Procedure for Analysis and Interpretation of Y-STR Amplification

1. **Purpose**-This procedure specifies the steps for performing analysis on DNA samples amplified with Promega PowerPlex® Y23 using the GeneMapper® ID-X (GMID-X) software and to provide guidelines for the interpretation and reporting of Y-STR DNA results.

2. **Scope**- This procedure applies to casework analysts and trainees in the Forensic Biology Section who perform or review Y-STR DNA analysis.

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

4. **Equipment and Materials**

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

5. **Procedure**

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

   5.1.1 Y-STR testing is an additional tool used in cases where recovery of the male DNA profile in male:female mixtures is necessary. This type of testing will be utilized only on intimate items in sexual assault type cases where a male DNA profile is not expected with PowerPlex® Fusion DNA testing due to the nature of the sample. As there is no searchable database for unknown Y-STR profiles, these cases must be accompanied by standards from the males involved in the case (i.e. suspect or elimination standards) in order for Y-STR analysis to be performed, and no standards identified as coming from females will be tested.

   5.1.2 Y-STR testing using the PowerPlex® Y23 amplification kit is an extremely sensitive analysis method. The pattern of Y-STR peaks obtained during this testing is defined as the Y-STR haplotype of the individual. Due to this linked inheritance, the product rule cannot be applied to estimate the rarity of the haplotype and a counting method must be applied.

   5.1.3 Results are interpreted by confirming controls functioned properly, determining which alleles are present, taking artifacts into consideration, determining if the profile is consistent with being from a single male or a mixture, and designating which loci are interpretable.

   5.1.4 If individuals are from the same paternal lineage, they are expected to have the same Y-STR haplotype, barring mutation. It may be impossible to determine the difference between these

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

individuals. As a result, for cases where a direct standard cannot be obtained (e.g. missing person case) an alternate standard from a paternal relative may be utilized as a secondary standard.

5.2 Creating a GMID-X Project

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

5.2.2 Add samples to be analyzed to the project.

5.2.3 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."

5.2.4 In the Samples pane, the category Sample Type shall have the samples labeled as follows when amplified with PowerPlex® Y23:

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

5.2.5 The remaining categories shall be filled out as follows:

- Specimen Category: no export
- Analysis Method: PowerPlex Y23 AnalysisMethod_AT
- Panel: PowerPlexY23_IDX_v2.0
- Size Standard: WEN_ILS_500_CS
- Instrument Type: ABI3500

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

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

5.2.6.2 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 previously.

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Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

