District of Columbia Department of Forensic Sciences

FBS39 – GlobalFiler™ Data Analysis Using GeneMapper™ *ID-X* 1.6

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1. Scope

1.1.   This method describes the process by which amplified DNA fragment genotypes are established using the Applied Biosystems GeneMapper™ *ID-X* Software (GMID-X) version 1.6.

2. Background

2.1.   The GlobalFiler™ PCR Amplification Kit is a 6-dye multiplex polymerase chain reaction system that facilitates the identification and interpretation of specific short tandem repeat regions in human DNA. A multiplex PCR amplification procedure is followed by a size separation of the individual amplified fragments

via capillary electrophoresis (CE). An internal size standard is included within the mixture that is used to prepare the amplicons for electrophoresis. This internal standard in conjunction with the simultaneous electrophoresis of allelic ladders allows for reliable genotyping of the controls and forensic samples by GMID-X.

3. Safety

3.1. Not applicable

4. Materials Required

4.1. GeneMapper™ *ID-X* Software, version 1.6

4.2. Windows-based computer capable of running the software

5. Standards and Controls

5.1. GlobalFiler™ Loci

Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	DNA Control 007
D3S1358	3p21.31	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20	6-FAM™	15, 16
vWA	12p13.31	11,12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24		14, 16
D16S539	16q24.1	5, 8, 9, 10, 11, 12,13, 14, 15		9, 10
CSF1PO	5q33.3-34	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		11, 12
TPOX	2p23-2per	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15		8, 8
Y indel	Yq11.221	1, 2	VIC™	2
Amelogenin	X: p22.1-22.3 Y: p11.2	X, Y		X, Y
D8S1179	8q24.13	5, 6, 7, 8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 19		12, 13
D21S11	21q11.2-q21	24, 24.2, 25, 26, 27, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 37, 38		28, 31
D18S51	18q21.33	7, 9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27		12, 15
DYS391	Yq11.21	7, 8, 9, 10, 11, 12, 13		11
D2S441	2p14	8, 9, 10, 11, 11.3, 12, 13, 14, 15, 16, 17	NED™	14, 15
D19S433	19q12	6, 7, 8, 9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, 19.2		14, 15
TH01	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3		7, 9.3
FGA	4q28	13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2		24, 26

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Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	DNA Control 007
D22S1045	22q12.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	TAZ™	11, 16
D5S818	5q21-31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18		11, 11
D13S317	13q22-31	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16		11, 11
D7S820	7q11.21-22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		7, 12
SE33	6q14	4.2, 6.3, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20.2, 21, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 27.2, 28.2, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35, 35.2, 36, 37		17, 25.2
D10S1248	10q26.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	SID™	12, 15
D1S1656	1q42.2	9, 10, 11, 12, 13, 14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18.3, 19.3, 20.3		13, 16
D12S391	12p13.2	14, 15, 16, 17, 18, 19, 19.3, 20, 21, 22, 23, 24, 25, 26, 27		18, 19
D2S1338	2q35	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28		20, 23

5.2. **Analytical Controls** – A variety of controls are required to assess the quantity and quality of each sample as well as the effectiveness, accuracy and precision of the analytical procedures. Evaluation of controls is essential to the proper interpretation of the data.

5.2.1. **GeneScan™ 600 LIZ™ Size Standard (ILS)** – This standard contains DNA fragments of known sizes (20, 40, 60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, 460, 480, 500, 514, 520, 540, 560, 580 and 600) that are used in-lane to estimate sizes (base pair (bp)) of STR products.

5.2.1.1. Each sample tested must have the correct peak sizes assigned for its in-lane size standard before proceeding further with interpretation.

5.2.1.2. Samples requiring interpretation with failed ILS/insufficient ILS peak heights must be re-injected or re-run to obtain a suitable result. Documentation of re-injection or re-run will be made on the appropriate pages in the case file.

5.2.1.3. When the ILS in a corresponding reagent blank or negative control meets requirements, the reagent blank or negative does not have to be re-injected or re-run.

