

## Deviation Request Form (DRF)

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

<b>Initiator</b>	Cori Martin	<b>Date</b>	4/26/2018
<b>A. Requested deviation applies to (Technical Procedure – include specific section):</b>			
Procedure for GeneMapper ID-X and STR Interpretation with PowerPlex Fusion			
<b>B. Requested deviation:</b>			
See additional documentation.			
<b>C. Necessity for the deviation:</b>			
Updated to adjust the way analysts interpret samples which exhibit single allele sizing shifts at D12S391.			
<b>D. Technical review and Authorization (to be completed by the Quality Manager and/or Technical Leader)</b>			
<b>Comments(to include merits and impacts):</b>			
Approved	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No	Duration
			Until next procedure revision.
Signature	Cori Martin	Digitally signed by Cori Martin Date: 2018.04.26 09:13:39 -04'00'	Date
4/26/2018			
<b>E. Quality Assurance Authorization (to be completed by the Quality Manager, Forensic Scientist Manager or designee)</b>			
Acceptable within general QA guidelines and good laboratory practice?		<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Significant negative impact to Crime Laboratory Quality System?		<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No
<b>Restrictions/limitations:</b>			
<input checked="" type="checkbox"/>	Authorized	<input type="checkbox"/> Rejected	Signature
		Zach Kallenbach	Digitally signed by Zach Kallenbach DN: cn=Zach Kallenbach, o=North Carolina State Crime Laboratory, email=zkallenbach@ncdoj.gov, c=US Date: 2018.05.01 08:27:21 -04'00'
		Date	5/1/2018

5.12. **Single Allele Sizing Shifts:** Samples which have one allele that does not fall in bin and sizes approximately 0.5 base pairs away from the ladder allele are considered to have a single allele sizing shift. These shifts may be due to a sequence variation which causes the allele to migrate differently than the ladder allele. These samples shall be reamplified in order to size the allele.

5.12.1. For samples which exhibit a single allele sizing shift at D12S391, the allele shall be called in bin after the first instance of the allele sizing in bin. The sample may be amplified up to three times due to the presence of a single allele sizing shift. If the allele fails to fall in bin after three amplifications, it shall be called out of bin and shall be sized as described in the Off-Ladder Alleles and Microvariants section of this procedure.

5.12.2. For samples which exhibit a single allele sizing shift at loci other than D12S391, the allele shall be named with the result that occurs twice in no more than three amplifications. If the allele falls so that it shall be called out of bin, it shall be sized as described in the Off-Ladder Alleles and Microvariants section of this procedure.

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## Procedure for GeneMapper® ID-X and STR Interpretation with PowerPlex® Fusion

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

**2.0 Scope** – This procedure applies to DNA Database Section analysts and trainees who perform DNA analysis using GMID-X. The guidelines outlined herein are based upon this Laboratory's validation studies, review of literature, and over 20 years of forensic DNA Database experience. These guidelines are to be used in conjunction with the DNA Database Forensic Scientist's training and experience to provide scientific interpretation of the STR results.

### 3.0 Definitions

- **Allele:** An alternative form of a gene; allele designation is used to designate a specific size fragment of DNA for a specific locus in STR analysis.
- **Allelic Dropout:** An occurrence where one or more alleles from an individual's DNA profile fail(s) to amplify during PCR and as a result(s) is (are) not detected in the profile.
- **Amelogenin** – Gender-determining locus.
- **Analytical Threshold (AT):** The minimum height (RFU) requirement at and above which detected peaks may be reliably distinguished from background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles. The threshold for this Laboratory is internally derived by empirical data
- **Artifact** – Non-allelic byproducts of PCR technology (e.g., stutter, etc.), anomalies which occur during capillary electrophoresis (e.g., pull-up, spike, etc.), or byproducts of primer synthesis (e.g., dye blob, etc.).
- **Bin** – An expected location for a particular allele within a locus; a binset is a collection of expected locations for alleles at loci amplified as a set.
- **Consensus Allele** – An allele that conforms to an incremental repeat pattern at an STR locus.
- **Core Loci:** The loci defined by the FBI and required for inclusion within NDIS.
- **DNA Profile:** The combination of genotypes obtained from DNA analysis testing of multiple loci.
- **Electropherogram (egram)** - The computer generated electronic/visual result from an analysis performed by electrophoresis.
- **Full Profile:** A DNA profile that exhibits genotypic information at each locus tested and there is no evidence of allelic dropout, degradation, or preferential amplification.
- **Heterozygote Peak Balance** – A difference of greater than approximately 70 % between two peaks of a single source sample at a given locus.
- **.hid File** - A raw data file generated during sample electrophoresis as part of a run; only viewable through GMID-X software (or equivalent).
- **Incomplete Spectral Separation:** A signal from an allele labeled with one dye set affects the same x-axis location of the electropherogram in another dye set.
- **Inhibition:** The total or partial suppression of the PCR process that would result in partial or no DNA profile being obtained.
- **Injection:** When a DNA sample is electrokinetically introduced into a capillary for electrophoretic separation.

