenin marker does not amplify). Typically, it only has one allele per male donor, but there are mutations that can cause more than one peak to appear.

8.8. When a single-source DNA profile is compared to known references and/or other evidence items, it is considered a "match" if all the alleles in one item's profile are present in the other item's profile at the overlapping loci.

8.9. When a single-source DNA profile is compared to known references and/or other evidence items, it is considered an exclusion if any of the alleles in one item's profile are missing from the other item's profile at the overlapping loci (assuming there aren't other reasonable explanations, such as dropout).

8.10. If a single-source match between evidence and a known reference is concluded, statistical probabilities that represent the strength of the match MUST be reported. For single-source profiles, a random match probability (RMP) statistic is used (see Genetic Analysis method).

8.11. When the RMP does **not** meet the "identity" threshold:

8.11.1. The match statement in the RESULTS should include wording similar to the following:

> **"The DNA profile obtained from the** questioned sample **(Item #) matches the DNA profile of** individual's name **(Item #). The estimated probability of randomly selecting an unrelated individual from either the FBI Caucasian or the FBI African American population databases matching this ** locus DNA profile is 1 in **."**

8.11.2. The match statement in the CONCLUSION should include wording similar to the following:

"Name **is included as a potential donor for the DNA profile obtained from the** sample **(Item #)."**

8.12. When the RMP is **equal to** or **exceeds** the "identity" threshold:

8.12.1. The match statement in the RESULTS should include wording similar to the following:

"The DNA profile obtained from the questioned sample **(Item #) matches the DNA profile of** individual's name **(Item #). The estimated probability of randomly selecting an unrelated individual from either the FBI Caucasian or the FBI African American population databases matching this DNA profile is less than 1 in 36 billion."**

8.12.2. The identity statement in the CONCLUSION should include wording similar to the following:

"With the exception of identical twins or close relatives, it is concluded with a high degree of statistical confidence that the DNA from the questioned sample **(Item #) came from** individual's name**."**

9. Recognition of Mixtures

9.1. The detection of three or more alleles at two or more loci is strong evidence of a mixture.

9.2. Peak balance less than 60% at two or more loci may indicate a mixture (assuming peaks are above the stochastic threshold) but additional information at other loci may be necessary to confirm a mixture. Heterozygous peak balance (typically ≥60%) may be in the range of 30-60% due to slight degradation and/or borderline stochastic effect. Additional information from other loci may be of assistance.

9.3. Peaks in the stutter position that are greater than the stutter threshold may indicate a mixture (assuming peaks are above the stochastic threshold). Additional information is necessary to confirm a mixture.

9.4. Evidence of a mixture at only one locus will typically be reported as a single-source profile.

9.5. It is sometimes possible to deduce the relative amounts of each contributor in a mixture based on the peak height ratios, including major/minor profiles and the minimum number of contributors. The ratios of DNA from the various contributors should be consistent across most of the loci in a profile.

9.6. The peak height ratio of X and Y peaks in Amelogenin can provide information about relative amounts of male and female DNA in a mixture (assuming peaks are above the stochastic threshold).

9.7. Re-amplifying with more input DNA to increase the peak heights of low-level peaks and minor contributors may aid in interpretation.

9.8. Peaks that are below the analytical threshold can be considered in recognizing a mixture in a low-level DNA profile, but those alleles cannot be matched or reported.

9.9. Be aware of overlapping alleles from different contributors "masking" the fact more than one person's DNA is present.

9.10. A locus with two homozygous contributors can look like a single heterozygous contributor.

9.11. If contributors share an allele (i.e., overlap at an allele), the peak height of the overlapping alleles will be the sum of the peak heights from each individual.

10. Interpretation of Mixtures

10.1. **Major/Minor Mixture:**

10.1.1. In a two-person mixture, the relative ratio of the contributors in a mixture can be estimated by examining loci with four alleles. To determine if there is a clear major contributor, peak heights are compared mathematically. For example: in a locus where four alleles are present, the larger

peak in the minor component is divided by the height of the smaller peak in the major component and then multiplied by 100 to give a percent. If a mixture appears to have a clearly predominant contributor within a mixed DNA profile (e.g., the minor peaks are generally ≤30% of the major peaks), then the mixture may be deemed a "major/minor" mixture and an RMP for the predominant ("major") DNA profile can be reported. **Note: "**30%" is an approximation, as the quality of the DNA and allele overlap can affect peak height ratios.

10.1.2. If a mixture appears to have a single contributor in the minor component, an RMP for the minor contributor's DNA profile can only be reported for the loci that have two alleles present in the minor component (i.e., no overlap between major and minor alleles).