5.2.2. **Allelic Ladder(s)** – the allelic ladder provided in the kit is used to ascertain the genotypes of the samples. While the ladder consists of the

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common alleles in the population for each locus, additional atypical alleles may also be included.

5.2.2.1.   Sample profiles require at least one valid/passing allelic ladder in a run to be considered for interpretation. All passing ladders used in a run that pertain to a case (i.e. are in injections with case samples and/or controls) should be included in the case file or core binder.

5.2.2.2.   Allelic ladders missing any alleles, having off-ladder alleles, insufficient peak heights or are consistent with a poor injection will be documented on the appropriate page(s) in the case file or core binder. The injection may be reanalyzed using another/other ladder(s) within the plate.

5.2.2.3.   In the event all ladders in the run fail or no ladder in the run meets the migration criteria, all samples must be re-injected or re-run. Documentation of re-injection or re-run will be made on the appropriate page(s) in the case file and core binder.

5.2.3. Negative Controls

5.2.3.1.   Reagent Blank (extraction control): The reagent blank is a check for possible contamination of the extraction reagents by human DNA or by amplified STR product. The reagent blank is created by carrying out the DNA extraction in a tube containing no sample. This "blank" extract is then processed in the same manner as the corresponding sample(s) through PCR amplification and run along with the test samples.

5.2.3.1.1. The appearance of typeable test results in the reagent blank may indicate that a sample preparation reagent(s) has/have been contaminated, cross-contamination between samples occurred during testing, or human DNA or STR product got into a sample(s) from some other source.

5.2.3.1.2. When a reagent blank performs appropriately and passes analysis, but a corresponding sample(s) needs to be re-injected or re-run, the reagent blank does not have to be re-injected or re-run.

5.2.3.1.3. If a sample needs re-amplification, the reagent blank does not need to be re-amplified unless its prior amplification concentration(s) no longer meets or exceeds the sample's new amplification concentration.

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5.2.3.2.   Negative Amplification Control: The negative amplification control is a check for contamination during set up of the PCR amplification reaction. It essentially monitors the "environment" in that process for possible sources of contamination.

5.2.3.2.1. If typing results reproducibly occur in this control, then the PCR reaction set up area, equipment and amplification reagents should be considered possible sources of contamination.

5.2.3.2.2. When a negative amplification control performs appropriately and passes analysis, but a corresponding sample(s) needs to be re-injected or re-run, the negative amplification control does not have to be re-injected or re-run.

5.2.3.3.   Any typeable peak (a reproducible peak above the analytical threshold) appearing in negative control samples (reagent blank or negative amplification control) in the defined analysis range of 60 – 460bp that is not attributable to an artifact, will be resolved as listed below. The ultimate determination regarding repeated testing or use of the control/affected sample results will be determined by the Technical Leader (or designee). All corresponding electropherograms and appropriate worksheets will be documented appropriately. Listed below are possible steps for resolution of typeable peaks in a sample.

5.2.3.3.1. Typeable peaks occurring in a negative control may indicate a critical contamination problem. When possible, steps must be taken to attempt to determine the source of contamination before further testing is conducted. When necessary, at the direction of the Technical Leader, all testing in the Unit will cease and steps will be taken to ensure the contamination event has been contained/resolved.

5.2.3.3.2. The appearance of a peak(s) in a negative control does not necessarily mean that the types obtained from the test samples are incorrect. The contamination might be due to a single event limited to the control. Alternatively, even if a reagent used to prepare all the samples was contaminated, the level of contamination might be inconsequential compared to the amount of DNA being amplified and typed in the test samples. It is noted, however, that further testing may be necessary to support these hypotheses.

5.2.3.3.3. The analyst will ensure the Technical Leader (or designee) has been apprised of the contamination event as soon as practicable and initiate an evaluation to determine the source of the contamination. The analyst will ensure the Technical Leader/designee remains apprised of the status of the contamination event evaluation until such a time as the matter has been resolved. The initial evaluative step should include a determination regarding whether a sample/tube switch may have occurred or whether DNA from a sample in the run may have been inadvertently introduced into the negative control resulting in the contamination event. This assessment will help to inform whether cessation of testing in the Unit needs to be considered.