- **Locus (plural, loci)** - The chromosomal location or location of a gene or DNA marker.
- **Match:** DNA profiles are considered to match if their patterns are the same after taking into consideration the properties of the substrate tested and limitations of the specific techniques used.
- **Microvariant** - An allele that varies by less than the consensus repeat unit.
- **Mixture:** A DNA typing result originating from more than one individual. Samples containing DNA from more than one individual typically have more than two alleles at multiple loci and extreme peak height imbalance. NOTE: If a DNA profile is observed to have more than two peaks at more than one locus, then there is a high possibility that there is a mixture of two or more individuals' DNA profiles. If three peaks are observed at only one locus, then there may not be a mixture; the individual contributor may have a triallelic pattern at that locus.
- **Noise** – Background signal detected by a data collection instrument.
- **Non-Match:** Assuming a single source from a sample, two DNA profiles are considered to be a non-match if there is a difference of one allele after taking into consideration the circumstances of collection and preparation of samples and knowledge of the properties of the substrate tested and limitations of the specific techniques used.
- **Off-Ladder Allele** - Alleles that size within a locus marker size range but outside the allele categories (bins) represented in the ladder.
- **Off-Scale Data** - The result of excess DNA present in an electrophoresed sample, typically visualized by excessive artifacts as a result of peak heights consistently greater than 12,000 RFUs.
- **Outside Marker Range (OMR)** – Indicates if labeled peaks are detected between two marker size ranges defined in the panel.
- **Panel** – A collection of markers specific to an amplification kit, such as PowerPlex® Fusion.
- **Partial DNA Profile:** A DNA profile that does not produce DNA typing results for all loci tested.
- **Peak:** A well-defined point on an electropherogram that is within bin (on-ladder). See “Microvariant” and “Off-Ladder Alleles” for exceptions to the “on-ladder” requirement.
- **PowerPlex® Fusion** - A commercially produced amplification kit which contains the following loci: Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, DYS391, D8S1179, D12S391, D19S433, FGA, and D22S1045.
- **Project** – A set of data saved as a single entity in GMID-X.
- **Relative Fluorescence Units (RFUs)** – A unit of measurement in electrophoresis when fluorescence detection is used; determines peak height.
- **Run** - Each set of 24 samples that are injected and separated electrophoretically on the Capillary Electrophoresis Unit (ABI 3500xL or equivalent).
- **Shoulder and Tail:** Elongated or raised areas to the immediate left and right, respectively, of a main peak, but not separated from the main peak.
- **Single Source Profile:** A combination of genotypes obtained from STR DNA testing that could only originate from a single individual. A sample may be considered to consist of a single contributor when no more than two alleles are observed at each locus. All loci are to be evaluated in making this decision. If three alleles are observed at one locus, then there may not be a mixture; the individual contributor may have a triallelic pattern at that locus.
- **Spike/Electrical Spike:** An artifact believed to be caused by a spike in the current within a capillary that causes a sharp increase in signal. This artifact lacks the defined morphology of a peak.

- **Split Peaks** - A split peak is where one allele is represented by two peaks. Lack of full nucleotide A addition may be observed when the amount of input DNA is greater than the recommended protocol. In this case, more time is needed for Taq Polymerase to add the A nucleotide to all molecules. Amplification of too much input DNA also results in off-scale data (saturation of signal) and may be manifested as split peaks.
- **Stochastic Effects:** The observation of intra-locus peak imbalance and/or allele drop-out resulting from random, disproportionate amplification of alleles in low-quantity template samples.
- **Stochastic Threshold (ST):** The peak height value above which it is reasonable to assume that, at a given locus, allelic dropout of a sister allele of a heterozygotic pair has not occurred. The threshold for this Laboratory is internally derived through the use of empirical data.
- **Stutter:** An artifact of PCR amplification that is typically one repeat unit less (may also be one repeat unit more) than the corresponding main allele peak resulting from strand slippage during amplification.
- **Triallelic Pattern:** Three peaks observed at a single locus and not the result of a mixture. These peaks may or may not be of equal intensity.
- **Unincorporated Dye:** Unincorporated dye (i.e., dye blobs) may be observed in an electropherogram and are distinct morphologically from a labeled DNA fragment. A dye blob does not exhibit the typical sharp, distinct peak that is produced by actual alleles and is observed as a wider, thicker peak and may be lacking the sharply defined slope to the apex of a peak.
- **WEN Internal Lane Standard 500:** A commercially produced set of DNA fragments of known size, used to determine the size of DNA fragments in an electrophoresed sample.
- **Y-Specific Marker:** A locus on the Y chromosome. Only samples containing male DNA will produce alleles for this type of marker.

#### 4.0 Equipment and Materials

- Computer with GeneMapper® ID-X (version 1.4 or most current) software
- Microsoft Excel, Word
- Print2PDF software

#### 5.0 Procedure

##### 5.1 Sample RFU Thresholds

**5.1.1 Analytical Threshold** - The analytical thresholds were established through validation and performance check studies using the PowerPlex® Fusion PCR Amplification kit.

**5.1.1.1** The analytical threshold for the orange dye channel (WEN ILS 500) shall be set at 100 RFU.

**5.1.1.2** The analytical threshold for 18s injections is set at 125 RFU for blue, green, yellow, and red dye channels. Anything present below 125 RFU is considered to be indistinguishable from background noise and shall not be considered for analysis.

**5.1.2 Stochastic Threshold** - The stochastic thresholds were established through validation and performance check studies using the PowerPlex® Fusion PCR Amplification kit.

- 5.1.2.1** The stochastic threshold for 18s injections is set at 180 RFU for blue, green, yellow, red, and orange dye channels.

NOTE: This threshold does not apply to the locus DYS391.

**5.2 GeneMapper® ID-X Analysis and Plot Settings** - Only the Technical Leader or the GMID-X Administrators may change settings within the Analysis Method Editor or the Table Setting Editor.

- 5.2.1** The Peak Detector tab of the Analysis Method Editor settings shall appear as below.

