Procedure for GeneMapper® ID-X

- **1.0 Purpose** This procedure specifies the steps for performing analysis on 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.

3.0 Definitions

- Amelogenin Gender-determining locus.
- 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.
- 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.
- Consensus Allele An allele that conforms to an incremental repeat pattern at an STR locus.
- Electropherogram (egram) The computer generated electronic/visual result from an analysis performed by electrophoresis.
- 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).
- Injection See the DNA Database Section Procedure for Use of the 3500xL Genetic Analyzer.
- Locus (plural, loci) The chromosomal location or location of a gene or DNA marker.
- Microvariant An allele that varies by less than the consensus repeat unit.
- Noise Background signal detected by a data collection instrument.
- **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, below the lowest marker size range defined the in the panel, or above the highest marker size range defined in the panel.
- **Panel** A collection of markers specific to an amplification kit, such as PowerPlex® Fusion.
- 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, THO1, 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 See the DNA Database Section Procedure for Use of the 3500xL Genetic Analyzer.
- **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.

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 Creating a Project

- **5.1.1** 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.
- **5.1.2** Select "Edit" and "Add Samples to Project" to add samples to be analyzed to the project.
- **5.1.3** 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."
- **5.1.4** Verify that the table setting (in top center of the main GMID-X window) is set to 3500 Data Analysis. 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 ($\overline{\$}$) present next to it.

- **5.1.5** The remaining categories shall be filled out as follows:
 - Specimen Category: Arrestee (Arrestee samples for CODIS upload) Arrestee-State (Arrestee samples for SDIS level upload) Convicted Offender (Convicted Offender samples for CODIS upload) Offender-State (Convicted Offender samples for SDIS level upload) No Export (Samples that will not be uploaded into CODIS) Staff (Staff samples for CODIS upload)
 - Analysis Method: PowerPlex Fusion 18s WEN (for analysis of 18s injections)
 - Panel: PowerPlex Fusion
 - Size Standard: WEN_ILS_500_IDX
 - Instrument Type: ABI3500, or similar (NOTE: automatically filled in by the software and instrument)
- **5.1.6** Once all samples and categories are 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 "Databasing Security Group" and select "OK."
 - **5.1.6.1** The Analysis Summary and Genotypes tabs become available in the main GMID-X window once analysis is complete.
 - **5.1.6.2** 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**.
 - **5.1.6.3** For CODIS hit confirmations, Forensic Scientists shall delete their projects from the software only after a copy of the project has been added to the associated case record. For in-house sample analysis, the project shall be deleted once it has been moved to the network.
- **5.1.7** Analysis and Plot Settings
 - **5.1.7.1** Select the Samples tab and highlight a single sample. Access the Analysis Method Editor by selecting "Tools" from the menu in the main GMID-X window; then select "Analysis Method Editor."
 - **5.1.7.2** Select the "Peak Detector" tab and ensure that the settings for Peak Detection, Smoothing and Baselining, Size Calling Method, and Normalization appear as below when the samples have been amplified with PowerPlex® Fusion.

Start Pt: 0 Start Size: 0 Stop Pt: 10000 Stop Size: 1000 Smoothing and Baselining Smoothing None Polynomial Degree Smoothing None Peak Window Size Peak Window Size Baseline Window: 51 pts Slope Threshold Size Calling Method Peak Start: Peak End:	R: 125 P: 125 O: 100 idth: 2 e: 3	pts			
Full Range All Sizes Start Size: B: 125 Start Pt: 0 Start Size: 0 G: 125 Stop Pt: 10000 Stop Size: 1000 Y: 125 Smoothing and Baselining None None Polynomial Degree Peak Window Size Baseline Window: 51 pts Slope Threshold Peak Start: Peak Start: Size Calling Method 2nd Order Least Squares Peak End: Peak End: Peak End:	R: 125 P: 125 O: 100 idth: 2 e: 3	pts			
Stop Pt: 10000 Stop Size: 1000 G: 125 Smoothing and Baselining Smoothing ONONE O Light O Heavy Baseline Window: 51 pts Size Calling Method O 2nd Order Least Squares	O: 100 idth: 2 e: 3	pts			
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Order Least Squares					
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Local Southern Method	ition, ir applic	able			
Global Southern Method					
F	Eactory Defa	ults			

