# Deviation Request Form (DRF)

Directions: The Initiator will complete Sections A through C. Additional continuation pages can be included if necessary.

| **Initiator** | MJ DeHaan | **Date** | 1/25/2019 |
|---|---|---|---|

A. Requested deviation applies to (Technical Procedure – include specific section):

Procedure for Fusion 6C Analysis and Interpretation 5.6.13.4

B. Requested deviation:

See attached wording

C. Necessity for the deviation:

To incorporate results of additional studies performed within the Forensic Biology Section to allow Forensic Scientists to interpret 4 contributor mixtures.

D. Technical review and Authorization (to be completed by the Quality Manager and/or Technical Leader)
Comments(to include merits and impacts):

Following additional mixture studies results, the scientist may interpret 4 person mixtures by following the steps laid out in the study summary and in the procedure.

| Approved | ☑ | Yes | ☐ | No | Duration | until next version |
|---|---|---|---|---|---|---|
| Signature | Mackenzie G DeHaan | Digitally signed by Mackenzie DeHaan DN: cn=Mackenzie DeHaan, o=NC SCL, ou=FB, email=mdehaan@ncdoj.gov, c=US Date: 2019.01.25 08:16:13 -05'00' | | | Date | 1/25/2019 |

E. Quality Assurance Authorization (to be completed by the Quality Manager, Forensic Scientist Manager or designee)

| | | | | |
|---|---|---|---|---|
| Acceptable within general QA guidelines and good laboratory practice? | ☑ | Yes | ☐ | No |
| Significant negative impact to Crime Laboratory Quality System? | ☐ | Yes | ☑ | No |

Restrictions/limitations:

| ☑ | Authorized | ☐ | Rejected | Signature | Jody H. West | Digitally signed by Jody H. West DN: cn=Jody H. West, o=DOJ, ou=State Crime Laboratory, email=jwest@ncdoj.gov, c=US Date: 2019.01.25 08:21:57 -05'00' | Date | 1-25-19 |
|---|---|---|---|---|---|---|---|---|

Form approved for use by: Timothy G. Suggs

Digitally signed by Timothy G. Suggs DN: cn=Timothy G. Suggs, o=North Carolina State Crime Laboratory, ou=Quality Manager, email=tsuggs@ncdoj.gov, c=US Date: 2014.12.19 09:01:52 -05'00'

**5.6.13.4 Four (or greater) Contributor Mixtures -** Due to the complexity resulting from allele sharing in mixtures with >3 contributors, these mixtures will be generally deemed as inconclusive. The only exception will be if a single major contributor or multiple major group (consisting of 2 contributors) can be clearly discerned. Determination of the possibility for interpretation of the major profile will be based upon the evaluation of multiple factors, including the overall contribution of the minor profiles to the mixture, the peak height balance between peaks marked as major, and the mixture ratio between the major and the minor profiles. In mixtures with >3 contributors, a multi-step approach must be taken to interpret the major contributor profile in the mixture. The major contributor must demonstrate a PHR of 70% for the major contributor to discern using Fusion 6C. The overall mixture ratio should be a minimum of 3:1 (between the major and the next highest minor contributor) to determine the major/minor(s) profiles. Additionally, the overall major contributor contribution should be ≥ 70% to discern the profile. Loci which do not meet the overall major contribution or PHR should not be included within the major profile. In a multiple major, the major group contributor should be indistinguishable. Interpretation that does not meet the above multi-step approach would require approval in writing by the DNA TL.

# Procedure for Analysis and Interpretation of Promega PowerPlex® Fusion 6C Samples

**1.0 Purpose**-This procedure specifies the steps for performing analysis and interpretation of DNA samples amplified with Promega PowerPlex® Fusion 6C using the GeneMapper® ID-X (GMID-X) software.

**2.0 Scope**- This procedure applies to casework analysts and trainees in the Forensic Biology Section who perform DNA analysis using GMID-X and interpret samples amplified with Promega PowerPlex® Fusion 6C.

**3.0 Definitions** – See Section Definition list.

## 4.0 Equipment and Materials

- Computer with GeneMapper® ID-X (version 1.4 or most current) software
- Microsoft Excel, Word
- Print2PDF software
- CODIS software program (Analyst Workbench) located on CODIS computer(s)

## 5.0 Procedure

**5.1 Introduction** – The interpretation of results in casework is a matter of professional judgment and expertise. Not every situation can or should be covered by a pre-set rule. The criteria are based on validation studies and literature references and set a minimum criteria developed by the laboratory for which analysts should adhere. These guidelines establish a framework to ensure that interpretation is made objectively and consistently between analysts, reporting conclusions that are supported by analytical data.

**5.2 Documentation –** Analysts shall document on the allele call tables any assumptions used (e.g., use of donor for intimate samples, number of contributors), determination of major profiles (to include listing the specific loci used), the non-use of loci for interpretation and reason (e.g., imbalance, stochastic effects), and list loci which may be used to perform statistical interpretation. Additionally, any other notes, remarks, and observations used to make an interpretation and/or conclusion regarding unknown samples shall be documented on the allele call tables (or on data created in the ArmedXpert™ program).

### 5.3 Basic Interpretation Steps

**5.3.1** Identify presence of mixture.

**5.3.2** Designate allele peaks.

**5.3.3** Identify number of potential contributors.

**5.3.4** Estimate mixture ratio.



***All copies of this document are uncontrolled when printed.***

**5.3.5** Consider possible genotype combinations.

**5.3.6** Compare reference samples.

## 5.4 Creating a Project

**5.4.1** Open GMID-X and log in under the Forensic Scientist's name/log-in. Forensic Scientists shall log into and use the Casework Database Host.

**5.4.2** To add samples to be analyzed to the project, locate the run folder containing the samples to be added to the project under the "Files" tab. Highlight the run folder (or select the specific samples to be analyzed) and select add to List. The file(s) then appears on the right-hand side. Select "Add."

