# Procedure for GeneMapper® ID-X and Y-STR Interpretation with PowerPlex® Y23

- **1.0 Purpose** This procedure specifies the steps for performing analysis and interpretation of DNA samples amplified with the PowerPlex® Y23 System using GeneMapper® ID-X (GMID-X).
- 2.0 Scope This procedure applies to DNA Database Section analysts and trainees who perform Y-STR 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** See Section Definitions List

## 4.0 Equipment and Materials

- Computer with GeneMapper® ID-X (version 1.6 or most current) software
- Microsoft Excel, Word
- Print2PDF software

## 5.0 Procedure

# 5.1 Sample RFU Thresholds

- **5.1.1 Analytical Threshold** The analytical threshold was established through validation and performance check studies using the PowerPlex® Y23 PCR Amplification kit.
  - **5.1.1.1** The analytical threshold for 18s injections is set at 100 RFU for blue, green, yellow, red, and orange dye channels. Anything present below 100 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® Y23 PCR Amplification kit.
  - **5.1.2.1** The stochastic threshold for 18s injections is set at 200 RFU for blue, green, yellow, red, and orange dye channels.

NOTE: This threshold applies to DYS385.

- **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.

| Peak Detection Algorithm: Advanced<br>Ranges<br>Analysis Sizing<br>Full Range Sizing<br>Start Pt: 0<br>Stop Pt: 10000<br>Stop Size: 1000<br>Smoothing and Baselining<br>Smoothing None<br>© Light<br>© Heavy<br>Baseline Window: 51 pts<br>Size Calling Method<br>© 2nd Order Least Squares<br>© Stop Size: 0<br>Stop Size: 1000<br>Stop Size: 1000<br>Sto | Peak Detection         Peak Amplitude Thresholds:         B:       100         G:       100         P:       100         Y:       100         Y:       100         Min. Peak Half Width:       2         Polynomial Degree:       3         Peak Window Size:       15         Slope Threshold       0.0         Peak Start:       0.0         Normalization       Use Normalization, if applicable |
|--|---|
| ○ Global Southern Method           Save As         Save  | Eactory Defaults  |

| 5.2.2 | The Allele | tab of the | Analysis   | Method Editor | settings shall | appear as below  |
|-------|------------|------------|------------|---------------|----------------|------------------|
| J     | The Thiere | tuo or the | 7 mary 515 | memou Lunoi   | sounds shan    | appear as below. |

| Analysis Method Editor                                     |  |  |  |  |  |  |
|--|--|--|--|--|--|--|
| General Allele Peak Detector Peak Quality SO & GO Settings |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| Bin Set: PowerPlexY23_Bins_IDX_v2.0                        |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| tter ratio   | and dista  | nce if availa  | ble  |  |  |  |
|  | Tri  | Tetra  | Penta  | Hexa   |  |  |
|  | 0.2  | 0.2  | 0.2  | 0.2  |  |  |
|  | 0.0  | 0.0  | 0.0  | 0.0  |  |  |
| From   | 0.0  | 0.0  | 0.0  | 0.0  |  |  |
| То   | 0.0  | 0.0  | 0.0  | 0.0  |  |  |
|  | 0.0  | 0.0  | 0.0  | 0.0  |  |  |
| From   | 0.0  | 3.25   | 0.0  | 0.0  |  |  |
| То   | 0.0  | 4.75   | 0.0  | 0.0  |  |  |
|  | 0.0  | 0.0  | 0.0  | 0.0  |  |  |
| From   | 0.0  | 0.0  | 0.0  | 0.0  |  |  |
| То   | 0.0  | 0.0  | 0.0  | 0.0  |  |  |
| Amelogenin Cutoff 0.05                                     |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  | <u>E</u> actor   | y Defaults   |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| Save As Save Cancel Help                                   |  |  |  |  |  |  |
|  | Peak Q<br>ns_IDX_v<br>tter ratio<br>From<br>To<br>From<br>To<br>From<br>To<br>0.05 | Peak Quality S<br>ns_IDX_v2.0<br>tter ratio and dista<br>Tri<br>0.2<br>0.0<br>From 0.0<br>To 0.0<br>From 0.0<br>From 0.0<br>From 0.0<br>From 0.0<br>0.0<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solution<br>Solutio | Peak Quality       SQ & GQ Sett         ns_IDX_v2.0       Image: Constraint of the set of | Peak Quality       SQ & GQ Settings         ns_IDX_v2.0         tter ratio and distance if available         0.2       0.2         0.0       0.0         0.05       Eactor | r       Peak Quality       SQ & GQ Settings         ns_IDX_v2.0       •         Tri       Tetra         0.2       0.2       0.2         0.0       0.0       0.0         From       0.0       0.0         0.0       0.0       0.0         0.0       0.0       0.0         From       0.0       0.0         0.0       0.0       0.0         To       0.0       0.0         0.05       Eactory Defaults |  |