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

5.2.7 Analysis and Plot Settings

5.2.7.1 Access the Analysis Method Editor by selecting "Tools" from the menu in the main GMID-X window, and then select "Analysis Method Editor." Note that the user must have a single sample in the main GMID-X window selected in order to access the Editor.
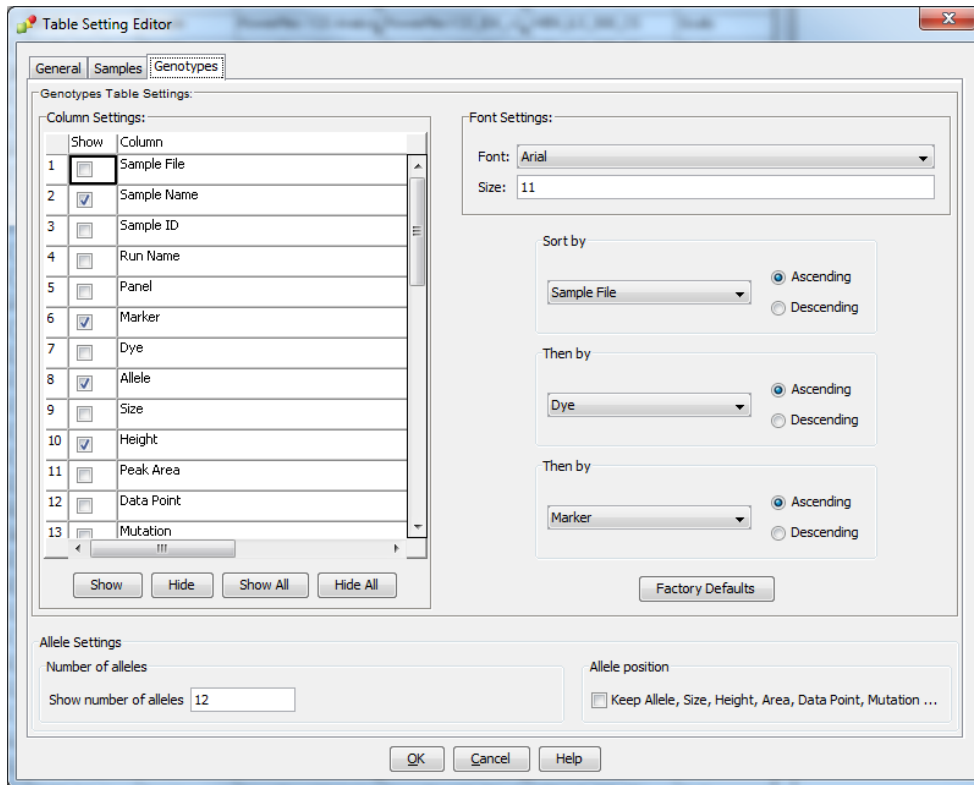
5.2.7.2 Select the "Peak Detector" tab and ensure that the settings for Peak Detection, Smoothing and Baselining, and Size Calling Method appear as below when the samples have been amplified with PowerPlex® Y23. The "Allele" tab contents should also appear as below. The analytical threshold was established through validation and performance check studies using the PowerPlex® Y23 PCR Amplification kit. The analytical threshold is dye channel specific. Anything present below these thresholds is considered to be indistinguishable from background noise and shall not be considered for analysis.
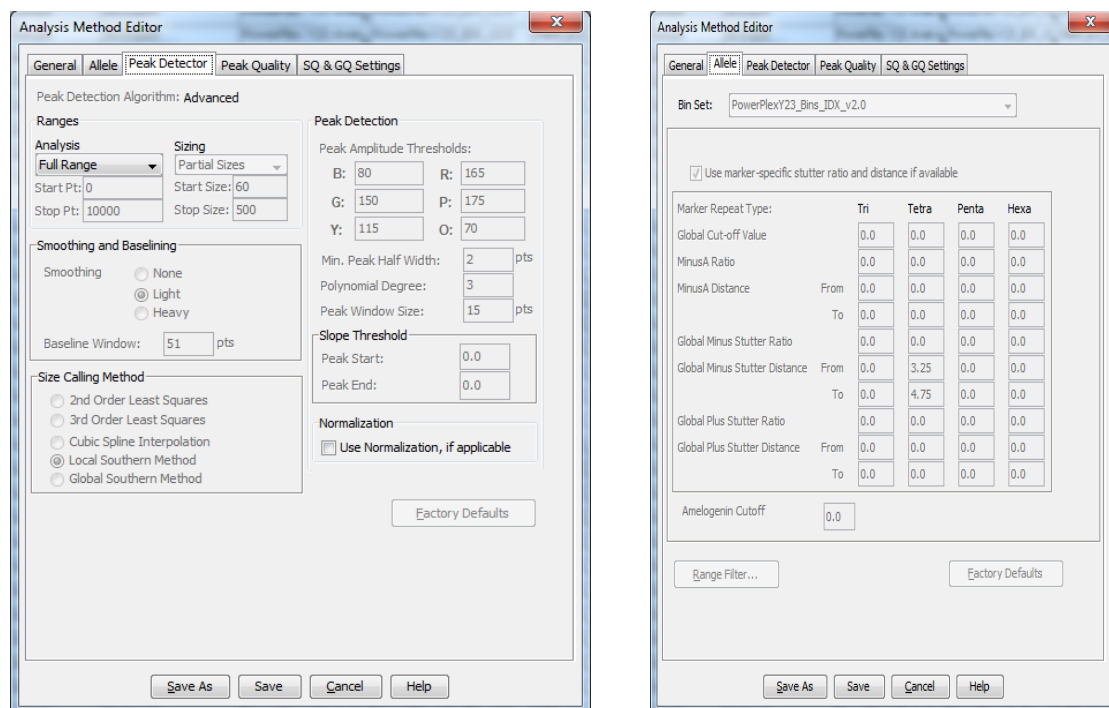
5.2.7.3 Marker-specific stutter filter percentages for PowerPlex® Y23 are listed below. All potential stutter peaks must be evaluated as GMID-X cannot set automatic stutter cutoffs for all stutter peaks noted in validation studies.