**5.4.3** In the Samples pane, the category Sample Type shall have the samples labeled as follows when amplified with Promega PowerPlex Fusion 6C®:

- Ladder: Allelic Ladder
- 2800M (Positive Amplification Control): Positive Control
- All Negative Controls (extraction and amplification): Negative Control
- All other samples shall be Samples
- Sample File names appear by well number (e.g., A12) followed by the sample name as entered by the Forensic Scientist prior to electrophoresis. Also, until the samples are analyzed, the Status icon to the left of the Sample File name has the unanalyzed icon (  ) present next to it. Verify that the table setting (in top center of the main GMID-X window) is set to 31XX Data Analysis.

**5.4.4** The remaining categories shall be filled out as follows:

- Specimen Category: no export
- Analysis Method: Fusion
- Panel: PowerPlex_Fusion_6C_Panels_IDX_v1.1
- Size Standard: WEN_ILS_500_CS
- Instrument Type: ABI3500, or similar (note: automatically filled in by the software and instrument)

**5.4.5** Once all samples and categories are properly labeled and identified, select the Analyze icon (  ) to analyze the data. When prompted, create a project name for the set of samples and select the "Casework Security Group" and select "OK."



***All copies of this document are uncontrolled when printed.***

**5.4.5.1** The Analysis Summary and Genotypes tabs become available in the main GMID-X window once analysis is complete.

**5.4.5.2** If any sample displays a solid red stop sign icon (  ) on the right-hand side of the GMID-X window, assess the data as described in **5.2**.

**5.4.5.3** Forensic Scientists shall delete their projects from the software only after a copy of the project has been added to the associated case record and the case record has been published.

### 5.4.6 Analysis and Plot Settings

**5.4.6.1** Access the Analysis Method Editor by selecting "Tools" from the menu in the main GMID-X window, and then select "Analysis Method Editor." Note that the user must have a single sample in the main GMID-X window selected in order to access the Editor.

**5.4.6.2** Select the "Peak Detector" tab and ensure that these settings for Full Range analysis, Peak Detection (see analytical threshold), Smoothing (Light) and Baselining (51 points), and Size Calling Method (Local Southern) have been chosen when the samples have been amplified with PowerPlex® Fusion 6C. The analytical threshold was established through validation and performance check studies using the PowerPlex® Fusion 6C amplification kit. The analytical threshold is set per dye channel (Blue (FL-6C) – 75 RFU, Green (JOE-6C) – 75 RDU, Yellow (TMR-6C) – 80 RFU, Red (CXR-6C) – 100 RFU, Purple (TOM-6C) – 60 RFU, and Orange (WEN) – 35 RFU). Anything present below these thresholds is considered to be indistinguishable from background noise and shall not be considered for analysis. The "Allele" tab contents should also appear using the stutter as seen below and bin set of PowerPlex_Fusion_6C_Bins_IDX_v1.1.

**5.4.6.3** Marker-specific stutter filter percentages for PowerPlex® Fusion 6C are listed below. All potential stutter peaks must be evaluated as GMID-X cannot set automatic stutter cutoffs for all stutter peaks noted in validation summaries. In addition to the N-4 stutter percentages listed below, N-8 and N+4 were evaluated in the validation studies. The maximum observed values were 1.1% for N-8 and 3.0% for N+4. Stutter filters in GMIDX are the validated values + 3 standard deviations. STR results shall not be considered inconclusive if stutter peaks are present in single source samples. In mixed samples with major/minor components, minor peaks in stutter position that are indistinguishable from stutter may be interpreted. Samples with overall peak height values within the stochastic range or close to overblown range should be evaluated carefully as increased stutter may be seen in these samples.



***All copies of this document are uncontrolled when printed.***

| Locus | N-4 Average Stutter % |
|---|---|
| D3S1358 | 9 |
| D1S1656 | 8.5 |
| D2S441 | 4.8 |
| D10S1248 | 8 |
| D13S317 | 4.9 |
| Penta E | 2.7 |
| D16S539 | 6.4 |
| D18S51 | 8.1 |
| D2S1338 | 8.5 |
| CSF1PO | 5.5 |
| Penta D | 1.9 |
| THO1 | 2.2 |
| vWA | 6.9 |
| D21S11 | 8.2 |
| D7S820 | 4.7 |
| D5S818 | 6.1 |
| TPOX | 2.5 |
| D8S1179 | 7.3 |
| D12S391 | 9.1 |
| D19S433 | 7 |
| SE33 | 10 |
| D22S1045 | 7.2 |
| DYS391 | 6.7 |
| FGA | 7.1 |
| DYS576 | 8.5 |
| DYS570 | 8.9 |

**5.4.6.4** In the main GMID-X window, ensure that the Traditional Allele Table is chosen; select "Tools" from the top menu; then select "Table Setting Editor." Under the "Genotypes" tab, at the bottom of the window, ensure that the "Number of Alleles" under Allele Settings is set to at least 12.

**5.4.6.5** Only the DNA Technical Leader or the GMID-X Administrators may change settings for the Analysis Method Editor or the Table Setting Editor.



***All copies of this document are uncontrolled when printed.***

### 5.4.7 Adding/Deleting samples

**5.4.7.1** If a Forensic Scientist must add additional sample(s) to a current project, follow **5.1.2** through **5.1.8**. The newly added sample shall be analyzed (select the Analyze icon) before the data may be viewed.

**5.4.7.1.1** If the sample is from a different injection (e.g., run folder), the associated Allelic ladder shall also be imported in order for the sample data to be sized properly.

**5.4.7.2** If a Forensic Scientist must remove a sample(s) that has been added to the project (e.g., a sample from an unrelated case), highlight the sample to be removed and in the menu bar, select Edit and Delete from Project. Select "OK" when prompted.

**5.4.7.2.1** Samples from the same case that are present in a project, but are not used (e.g., redundancy, sizing data issues or similar), shall not be deleted.

## 5.5 Analyzing/Preliminary Evaluation of Data from Each GMID-X Project

**5.5.1** Evaluate Sizing Data (i.e., WEN)

**5.5.1.1** WEN sizing data for all samples must be evaluated to ensure all peaks are present and correctly sized (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500-base pairs).

**5.5.1.2** If any sample or reagent blank in the project indicates no sizing data (e.g., WEN failure), then that sample or reagent blank shall not be used for comparison. This information shall be noted on the Casework Table Worksheet.