| Locus     | Stutter  | Stutter | Stutter  | Stutter | Stutter  | Stutter |
|-----------|----------|---------|----------|---------|----------|---------|
| Locus     | Position | %       | Position | %       | Position | %       |
| DYS576    | N-4      | 14.7%   |          |         |          |         |
| DYS389I   | N-4      | 8.1%    |          |         |          |         |
| DYS448    | N-6      | 4.0%    |          |         |          |         |
| DYS389II  | N-4      | 15.2%   |          |         |          |         |
| DYS19     | N-2      | 10.2%   | N+2      | 3.8%    | N-4      | 10.7%   |
| DYS391    | N-4      | 12.4%   |          |         |          |         |
| DYS481    | N-3      | 29.8%   | N+3      | 5.5%    |          |         |
| DYS549    | N-4      | 11.4%   |          |         |          |         |
| DYS533    | N-4      | 10.7%   |          |         |          |         |
| DYS438    | N-5      | 5.0%    |          |         |          |         |
| DYS437    | N-4      | 8.4%    |          |         |          |         |
| DYS570    | N-4      | 15.9%   |          |         |          |         |
| DYS635    | N-4      | 11.5%   |          |         |          |         |
| DYS390    | N-4      | 13.4%   |          |         |          |         |
| DYS439    | N-4      | 10.7%   |          |         |          |         |
| DYS392    | N-3      | 17.1%   | N+3      | 10.5%   |          |         |
| DYS643    | N-5      | 3.9%    |          |         |          |         |
| DYS393    | N-4      | 15.1%   |          |         |          |         |
| DYS458    | N-4      | 14.7%   |          |         |          |         |
| DYS385a/b | N-4      | 14.9%   |          |         |          |         |
| DYS456    | N-4      | 15.1%   |          |         |          |         |
| Y-GATA-HA | N-4      | 11.1%   |          |         |          |         |

**5.2.3** Marker-specific stutter filter percentages for PowerPlex® Y23 are listed below.

5.2.4 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.

| 2 | 🧨 Table Setting Editor            |          |                          |   |          |                  |  |  |
|---|-----------------------------------|----------|--------------------------|---|----------|------------------|--|--|
| G | General Samples Genotypes         |          |                          |   |          |                  |  |  |
|   | Geno                              | types T  | able Settings:           |   |          |                  |  |  |
|   | Colu                              | imn Set  | tings:                   |   | Font Set | ttings:          |  |  |
|   |                                   | Show     | Column                   |   |          |                  |  |  |
|   | 1                                 | 1        | Sample File              | ~ | Font:    | Arial            |  |  |
|   | 2                                 | <b>V</b> | Sample Name              |   | Size:    | 11               |  |  |
|   | 3                                 |          | Sample ID                | Ξ |          | Sort by          |  |  |
|   | 4                                 |          | Run Name                 |   |          |                  |  |  |
|   | 5                                 |          | Panel                    |   |          | Sample Name      |  |  |
|   | 6                                 | V        | Marker                   |   |          | Descending       |  |  |
|   | 7                                 |          | Dye                      |   |          | Then by          |  |  |
|   | 8                                 | <b>V</b> | Allele                   |   |          | Ascending        |  |  |
|   | 9                                 |          | Size                     |   |          | Dye   Descending |  |  |
|   | 10                                | 1        | Height                   |   |          |                  |  |  |
|   | 11                                |          | Peak Area                |   |          | Then by          |  |  |
|   | 12                                |          | Data Point               |   |          | Marker           |  |  |
|   | 13                                | •        | Mutation                 | - |          | Descending       |  |  |
|   |                                   | Sho      | w Hide Show All Hide All |   |          | Factory Defaults |  |  |
|   | Allele Settings                   |          |                          |   |          |                  |  |  |
|   | Number of alleles Allele position |          |                          |   |          |                  |  |  |
|   | Show number of alleles 12         |          |                          |   |          |                  |  |  |
|   | QK Cancel Help                    |          |                          |   |          |                  |  |  |