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Peak Detector' tab selected. The 'Peak Detection Algorithm' is set to 'Advanced'. The 'Ranges' section has 'Analysis' set to 'Full Range' and 'Sizing' set to 'All Sizes'. The 'Start Pt' is 0 and 'Stop Pt' is 10000. The 'Start Size' is 0 and 'Stop Size' is 1000. The 'Smoothing and Baseline' section has 'Smoothing' set to 'Light' and 'Baseline Window' set to 51 pts. The 'Size Calling Method' section has 'Local Southern Method' selected. The 'Peak Detection' section has 'Peak Amplitude Thresholds' set to B: 125, R: 125, G: 125, P: 125, Y: 125, O: 100. The 'Min. Peak Half Width' is 2 pts, 'Polynomial Degree' is 3, and 'Peak Window Size' is 15 pts. The 'Slope Threshold' section has 'Peak Start' and 'Peak End' both set to 0.0. The 'Normalization' section has 'Use Normalization, if applicable' unchecked. A 'Factory Defaults' button is located at the bottom right of the dialog box. At the very bottom of the dialog box are buttons for 'Save As', 'Save', 'Cancel', and 'Help'.

5.2.2 The Allele tab of the Analysis Method Editor settings shall appear as below.

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Allele' tab selected. The 'Bin Set' is set to 'PowerPlex\_Fusion\_Bins\_IDX\_v2.0'. A checkbox labeled 'Use marker-specific stutter ratio and distance if available' is checked. Below this is a table for 'Marker Repeat Type' with columns for Tri, Tetra, Penta, and Hexa. The table contains values for Global Cut-off Value, MinusA Ratio, MinusA Distance (From/To), Global Minus Stutter Ratio, Global Minus Stutter Distance (From/To), Global Plus Stutter Ratio, and Global Plus Stutter Distance (From/To). The 'Amelogenin Cutoff' is set to 0.05. At the bottom are buttons for 'Range Filter...', 'Factory Defaults', 'Save As', 'Save', 'Cancel', and 'Help'.

Marker Repeat Type:		Tri	Tetra	Penta	Hexa
Global Cut-off Value		0.05	0.05	0.05	0.0
MinusA Ratio		0.0	0.0	0.0	0.0
MinusA Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0
Global Minus Stutter Ratio		0.0	0.0	0.0	0.0
Global Minus Stutter Distance	From	0.0	3.25	0.0	0.0
	To	0.0	4.75	0.0	0.0
Global Plus Stutter Ratio		0.0	0.0	0.0	0.0
Global Plus Stutter Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0