5.2.3.3.4. If possible, the samples associated with the contaminated reagent blank or negative amplification control should be reanalyzed. Reanalysis should occur unless there are consumption issues. This reanalysis may initially consist of re-injecting or re-running all associated samples, followed by re-amplifying and/or re-extracting all associated samples, as needed and when possible. Results of the reanalysis will be used to determine if the contamination was most likely isolated or may be have been due to a contaminated reagent, consumable, work surface, or piece of equipment.

5.2.3.3.5. When re-injection, re-running, and/or re-amplification have not resolved the situation, and it is not possible to re-extract a sample associated with a contaminated reagent blank or negative amplification control, consult with the Technical Leader.

5.2.3.4. The raw data for all negative controls, including those with no signs of typeable peaks, must be examined for the presence of primer peaks to ensure the samples were properly amplified.

5.2.4. **Positive Amplification Control (DNA Control 007)** – the positive amplification control is a human male DNA sample with a known autosomal STR genotype (see Section 5.1 above) included in the GlobalFiler™ PCR Amplification Kit. The positive amplification control ensures that the amplification process is working properly.

5.2.4.1. Each amplification plate will include at least one positive control.

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5.2.4.2. Sample profiles require at least one valid/passing positive control on an amplification plate to be considered for interpretation. A passing positive control gives the proper typing results at all loci. See Appendix 14.2. All positive control(s) on a plate/run that pertain to a case should be included in the case file or core binder.

5.2.4.3. In the event that a positive control in the run fails or no positive control in the run gives the proper typing results at all loci, it may be re-injected or re-run when the analyst determines that doing so will be beneficial. Documentation of re-injection or re-run will be made on the appropriate page(s) in the case file and/or core binder.

5.2.4.4. If the positive control still fails, the entire batch of samples must be re-amplified. When it is not possible to re-amplify a corresponding sample(s), the sample(s) should be re-extracted whenever possible.

5.2.4.5. If it is not possible to re-amplify or re-extract a sample associated with the positive control, the original typing results may not be used.

6. Calibration

6.1. Not applicable

7. Procedures

7.1. Separation of Evidence and Reference Samples

7.1.1. Prior to analyzing a project, reference samples must be either run separately from evidence samples or if run together, they must be separated out into two separate run folders.

7.2. Overview of 1st and 2nd Read Responsibilities

7.2.1. 1st Reader:

7.2.1.1. Perform initial analysis with stutter filter on for all samples.

7.2.1.2. Confirm the Internal Lane Size Standards.

7.2.1.3. Evaluate the following controls: allelic ladders, positive controls, negative amplification controls and reagent blanks.

7.2.1.4. Edit samples as necessary.

7.2.1.5. Print and initial the electropherograms with stutter filters on.

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7.2.1.6. Save and export the GMID-X project (.ser) to the appropriate folder on the network.

7.2.1.7. Analyze samples with stutter filters off ensuring that the same edits are made as the first analysis, save as "Project Name_STRmix" and export the GMID-X project (.ser) and the STRmix™ table to the appropriate folder on the network.

7.2.1.8. Document (date/initial) the completion of the 1st Reader analysis review in the designated location on the Sample Tracking and Control Solutions (STACS) 3500 batch documentation.

7.2.2. 2nd Reader:

7.2.2.1. Analyze all samples with the stutter filter on.

7.2.2.2. Confirm the Internal Lane Size Standards.

7.2.2.3. Evaluate the following controls: allelic ladders, positive controls, negative amplification controls and reagent blanks.

7.2.2.4. Evaluate samples and make edits if necessary.

7.2.2.5. Resolve any discrepancies between the 1st Reader and 2nd Reader.

NOTE: Discrepancies may be resolved by handwritten corrections on the electropherograms; however, all edits must also be made by the 1st Reader in the "stutter filter on" and "stutter filter off" projects (.ser files).