For PowerPlex Fusion 18s WEN:

5.1.7.3 Next select the "Allele" tab; the contents for all PowerPlex® Fusion Analysis Methods shall appear as below:

Analysis Method Editor							
General Allele Peak Detector	Peak Q	uality S	Q & GQ Sett	ings			
Bin Set: PowerPlex_Fusion_Bins_IDX_v2.0							
Use marker-specific stut	tter ratio	and dista	nce if availal	ble			
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		
	То	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		
	То	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		
	То	0.0	0.0	0.0	0.0		
Amelogenin Cutoff	0.05						
Range Filter				Factor	y Defaults		
Save As Save Cancel Help							

		Minus Stutter	Plus Stutter
Locus	% Stutter	(base pairs)	(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

5174	Marker-sp	ecific stutte	r filter ne	ercentages	for Po	werPlex®	Fusion ar	e listed below.
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5.1.7.5 In the main GMID-X window, ensure that the Table Setting is set to "3500 Data Analysis;" 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. Repeat for the Table Setting of "Traditional Allele Table." The "3500 Data Analysis" Table Setting shall be used for analyzing data. The "Traditional Allele Table" Table Setting shall be used for exporting casework tables.

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	Show	Column	-1			
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2		Sample Name		Size:	11	
3		Sample ID			Sort by	
4		Run Name				
5		Panel			Sample Name	
6	V	Marker			() Descending	
7		Dye			Then by	
8	V	Allele	1			
9		Size			Dye Descending	
10	V	Height				
11		Peak Area			Then by	
12		Data Point			Marker	
13		Mutation	T		© Descending	
	Sho				Factory Defaults	
Allele	e Setting	JS				
	nber of				Allele position	
Sho	ow numb	per of alleles 12			Keep Allele, Size, Height, Area, Data Point,	Mutation
	QK <u>C</u> ancel Help					

5.1.7.6 Only the Technical Leader or the GMID-X Administrators may change settings within the Analysis Method Editor or the Table Setting Editor.

5.1.8 Adding/Deleting samples

- **5.1.8.1** If one or more additional samples must be added to a current project, follow **5.1.2** through **5.1.6**. The newly added sample(s) shall be analyzed (select the Analyze icon) before the data can be viewed.
 - **5.1.8.1.1** 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.
- **5.1.8.2** If a Forensic Scientist must remove a sample that has been added to the project (e.g., a sample from an unrelated CODIS confirmation), highlight the sample to be removed and, in the menu bar, select "Edit" and "Delete from Project." Select "OK" when prompted.
 - **5.1.8.2.1** 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 (refer to **5.2.3.2**).

5.2 Analyzing/Preliminary Evaluation of Data of Each GMID-X Project

- **5.2.1** Evaluate Sizing Data (WEN ILS 500)
 - 5.2.1.1 Select Edit from the GMID-X menu bar at the top of the window and Select All. Click on the Size Match Editor Icon (III). 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.
 - **5.2.1.2** If any sample or reagent blank in the project indicates no sizing data (i.e., WEN ILS 500 failure), then that sample or reagent blank shall not be used for comparison. This information shall be noted on the Casework Table Worksheet or the Exceptions/Notes Report. If there is no sizing data for an allelic ladder, then no data associated with that ladder can be sized.
 - **5.2.1.2.1** If the allelic ladder has no sizing data or otherwise 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.
 - **5.2.1.2.2** If an amplification 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.
 - **5.2.1.2.3** Any sample/reagent blank that has no sizing data shall be reinjected or replated.
 - **5.2.1.3** Samples and controls that show no sizing data, but contain all correctly labeled peaks may be used for comparison.
 - **5.2.1.3.1** From the samples tab select the sample and click on the Size Match Editor.
 - **5.2.1.3.2** Click Override SQ. Then click OK.
 - **5.2.1.3.3** With the samples still highlighted in the samples tab, click on Analyze Selected Samples. Examine all samples in the manner as described for the allelic ladders and controls.