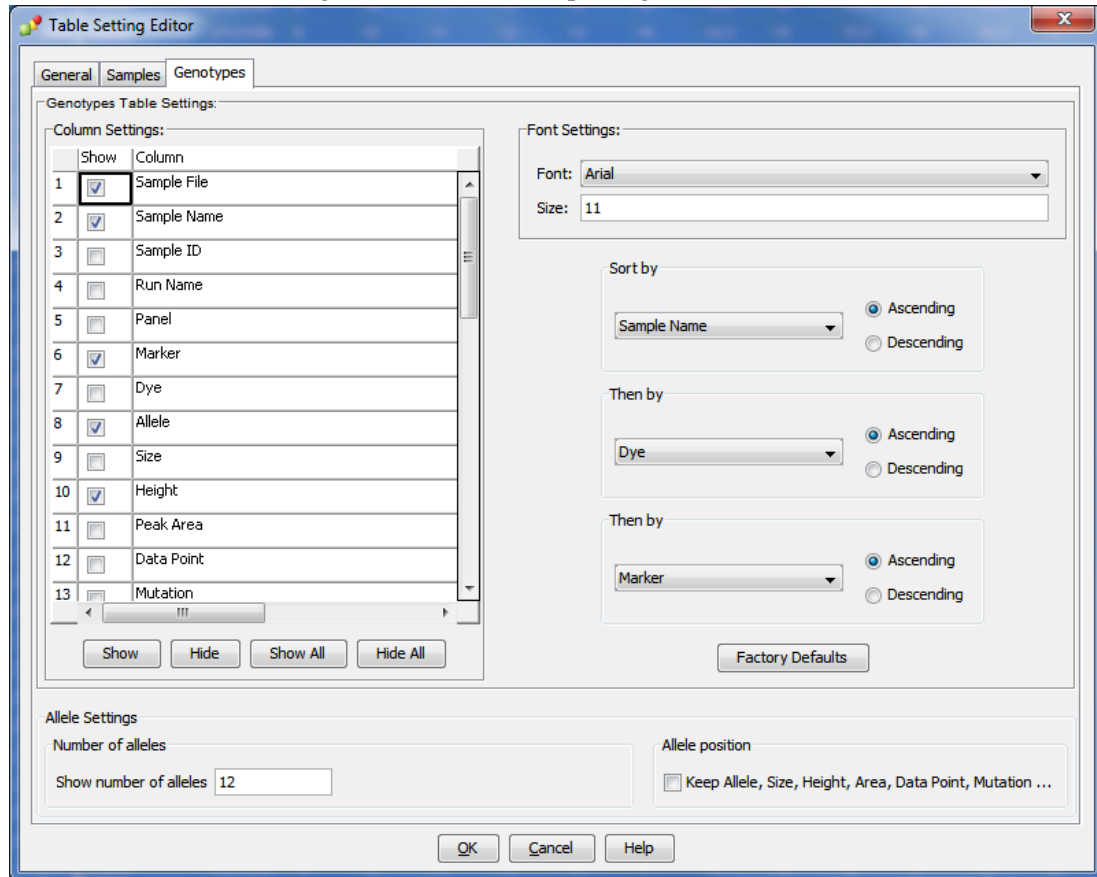
Amelogenin Cutoff: 0.05

**5.2.3** Marker-specific stutter filter percentages for PowerPlex® Fusion are listed below.

Locus	% Stutter	Minus Stutter (base pairs)	Plus Stutter (base pairs)
Amel	0.0	3.25 to 4.75	-
D3S1358	11.9	3.25 to 4.75	-
D1S1656	14.2	3.25 to 4.75	-
D1S1656	3.6	1.25 to 2.75	-
D2S441	9.2	3.25 to 4.75	-
D10S1248	12.4	3.25 to 4.75	-
D13S317	9.8	3.25 to 4.75	-
Penta E	7.6	3.25 to 5.75	-
D16S539	10.2	3.25 to 4.75	-
D18S51	14.6	3.25 to 4.75	-
D2S1338	13.9	3.25 to 4.75	-
CSF1PO	9.5	3.25 to 4.75	-
Penta D	6.8	3.75 to 5.75	-
TH01	4.6	3.25 to 4.75	-
vWA	11.2	3.25 to 4.75	-
D21S11	11.6	3.25 to 4.75	-
D7S820	11.0	3.25 to 4.75	-
D5S818	9.5	3.25 to 4.75	-
TPOX	5.5	3.25 to 4.75	-
DYS391	8.7	3.25 to 4.75	-
D8S1179	10.9	3.25 to 4.75	-
D12S391	15.8	3.25 to 4.75	-
D19S433	11.0	3.25 to 4.75	-
FGA	12.1	3.25 to 4.75	-
D22S1045	16.4	2.25 to 3.75	-
D22S1045	8.6	-	2.25 to 3.75




- 5.2.1** The Table Setting Editor for the “3500 Data Analysis” Table Setting and the “Traditional Allele Table” Table Setting shall both appear as below. The “3500 Data Analysis” Table Setting or the “Traditional Allele Table” Table Setting shall be used for analyzing data. The “Traditional Allele Table” Table Setting shall be used for exporting casework tables.



### 5.3 Creating a Project

- 5.3.1** Open GMID-X and log in under the Forensic Scientist’s name/log-in. Analysis under the generic “gmidx” log-in name is not permitted. Forensic Scientists shall log into and use the DNA Database Host.
- 5.3.2** Use the “Add Samples To Project” function to add samples to be analyzed to the project.
- 5.3.3** 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.”
- 5.3.4** Verify that the table setting (in top center of the main GMID-X window) is set to “3500 Data Analysis” or “Traditional Allele Table.” In the Samples pane, the category Sample Type shall have the samples labeled as follows when amplified with PowerPlex® Fusion:

- Ladder: Allelic Ladder
- Reagent Blank: Negative Control
- Negative Amplification Control: Negative Control
- 2800M (Positive Amplification Control): Positive Control
- All other samples: Sample

NOTE: Sample File names appear by the sample name as entered by the Forensic Scientist prior to electrophoresis followed by capillary number, well position (e.g., A12), and injection number. 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.

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

- Specimen Category: Arrestee (Arrestee samples for NDIS upload)  
Arrestee-State (Arrestee samples for SDIS level upload)  
Convicted Offender (Convicted Offender samples for NDIS upload)  
Offender-State (Convicted Offender samples for SDIS level upload)  
No Export (Samples that will not be uploaded into CODIS)  
Staff (Staff samples for SDIS upload)
- Analysis Method: PowerPlex Fusion 18s WEN
- Panel: PowerPlex Fusion
- Size Standard: WEN\_ILS\_500\_IDX
- Instrument Type: Automatically filled in by the software

**5.3.6** Once all samples and categories are labeled and identified, analyze the samples using the Analyze feature of the software. When prompted, create a project name for the set of samples and select the “Databasing Security Group” and select “OK.”

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

## **5.4 Adding/Deleting Samples**


**5.4.1** If one or more additional samples must be added to a current project, follow the steps listed under the Creating a Project section of this document. The newly added sample(s) shall be analyzed before the data can be viewed.

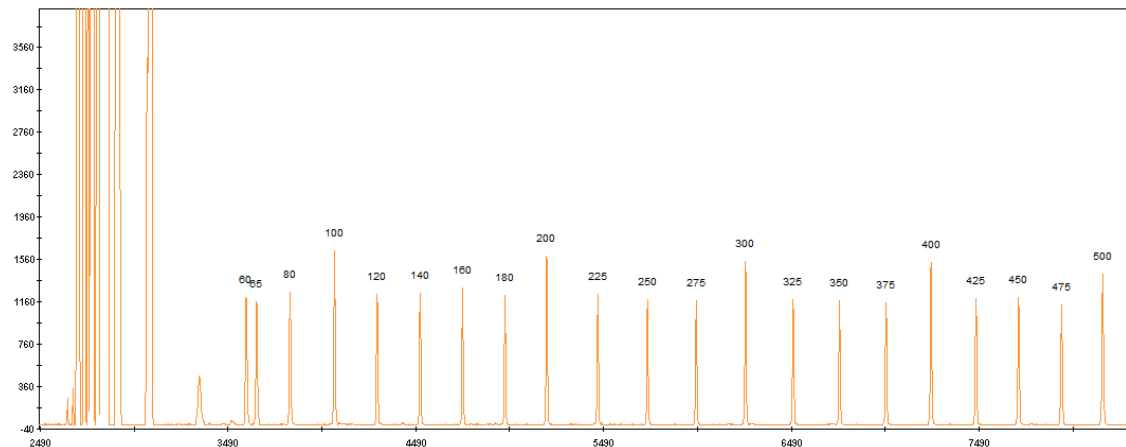
**5.4.1.1** If the sample is from a different injection, the associated Allelic Ladder shall also be imported in order for the sample data to be sized properly.