7.2.2.6. Initial the electropherograms printed by the 1st read.

7.2.2.7. Document (date/initial) the completion of the 2nd Reader analysis verification in the designated location on the STACS 3500 batch documentation.

7.2.2.8. Add control electropherograms (ladders, positives, negatives, reagent blanks) to the appropriate section of the core binder or to the case file (e.g., for proficiency tests and single cases not in a batch).

7.3. Starting a New Project:

7.3.1. Open GMID-X by double clicking on the GMID-X icon located on the desktop. At the prompt, make sure that the appropriate username and password are entered.

7.3.2. From the main menu, select **Edit > Add Samples to Project**.

7.3.3. In the pop-up window, highlight the appropriate run folder or sample files.

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7.3.4. Select the "**Add to List**" button.

7.3.5. The folder will move to the right-hand column under "Samples to Add." Repeat this action for any additional folder(s) or samples you wish to combine into one project.

7.3.6. After the folder moves, select the "**Add**" button. The project will automatically be populated with the chosen samples and a new screen will appear.

7.3.6.1. Ensure proper table setting is displayed (3500 Data Analysis).

7.3.7. Ensure the appropriate Sample Type for each sample is selected: Sample, Positive Control, Allelic Ladder or Negative Control.

7.3.8. Select the "**GlobalFiler_v1.6**" Analysis Method to analyze all samples.

7.3.9. Select the appropriate Panel.

7.3.9.1. For each sample select "**Panels**" and then select "**GlobalFiler_Panel_DFS**".

7.3.9.2. After initial analysis with stutter filters on, the project will be re-analyzed using the "**Globalfiler_Panel_DFS_strmix**". See step 7.8 for further instruction.

7.3.9.3. For each sample select the appropriate Size Standard: "**GS600_LIZ_(60-460)"** size standard.

7.4. Beginning Analysis

7.4.1. Select the green triangle button in the toolbar to begin analysis.

7.4.2. Save Project will appear. Enter the project name.

7.4.2.1. Project name will be saved as "RUN-YYMMDD-XX" (e.g. RUN-160606-07). Add "_Q" or "_K" when applicable and initials of the analyst performing the 1st Read.

7.4.2.2. Select "**OK**".

7.4.2.3. At completion of the software analysis an Analysis Summary window will appear.

7.4.2.4. **NOTE**: The Analysis Summary is an assessment of the quality checks of the samples in the project. Each sample will be reviewed by the 1st and 2nd readers during examination of the data.

7.5. Genotyping Quality Labels:

7.5.1. GMID-X includes a series of flags to alert an analyst that there may be problems with the sample data. The flags will display green if the sample data has passed all quality checks, yellow if the sample data needs to be

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evaluated further, and red if the sample data is low quality. For a list of abbreviations, please see page 41 of the GeneMapper® *ID-X* Software Version 1.5 Reference Guide.

7.6.   Viewing the Raw Data:

7.6.1. In the main analysis screen, the column to the left will contain the run folder.

7.6.2. Open the run folder by double-clicking on it. All the sample files will appear below.

7.6.3. Click on a sample file to open it. Depending on which tab is selected, the sample information, raw data, or EPT data will appear on the screen to the right.

7.6.4. Select the "**Raw Data**" tab to verify that the baseline is stable, the peak heights are reasonable in the raw data and all primer peaks are present.

7.7.   Examining and Editing Results:

7.7.1. Examining the Size Standard:

7.7.1.1.   Select Edit > Select All.

7.7.1.2.   Select **Tools > Size Match Editor** or select the Size Match Editor icon in the toolbar.

7.7.1.3.   The Size Match Editor window will open displaying the size standard of each sample well individually.

7.7.1.4.   Evaluate each sample by clicking the sample name on the left and view the labeled size standard peaks displayed on the right. The 60bp – 460bp peaks will be observed (see Appendix section 14.1). All peaks should display good peak morphology/height and be labeled appropriately. (Note the sizing quality value assigned to each sample well. If the value is lower than 0.75, attention should be given to the quality of the injection.)