Marker	Stutter Position	Stutter %	Stutter Position	Stutter %	Stutter Position	Stutter %	Stutter Position	Stutter %
DYS576	N-4	14.7%	N+4	3.0%				
DYS389 I	N-4	8.1%	N+4	1.1%				
DYS448	N-5	4.0%	N+5	1.4%	N+6	0.6%		
DYS389 II	N-4	15.2%	N+4	2.0%				
DYS19	N-4	10.7%	N+4	1.3%	N-2	10.2%	N+2	3.8%
DYS391	N-4	12.4%	N+4	0.6%				
DYS481	N-3	29.8%	N+3	6.4%				
DYS549	N-4	11.4%	N+4	2.1%				
DYS533	N-4	10.7%	N+4	1.9%				
DYS438	N-5	5.0%	N+5	1.0%				
DYS437	N-4	8.4%	N+4	1.5%				
DYS570	N-4	15.9%	N+4	1.7%				
DYS635	N-4	11.5%	N+4	2.5%				
DYS390	N-4	13.4%	N+4	1.2%				
DYS439	N-4	10.7%	N+4	3.0%				
DYS392	N-3	17.1%	N+3	10.5%	N-5	3.9%	N+5	1.4%
DYS393	N-4	15.1%	N+4	3.4%				
DYS458	N-4	14.7%	N+4	0.8%	N-6	0.4%	N-8	1.0%
DYS385	N-4	14.9%	N+4	2.1%				
DYS456	N-4	15.1%	N+4	2.8%				
YGATAH4	N-4	11.1%	N+4	1.7%				

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

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

5.2.7.5 Only the DNA Technical Leader or the GMID-X Administrators shall change settings for the Analysis Method Editor or the Table Setting Editor.

5.2.8   Adding/Deleting samples

5.2.8.1 If a Forensic Scientist must add additional sample(s) to a current project, follow **5.2.2** through **5.2.7**. The newly added sample shall be analyzed (select the Analyze icon) before the data can be viewed.

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

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

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

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

5.3.1   Evaluate Sizing Data (e.g., WEN)

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Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

5.3.1.1 WEN sizing data for all samples must be evaluated to ensure all peaks are present and correctly sized (sizing peaks -60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 base pairs).
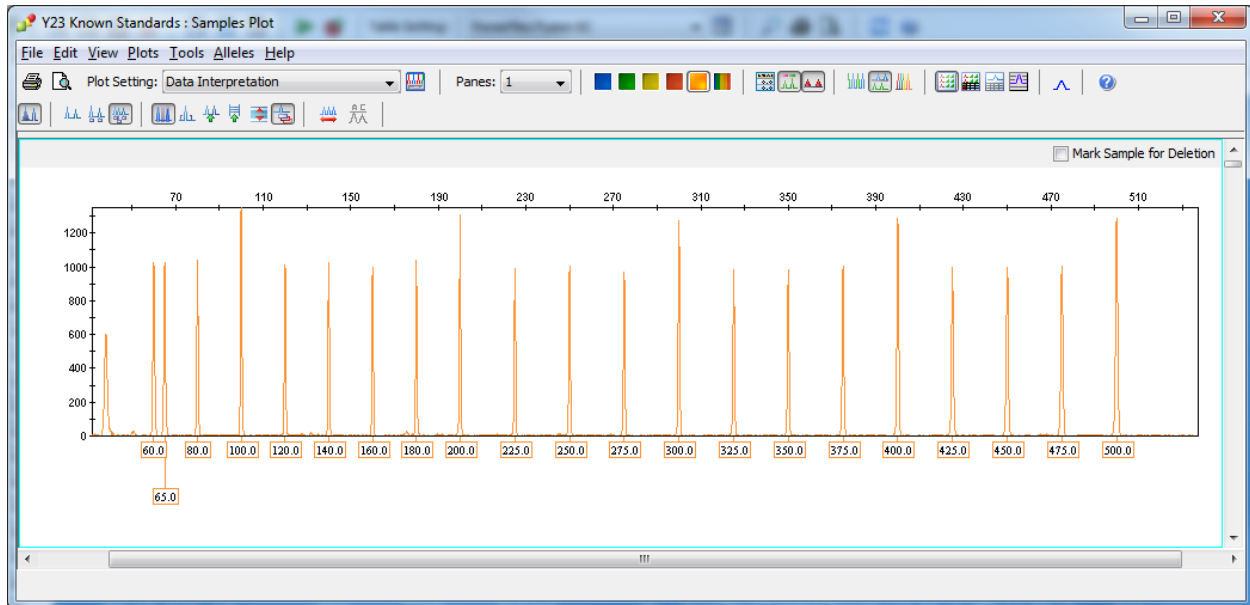
5.3.1.2 If any sample or reagent blank in the project indicates no sizing data (e.g., WEN failure), then that sample or reagent blank shall not be used for comparison. This information shall be noted on the Casework Table Worksheet. If there is no sizing data for an allelic ladder, then no data associated with that ladder can be sized.