**5.4.2** If a Forensic Scientist must remove a sample that has been added to the project (e.g., a sample from an unrelated CODIS hit confirmation), highlight the sample to be removed and, in the menu bar, select “Edit” and “Delete from Project.” Select “OK” when prompted.

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

## 5.5 Evaluate Sizing Data (WEN ILS 500)

- 5.5.1** Select all samples and controls within the project. Click on the Size Match Editor Icon (  ). The new window that opens displays the WEN sizing data for all selected samples; scroll through each one and confirm that all sizing peaks are correct (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, and 500-base pairs). Close this window.



- 5.5.2** If any sample or control in the project indicates no sizing data (i.e., WEN ILS 500 failure), then that sample or control shall not be used for comparison. This information shall be noted on the Casework Table or the Exceptions/Notes Report.
- 5.5.3** If there is no sizing data for an allelic ladder, then no data associated with that ladder can be sized.
- 5.5.4** If the allelic ladder has no sizing data, a ladder which was injected on the same plate on the same day by the same Forensic Scientist may be used to size the run. The .hid file for the ladder intending to be used must be added to the run folder prior to entering the run folder into GeneMapper® ID-X. This information shall be noted on the Casework Table or the Exceptions/Notes Report.
- 5.5.5** If a control has no sizing data, a duplicate control from the same plate/day/Forensic Scientist which originated from the same amplification product may be used.
- 5.5.6** Samples and controls that show no sizing data but contain all correctly labeled sizing peaks may be used for comparison after the Override SQ function has been applied.

- 5.5.6.1** From the samples tab select the sample and click on the Size Match Editor.


**5.5.6.2** Click Override SQ. Then click OK.

**5.5.6.3** Reanalyze selected samples. Examine all samples in the manner as described in the Assess Overall Quality of Data section of this procedure.

## **5.6 Assess Overall Quality of Data**

### **5.6.1 Allelic Ladders**

**5.6.1.1** 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.

**5.6.1.2** Select all ladders within the project and open the Samples Plot window by selecting the Display Plots icon (). In the Samples Plot window, ensure that the Plot Setting is set to “Data Interpretation” or “Traditional Genotype Plot.”

**5.6.1.3** Scroll through each of the color channels (blue, green, yellow, red) and verify the following for all loci tested.

**5.6.1.3.1** All expected alleles shall be equal to or greater than the analytical threshold.

**5.6.1.3.2** Each allele shall be in the expected position in order to use the associated samples and controls.

**5.6.1.4** If the allelic ladder fails, a ladder which was injected on the same plate on the same day by the same Forensic Scientist may be used to size the run. The .hid file for the ladder intending to be used must be added to the run folder prior to entering the run folder into GeneMapper® ID-X. This information shall be noted on the Casework Table or the Exceptions/Notes Report.

### **5.6.2 Negative Controls**

**5.6.2.1** Select all negative controls and open the Samples Plot window.

**5.6.2.2** Examine the negative controls and verify the following for all loci tested.

**5.6.2.2.1** Ensure there are no true alleles above the analytical threshold.

**5.6.2.2.2** Artifact peaks that are above the analytical threshold do not cause the negative control to fail and do not require the sample to be reinjected. Remove any artifacts as described in the Artifacts section of this procedure.

**5.6.2.3** Any instances of true alleles above analytical threshold in a negative control shall be reported to the Technical Leader.

**5.6.2.4** If any negative control has a true allele above the analytical threshold and there is not a duplicate passing negative control from the same amplification, the negative control shall be reanalyzed (i.e., reinjected or replated) or the plate shall be reamplified.

### **5.6.3 Positive Controls**

**5.6.3.1** Select all positive controls and open the Samples Plot window.

**5.6.3.2** Examine the positive controls and verify the following for all loci tested.

**5.6.3.2.1** All positive amplification controls shall be void of extraneous, detectable alleles. Artifacts do not cause a positive control to fail. Remove any artifacts as described in the Artifacts section of this procedure.

**5.6.3.2.2** If multiple positive amplification controls are run, at least one must yield a complete profile with only the expected allele calls.

**5.6.3.3** If no positive amplification controls produce a complete profile, data from the associated runs shall not be used.

### **5.6.4 Samples**

**5.6.4.1** Select all samples and open the Samples Plot window.

**5.6.4.2** Assess the quality of the peaks including RFU values and determine if artifacts are present.

**5.6.4.3** Single alleles which fall below the stochastic threshold shall be considered partial for purposes of interpretation and CODIS entry. In such instances loci shall be noted. Single alleles which are above the stochastic threshold shall be considered true homozygotes and not potential candidates for allelic dropout unless there are other indications of potential allelic dropout.

**5.6.4.4** The profile shall contain the 20 core loci for upload into NDIS. Profiles that do not contain all core loci may be uploaded to SDIS at the discretion of the CODIS Administrator.

**5.6.4.5** For samples with dropout, at a minimum a second amplification of the sample shall be completed in an attempt to obtain a full profile.