**5.5.1.2.1** If there is no sizing data for an allelic ladder, then no data associated with that ladder can be sized unless a ladder is imported from another run injected on the same instrument on the same day. Note: The source of this imported ladder shall be documented in the case file. When using an imported ladder, data must be evaluated for migration shift (e.g. numerous OL calls seen with imported ladder use).

**5.5.1.2.2** If an amplification control has no sizing data, a duplicate control from the same amplification may be used.

**5.5.1.2.3** Any sample/reagent blank that has no sizing data shall be reanalyzed (e.g. injected again on 3500, re-amplified, or re-extracted).



***All copies of this document are uncontrolled when printed.***

### 5.5.2 Assess Overall Quality of Data

#### 5.5.2.1 Allelic Ladders and Controls

**5.5.2.1.1** Once all samples have been analyzed, the Analysis Summary tab will appear. This tab shows an overview of all samples, ladders, and controls for the run.

**5.5.2.1.2** Verify that all the alleles are sized and labeled correctly according to the expected calls for the PowerPlex® Fusion 6C Allelic ladder. All expected peaks must be present and above the appropriate analytical threshold.

**5.5.2.1.3** All positive control(s) must have the expected allelic results. If these expected alleles are not in the correct position or are below the analytical threshold, then the control shall be re-injected. If re-injection is unsuccessful, then the controls and all associated samples shall be re-amplified and analyzed before that locus may be used for analysis.

**5.5.2.1.4** If any peaks not attributable to artifacts are present above the analytical thresholds, the controls shall be reanalyzed (i.e., re-injected or re-amplified.) If further examination is necessary, then the control(s) and associated samples shall be re-extracted. If reanalysis is not possible, then the samples may be interpreted upon consultation with the DNA Technical Leader (TL). The TL shall consider the peak height and number of peaks with respect to the profile. This consultation shall be documented.

**5.5.2.2 Samples-** Examine all samples to assess the quality and quantity of DNA present.

**5.5.2.2.1 Artifacts:** If any peaks are to be removed from consideration as true alleles due to their presence as a known artifact (e.g., pull-up, dye-blob, stutter, spike), highlight the sizing box associated with the peak and right-click the mouse. The Forensic Scientist shall evaluate peaks before deleting to ensure they are artifacts and not peaks that are a part of a minor/low level profile. Select Delete Label(s) (multiple peaks can be deleted simultaneously by selecting them at the same time-when the warning dialog box appears, select OK). When prompted, type in artifact as the Reason(s) for Change. The Forensic Scientist should not relabel the peak if the GMID-X software automatically labels the peak (pink label).

**5.5.2.2.2 Non-specific amplification peaks:** Non-specific amplification is known to occasionally occur with STR kits and is documented in developmental validation and through on-going technical support from the manufacturer.



***All copies of this document are uncontrolled when printed.***

The Forensic Scientist shall evaluate these peaks. Peaks may be designated as artifacts if they meet any of the following criteria: atypical peak morphology; OL designation without proper sizing (outside of +/- 0.5 bp bin); rfu value inconsistent with remainder of profile (e.g. 7000 rfu peak noted when remainder of profile is ~500 rfu); manufacturer documented non-specific amplification peak; and/or lack of stutter peak.

**5.5.2.2.3 Off-ladder Alleles, Tri-alleles and Microvariants:** If a sample(s) contains an off-ladder allele that is not an artifact or a microvariant is present in a sample and GMID-X has not automatically assigned it an allelic value, Forensic Scientists shall document the proper sizing/locus assignment of the off-ladder alleles, tri-alleles, and/or microvariants as follows:

**5.5.2.2.3.1** In a single source sample, if the allele in question lies outside the marker range of a locus (e.g., between two loci, above or below the end of the color channel), the Forensic Scientist shall attempt to determine which locus the allele should be assigned based upon the presence or absence of heterozygosity at the loci in question.

**5.5.2.2.3.2** If one locus exhibits homozygosity and the second heterozygosity, the off-ladder allele shall be assigned to the homozygous locus.

**5.5.2.2.3.3** If both loci exhibit homozygosity or heterozygosity, the Forensic Scientist shall document either on the allele call table or the electropherograms that the allele was observed, but not assign the allele to a particular locus, and mark the allele as inconclusive (INC). The called (non off-ladder) alleles at these loci shall be reported and uploaded into CODIS.

**5.5.2.2.3.4** In some instances, an off-ladder allele from one locus may appear within an adjacent locus (i.e., appears to be a potential tri-allele next to a homozygous locus). If the Forensic Scientist believes such a situation has occurred, the entire locus containing that allele shall be marked as inconclusive.

**5.5.2.2.3.4.1** A microvariant or an off-ladder shall be named/assigned per NDIS standards. For CODIS eligibility, refer to the Forensic Biology Section Procedure for CODIS.



***All copies of this document are uncontrolled when printed.***

**5.5.2.2.3.4.2** In the main GMID-X window, select the sample(s) containing the microvariant/off-ladder allele and the allelic ladder. Display Plots. Zoom in to the locus containing the microvariant/off-ladder allele; the Sample Plot displays the allelic ladder in the top half of the Plot window and the sample is in the bottom half.

**5.5.2.2.3.4.3** Highlight the microvariant/off-ladder allele in the sample, along with the alleles in the allelic ladder that lie the closest in size to the microvariant/off-ladder allele (to select peaks simultaneously, hold down the Ctrl key on the keyboard while selecting). Select the sizing table icon (  ). Select Ctrl + G to make the sizing table display the selected alleles.

**5.5.2.2.3.4.4** Based upon the sizing information provided in the table, determine the appropriate allele call for the microvariant/off-ladder allele.

**5.5.2.2.3.4.4.1** The correct allele call for the microvariant/off-ladder allele is dependent upon the base pair size when compared to the allelic ladder.

**5.5.2.2.3.4.4.2** Microvariants that vary by less than the consensus repeat unit for that locus must be designated as an integer of that variation (e.g., the 9.3 allele at TH01).

**5.5.2.2.3.4.5** Rename the allele by right-clicking on it and selecting Rename Allele Label. Select Custom Allele Label and enter the call in the box. When prompted by the Reason(s) for Change box, type microvariant or off-ladder allele.