5.3.1.2.1 If the allelic ladder has no sizing data, a ladder may be imported from another run to size the run. Note: The source of this imported ladder shall be documented in the case file. When using an imported ladder, data must be evaluated for migration shift (e.g. numerous OL calls seen with imported ladder use.)

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

5.3.1.2.3 Any sample/reagent blank that has no sizing data shall be re-injected.

5.3.2   Assess Overall Quality of Data

5.3.2.1 Allelic Ladders and Controls

5.3.2.1.1 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.

All copies of this document are uncontrolled when printed.

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

5.3.2.1.2 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.

5.3.2.1.3 Verify that all the alleles are sized and labeled correctly according to the expected calls for the PowerPlex® Y23 Allelic ladder. All expected peaks must be present and above the appropriate analytical threshold. Close this Sample Plot window.

5.3.2.1.4 For any positive or negative control not showing a green square, select the appropriate control and open the sample plot window as described previously.

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

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

5.3.2.2 Samples

5.3.2.2.1 Examine all samples in the manner as described for the allelic ladders and controls. Failure of any locus to amplify shall not preclude interpretation at those loci which are present.

5.3.2.2.1.1 Artifacts: The PCR process produces artifacts that are known and well characterized. 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 the mouse. Select "Delete Label(s)" (multiple peaks can be deleted simultaneously by selecting them at the same time-when the warning dialog box appears, select "OK"). When prompted, type in artifact as the Reason(s) for Change.

5.3.2.2.1.2 Stutter: Results shall not be considered inconclusive if stutter peaks are present. Marker specific stutter percentages are

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

incorporated into the GMID-X analysis. Additional stutter positions (noted in previous table) should be considered during interpretation. Peaks in stutter positions with ratios greater than the stutter guidelines should be interpreted with caution. Excessive peak heights can cause stutter peaks in excess of the validated percentages.

5.3.2.2.1.3 **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 an artifact, the Forensic Scientist shall leave the peak labeled as OMR.

5.3.2.2.1.4 **Spike**: GMID-X software provides automatic labelling 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 an artifact, the Forensic Scientist shall leave the peak labeled as "spike."

5.3.2.2.1.5 **Off-ladder Alleles:** Any allele designations as off-ladder (OL) peaks must be evaluated to determine if the peak is a true OL allele, an artifact, or a result of electrophoretic shifting.

5.3.2.2.1.6 **OL Allele within locus range:** Microvariants may be observed and designated "Z.X", where the Z is the lower rung of the allelic ladder bracketing the microvariant. The "X" identifies the allele as having a size between alleles Z and Z+1.

5.3.2.2.1.6.1 Using the nearest allelic ladder peak will enable the microvariant to be defined accurately. The microvariant will be designated as Z.1, Z.2, Z.3, Z.4, or Z.5 as applicable.

5.3.2.2.1.6.2 Verification of OL: For single instance OL, the sample having alleles falling outside of allelic ladder bins will be reinjected in the same run as an allelic ladder for verification whether the allele is a true microvariant or the original designation was incorrect due to migration issues during electrophoresis. Multi-occurrence OLs do not require reinjection. Note: Questioned and known

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

samples shall not be used for verification of OL alleles.

5.3.2.2.1.7 OL Allele located between two loci: 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.

5.3.2.2.1.7.1 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.

5.3.2.2.1.7.2 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.

5.3.2.2.1.7.3 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.

5.3.2.2.1.7.3.1 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.

5.3.2.2.1.7.3.2 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

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

no data. Both loci in question will be designated as inconclusive.

5.3.2.2.1.7.4 Verification of OL: For single instance OL, the sample having alleles falling outside of allelic ladder bins will be reinjected in the same run as an allelic ladder for verification of whether the allele is a true microvariant or if the original designation was incorrect due to migration issues during electrophoresis. Multi-occurrence OLs do not require reinjection. Note: Questioned and known samples shall not be used for verification of OL.

5.3.2.2.1.8 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 has been observed.

5.3.2.2.1.8.1 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.

5.3.2.2.1.9 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.

5.3.2.2.1.10 **Off-scale Samples:** Off-scale samples (consistently >15000 RFUs) shall not be used for comparison. Such samples shall be re-amplified with a lower concentration.

5.3.3 Printing Egrams Electronically

5.3.3.1 The egrams for any well in which data is collected (e.g., blank, ladder, item, etc.) shall be printed and the egrams for each GMID-X project shall exist as an individual PDF file. Ensure the plot setting is set to "Traditional Genotype Plot" when printing to PDF. The egrams for the questioned items in a case shall be printed prior to the known items.