10.1.3. See "Interpretation of Single-Source" section for results and conclusion wording for a clear major and/or minor contributor.

10.2. **Deduced Profile from Mixture on Intimate Body Swabs:**

10.2.1. If one contributor's profile is from an intimate body sample (such as the female's epithelial cells on a vaginal swab) then the other contributor's profile may be deduced, even at overlapping alleles. A deduced profile is obtained by eliminating the alleles of the known (intimate) donor from the mixed profile; the remaining alleles can be attributed to the other contributor in the mixture. For overlapping alleles, the peak height contributed by the known (intimate) donor can be subtracted.

10.2.2. An RMP can be reported for the deduced profile. Random match probabilities for the intimate donor are not required since the origin is known.

10.2.3. Intimate body samples include but are not limited to the following: body cavity swabs, fingernail cuttings, and swabs of the skin's surface.

10.2.4. See "Interpretation of Single-Source" section for results and conclusion wording for a deduced profile.

10.3. **Mixture with No Clear Major or Minor:**

10.3.1. If a mixture does not fit the first two types of mixtures (no clear major or minor and is not an intimate body swab) a Combined Probability of Inclusion (CPI) may be calculated using the Mixture module in PopStats.

Examples of this would be:

10.3.2. A biological stain from two people with approximately the same relative proportions of DNA from each person. In these cases where major and minor contributors cannot be reliably separated (e.g., a 1:1 or 2:1 ratio mixture that is not an intimate sample), a CPI can be calculated for the mixture.

10.3.3. A biological stain from two individuals where there is a major contributor and one minor contributor, but certain loci for the minor component do not fit the definition of a major/minor mixture. In these cases, a CPI can be calculated for the minor contributor; an RMP can be calculated for the major contributor's DNA profile.

10.3.4. A biological stain from three people where there is no clear major or clear minor contributor. In these cases, a CPI can be calculated for the entire mixture.

10.3.5. A biological stain from three people where there is one major contributor and two minor contributors. A CPI for minor contributors would be calculated using all the alleles in the mixture (to account for overlap) and an RMP can be calculated for the major contributor's DNA profile.

When CPI is employed, the following conditions must be met:

10.3.6. Only loci where all the alleles are above the stochastic threshold are eligible for CPI.

10.3.7. Only profiles where six or more loci are eligible for CPI should be calculated for CPI.

10.3.8. Mixtures that contain four or more contributors would provide very limited statistics and should not be interpreted at all (see mixture types below).

10.3.9. The exception to calculating CPI for loci that have alleles below the stochastic threshold is if there is a "mixture in the major". A mixture in the major is defined as follows:

- the "major" alleles are peaks that are twice the stochastic threshold,
- any "minor" alleles present are below the stochastic threshold, or at least one-half the heights of the "major" alleles,
- and there are no alleles in the region between those two ranges.

The CPI statistic would apply to the "mixture in the major". Any "minor donors" would be uninterpretable and inconclusive.

CPI statistics are reported in the RESULTS with wording similar to the following:

10.3.10. Two people, no clear major or minor:

"**The mixture of DNA profiles obtained from the** questioned sample **(Item #) is consistent with the DNA profiles of** individual's name **(Item #) and** individual's name **(Item #).**

- **Based on the FBI Caucasian database, it is estimated that only 1 in * people is a potential contributor to this profile.**
- **Based on the FBI African American database, it is estimated that only 1 in * people is a potential contributor to this profile.**"

10.3.11. Three people, no clear major or minor:

"The mixture of DNA profiles obtained from the questioned sample **(Item #) is consistent with the DNA profiles of** individual's name **(Item #) and** individual's name **(Item #) and at least one additional unknown individual.**

- **Based on the FBI Caucasian database, it is estimated that only 1 in * people is a potential contributor to this profile.**
- **Based on the FBI African American database, it is estimated that only 1 in * people is a potential contributor to this profile.**"

10.3.12. Three people, clear single-source major, and a mixture in the minor:

"**A mixture of DNA profiles was obtained from the** questioned sample **(Item #):**

- **The major contributor matches the DNA profile of** individual's name **(Item #). The random match probability…**
- **The minor component is consistent with the DNA profile of** individual's name **(Item #) and at least one additional individual.**
- **Based on the FBI Caucasian database, it is estimated that only 1 in * people is a potential contributor to the minor component of this profile.**
- **Based on the FBI African American database, it is estimated that only 1 in * people is a potential contributor to the minor component of this profile.**"

10.3.13. Mixture of two or three people in major, and uninterpretable in the minor:

"**A mixture of DNA profiles was obtained from the** questioned sample **(Item #):**