**5.5.2.2.3.4.6** With the alleles highlighted still present in the sizing table, add a copy to the appropriate Case Record Object Repository in FA.



***All copies of this document are uncontrolled when printed.***

**5.5.2.2.3.5 Verification of Off-Ladder Alleles, Tri-alleles, Microvariants:**

**5.5.2.2.3.5.1** Off-ladder alleles and microvariants do not require verification as long as they can be properly sized and there is no shifted alleles or injection issues.

**5.5.2.2.3.5.2** Tri-alleles shall be re-amplified in order to verify their presence and size (if applicable) if the allele is to be used for interpretation.

**5.5.2.2.3.5.3** When multiple occurrences of the same tri-allele are seen within the questioned samples of the same case, no verification is required.

**5.5.2.2.3.5.4** Shifted alleles/loci shall not be used for comparison in any control, ladder or sample.

**5.5.2.2.3.5.5** A known sample cannot be used to verify the presence of a tri-allele in a questioned sample.

**5.5.2.2.3.6 Off-scale Samples:** Off-scale samples shall not be used for comparison. Such samples shall be re-amplified with a lower concentration.

### 5.5.3 Printing Egrams Electronically

**5.5.3.1** The egrams for any well in which data is collected (e.g., blank, ladder, item, etc.) shall be printed and the egrams for each GMID-X project shall exist as an individual PDF file. Ensure the plot setting is set to "Traditional Genotype Plot" when printing to PDF. The egrams for the questioned items in a case shall be printed prior to the known items. The egrams for the negative controls and samples with no DNA peaks detected shall be printed scaled to approximately 100 rfus.

**5.5.3.1.1** GMID-X projects are added to the FA case record object repository by exporting the projects: in GMID-X, select "Tools," "GeneMapper® ID-X Manager," select the applicable project and select "export." The file is exported/saved as a .ser file.

**5.5.3.2** Any individual sample/control/ladder with the case that is not used for comparison purposes (e.g., WEN failure, redundancy, further analysis) shall be added to the appropriate



***All copies of this document are uncontrolled when printed.***

FA Case Record Object Repository. Such samples/controls/ladders shall appear in the Casework Table explaining why it was not used for comparison (e.g., WEN failure, redundancy, re-analysis).

**5.5.3.2.1** Even if not used for comparison purposes, all instrument files (e.g. .hid) associated with such samples/controls/ladders shall be present in the run folder containing the electronic data in the FA case record object repository.

**5.5.3.2.2** For any sample/control/ladder that is not used for comparison purposes, corrections (labeling of artifacts) are not required.

**5.5.3.3** If an entire run or project for a case is not used for comparison purposes, it shall be added to the case record object repository. Corrections (e.g., labeling of artifacts) are not required in this instance. A Casework Table shall be generated for this instance and the reason for not using the entire run/project shall be noted on this page (also Print2PDF; see **5.3.1**). An Allele Call Table is not required for such runs/projects.

**5.5.3.4** The WEN sizing standard shall be printed along with the overall egram PDF file for each project. The WEN must be present and labeled correctly for all samples/controls/ladders, and all peaks (60 through 500) must be visible. The full width of all peaks must be visible; however, it is acceptable to zoom in on the heights of the peaks when printing.

### 5.5.4 View Raw Data

**5.5.4.1** In order to verify the addition of amplification kit primers to all samples and controls which do not show amplification of at least one allele (which includes instances of WEN or injection failure), the raw data for these samples/controls shall be added to the case record object repository. Any sample/control that shows at least one allele is not required to be printed, as it may be inferred that the presence of an allele indicates primers were added to the sample/control. Note: If the entire run is not used for analysis, then the negative raw data is not required to be printed.

**5.5.4.1.1** In the main GMID-X window, highlight the sample/control and select Raw Data from the View option in the main GMID-X menu.

**5.5.4.1.2** The subsequent window displays the raw data on the right-hand side and the sample and run information on the left-hand side. Expand the left-hand window so that the full run file name is clearly visible. The right-hand window shall show the maximum vertical and horizontal axis.



***All copies of this document are uncontrolled when printed.***

**5.5.4.1.3** Using the "Alt" and "Print Screen" buttons on the computer, take a snapshot of the raw data. Open up a Word document and paste this snapshot into the document. Repeat this process for all samples/controls in each project that do not show amplification of at least one allele.

**5.5.4.1.3.1** This raw data Word document shall contain at least the following information in the header: full case number, date, and Forensic Scientist's initials.

**5.5.4.1.4** The Word document(s) containing the snapshots of the raw data shall be saved/printed as a .pdf file and included in case record object repository.

## 5.6 Sample Interpretation

**5.6.1** Failure of any locus (loci) to amplify shall not preclude the Forensic Scientist from interpreting/reporting those loci that are present.

**5.6.2** Reasoning for the determination of whether or not a locus is used for statistical interpretation shall be documented. Reasoning may be applied to the entire locus or to a portion of the locus (e.g., minor contributor(s)). Inconclusive loci (or portions) shall not be used for comparison purposes.

**5.6.3** If no profile above analytical threshold is present, the scientist shall interpret that sample as no DNA profile obtained.

**5.6.4** The presence of apparent degradation, inhibition, or significant stochastic effects in a profile shall be considered during the interpretation process and may influence the assessments made at each locus. Additionally, the scientist may use additional criteria including quantitative values and/or the totality of the profile to determine if potential stochastic amplification has occurred. The stochastic threshold is set at 300 RFU.

**5.6.5** Data generated from quantitation results or electropherogram results may be used in the determination of the presence of a male contributor in a sample. Data obtained at Amelogenin and/or the YSTR loci (DYS391, DYS570, and DYS576) may be utilized for this determination even if the sample (or portion of sample) is interpreted as inconclusive. This data may be used to clarify which samples would be recommended for YSTR processing following our current guidelines.

**5.6.6** DYS391, DYS570, and DYS576 will be considered information only loci and can be used to assist in the determination of the number of contributors in the sample. These loci will not be used for inclusion or exclusion of a known standard or for statistical calculations. Lack of signal at these loci will not result in the profile being called "partial" and will not negate identity if



***All copies of this document are uncontrolled when printed.***

all other loci meet the criteria. Results are required in the positive control to ensure appropriate amplification. These loci may be compared to any profiles generated with Y23 to ensure the concordance of samples. Marking alleles at these loci as a part of a major profile should be done with caution in profiles where >2 males are present.