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

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

5.3.3.2 Any individual sample/control/ladder with the case that is not used for comparison purposes (e.g., WEN failure, redundancy, further analysis) shall be added to the appropriate FA Case Record Object Repository. Such samples/controls/ladders shall appear in the Casework Table explaining why it was not used for comparison.

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

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

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

5.3.3.4 The WEN sizing standard shall be printed as a separate set of egrams and added to the overall egram PDF file for each project. The WEN must be present and labeled correctly for all samples/controls/ladders and all peaks (60 through 500) must be visible.

5.3.4   View Raw Data

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

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

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

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

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

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

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

5.4 Interpretation Guidelines for PowerPlex® Y23

5.4.1 After artifacts have been removed from consideration, the sample shall be evaluated to assess the quality of the data and status as a single-source or mixture sample. The entire profile must be taken into consideration when determining if the sample is a mixture.

5.4.2 Interpretation of all questioned items shall be performed prior to comparison to known profiles.

5.4.3 Samples with less than an optimal amount of template DNA may be affected by stochastic effects. It is possible in these samples that complete amplification of the sample has not occurred and not all alleles are being detected. Stutter may additionally influence the RFU values seen, especially in samples that contain a mixture.

5.4.4 Any and all information used in the interpretation of a sample shall be documented in the case file. This would include: determination of number of contributors, any assumptions based on expected contributors, ratio of contributors, and peak height ratios.

5.4.5 For a profile to be interpreted there must be a minimum of six loci with data above the analytical threshold.

5.4.6 Single Source Samples

5.4.6.1 A sample can be considered to be single source if only one allele is present at all loci except for DYS385 (which may have one or two alleles).

5.4.6.2 In rare occurrences two peaks may be detected at additional loci. This is due to gene duplication. In addition to duplications, triplications and deletions are known to occur. If this is believed to be the case, the sample will be re-amplified for confirmation, sample permitting.

5.4.6.3 The stochastic threshold for DYS385 is 650 rfu. A single peak below this threshold must not be designated as a homozygote for statistical calculations.

All copies of this document are uncontrolled when printed.

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

5.4.6.4 If two alleles are detected at locus DYS385, the peak height ratio between the two alleles should be ≥65% from a single contributor.

5.4.7   Mixed Samples

5.4.7.1   General

5.4.7.1.1 The entire profile must be taken into consideration when determining the number of contributors, to include: the presence of two or more alleles at two or more single copy loci in a sample (and/or more than two alleles at DYS385), a peak present in stutter position that is significantly above the typical stutter percentage, and imbalanced peak heights at DYS385. However, these factors do not by themselves necessitate the interpretation that the profile is a mixture.

5.4.7.1.2 Number of Contributors

5.4.7.1.2.1   A mixture of two males is expected to contain no more than 2 alleles per locus (up to 4 alleles at DYS385).

5.4.7.1.2.2   A mixture of three males is expected to contain no more than 3 alleles per locus (up to 6 alleles at DYS385).

5.4.7.1.2.3   Peak height variation across the profile may indicate a higher number of contributors than allele count alone.

5.4.7.1.3 For loci with possible allele sharing, the ratio of contributors (mixture ratio) from the entire profile must be considered in determining predominance.

5.4.7.1.4 Indistinguishable mixtures

5.4.7.1.4.1   Indistinguishable mixtures may be used for exclusion when allelic dropout is not likely.

5.4.7.1.4.2   A mixture profile must have a minimum of 10 loci demonstrating major and minor components to be classified as having a major single source contributor.

5.4.7.1.4.3   For 2 male mixtures where the presence of an intimate contributor may be assumed and there is no reasonable expectation of undetected data based on peak heights and allele number, the haplotype of the second contributor may be derived by deconvolution.

5.4.7.1.5 Minor Contributors - Minor contributors may be used for exclusion when allelic dropout is not likely and the number of contributors is stated. Since no

All copies of this document are uncontrolled when printed.

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

statistical interpretations can be made, no inclusions will be rendered for the minor contributors to a mixture unless deconvolution of the mixture shows an interpretable minor profile.

5.4.7.1.5.1 Inclusions to a minor contributor (of a two person mixture) may be rendered when there is no likelihood of dropout (utilizing a 650 RFU peak height for single alleles in the minor).

5.4.7.1.5.2 Peak height ratio should be ≤45% when 2 alleles are present at a single copy locus and ≤45% between major and minor contributors and the peak height ratio for the major contributor pair should be ≥75% for the multi-copy locus.

5.4.7.2 Mixture of Two Males

5.4.7.2.1 A minimum mixture ratio of 3:1 should be seen between contributors to determine if the profile contains a single major contributor (distinguishable mixture).