#### 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® Y23:

- 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 ( $\mathfrak{F}$ ) 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 Y23 100 RFU 20 GF
- Panel: PowerPlexY23\_IDX\_v2.0
- 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, 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 Y23)

5.5.1 Select all samples and controls within the project. 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.



- **5.5.2** If any sample or control in the project indicates no sizing data (i.e., WEN ILS 500 Y23 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 "DB 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 must be equal to or greater than the analytical threshold.
  - **5.6.1.3.2** Each allele must 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 data associated with the failed negative control shall not be used. The negative control shall be reanalyzed (i.e., reinjected or replated) or the sample set 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 must 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 At DYS385 Single alleles which fall below the stochastic threshold shall be considered partial for purposes of interpretation and CODIS entry. In such instances, the locus 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** For samples with dropout, at a minimum a second amplification of the sample shall be completed in an attempt to obtain a full profile.
- **5.6.4.5** 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.
- **5.6.4.6** 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.

- **5.6.4.6.1** Microvariant not automatically sized by the ladder
- 5.6.4.6.2 Off-Ladder Allele
- 5.6.4.6.3 OMR
- **5.6.4.6.4** More than one peak is observed at a locus or more than two peaks are observed at DYS385
- **5.6.4.7** Heterozygote Peak Balance Database samples generally exhibit a balance of >75% between DYS385 heterozygous alleles. A database sample shall be rerun if the imbalance does not allow for distinction between true alleles and artifacts.
- **5.6.4.8** Mixtures Any mixture profiles shall be reported to the Technical Leader. The Technical Leader shall determine root cause and further action.
- **5.6.4.9** 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.
- **5.6.4.10** It is permissible to combine results from different injections and amplifications of the same sample when determining a final DNA profile.
- **5.6.4.11** For any sample/control/ladder/injection that is not used for comparison purposes, labeling of artifacts is not required.

## 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.
  - **5.7.2.1** N±3 peaks: For trinucleotides, a stutter peak may appear in the n±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.

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- **5.7.2.4** N $\pm$ 6 peaks: For hexanucleotides, a stutter peak may appear in the n $\pm$ 6 position.
- 5.7.3 Known Artifacts: Promega has noted reproducible artifacts that may appear in samples amplified with PowerPlex<sup>®</sup> Y23. For a list of these artifacts, see the PowerPlex<sup>®</sup> Y23 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 xaxis 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-DNA Database.
- **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.
- **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** Documentation of the microvariant/off-ladder allele sizing information may be retained. 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** If the DNA profile has data at one locus bracketing the off-ladder allele, and no data at the other bracketing locus, then the off-ladder allele will be assigned to the locus with no data.
  - **5.9.2** Alleles which are located outside the range of the ladder or bin set (above or below) shall be described as "<" or ">" the largest or smallest allele for that locus with a set of () placed around the off ladder allele. For example, if a band is located above the largest allele for the DYS19 locus, it would be designated as "(>19)". This should be clear when used and can be verified

with a locus review of the egram.