**5.6.7** Peak height ratio - Samples shall be examined for balance at each locus. Based upon validation studies, single source samples/single major contributor (predominant profile) should exhibit heterozygote peak height ratios (PHR) greater than or equal to 60 % when both peaks are at/above the stochastic threshold. Low PHR may indicate a null allele, a primer binding site mutation, degradation, or the presence of inhibition.

**5.6.8** A minimum of 5 loci (without potential dropout) must be present for the profile to be interpretable. Profiles (e.g. minor profiles) that do not meet this threshold may be interpreted with the use of mixture interpretation software. These instances would include profiles whose interpretation combines "Allele, Any" with loci with no dropout. This determination will be made upon consultation with the DNA Technical Leader.

**5.6.8.1** ArmedXpert software will be used to assist the Forensic scientist in evaluating mixtures. If a mixture profile is being interpreted and there are ≥5 alleles present in the minor/additional contributor then the scientist will use the data obtained in ArmedXpert to assist in the interpretation of the mixture components. This allows the analyst to fully consider effects of peak heights and allele sharing during the mixture interpretation.

**5.6.9** Reinterpretation of Legacy Data – Comparison to single source (to include major profiles) is not considered reinterpretation. Additionally, it is not reinterpretation if documentation of previous interpretation includes possible contributor genotypes. Any assessment of allele calls, removing of alleles/loci for statistical calculations, or a change in assumptions is considered reinterpretation. Any reinterpretation shall require review by the Technical Leader and CODIS Administrator.

**5.6.10** Reference standards that are submitted for comparison to outsource data that has been accepted by the Laboratory for upload shall be compared only to the profile(s) entered in CODIS. No other profiles/data from the case that was analyzed by an external laboratory (outsourced data) shall be reviewed. If a request is received to review additional profiles, the Forensic Biology Manager, Technical Leader, and CODIS Administrator shall make a determination of what items, if any, may be compared.

**5.6.11** Reference standards submitted for comparison to profiles generated within the Forensic Biology Section shall be compared to the profiles in the case (and listed cross-reference cases). No comparison is required if no DNA profile was generated from an item or if the profile (or component) was previously reported as inconclusive.



***All copies of this document are uncontrolled when printed.***

### 5.6.12 Single Source Profiles

**5.6.12.1** Generally, a sample is considered to have originated from a single individual if no more than two alleles are present at all loci for which typing results were obtained (although tri-allelic loci may occur).

**5.6.12.2** The peak height ratios for all heterozygotes should meet or exceed the empirically determined PHR. It is noted that peak height imbalances may be seen as a result of a primer binding site mutation or also as peak heights are closer to the stochastic threshold.

**5.6.12.3** Homozygote alleles will usually appear as peaks with peak heights approximately twice the height of heterozygote alleles.

### 5.6.13 Mixture Profiles

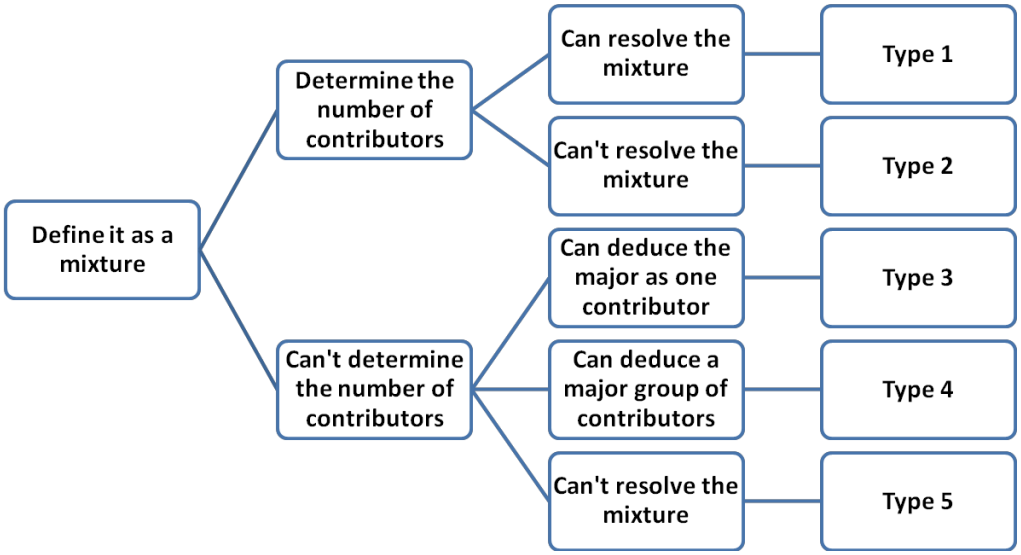
**5.6.13.1** **General -** To assist interpretation, mixtures can be grouped into categories based on the strategy employed. Mixtures may not always fit into these mixture types and often can be a combination of types across loci. This scheme is mainly used as a guide for analysts to stay consistent with interpretation. The below guide is not all inclusive for every mixture that will be encountered during casework analysis. The below should be used in conjunction with other factors, including an evaluation of the profile for peak height imbalance and possibility of dropout when determining number of contributors. For some mixtures, basing a number of contributors solely on the allele count at a locus will not fully represent the interpretation of the mixture (e.g. a mixture with a maximum of 4 alleles seen at loci may be interpreted as a mixture with >2 contributors based on these additional factors).

There are three basic steps to the application of the scheme: evaluate the overall quality of the profile, determine if the number of contributors can be inferred and decide if contributor genotypes at a particular locus can be restricted based on certain criteria.

Mixture interpretation will be performed utilizing the below steps and may be combined with the use of the Armed Xpert software (see Procedure for Armed Xpert).



***All copies of this document are uncontrolled when printed.***




**5.6.13.1.1** A sample is considered to have originated from more than one individual if three or more alleles are present at one or more loci (excepting tri-allelic loci) and/or the peak height ratios between a single pair of allelic peaks for one or more loci are below the empirically determined heterozygous peak height ratio expectation. The entire profile must be evaluated before defining it as a mixture, especially when the profile is low-level and/or partial.