5.4.7.2.2 Single peaks at single-copy loci should be at a minimum of 650 RFUs to be considered for the interpretation of a major contributor.

5.4.7.2.3 Application of Peak Height Ratios to determine major contributor:

5.4.7.2.3.1 Single copy loci – Peak height ratio between the two males should be ≤45% when 2 alleles are present at a locus.

5.4.7.2.3.2 Multi-copy locus (DYS385) – Peak height ratio should be ≤ 45% between major and minor contributors and the peak height ratio for the major contributor pair should be ≥75%.

5.4.7.2.3.3 For samples amplified at lower template amounts (<0.5 ng), the minimum peak height ratio should be ≤30% (reduced from 45%). This increase in stringency is due to dropout considerations and possible stutter elevation seen in these profiles.

5.4.7.2.4 If a single major contributor cannot be determined (indistinguishable mixture) and a reference profile can be applied (elimination standard), then the profile may be deconvoluted in the following manner:

5.4.7.2.4.1 Loci with two alleles above 650 RFU: the allele not attributed to the elimination standard may be assigned to the unknown haplotype.

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

5.4.7.2.4.2   Loci with two alleles where only one is above 650 RFU and assigned to the elimination standard: the remaining allele may be assigned to the unknown haplotype.

5.4.7.2.4.3   Loci with one allele above 650 RFU: that allele may be assigned to the unknown haplotype.

5.4.7.2.4.4   Loci with one allele below 650 RFU: this locus is considered inconclusive with regard to the unknown haplotype if the allele can be assigned to the elimination standard.

5.4.7.3 Mixture of Three Males

5.4.7.3.1 A minimum mixture ratio of 5:1 should be seen between contributors to determine if the profile contains a single major contributor (distinguishable mixture).

5.4.7.3.2 If no single major contributor can be determined, then this mixture is deemed as indistinguishable and may be suitable only for exclusionary purposes.

5.4.7.3.3 Single peaks at single-copy loci should be at a minimum of 650 RFUs to be considered for the interpretation of a major contributor.

5.4.7.3.4 Peak Height Ratio

5.4.7.3.4.1   Single copy loci – Peak height ratio between the major and minor contributors should be $\leq 30\%$.

5.4.7.3.4.2   Multi-copy locus (DYS385) – Peak height ratio between the major and minor contributors should be $\leq 40\%$ and peak height ratio between the major pair should be $\geq 80\%$.

5.4.7.4 Mixture of More than Three Males

5.4.7.4.1 A clear major profile should be present to determine a major contributor. The possibility for allele sharing having an additive effect and causing a false major contributor must be considered with mixtures of more than three males.

5.4.7.4.2 Single peaks should be at a minimum of 650 RFUs to be considered for interpretation of the major contributor.

5.4.7.4.3 Peak Height Ratio

5.4.7.4.3.1   Single copy loci – Peak height ratio between the major and minor contributors should be $\leq 40\%$.

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

5.4.7.4.3.2 Multi-copy locus (DYS385) – Peak height ratio between the major and minor contributors should be ≤ 40% and peak height ratio between the major pair should be ≥ 80%.

5.4.7.4.4 Indistinguishable mixtures – When there is no indication of dropout, these profiles may be used for exclusionary purposes. If dropout is indicated, then the mixture is inconclusive overall.

5.5 Exporting and Creating Tables

5.5.1 Casework Table

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

5.5.1.2 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.

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

5.5.1.4 When the Text Import Wizard opens, select "Finish."

5.5.1.5 Adjust the columns to accommodate the full text for each line.

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

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

5.5.1.8 The Casework Table Worksheet shall exist independently of the egrams and the Allele Call Table Worksheet(s). Save/print as a .pdf file, merging it with the 3500 Setup table.

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

5.5.2 Allele Call Table

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

5.5.2.1 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.

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

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

5.5.2.4 When the Text Import Wizard opens, select "Finish."

5.5.2.5 Open the Allele Call Table Excel file in the Forensic Biology Shared Folder. Copy and paste the allele calls generated from the .txt file into the Results tab in the appropriate Allele Call Table Worksheet.

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

5.5.2.7 If minor alleles or profiles are to be marked, the Forensic Scientist shall indicate these with parentheses. Alleles that fall within the stochastic range (i.e., between the AT and the ST) shall have their cells highlighted. Minor alleles shall be marked if an analyst is calling that/those locus/loci predominant in a mixture. Analysts shall not mark minor alleles if predominance is not being reported.