- 5.6.4.6** Off-scale samples which exhibit peak heights consistently above 12,000 RFUs and which contain excessive artifacts which affect the ability to successfully interpret the profile shall not be used for analysis.
- 5.6.4.7** Samples shall be reamplified as a second, confirmatory analysis if they contain one of the following and if there is no previous data for the sample/locus or the previous data is not consistent.
  - 5.6.4.7.1** Microvariant not automatically sized by the ladder
  - 5.6.4.7.2** Off-Ladder Allele
  - 5.6.4.7.3** OMR
  - 5.6.4.7.4** Trialallele
- 5.6.4.8 Heterozygote Peak Balance** – Database samples generally exhibit a balance of >70% between heterozygous alleles. A database sample shall be rerun if the imbalance does not allow for distinction between true alleles and artifacts.
- 5.6.4.9** Mixtures – Any mixture profiles shall be reported to the Technical Leader. The Technical Leader shall determine root cause and further action.
- 5.6.4.10** A sample that will not be reported with a particular GMID-X file shall be marked as “no export” in the GeneMapper® ID-X project so that it is not uploaded to CODIS.
- 5.6.4.11** It is permissible to combine results from different injections and amplifications of the same sample when determining a final DNA profile.

## **5.7 Artifacts**

- 5.7.1** 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), highlight the sizing box associated with the peak and right click. Select “Delete Label(s)” (multiple peaks can be deleted simultaneously by selecting them at the same time-when the warning dialog box appears, select “Yes”). When prompted, type in “artifact” as the Reason(s) for Change. The individual artifact classification shall not be entered on the electropherogram.
- 5.7.2 Stutter:** The GeneMapper® ID-X software contains designated cutoffs for peaks in stutter positions and shall be used for designating stutter. Stutter products are most often observed one repeat unit below the true allele peak. Note that D22S1045 is a trinucleotide and that Penta D and Penta E are pentanucleotides.
  - 5.7.2.1  $N \pm 3$  peaks:** For trinucleotides, a stutter peak may appear in the  $n \pm 3$  position.

**5.7.2.2 N±4 peaks:** For tetranucleotides, a stutter peak may appear in the n±4 position.

**5.7.2.3 N±5 peaks:** For pentanucleotides, a stutter peak may appear in the n±5 position.

**5.7.3 Known Artifacts:** Promega has noted reproducible artifacts that may appear in samples amplified with PowerPlex® Fusion. These artifacts may be observed in the locations specified below.

**5.7.3.1 N±2 peaks:** D1S1656, D13S317, D18S51, D21S11, D7S820, D5S818, D12S391 and D19S433

**5.7.3.2 N-1 peaks:** Amelogenin and D2S441

**5.7.3.3 N-3 peaks:** D12S391

**5.7.3.4** For a list of additional artifacts that are typically below common minimum thresholds, see the PowerPlex® Fusion System Technical Manual.

#### **5.7.4 Incomplete Spectral Separation**

**5.7.4.1 Pull Up:** Pull up may be noted when a true allele causes an artifact in the same x-axis location of another dye channel. It is observed as a relatively small peak located directly under the larger peak. Scientists shall be knowledgeable of this phenomenon and use the computer software to aid in discerning true alleles from pull up.

**5.7.4.2 Pull Down:** Pull down may be noted when more than one true allele is at the same x-axis location in multiple dye channels. The larger RFU allele alters the peak morphology of the smaller RFU allele. Scientists shall be knowledgeable of this phenomenon and use the computer software to aid in discerning true alleles from artifacts.

**5.7.5 Spike:** GMID-X software provides automatic labeling of artifacts known as “Spikes” based upon peak morphology and typical presence in multiple dye channels. Peaks automatically labeled by the software as “Spikes” can also include spectral pull up. Forensic Scientists shall assess all instances of “Spike” to ensure that a true allele was not mislabeled by the software. If the “Spike” call is appropriate (to include stemming from instances of pull up), the Forensic Scientist shall leave the peak labeled as “Spike.” The pink box around the “Spike” label indicates the software recognizes that peak as an artifact. GMID-X does not consider this peak to be a true allele, so deleting the “Spike” is not necessary. If the “Spike” call is for an allele that is not an artifact and should be labeled as an allele, left click on the “Spike” label. Right click on the “Spike” label and select “Add Allele Label.” Appropriately name the allele in the Custom Allele Label field and click OK. Type “Allele” as the Reason(s) for Change.


**5.7.6 Unincorporated Dye:** Scientists shall not call dye blobs as an actual allele. Dye blobs shall not be considered for interpretation.

**5.7.7 Shoulder and Tail:** Shoulders and tails do not prevent the scientist from assigning the specific peak an allelic value.

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

**5.8.1** An off-ladder allele or a microvariant shall be named/assigned per NDIS standards. For CODIS eligibility, refer to the Procedure for CODIS.

**5.8.2** In the main GMID-X window, select the sample(s) containing the microvariant/off-ladder allele and the allelic ladder from the same injection. View them in Display Plots. Zoom in to the locus containing the microvariant/off-ladder allele.

**5.8.3** 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 () or select Ctrl + A on the keyboard to bring up the sizing table. View the sizing table with only the selected alleles present (keyboard shortcut: Ctrl + G).

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

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

**5.8.4.2** Alleles 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).

**5.8.5** Rename the allele by selecting the allele with a left-click, 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.”

**5.8.6** CODIS hits require retained documentation of the microvariant/off-ladder allele sizing information.

**5.8.6.1** With the microvariant/off-ladder allele and the closest ladder allele(s) present in the sizing table (remove any extraneous), select “File” and “Print” and select “Print2PDF.”