- **The major component of this profile is consistent with the DNA profile of** individual's name **(Item #) and at least one additional individual.**
- **Based on the FBI Caucasian database, it is estimated that only 1 in * people is a potential contributor to the major component of this profile.**
- **Based on the FBI African American database, it is estimated that only 1 in * people is a potential contributor to the major component of this profile.**
- **Due to the limited genetic information in the minor component of this profile, no meaningful comparison can be made to the minor donors.**"

10.4. **Complex Mixture:**

10.4.1. If a mixture appears to contain more than three contributors that cannot be resolved, the CONCLUSION should have wording similar to the following:

"Due to the complexity of the low-level mixture obtained from the questioned sample **(Item #), this DNA profile is not suitable for comparison to known reference samples."**

11. Limited Genetic Information:

11.1.1. If a mixture appears to be uninterpretable (i.e., profile shows excessive allelic dropout, degradation and/or stochastic effect), the CONCLUSION should have wording similar to the following:

"Due to the limited genetic information in the DNA profile obtained from the questioned sample **(Item #), this DNA profile is not suitable for comparison to known reference samples."**

12. Expected Control Values

12.1. Internal Lane Size (ILS) standard

12.1.1. The ILS is a set of DNA fragments (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, and 500 bp) labeled with orange (WEN).

12.1.2. GeneMapper uses these known bp sizes to determine the bp size of the other peaks in a sample.

12.2. Positive PCR Control:

Blue (FL)		Green (JOE)		Yellow (TMR)		Red (CXR)	
Locus	**2800M**	**Locus**	**2800M**	**Locus**	**2800M**	**Locus**	**2800M**
Amelogenin	**X, Y**	D16s539	**9, 13**	TH01	**6, 9.3**	D8s1179	**14, 15**
D3s1358	**17, 18**	D18s51	**16, 18**	vWA	**16, 19**	D12s391	**18, 23**
D1s11656	**12, 13**	D2s1338	**22, 25**	D21s11	**29, 31.2**	D19s433	**13, 14**
D2s441	**10, 14**	CSF1PO	**12**	D7s820	**8, 11**	FGA	**20, 23**
D10s1248	**13, 15**	Penta D	**12, 13**	D5s818	**12**	D22s1045	**16**
D13s317	**9, 11**			TPOX	**11**		
Penta E	**7, 14**			DYS391	**10**		

12.2.1. Ideally, all of the alleles will be detected, but the positive control is considered appropriate even if some alleles dropout or fall below the detection threshold, as long as the interpretable loci are consistent with the expected profile.

12.2.2. If the positive control does not work or does not type correctly, repeat the injection.

12.2.3. If the positive control does not work or types incorrectly repeatedly, the test results for that set of amplifications will be rendered inconclusive and should be re-amplified.

12.2.4. Possible explanations for an incorrect or failed positive control include faulty control DNA, contamination, failure to add control DNA, or instrument failure.

12.3. Negative PCR Control:

12.3.1. There should be no interpretable DNA profile in the Negative PCR Control.

12.3.2. If the Negative PCR Control gives an interpretable STR profile repeatedly, the test results for that set of amplifications will be rendered inconclusive and need to be re-amplified.

12.4. Reagent Blank Control:

12.4.1. There should be no interpretable DNA profile in the Reagent Blank Control.

12.4.2. If the Reagent Blank Control gives an interpretable STR profile, the Reagent Blank Control should be re-injected. If the profile is not repeated, the results can be reported, but if it gives an interpretable profile repeatedly, the Reagent Blank should be re-amplified (sample permitting).

12.4.3. If any interpretable profile is detected in multiple amplifications, then the samples extracted with that particular Reagent Blank may be rendered inconclusive. If there is no further sample to re-test, and the source of the contamination can be identified and explained, the result may be reported along with full disclosure of the contamination event.

12.4.4. If sample size permits, DNA can be freshly extracted from the pertinent test samples with a new Reagent Blank Control. If additional evidence is consumed (e.g., additional cuttings), a Quality Assurance Report may need to be initiated and the event explained in the final report.

12.4.5. Bottles of reagents that were used should be considered possible sources of contamination and may need to be discarded or QC tested as soon as possible.

13. Statistical Analysis

13.1. Follow "Genetic Analysis" method to calculate the statistical significance of any matches or inclusions.

14. Glossary

14.1. Autosomal loci = the 22-loci in a DNA profile from locations NOT on the X- or Y-chromosomes (all loci except Amelogenin and DYS391).

14.2. Complete DNA profile = when a single-source profile (or deduced profile) has comparable data for all of the autosomal loci (i.e., no alleles are dropping out or inconclusive alleles attributed to that individual).