7.7.1.5.   If the sizing quality value is lower than 0.75 and is still considered suitable for further analysis, select **Override SQ**. Alternately, all size quality checks can be overridden at once by selecting **Edit > Override all SQ**, when finished hit **OK**. If a size quality check is overridden, a note should be made on the sample's electropherogram.

7.7.1.6.   When sizing quality is low the size match editor can be used to adjust the size standard. Typical problems include a peak shift, missing or extra peak(s). To edit a particular size standard peak, conduct the following:

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7.7.1.6.1. Click the peak to be edited

7.7.1.6.2. Right click the peak to open the editing pop-up window and choose to add, delete or change the selected peak.

7.7.1.6.3. If adding or changing a peak, move the cursor to the right to open the select size sub-menu. Choose the desired value from this menu. **Tools > Check Sizing Quality**. If the peaks are sized correctly after the edit but the quality score is still below passing, click the override SQ button to set the SQ to 1.0.

7.7.1.6.4. If deleting a label from a peak, select **Tools > Auto Adjust Sizes** to adjust the size of other peaks automatically to the right of the selected peak. To delete all labels and manually assign peak values select **Edit > Delete** all Size Labels.

7.7.1.6.5. Click **Apply** if editing a second size standard peak or click **OK** if finished.

7.7.1.6.6. Exit out of the Size Match Editor window.

7.7.2. Viewing and Printing Analyzed Samples:

7.7.2.1. In the samples tab highlight the sample(s) to be viewed. Begin by assessing the ladder(s), followed by controls and then samples.

7.7.2.2. View > Display Plots or click the Display Plots button.

7.7.2.3. In the Plot Settings drop down menu, select **Casework Genotype VIEW ONLY**.

7.7.2.4. Select and evaluate that the Ladders are labeled with correct allele designation (see Appendix section 14.3).

7.7.2.5. Select and evaluate that the Positive controls contain the appropriate allele calls (see Appendix section 14.2).

7.7.2.6. Select and evaluate that the Negative amplification controls and reagent blanks are free of contamination.

7.7.2.7. **NOTE**: To edit Reagent Blanks, Positive controls and Negative controls for artifacts, see section 7.7.3.

7.7.2.8. Assess each sample using FBS31- GlobalFiler™ Interpretation. The following features will be used to aid in the process:

7.7.2.8.1. View sample in sample plot window (all dye channels).

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7.7.2.8.2. **OPTIONAL**- View peaks one dye at a time beginning with blue. Click the all dye channel icon in the toolbar to deselect all other dye channels. Then select the blue dye icon in the tool bar. Click through each sample to assess the size call with the corresponding peak on the samples plot. Repeat for each color. Multiple dye channels will need to be displayed simultaneously to determine if non-characteristic peak(s) (i.e. a spike or pull-up) are present. To view all dye channels, select the all dye channels icon in the toolbar.

7.7.2.8.3. The y-axis can be magnified to assess the baseline of all peaks for a thorough analysis.

7.7.2.9. Evaluate all samples for correct allele calls and remove all artifacts. To edit a sample, see section 7.7.3.

7.7.2.10. Ensure that the "Casework Genotype PRINT" plot setting is selected and print the Genotypes plot for each sample using "Ctrl" + "P" or the following instructions:

7.7.2.10.1. Within the samples tab of the main project window, select the sample in the table you want to display in the printouts.

7.7.2.10.2. Select View > Display Plots.

7.7.2.10.3. Select **Casework Genotype PRINT** from the Plot Setting drop-down list.

7.7.2.10.4. **NOTE**: You have the option to view the electronic data using the Casework Genotype VIEW ONLY. This plot setting will not be used for printing.