**5.6.13.1.1.1** When no more than 4 peaks are present at any locus (above analytical threshold) the profile should generally be considered a mixture consistent with two contributors.

**5.6.13.1.1.2** When 5 or 6 peaks are present at any locus (above analytical threshold) the profile should be considered a mixture consistent with three contributors.

**5.6.13.1.1.3** When 7 or more peaks are present at any locus (above analytical threshold) the mixture should be considered a complex mixture (four or more contributors).

**5.6.13.1.2** Alleles between the analytical threshold and the stochastic threshold may be used in the assessment of the number of contributors.

**5.6.13.1.3** An estimation of the minimum number of contributors to a mixture should not be construed as designation of an absolute number of individuals that must have



***All copies of this document are uncontrolled when printed.***

contributed to a mixed specimen. Rather, this estimation is provided to describe the fewest number of individuals who must have contributed to a mixture.

**5.6.13.1.4** Allele sharing can exist between multiple contributors which may cause the PHR expectation to vary from the empirically determined value. This may be seen as allele stacking which could create imbalance between heterozygotic peaks of a major contributor. Alternatively, allele(s) attributable to minor contributor(s) may be masked by the major contributor(s). Generally, the more dissimilar the respective contributions of the major and minor contributors, the lesser the potential impact of allele stacking/masking.

**5.6.13.1.5** Determining the mixture ratio – The average mixture ratio should be determined (e.g., for loci with 4 alleles present calculate by dividing the sum of the largest alleles by the sum of the smallest alleles).

**5.6.13.1.6** When a contributor to an intimate mixture is known, the known profile may be used to determine the genotype of the foreign profile.

**5.6.13.1.7** Type 1 – Profile contains 2 or 3 contributors and data can be resolved into respective major/minor contributors. Mixtures may be conditioned using assumed contributors. In some 3 contributor mixtures, the minor profiles may not be able to be resolved into respective genotypes.

**5.6.13.1.8** Type 2 – Profile contains 2 or 3 contributors, but cannot be resolved into major/minor contributors.

**5.6.13.1.9** Type 3 – Number of contributors cannot be assumed, however, mixture is consistent with 2 or 3 contributors and major contributor can be resolved. Minor component(s) has minimal effect on major contributor. Minor components are generally considered inconclusive.

**5.6.13.1.10** Type 4 - Number of contributors cannot be assumed, however, mixture is consistent with 3 contributors and major contributor group can be resolved. Minor component(s) has minimal effect on major contributor. Minor components are generally considered inconclusive.

**5.6.13.1.11** Type 5 – Mixtures are generally considered inconclusive either due to low quantity and not enough data to assume a number of contributors or resolve contributors, or mixture consists of 4 or more contributors with no resolvable major contributor or major group.



***All copies of this document are uncontrolled when printed.***

**5.6.13.2 Two Contributor Mixtures**

**5.6.13.2.1** A two contributor mixture can demonstrate a major and minor contributor, a mixture containing a known contributor (Type 1), or an indistinguishable mixture (Type 2).

**5.6.13.2.2** In order to determine a major and minor contributor, the average mixture ratio should be at least 3:1. Forensic Scientist should remember that as the peak heights fall close/below ST, mixture ratios may vary.

**5.6.13.2.3** The major contributor must be unambiguous based upon the mixture profile in its entirety, considering peak height ratio, mixture ratio, and the potential for drop-out.

**5.6.13.2.4** In mixtures with a resolvable major and minor, each profile shall be treated as a single source profile.

**5.6.13.3 Three Contributor Mixtures -** Generally the scheme to resolve 2 contributor mixtures can be extended to 3 contributor mixtures, but the greater potential for allele sharing must be considered. As a result, while a major contributor may be deduced, for some mixtures the minor contributors will be inconclusive.

**5.6.13.4 Four (or greater) Contributor Mixtures -** Due to the complexity resulting from allele sharing in mixtures with ≥4 contributors, these mixtures will be deemed as inconclusive. The only exception will be if a single major contributor can be clearly discerned and is approved in writing by the DNA TL. Determination of the possibility for interpretation of the major profile will be based upon the evaluation of multiple factors, including the overall contribution of the minor profiles to the mixture, the peak height balance between peaks marked as major, and the mixture ratio between the major and the minor profiles.

**5.6.13.5 Unknown Number of Contributors -** For mixtures where a number of contributors cannot be determined, interpretation can be made for the major contributor.

**5.6.13.5.1** Mixtures with one major contributor (Type 3)

**5.6.13.5.1.1** The major should be clearly discernible from the rest of the profile.

**5.6.13.5.1.2** If proportions cannot be reliably assessed (e.g. due to peak heights) then the major contributor should be 4X greater than the largest peak of the minor group.



***All copies of this document are uncontrolled when printed.***

**5.6.13.5.2** Mixtures with a major group (Type 4)

**5.6.13.5.2.1** The major group can be deduced from the mixture, generally with 2 main contributors and low-level contributor(s).

**5.6.13.5.2.2** The major group should be supported by peak heights and proportions throughout the profile.

**5.6.13.5.2.3** As peak heights approach the ST or if alleles are below the ST, caution must be taken in calling a major group to ensure that the additional contributors are not influencing the major group.

**5.6.13.5.3** Unresolvable mixtures (Type 5) – These mixtures are inconclusive for comparison purposes, as they have no resolvable major contributor and/or do not have sufficient quality to reliably include or exclude known individuals.

## 5.7 Comparison of Profiles

**5.7.1** When interpreting DNA typing results, the analyst must interpret the results from evidentiary items prior to comparison of any known samples, other than those of assumed contributors (e.g., victim standard on vaginal swabs). This includes whether each locus meets the analytical threshold or stochastic threshold, is single-source, mixed (distinguishable or indistinguishable), meets PHR expectations, and/or has potential allelic dropout.

**5.7.2** Those loci which will be used in statistical calculations shall be determined prior to comparison to known reference samples.

**5.7.3** The donor's known profile may be used during the interpretation of intimate samples in order to deduce an additional contributor of the mixed profile.