5.5.2.8 If multiple injections are present in a case file due to re-extraction, re-amplification, or re-injection (non-serial in nature), the Forensic Scientist shall indicate from which run the sample came. Item descriptions shall also be typed into the designated areas in the worksheet. The Forensic Scientist shall ensure their initials are typed on each page of the worksheets, as well as the full case number and the date. All Allele Call Table Worksheets may be combined into one Excel spreadsheet.

5.5.2.9 Allele call tables for the question and known samples shall be generated (controls, ladders, and unlabeled/blank tables shall not be included) and included as separate .pdf files in the appropriate Case Record Object Repository.

5.6 Conclusions

5.6.1 Following the assessment of the data obtained and comparison of questioned profiles to known standards, the following are the four possible results:

5.6.1.1 **No results** – No peaks were detected on the electropherogram.

5.6.1.2 Inconclusive - Peaks were obtained at one or more loci; however, no conclusive result can be obtained. This may result when no statistical interpretation is able to be performed (e.g. mixture profile with indistinguishable contributors) or a limited amount of information is obtained from the profile (e.g. less than six loci with peaks above analytical threshold).

5.6.1.3 Exclusion - The profile of the known standard is not represented in the questioned sample. Any unknown profile will be compared to the Quality Control database.

5.6.1.4 Inclusion/Match – The profile of the questioned items has no explainable differences when compared to the known standard. This may be the result of a comparison of a single source profile, the major contributor, or the deconvoluted contributor of a mixture. At least ten loci must be eligible for statistical calculations to draw this conclusion.

5.7 Statistical Calculations

5.7.1 Once a questioned sample is compared to a known reference standard and an inclusion is made, the frequency estimation of the haplotype must be determined. No calculations are required for known standards that are included on an intimate sample. The basis for haplotype frequency estimation is the counting method. This frequency estimation would be the same for all paternally related males as to the standard that is included.

5.7.2 Statistical calculations shall be performed for single source and single major contributors with a minimum of 10 loci. For a single source profile with a single peak seen at DYS385 (below 650 rfu) "allele, any" will be utilized in the search of the database. No statistical calculations for Y-STR mixtures have been validated for use at this time.

5.7.3 A Y-STR haplotype frequency estimation is calculated from the observed haplotype frequency by attaching a confidence interval (generally 95% or greater) to the haplotype frequency estimate to capture the effect of the database size. The sampling variance decreases as the database size increases.

5.7.3.1 Haplotype Frequency (p) = x/N where x is the number of times the haplotype is observed in a database containing N number of haplotypes.

5.7.3.2 If the haplotype has not been previously observed in the database, the formula for calculating the upper 95% confident limit is: $1 - (0.05)^{1/n}$ where n = database size.

5.7.3.3 If the haplotype has been observed in the database, the formulae used for calculating the 95% confidence limit is that of an exact confidence interval, utilizing the formulae as described by Clopper and Pearson.

5.7.4 The consolidated U.S. YSTR Database shall be utilized for haplotype frequencies (http://usystrdatabase.org) unless a population other than that provided in the U.S. YSTR

Database is required. Then the Y-Chromosome Haplotype Reference Database (YHRD) may be used (https://yhrd.org). A list of other Y-STR haplotype databases can be found at http://cstl.nist.gov/biotech/strbase/y_strs.htm and utilized as necessary.

5.7.5 Entry of the haplotype profiles into the search must be performed per the User Directions provided on the www.usystrdatabase.org website; frequency and match estimates using the confidence interval are calculated by the website as described in the User Directions.

5.7.6 The populations searched must be selected for all, and results will be reported for Caucasians, African Americans, and Hispanics.

5.7.7 The statistic which provides the most genetic information and/or the highest discrimination potential must be reported. This "either/or" scenario comes about due to databases containing different population sizes for different loci, as they are comprised of data from different multiplexes (for example, more population data exists for loci shared between the Applied Biosystems AmpF*l*STR® Yfiler® amplification kit and the Promega PowerPlex® Y23 than for the loci contained exclusively within the PowerPlex® Y23 kits). (As recommended by SWGDAM YSTR Committee_2014)

5.7.8 The statistical calculations must be reported to the same significant figure as displayed on the results sheet.

5.7.9 The statistic results sheet for both searches shall be printed out for each sample and saved as a .pdf file and imported into the Case Record OR.

5.8 Reporting

5.8.1 **Introductory Paragraph** – The following wording shall be added as applicable to the report.

YSTR DNA testing was performed on the extracts from Items _____ as well as on the extract(s) from the known DNA standard from _____ (Item _____). These extracts were previously quantitated, and then amplified, and tested with YSTR DNA genetic markers DYS576, DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385a/b, DYS456 and Y-GATA-H4.