5.2.2 Assess Overall Quality of Data

5.2.2.1 Allelic Ladders and Controls

- **5.2.2.1.1** 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.
- **5.2.2.1.2** For any ladder not showing a green square, select the allelic ladder(s) and open the sample plot window by selecting the Display Plots icon (). In the Samples Plot window, ensure that the Plot Setting is set to "Data Interpretation."
- **5.2.2.1.3** Scroll through each of the color channels (blue, green, yellow, red) and verify that all the alleles are sized and labeled correctly according to the expected calls for the PowerPlex® Fusion allelic ladder. All expected peaks must be present and above the appropriate analytical threshold. Close this Sample Plot window.
- **5.2.2.1.4** For any positive or negative control not showing a green square, select the appropriate control and open the sample plot window as described previously.
- **5.2.2.1.5** Examine the positive and negative control(s) and ensure that the controls meet the required interpretive guidelines (see the DNA Database Procedure for Autosomal DNA STR Interpretation with PowerPlex® Fusion). Remove any artifacts as described in **5.2.2.2.1**.
- **5.2.2.2 Samples** Examine all samples in the manner as described for the allelic ladders and controls.
 - **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. 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.
 - **5.2.2.2 OMR**: GMID-X software flags peaks that fall outside the range of known alleles for a locus with the label "OMR" (Outside Marker Range). 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 will prevent the call from appearing on the allele call table, so deleting the "OMR" is not necessary. If the "OMR" call is for an allele that should be associated with a surrounding marker, left click on the "OMR" label, hit Ctrl on the keyboard, and left click on the appropriate marker label. 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.

- **5.2.2.2.1** 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 to which locus the allele should be assigned based upon the presence or absence of heterozygosity at the loci in question.
- **5.2.2.2.2** If one locus exhibits homozygosity and the second heterozygosity, the OMR allele shall be assigned to the homozygous locus.
- **5.2.2.2.3** If both loci exhibit homozygosity or heterozygosity, the Forensic Scientist shall document on the allele call table 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 (so it does not appear on the allele call table but remains on the electropherogram) and typing "INC" as the Reason(s) for Change. The called alleles at these loci shall be reported and uploaded into CODIS.
- **5.2.2.2.4** 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.
- **5.2.2.2.5** In the main GMID-X window, select the sample(s) containing the allele mislabeled as an "OMR" and the allelic ladder. View them in Display Plots. Zoom in to the loci surrounding the allele mislabeled as an "OMR"; the Sample Plot displays the allelic ladder in the top half of the Plot window and the sample is in the bottom half.
- 5.2.2.2.6 Highlight the allele mislabeled as an "OMR" in the sample, along with the alleles in the allelic ladder that lie the closest in size to the allele mislabeled as an "OMR" (to select peaks simultaneously, hold down the Ctrl key on the keyboard while selecting). Select the sizing table icon (I). Select Ctrl + G to make the sizing table display the selected alleles.
- **5.2.2.2.7** Based upon the sizing information provided in the table, determine the appropriate allele call for the OMR.