14.3. Partial DNA profile = a single-source or deduced profile that is missing an allele or alleles at any of the autosomal loci. This definition is **different** than a "partial profile" in the CODIS database (a profile that is missing at least one of the original 13 CODIS core loci).

14.4. Mixture of DNA profiles = when a profile appears to have DNA from two or more contributors, it is called a "mixture" or "mixed DNA profile". It is sometimes possible to deduce a single-source profile from a mixture on an intimate body swab.

15. PowerPlex Fusion loci:

Dye	Locus	Stutter	Repeats	Repeat Sequence	Chromosomal location	Ladder Bins
Blue (FL)	**Amelogenin***	**0 %**	n/a	n/a *	Xp22.1–22.3 and Y	X, Y
	D3s1358	**11.9 %**	N – 4	TCTA Complex	3p21.31	8 – 21
	D1s11656	**14.2 %**	N – 4	TAGA Complex	1q42	8 – 21
		3.6 %	**N – 2**			
	D2s441	**9.2 %**	N – 4	TCTA	2p14	8 – 17
	D10s1248	**12.4 %**	N – 4	GGAA	10q26.3	8 – 19
	D13s317	**9.8 %**	N – 4	TATC	13q31.1	5 – 17
	Penta E	**7.6 %**	**N – 5**	AAAGA	15q26.2	4 – 25
Green (JOE)	**D16s539**	**10.2 %**	N – 4	GATA	16q24.1	4 – 16
	D18s51	**14.6 %**	N – 4	AGAA	18q21.33	7 – 27
	D2s1338	**13.9 %**	N – 4	TGCC/TTCC	2q35	10 – 28
	CSF1PO	**9.5 %**	N – 4	AGAT	5q33.1	5 – 16
	Penta D	**6.8 %**	**N – 5**	AAAGA	21q22.3	2.2 – 18
Yellow (TMR)	**TH01**	**4.6 %**	N – 4	AATG	11p15.5	3 – 13.3
	vWA	**11.2 %**	N – 4	TCTA Complex	12p13.31	10 – 25
	D21s11	**11.6 %**	N – 4	TCTA Complex	21q21.1	23.2 – 39
	D7s820	**11.0 %**	N – 4	GATA	7q21.11	5 – 16
	D5s818	**9.5 %**	N – 4	AGAT	5q23.2	5 – 19
	TPOX	**5.5 %**	N – 4	AATG	2p25.3	4 – 16
	DYs391**	**8.7 %**	N – 4	TCTA	Y	5 – 16
Red (CXR)	**D8s1179**	**10.9 %**	N – 4	TCTA Complex	8q24.13	6 – 20
	D12s391	**15.8 %**	N – 4	AGAT/AGAC Complex	12p12	13 – 27
	D19s433	**11.0 %**	N – 4	AAGG Complex	19q12	5.2 – 19.2
	FGA	**12.1 %**	N – 4	TTTC Complex	4q28	12.2 – 50.2
	D22s1045	**16.4 %**	**N – 3**	ATT	22q12.3	6 – 20
		8.6 %	**N + 3**			

* Amelogenin is not an autosomal locus. It should have an "X" band for females, and an "X" and "Y" band for males.

** DYS391 is not an autosomal locus. This locus should only have one allele from the male Y-chromosome.

16. Artifacts Other Than Stutter:

Locus or Dye Label	DNA-*Dependent* Artifacts	DNA-*Independent* Artifacts
Fluorescein (blue)	~ 63 – 68 bases [1] ~ 88 – 112 bases [2]	~ 58 – 59 bases [1] ~ 61 – 63 bases [1] ~ 83 – 86 bases [1]
JOE (green)	~ 73 – 85 bases [1] ~ 214 bases [1] ~ 247 bases [1]	~ 62 – 67 bases [1]
TMR (yellow)	~ 66 – 72 bases [1] ~ 172 – 176 bases [1]	~ 58 – 62 bases [1]
CXR (red)	~ 175 – 183 bases [1]	
Amelogenin	n–1	
D1S1656	n–2 n+2	
D2S441	n–1	
D13S317	n–2, n+2	
D18S51	n–2, n+2	
D21S11	n–2, n+2	
D7S820	n–2, n+2	
D5S818	n–2, n+2 n–8 to n–9 [3]	
D12S391	n–2 n+2 n–3	
D19S433	n–2, n+2	

[1] Artifact sizes may vary depending on instrumentation and environmental conditions.

[2] For artifacts in this size range, rfu approximately 1.5% or less of the main peaks may be observed at the D3S1358 locus.

[3] Low intensity peaks (50–200 rfu) that migrate approximately 8–9 bases to the left of the main allele may represent DNA secondary structure.