## 5.8 Results

**5.8.1** **Inclusion –** A known individual's profile is included in a single source or mixed sample if the genotype is present at all loci at which DNA typing results are deemed interpretable with no unexplained differences. The loss of an allele due to preferential amplification, stochastic effects, mutation, or other factors must be considered and does not necessarily indicate an exclusion.

**5.8.2** **Exclusion** – A known individual's profile could not have contributed to or is not a part of the questioned profile from a single source or mixed sample if the genotype is missing at any loci at which the DNA typing results are deemed complete.



***All copies of this document are uncontrolled when printed.***

**5.8.3 Inconclusive –** Inconclusive results which are not suitable for comparison may be narrowed to the following:

**5.8.3.1** The profile has limited data available due to the possibility of allelic dropout, degradation, preferential amplification, and/or the potential for the masking of minor alleles by the major profile or minor alleles located in stutter positions in the mixture.

**5.8.3.2** The profile is too complex due to the total number of possible contributors present, the possibility of allele sharing between multiple contributors, and/or the possibility of allelic dropout of lower level contributors. For example, a mixture of at least four contributors with no single unambiguous major contributor.

**Note:** The inconclusive conclusion will be applied to the profile as a whole or minor contributor(s) and no comparison shall be performed between an inconclusive profile/portion of profile and any reference standard. Such data is not suitable for inclusion or exclusion due to insufficient information.

**5.8.4** Incidences of employee, vendor or batch case matches/associations shall be immediately conveyed to the DNA TL. Any incidences of the unintentional introduction of exongenous DNA into a control shall also be immediately conveyed to the DNA TL.

**5.8.5** With unknown profiles in a case, comparisons between unidentified single source or predominant profiles to mixtures is not permitted (except for numbering of unknown profiles seen within a case).

**5.8.6** DNA profiles for questioned items generated under prior technology (e.g., Identifiler, Quantifiler, etc.) may be compared to standards generated using new technology only after consultation with the DNA Technical Leader.

## 5.9 Exporting and Creating Tables

### 5.9.1 Casework Table

**5.9.1.1** In the main GMID-X window, select the "Traditional Allele Table" from the Table Setting menu.

**5.9.1.2** Select all samples and under "File" in the main GMID-X menu, select "Export Table." Save the table to a location easily located by the Forensic Scientist (e.g., desktop or thumb drive). Ensure the file is exported/saved in the Tab-delimited Text (.txt) format.

**5.9.1.3** Open Excel; open the .txt file saved in the previous step (note: the Forensic Scientist may have to change the file of type to All Files in order for the software to access the .txt file).



***All copies of this document are uncontrolled when printed.***

**5.9.1.4** When the Text Import Wizard opens, select "Finish."

**5.9.1.5** Adjust the columns to accommodate the full text for each line.

**5.9.1.6** Select "Page Setup." Under the Page tab, select landscape and fit to 1 page wide by 1 page tall; under the Header/Footer tab, select Custom Header – at a minimum, the Forensic Scientist shall enter the full case number, initials and the date; under the Sheet tab, select only the Gridlines box. Finish by selecting "OK."

**5.9.1.7** Forensic Scientists shall note on this page any samples that are not to be used for comparison and why, or those that are to have additional analysis performed on them (see **5.2.3.2**).

**5.9.1.8** The Casework Table Worksheet shall be saved/printed as the first page of the egram file.

**5.9.1.9** Only samples and controls specific to the case shall be present in the Casework Table.

### 5.9.2 Allele Call Table

**5.9.2.1** In the main GMID-X window, select the "Traditional Allele Table" from the Table Setting menu. With all samples selected, select the "Genotypes" tab in the main GMID-X menu.

**5.9.2.2** Under the "File" menu in the main GMID-X window, select "Export Table." Save the table to a location easily located by the Forensic Scientist on his/her computer (e.g., desktop or thumb drive). Ensure the file is exported/saved in the Tab-delimited Text (.txt) format.

**5.9.2.3** Open Excel; open the .txt file saved in the previous step (note: the Forensic Scientist may have to change the files of type to All Files in order for the software to access the .txt file).

**5.9.2.4** When the Text Import Wizard opens, select "Finish."

**5.9.2.5** Open the Allele Call Table Excel file in the Forensic Biology Shared Folder. Copy and paste the allele calls generated from the .txt file (Select All) into the Results tab in the appropriate Allele Call Table Worksheet.

**5.9.2.6** Return to the Allele Call Table tab (or the Mixture tabs if any samples have more than 2 alleles at any given locus) and verify that the copy and paste function worked properly.

**5.9.2.7** If minor alleles or profiles are to be marked, the Forensic Scientist shall indicate these with parentheses. Alleles that fall within the stochastic range (i.e., between the AT and the ST)



***All copies of this document are uncontrolled when printed.***

shall have their cells highlighted. Minor alleles shall be marked if an analyst is calling that/those locus/loci major in a mixture. Analysts shall not mark minor alleles if a major contributor is not being reported.

**5.9.2.8** If multiple injections are present in a case file due to re-extraction, re-amplification, or re-injection (non-serial in nature), the Forensic Scientist shall indicate from which run the sample came. Item descriptions shall also be typed into the designated areas in the worksheet. The Forensic Scientist shall ensure the full case number and the date are present.

**5.9.2.9** It is permissible to combine results from different injections, dilutions and amplifications of the same sample when determining a final DNA profile. In order to call predominance at a locus, all results shall show the same predominance. When separate extracts from different locations on a given evidentiary item are combined prior to amplification, the resultant DNA profile is NOT considered a composite profile. Unless there is a reasonable expectation of sample(s) originating from a common source (e.g., duplicate vaginal swabs or a bone), allelic data from separate extractions from different locations on a given evidentiary item should not be combined into a composite profile.

**5.9.2.10** It is permissible for the Forensic Scientist to combine all Allele Call Table Worksheets into one Excel spreadsheet.

**5.9.2.11** Any and all notes, remarks, and observations used by the Forensic Scientist to make an interpretation and/or conclusion regarding a question sample shall be noted on the Allele Call Table Worksheet(s). Full, single source samples above stochastic threshold and samples that produce no DNA profiles do not have to be noted.