- **5.2.2.2.7.1** The correct allele call for the OMR is dependent upon the base pair size when compared to the allelic ladder.
- **5.2.2.2.8** With the alleles highlighted in **5.2.2.2.2.6** still present in the sizing table (remove any extraneous), select "File" and "Print" and select "Print2PDF" (for samples associated with CODIS hits).
- 5.2.2.3 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 will prevent the call from appearing on the allele call table, 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.
- **5.2.2.2.4 Off-Ladder Alleles and Microvariants:** If a sample(s) contains an offladder 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 reinjection (or reamplification, if necessary) of the sample(s) and document the sizing/locus assignment of the off-ladder alleles and/or microvariant as follows:
 - **5.2.2.4.1** When a locus contains three alleles (one of which is off-ladder) and the adjacent locus is homozygous, the DNA Database Forensic Scientist shall call the off-ladder allele inconclusive by deleting the allele at that locus (so it does not appear on the allele call table but remains on the electropherogram) and typing "INC" as the Reason(s) for Change.
 - **5.2.2.4.2** An off-ladder allele or a microvariant shall be named/assigned per NDIS standards. For CODIS eligibility, refer to the Procedure for CODIS.
 - **5.2.2.4.3** In the main GMID-X window, select the sample(s) containing the microvariant/off-ladder allele and the allelic ladder. View them in 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.

- 5.2.2.4.4 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.
- **5.2.2.4.5** Based upon the sizing information provided in the table, determine the appropriate allele call for the microvariant/off-ladder allele.
 - **5.2.2.4.5.1** The correct allele call for the microvariant/off-ladder allele is dependent upon the base pair size when compared to the allelic ladder.
 - **5.2.2.4.5.2** 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).
- **5.2.2.4.6** 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."
- **5.2.2.4.7** With the alleles highlighted in **5.2.2.4.4** still present in the sizing table (remove any extraneous), select "File" and "Print" and select "Print2PDF" (for samples associated with CODIS hits).
- **5.2.2.5 Sizing Shifts:** If an allele at any locus has been sized outside of the appropriate bin which results in an "off ladder" call by GMID-X, the sample shall be re-injected (or reamplified, if necessary) in order to size the allele(s). Shifted alleles/loci shall not be used for comparison in any control, ladder or sample.
- **5.2.2.2.6 Off-scale Samples:** The use of off-scale Database samples shall be determined by the DNA Database Forensic Scientist based upon his or her training and experience.

5.2.3 Printing Egrams Electronically

- **5.2.3.1** The egrams associated with CODIS hits shall be printed as a .pdf file. The egrams for each GMID-X project shall exist as its own .pdf file. Ensure the plot setting is set to "Traditional Genotype Plot" when printing to PDF.
 - **5.2.3.1.1** GMID-X projects are added to the FA case record object repository (or equivalent folder on the server if analysis is not part of a case record) 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.
- **5.2.3.2** 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).
 - **5.2.3.2.1** Even if not used for comparison purposes, all .hid files associated with such samples/controls/ladders shall be present in the run folder containing the electronic data in the FA case record object repository (or equivalent folder on the server if analysis is not part of a case record).
 - **5.2.3.2.2** For any sample/control/ladder that is not used for comparison purposes, corrections (labeling of artifacts) are not required.
- **5.2.3.3** If an entire run or project is not used for comparison purposes, it shall be printed to PDF and added to the case record object repository (or equivalent folder on the server if analysis is not part of a case record) as described below. Corrections (e.g., labeling of artifacts) are not required in this instance.
 - **5.2.3.3.1** 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.
 - **5.2.3.3.2** 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 peaks (60 through 500) must be visible.
 - **5.2.3.3.** Raw data for negatives associated with a CODIS hit shall be printed (PDF; see **5.2.4**).

- **5.2.3.3.4** A Casework Table shall be generated and the reason for not using the entire run/project shall be noted on this page (PDF; see **5.3.1**).
- **5.2.3.3.5** An Allele Call Table is not required for such runs/projects.

5.2.4 View Raw Data

- **5.2.4.1** The raw data for all samples from each project must be present in the corresponding case record object repository (or equivalent folder on the server if analysis is not part of a case record). 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 screenshot of each negative control and injection failure in the case record object repository.
 - **5.2.4.1.1** In the main GMID-X window, highlight the negative control or negative sample and select "Raw Data" from the "View" option in the main GMID-X menu.
 - **5.2.4.1.2** 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 (including run number) is clearly visible. The right-hand window shall show the maximum vertical and horizontal axis.
 - **5.2.4.1.3** Using the "Alt" and "Print Screen" buttons on the computer, 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.
 - **5.2.4.1.3.1** This raw data Word document shall contain at least the project name (refer to the DNA Database Section Procedure for DNA Database Operations), DNA Database Forensic Scientist's initials, and the date.
 - **5.2.4.1.4** The Word document(s) containing the screenshots of the raw data shall be saved/printed as a .pdf file and included in the case record object repository.