And/or

DNA extractions were performed on Items _____ as well as on the known DNA standard from _____ (Item _____). These extracts were then quantitated, amplified, and tested with YSTR DNA genetic markers DYS576, DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385a/b, DYS456 and Y-GATA-H4.

And/or

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

DNA extractions were performed on Items _____ as well as on the known DNA standard from _____ (Item _____). These extracts were then quantitated, amplified, and tested with <u>nuclear and </u>YSTR DNA genetic markers DYS576, DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385a/b, DYS456 and Y-GATA-H4.

5.8.2   Single Source Profiles

5.8.2.1 Match - If the YSTR DNA profile from a known sample is included in the single source questioned sample, then the results shall be reported as follows:

The YSTR DNA profile obtained from ____ (Item __) matches the YSTR DNA profile obtained from ___ (Item ___).

If the sample is a partial profile, then the qualifying word partial shall be added to the above statement and matches will be reported as consistent with.

5.8.2.2 Exclusion – If the YSTR DNA profile from a known sample is excluded as being a contributor, then the results shall be reported as:

The YSTR DNA profile(s) obtained from ___ (Item __) is/are excluded as contributors to the YSTR DNA profile obtained from _____ (Item __).

5.8.3   Mixture Profiles

5.8.3.1 Primary Statement – For profiles containing a mixture of multiple males, the following statement shall be reported.

The YSTR DNA profile obtained from ___ (Item __) is consistent with a mixture from # male contributors.

5.8.3.2 Major – When a major contributor can be ascertained from a mixture, the following statement shall be reported.

The YSTR DNA profile obtained from ___ (Item __) matches the major YSTR DNA profile of this mixture.

5.8.3.3 Minor – For profiles where the minor profile can be derived from a mixture the following statement shall be reported.

The YSTR DNA profile obtained from ___ (Item __) matches the minor profile of this mixture.

Procedure for Y23 Interpretation
Forensic Biology Section
Issued by Forensic Biology Forensic Scientist Manager and DNA Technical Leader

Version 1
Effective Date: 12/20/2016

5.8.3.4 If the owner of the intimate sample is used to derive the remaining contributor of the mixture, the following statement shall be reported.

Assuming the YSTR DNA profile obtained from _____ (Item ___) as a contributor to the mixture obtained from _____ (Item __), the remaining component of the mixture matches the YSTR DNA profile obtained from _____ (Item __).

5.8.4 Inconclusive – For YSTR DNA profiles (or components of the profile) obtained whose results are not suitable for comparisons, the following statement shall be reported:

The YSTR DNA profile obtained from ___ (Item __) is inconclusive due to insufficient quality and/or quantity.

5.8.5 No DNA result – If no YSTR DNA was obtained, the result shall be reported as:

No YSTR DNA profile was obtained from ___ (Item __).

5.8.6 Statistics -The result of the database search, including the number of matches and the 95% upper confidence intervals shall be documented in the report. The results shall be reported as follows:

The YSTR DNA profile developed from Item _ matches the YSTR DNA profile developed from __ (Item _). Therefore, (name) and any male paternal relatives cannot be excluded as potential DNA donors to this sample. Utilizing the consolidated U.S. YSTR Database, this YSTR profile has been observed as follows:

_ in ___ Caucasian males (Applying the 95% upper confidence interval results in approximately 1 in every ___ male individuals.),

_ in __ African American males (Applying the 95% upper confidence interval results in approximately 1 in every ___ male individuals.), and

_ in ___ Hispanic males (Applying the 95% upper confidence interval results in approximately 1 in every ___ male individuals.).

If a match is made to the owner of an intimate sample, then no search of the database is required, and the following statement will be used.

No search of the YSTR database was performed for Item ___.

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

7.0 Safety - N/A

8.0 References

All copies of this document are uncontrolled when printed.

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

Promega PowerPlex® Y23 PCR Amplification Kit User's Manual.

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

NC SCL Validation Studies of Promega PowerPlex® Y23 Amplification Kit

US YSTR Database

Clopper, C. and Pearson, E. *The use of confidence or fiducial limits illustrated in the case of binomial,* Biometrika (1934) 26:404-413.

SWGDAM Y-STR Interpretation Guidelines_approved 01/09/2014

9.0 Records

- Promega PowerPlex® Y23 Plus Allelic Ladder Expected Results
- PowerPlex® Y23 2800M Expected Results

10.0 Attachments - N/A

Revision History		
Effective Date	Version Number	Reason
12/20/2016	1	Original Document