- **5.9.3** If the DNA profile has data at both bracketing loci, or if the DNA pattern is a mixture, then the locus to which the off-ladder allele belongs may not be determined. The off-ladder allele will not be given a locus designation. Both bracketing loci will be designated as inconclusive.
- **5.9.4** It is possible for alleles to fall outside of the range of their locus and to be detected in the size range of a neighboring locus. (e.g. an allele 20 in locus DYS391 would be detected in the range of locus DYS481) Such possibilities should be considered when a locus has a bi-allelic pattern and is adjacent to a locus with no data.
  - **5.9.4.1** If the bi-allelic pattern has one allele within a bin, and one allele designated as "off-ladder", the "off-ladder" allele will be designated as belonging to the locus showing no other data.
  - **5.9.4.2** If the bi-allelic pattern has both alleles within allelic bins, then it is not possible to determine which allele belongs to the locus showing no data. Both loci in question will be designated as inconclusive.
- **5.9.5** 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-DNA Database.
- **5.9.6** An "OMR" shall be sized as described in the Off-Ladder Alleles and Microvariants section of this document.
  - **5.9.6.1** The correct allele call for the OMR is dependent upon the base pair size when compared to the allelic ladder.
  - **5.9.6.2** 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.
- **5.9.7** Documentation of the OMR allele sizing information may be retained. 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 Duplications

**5.10.1** A duplication occurs when multiple alleles are present at a locus/loci. Duplications have been documented with the PowerPlex® Y23 amplification kit. Instances of multiple duplications within a sample have been documented as well. Most duplications have a one repeat difference; however two, three, and four repeat unit differences have been observed.

- **5.10.1.1** To distinguish between a mixture and a duplication: determine the number of loci containing more than one peak; consider the position on the Y-chromosome if multiple loci have two peaks; determine if the repeat spread is >1 repeat unit; examine DYS385 a/b for the presence of >2 peaks; and consider autosomal testing to confirm single-source sample.
- **5.10.2** A deletion occurs when there is a lack of a peak at a single locus/loci. A deletion has been reported at the DYS448 locus. Samples with this deletion will show two peaks (duplication) in DYS576 and a null allele in DYS448.
- **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 Printing Egrams Electronically

- **5.12.1** PDF egrams shall be retained.
- 5.12.2 Ensure the size range captured contains all loci and alleles.
- **5.12.3** The WEN sizing standard for samples/control/ladders may 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 View Raw Data

- **5.13.1** 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. Documentation of the raw data primer peaks of negative controls and negative samples shall be retained.
  - 5.13.1.1 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).
  - **5.13.1.2** 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.
  - 5.13.1.3 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.
    - **5.13.1.3.1** This raw data Word document shall contain at least the project name, DNA Database Forensic Scientist's initials, and the date.

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

#### 5.14 Exporting and Creating Tables

- **5.14.1 Casework Table**: The casework table shows all samples and controls run in a project and the GMID-X analysis settings used in the project.
  - **5.14.1.1** In the main GMID-X window, select the "Traditional Allele Table" from the Table Setting menu.
  - **5.14.1.2** 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.
  - **5.14.1.3** Import the .txt file into Excel.
  - **5.14.1.4** Adjust the columns to accommodate the full text for each line.
  - **5.14.1.5** The header shall include section name, project name, date, and initials.

**5.14.1.6** Print the Casework Table using print to PDF.

- **5.14.2** Allele Call Table: The allele call table shows reported allele calls for samples. It may also show the expected results for the positive control.
  - **5.14.2.1** 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.
  - **5.14.2.2** 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.
  - **5.14.2.3** Open the appropriate DNA Database Allele Call Table Worksheet. Populate this worksheet using the .txt file that was generated from GMID-X.
  - **5.14.2.4** Save the Allele Call Table Worksheet as a PDF document.

## 5.14.3 CODIS Table

**5.14.3.1** For CODIS sample export and specimen upload, refer to the Procedure for CODIS-DNA Database.

## 5.15 Exporting Projects

- **5.15.1.1** 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*<sup>™</sup>. *GeneMapper*<sup>®</sup> *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 Sample 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)* 

PowerPlex Y23 System for Use on the Applied Biosystems Genetic Analyzers Instructions for Use of Products DC2305 and DC2320. 2017 Promega Corporation. Part Number TMD035 Rev.4/17. (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  |  |  |  |
| 08/09/2021       | 3              | 4.0-Updated software version; 5.6.1.2-Updated plot setting name; 5.12.2-Removed plot setting requirement; 5.14.1.5-Removed 3500xL instrument name requirement; Updated References |  |  |  |