5.3 Exporting and Creating Tables

5.3.1 Casework Table

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

- **5.3.1.2** 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.
- **5.3.1.3** Open Excel; open the .txt file saved in the previous step.
- 5.3.1.4 When the Text Import Wizard opens, select "Finish."
- **5.3.1.5** Adjust the columns to accommodate the full text for each line.
- **5.3.1.6** Select "Page Setup." Under the "Page" tab, select landscape; under the "Header/Footer" tab, select "Custom Header" at a minimum, the DNA Database Forensic Scientist shall enter the project name, initials, and the date; under the "Sheet" tab, select only the "Gridlines" box. Finish by selecting "OK."
- **5.3.1.7** For CODIS confirmations, DNA Database 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**). For in-house runs, samples not used in analysis shall be noted on the Notes/Exceptions sheet.
- **5.3.1.8** Print the Casework Table Worksheet by using PDF. The Casework Table Worksheet shall exist independently of the egrams and the Allele Call Table Worksheet(s). It may be merged with the 3500xL setup sheet.
- **5.3.1.9** For CODIS confirmations, only samples and controls specific to the CODIS confirmation shall be present in the Casework Table.

5.3.2 Allele Call Table

- **5.3.2.1** 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.
- **5.3.2.2** Under the "File" menu in the main GMID-X window, select "Export Table." Save the table to a location easily located by the DNA Database Forensic Scientist (e.g., desktop or thumb drive). Ensure the file is exported/saved in the Tab-delimited Text (.txt) format.
- 5.3.2.3 Open the appropriate Allele Call Table Worksheet in the DNA Database Shared Folder. For in-house runs, use the PPF Allele Call Table. For CODIS confirmations, use the PPF Allele Call Table – CODIS Hits. Populate this worksheet using the .txt file that was generated from GMID-X.

- **5.3.2.4** Save the Allele Call Table Worksheet in Excel. This file must be separate from the file containing the egrams or the Casework Table.
- **5.3.2.5** For CODIS confirmations, only samples specific to the CODIS confirmation shall be present in the Allele Call Table.

5.3.3 CODIS Table

- **5.3.3.1** For CODIS sample export and specimen upload, refer to the Procedure for CODIS and the DNA Database Section Procedure for DNA Database Analysis and Technical Review of Database Samples Amplified with PowerPlex® Fusion.
- 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.

DNA Database Section Procedure for Autosomal DNA STR Interpretation with PowerPlex® Fusion

DNA Database Section Procedure for Use of the 3500xL Genetic Analyzer

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

9.0 Records

• 2800M and ZJK Expected Results

10.0 Attachments - N/A

Revision History						
Effective Date	Version Number	Reason				
08/03/2015	1	Original Document				
10/09/2015	2	5.2.2.2.3 - clarified "spike" call; 5.2.2.2.4- added reinjection requirement for off-ladder alleles/microvariants; deleted 5.2.2.2.4.8				
12/21/2015	3	5.1.1 SDIS levels added; 5.1.5 change Panel name; 5.1.7.3 updated picture 5% AMEL stutter; 5.2.1.2.1 clarify use of another ladder; 5.2.1.3 to 5.2 added procedure to override SQ; 5.3.2.7 Allele call saved as Excel; 9.0 replace MJB with ZJK				
4/18/2016	4	5.1.5 and 5.1.7.2 removed 24 sec injection parameters, updated from CC5 to WEN size standard ; 5.1.6.3 project shall be deleted once added to FA				