**5.9.2.12** Allele call tables generated within the Armed Xpert™ software program may be used instead of or in conjunction with allele call tables generated through export into an Excel spreadsheet.

**5.9.2.13** Allele call tables for the question and known samples shall be generated and included as separate .pdf files in the appropriate Case Record Object Repository.

**5.9.2.14** Only samples specific to the case shall be present in the Allele Call Table (i.e., controls, unlabeled/unassigned allele call tables, and ladders shall not be printed).

**5.9.3 CODIS Table -** Refer to the Procedure for CODIS for CODIS Table Export and Specimen Upload.



***All copies of this document are uncontrolled when printed.***

## 5.10 Statistical Calculations

**5.10.1** Statistics shall be calculated on questioned items if comparison to a known item results in an inclusion. The exception to this requirement is for intimate samples where this association is made to the owner or a consensual partner.

**5.10.2** In order to perform a statistical calculation, a minimum of 6 loci where frequency data has been determined must be present and eligible for use in the question sample(s). Refer to Section Procedure for Report Writing for reporting statements.

**5.10.3** Amelogenin, DYS391, DYS570, and DYS576 frequencies are not used for calculations.

**5.10.4** Failure of a locus to amplify in either the questioned or known sample does not preclude the generation of statistical results at loci where complete amplification occurred.

### 5.10.5 Random Match Probability

**5.10.5.1** RMP shall be used for single source or single predominant profiles. In this case, RMP may be calculated by use of the PopStats program. For mixture profiles, Armed Xpert™ will be used to generate frequency calculations (See Procedure for Use of Armed Xpert™). If a mixture contains a major contributor with a minor that is uninterpretable, then either program may be used to calculate frequency statistics. Frequency statistics are generated using the NIST population database.

**5.10.5.2** The formulas used in the calculation of the frequency of a DNA profile shall be in accordance with those published in the NRC II guidelines and in PopStats.

**5.10.5.2.1** Heterozygote frequencies: 2pq (p and q are the frequencies of the alleles observed.)

**5.10.5.2.2** Homozygote frequencies: $p^2 + p(1-p)\theta$, where $\theta = 0.01$ (p is the frequency of the allele observed and $\theta$ is a correction factor for expected levels of population substructure in a given population).

**5.10.5.2.2.1** If a locus contains a homozygote allele which falls below the stochastic threshold, that locus shall not be used.

**5.10.5.2.3** Multi-locus frequencies: the product rule shall be used.



***All copies of this document are uncontrolled when printed.***

#### 5.10.5.3Performing RMP using PopStats

**5.10.5.3.1** Log onto a CODIS computer, open the Analyst Workbench program, and click PopStats located on the bottom left.

**5.10.5.3.2** In the Specimen ID box type in the full case number (note: the item number may be entered along with the case number or separately within the comments box).

**5.10.5.3.3** In the Specimen Details block, type the alleles and click Calculate. Note: Analysts shall check PentaE for the correct import of the "5" allele to confirm it was not converted upon import to a <6 designation.

**5.10.5.3.4** Print the Forensic/Broward report using the Print to PDF and add it to the FA case record object repository.

**6.0 Limitations** - These guidelines are not meant to cover all situations and shall not be applied retroactively to analysis performed under previous versions of this procedure without the documented authorization of the DNA Technical Leader. Interpretation of low level samples should be approached with caution due to the limits in sensitivity of the STR typing testing procedure. Interpretation of low level results must also factor in the potential loss of data due to the inability to detect all genotypes present in a sample in an effort to ensure a reliable result is obtained.

**7.0 Safety** - N/A

**8.0 References**

Applied Biosystems™. GeneMapper® ID Software Version 3.1 User's Guide. P/N 4338775 Rev. C.

Promega PowerPlex® Fusion 6C Amplification Kit User's Guide.

Forensic Biology Section Procedure for Use of the 3500XL Genetic Analyzer for Casework

Forensic Biology Section Procedure for CODIS

Forensic Biology Section Procedure for Report Writing

NDIS STR Data Entry Software (accessible by registered CODIS users only)

National Research Council Committee on DNA Forensic Science (1996) An Update: The Evaluation of Forensic DNA Evidence. Washington, D.C., National Academy Press.

Butler, J.M. *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers*. 2nd ed. Burlington, MA: Elsevier Academic Press, 2005.

Federal Bureau of Investigation. "QUALITY ASSURANCE KNOWN SAMPLES FOR FORENSIC DNA TESTING LABORATORIES." *Forensic Science Communications, October 2008, Volume 10, Number 4.*

**9.0 Records**

- PowerPlex® Fusion 6C Allelic Ladder Expected Results
- PowerPlex® Fusion 6C 2800M Expected Results

**10.0 Attachments** - N/A

| **Revision History** | | |
|---|---|---|
| Effective Date | Version Number | Reason |
| 12/20/2016 | 1 | Original Document |
| 03/23/2018 | 2 | 5.2 – change tables to data; 5.4.6.3 – added comment about increased stutter; 5.5.2.2 – added wording for artifact evaluation, removed examples (in definition list); 5.2.2.1.1.1 – clarified for single source samples; 5.5.2.2.1.2 – added wording for verifying only if using to interpret; 5.5.3.1 – added scale printing for negatives; 5.6.5 – added wording about marking of minor/major for Y loci; 5.6.7 – added wording to clarify use of ArmedXpert; 5.6.9.1 – clarified allele count not only measure of # contributors; 5.6.9.1.7 – clarified minors may not be resolvable; 5.6.9.4 – added wording for determination of use of major |
| 01/25/2019 | 3 | 5.4.4 – updated category name; 5.4.6.2- update thresholds; 5.4.6.3 – added comment about stutter filters; 5.4.6.4, 5.5.1.1 – removed screenshots; 5.5.2.2.2 – added wording for non-specific amplification peaks; 5.5.2.2.3.5 – remove requirement for reinject microvariants; 5.5.3.4 – clarified WEN peak printing; 5.6.5 – added use of quant and AMEL data for determination of male contributor; 5.6.8, 5.6.9, 5.6.10 – add wording for previously compared profiles; 5.10.5.3.3 – add note for popstats PentaE; |



***All copies of this document are uncontrolled when printed.***