**5.9 OMR:** GMID-X software flags peaks that fall between loci with the label “OMR.” Forensic Scientists shall assess all instances of “OMR” to ensure that a true allele was not mislabeled by the software. If the “OMR” call is appropriate, the Forensic Scientist shall leave the peak labeled as “OMR.” The pink box around the “OMR” label indicates the software recognizes the peak as an artifact. GMID-X does not consider this peak to be a true allele, so deleting the “OMR” is not necessary. If the “OMR” call is for an allele that should be associated with a surrounding marker, follow the instructions below.

**5.9.1** The Forensic Scientist shall attempt to determine to which locus the allele should be assigned based upon the presence or absence of heterozygosity at the loci in question.

**5.9.2** If both loci exhibit homozygosity or heterozygosity, the Forensic Scientist shall document that the allele was observed. The allele shall not be assigned to a particular locus and shall be marked as inconclusive (INC) by deleting the allele and typing “INC” as the Reason(s) for Change. The called alleles at these loci shall be reported and uploaded into CODIS.

**5.9.3** If one locus exhibits homozygosity and the second heterozygosity, the OMR allele shall be assigned to the homozygous locus.

**5.9.4** When an OMR is a true allele and can be assigned to a locus, it shall be named/assigned per NDIS standards. For CODIS eligibility, refer to the Procedure for CODIS.

**5.9.5** An “OMR” shall be sized as described in the Off-Ladder Alleles and Microvariants section of this document.

**5.9.5.1** The correct allele call for the OMR is dependent upon the base pair size when compared to the allelic ladder.

**5.9.5.2** Left click on the “OMR” label, hold Ctrl on the keyboard, and left click on the locus marker label to which the allele shall be assigned. Right click on the “OMR” label and select “Add Allele Label.” Appropriately name the allele in the Custom Allele Label field and click OK. Type “Allele” as the Reason(s) for Change.

**5.9.6** CODIS hits require retained documentation of the OMR allele sizing information.

**5.9.6.1** With the OMR allele and the closest ladder allele(s) present in the sizing table (remove any extraneous), select “File” and “Print” and select “Print2PDF.”

## **5.10 Trialles**

**5.10.1** When a locus contains three alleles that all fall on the ladder, the Forensic Scientist shall confirm the trialles pattern by reamplification of the sample.

**5.10.2** When a locus contains three alleles (one of which is off-ladder or a microvariant) and the adjacent locus is homozygous, the DNA Database Forensic Scientist shall upload two variations of that profile.

**5.10.2.1** In the raw data folder, make two additional copies of the sample. Add the designation A after one sample name and the designation B after the other sample name (NCXXXXXXXXA\_capillary\_well\_injection).

**5.10.2.2** Add the two additional samples to the GeneMapper® ID-X project. Edit the Sample Name to match the Sample File name by clicking within the sample name box and adding the additional designation.

**5.10.2.3** Analyze both the A and B samples.

**5.10.2.4** One variation of the sample should reflect the off-ladder/microvariant allele labeled as described in the Off-Ladder Alleles and Microvariants section of this document (assigned to the locus in which it is present). In the second variation of the sample, the DNA Database Forensic Scientist shall label the allele as if assigning it to the adjacent locus. The DNA Database Forensic Scientist shall highlight the off-ladder allele/microvariant and select Delete Labels. When prompted by the Reason(s) for Change box, type the adjacent locus name and the allele call based on the ladder for that locus (< or > the closest allele call for that ladder).

**5.10.2.5** The original sample shall be marked as “No Export” in the GMID-X project. The A and B versions of the sample shall be appropriately marked for export in the GMID-X project and shall be uploaded to CODIS via cmf.

**5.10.2.6** The version of the sample where the off-ladder allele/microvariant has been deleted in GMID-X requires additional hand entry into CODIS. The information in the comments box of the deleted allele shall be appropriately updated in CODIS. This hand entry shall be witnessed by a DNA Database Forensic Scientist, Supervisor, or Manager.

**5.10.3** Additional samples in which a DNA Database Forensic Scientist believes an allele has shifted from one locus into another shall be discussed with the CODIS Administrator and the Technical Leader.

**5.11 Poor Injection Sizing Shifts:** Poor injections may cause off-ladder alleles to be present throughout a sample. Ladders, positive controls, and samples that exhibit shifted alleles shall not be used for comparison. Samples that exhibit shifted alleles shall be reinjected or reamplified in order for proper sizing of the allele(s).

**5.12 Single Allele Sizing Shifts:** Samples which have one allele sizing approximately 0.5 base pairs away from the ladder allele are considered to have single allele sizing shifts. These shifts may be due to a sequence variation which causes the allele to migrate differently than the ladder allele. These samples shall be reamplified in order to size the allele. The allele shall be named with the result that occurs twice in no more than three amplifications. If the allele falls so that it shall be called out of bin, it shall be sized as described in Off-Ladder Alleles and Microvariants section of this procedure.

### 5.13 Printing Egrams Electronically

**5.13.1** PDF egrams must be retained for CODIS hits. PDF egrams are not required to be maintained for in-house runs.

**5.13.2** The egrams associated with CODIS hits shall be printed as a .pdf file. Ensure the plot setting is set to “Traditional Genotype Plot” when printing to PDF.

**5.13.3** Any individual sample/control/ladder associated with a CODIS hit that is not used for comparison purposes (e.g., size standard failure, redundancy, further analysis) shall be printed in PDF format. Such samples/controls/ladders shall appear in the Casework Table explaining why it was not used for comparison (e.g., size standard failure, redundancy, re-analysis).

**5.13.3.1** For any sample/control/ladder that is not used for comparison purposes, labeling of artifacts is not required.

**5.13.4** If an entire CODIS hit run or project is not used for comparison purposes, it shall be printed to PDF and retained as described below. Labeling of artifacts is not required in this instance.

**5.13.4.1** Egrams shall be printed (PDF) for all samples/controls/ladders associated with a CODIS hit. Ensure the size range captured contains all loci and alleles.

**5.13.4.2** The WEN sizing standard for all samples/control/ladders associated with a CODIS hit shall be printed (PDF) as a separate set of egrams which may be added to the overall egram .pdf file for each project, or kept separately as an independent document. All available peaks (60 through 500) must be visible.

**5.13.4.3** Raw data for negative controls associated with a CODIS hit shall be printed as described in the View Raw Data section of this procedure.

**5.13.4.4** A Casework Table shall be generated and the reason for not using the entire run/project shall be noted on this page.

**5.13.4.5** An Allele Call Table is not required for such runs/projects.

**5.14 View Raw Data:** Raw data is viewed to ensure the addition of primers to all negative controls and negative samples. Documentation of the raw data primer peaks of negative controls and negative samples is required for CODIS hits.

**5.14.1** The raw data for all samples from each project must be retained. In order to verify the addition of amplification kit primers to all negative controls and negative samples, view the raw data of the negatives and ensure primer peaks are present. For CODIS hit confirmations, include a raw data screenshot for each negative control and each sample which fails to produce alleles.

**5.14.1.1** In the main GMID-X window, highlight the control or sample and select “Raw Data” from the “View” option in the main GMID-X menu (keyboard shortcut: Ctrl + F2).

**5.14.1.2** 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 (including run number) is clearly visible. The right-hand window shall show the maximum vertical and horizontal axis.

**5.14.1.3** Take a screenshot of the raw data. Open up a Word document and paste this screenshot into the document. Repeat this process for all negative samples and negative controls in each project.

**5.14.1.3.1** This raw data Word document shall contain at least the project name, DNA Database Forensic Scientist’s initials, and the date.

**5.14.1.4** The Word document(s) containing the screenshots of the raw data shall be saved/printed as a .pdf file.

## **5.15 Exporting and Creating Tables**

**5.15.1 Casework Table:** The casework table shows all samples and controls run in a project and the GMID-X analysis settings used in the project. The casework table is required for CODIS hits.

**5.15.1.1** In the main GMID-X window, select the “Traditional Allele Table” from the Table Setting menu.

**5.15.1.2** Under “File” in the main GMID-X menu, select “Export Table” (keyboard shortcut: Ctrl + E). Save the table in the Tab-delimited Text (.txt) format.

**5.15.1.3** Import the .txt file into Excel.

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

**5.15.1.5** The header shall include section name, 3500xL instrument name, project name, date, and initials.

**5.15.1.6** For CODIS confirmations, 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.

**5.15.1.7** Print the Casework Table using print to PDF. The Casework Table shall exist independently of the egrams and the Allele Call Table Worksheet(s). It may be merged with the 3500xL setup sheet.

**5.15.2 Allele Call Table:** The allele call table shows reported allele calls for samples. It may also show the expected results for the positive control. The allele call table is required for CODIS hits.

**5.15.2.1** In the main GMID-X window, select the “Traditional Allele Table” from the Table Setting menu. Select the “Genotypes” tab in the main GMID-X menu.

**5.15.2.2** Under the “File” menu in the main GMID-X window, select “Export Table” (keyboard shortcut: Ctrl + E). Save the table in the Tab-delimited Text (.txt) format.

**5.15.2.3** Open the appropriate DNA Database Allele Call Table Worksheet. Populate this worksheet using the .txt file that was generated from GMID-X.

**5.15.2.4** Save the Allele Call Table Worksheet as a PDF document. This file must be separate from the file containing the egrams or the Casework Table.

**5.15.2.5** For CODIS confirmations, only samples specific to the CODIS confirmation shall be present in the Allele Call Table.

### **5.15.3 CODIS Table**

**5.15.3.1** For CODIS sample export and specimen upload, refer to the Procedure for CODIS.

## **5.16 Exporting Projects**

**5.16.1.1** GMID-X projects shall be exported from the software and retained. The GMID-X project shall be removed from the GeneMapper® Manager database prior to technical review.

**6.0 Limitations** - GMID-X cannot be used on computers operating on Windows XP or older.

**7.0 Safety** - N/A

## **8.0 References**

*Applied Biosystems™. GeneMapper® ID-X Software Version 1.0 User's Guide. P/N 4375574 Rev. A.*

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

DNA Database Administrative Policy and Procedure

DNA Database Section Procedure for CODIS Hits

DNA Database Section Procedure for Sample Accessioning and Processing

DNA Database Section Procedure for Samples Analyzed In-House

“QUALITY ASSURANCE KNOWN SAMPLES FOR DNA DATABASING LABORATORIES.”  
*September 1, 2011.*

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

*PowerPlex Fusion System: Instructions for Use of Product DC2402 and DC2408. 2012 Promega Corporation. Part Number TMD039 Rev.10/12. (or most recent revision)*

Procedure for CODIS-DNA Database

State Crime Laboratory Quality Manual

State Crime Laboratory Safety Manual

## 9.0 Records

- 2800M and NIST Traceable Standard Expected Results

## 10.0 Attachments - N/A

Revision History		
Effective Date	Version Number	Reason
03/03/2017	1	Original Document
03/12/2018	2	5.5.1-Added picture; Added 5.6.2.3-Contamination of a negative control must be reported to the TL; Added 5.6.4.9-Mixtures must be reported to the TL; 8.0-Updated CODIS procedure name