7.7.2.10.5. Select print icon in tool bar.

7.7.2.10.6. Print out each casework sample separately. Controls may be grouped and printed.

7.7.2.10.7. Close the Samples Plot window and return to the Project window.

7.7.2.11. Select **File > Save Project** to save all changes.

7.7.2.12. Export the project (see section 7.10).

7.7.3. Making Changes to Alleles/Artifacts:

7.7.3.1. Edits to the sample can be made on GMID-X plots (e.g. pull-up/spikes/baseline problems/OMR (outside marker range)).

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See FBS31- GlobalFiler™ Interpretation for identification of artifacts.

7.7.3.2. Changes to alleles/artifacts can be made by highlighting the allele in the plots window and right clicking. Select "**Delete Allele**" or "**Rename Allele**". Include a reason for the change. Select **OK**.

7.7.3.3. If edits are made to the sample, before printing, make sure that the edits are displayed (i.e. make sure Show Allele Changes in the tool bar is selected).

7.7.3.4. Alleles not found in the ladder do exist. These off-ladder alleles may contain full and/or partial repeat units. An off-ladder allele is defined as an allele falling outside of the $\pm$ 0.5 bp window of any known allelic ladder allele or virtual bin. If a sample allele peak is called an off-ladder allele, then the sample needs to be re-injected or re-amplified to verify the result. See FBS31-GlobalFiler™ Interpretation, Section 6.2.1.2.3 for examples of when the sample does not need to be re-injected or re-amplified.

7.7.3.5. The designation of alleles containing an incomplete repeat unit, falling within the range spanned by the ladder alleles, includes the number of complete repeats, a decimal point, then the number of base pairs in the incomplete repeat (e.g. 9.3 for a TH01 allele with 9 full repeats plus three more bps). If no bin is designated by the software, the determination of the number of additional bp(s) present in a microvariant is made by comparing the bp size of the off-ladder peak with the bp size of the flanking alleles in the allelic ladder.

7.7.3.6. When an allele is seen above the largest or below the smallest allele of a locus's allelic ladder, the allele will be designated as either > or < the respective ladder allele (e.g., an allele which migrates below the D3S1358 12 allele will be designated as D3S1358 <12) on the electropherogram print-out. For STRmix™ purposes, the allele must be renamed a numerical value in the GeneMapper® ID-X software during re-analysis and the locus must be ignored during STRmix™ analysis.

NOTE: The designation of the off-ladder allele can also be made using the GeneMapper® ID-X software prior to printing out the electropherogram. Refer to section 7.7.3.2 for information on how to edit peaks.

7.8. Re-analyzing data for STRmix™ Input:

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7.8.1. This is applicable for evidence samples and associated ladders only. **NOTE**: Reference samples do not require re-analysis.

7.8.2. After initial analysis with stutter filters on, the project will be re-analyzed using the "**Globalfiler_STRmix_v1.6**" analysis method.

    7.8.2.1. For each sample select "Panels" and then select "**GlobalFiler_Panel_DFS_strmix**".

    7.8.2.2. Click **Ok.**

    7.8.2.3. Select the green triangle button in the toolbar to begin re-analysis.

    7.8.2.4. Re-edit all artifact peaks, if applicable. Refer to section 7.7.3 for information regarding editing artifact peaks. Ensure that all N-1/N+1 stutter peaks remain.

    7.8.2.5. The reporting analyst will print the electropherogram for samples that are to be entered into STRmix with stutter filters off.

7.9. Exporting the Table for STRmix™:

7.9.1. In the main window, in the Table Setting dropdown of the tool bar choose **STRMix Input v1.6**.

7.9.2. Select the Genotypes tab.

7.9.3. Select File > Export Combined Table.

7.9.4. Export File will remain as a Tab-delimited text (.txt).

7.9.5. One line per marker will be chosen in the merge section.

7.9.6. The "Included all marker information" box should be checked in the merge section.

7.9.7. Ensure File name is correct

7.9.8. Files of type will remain as Tab-delimited text (.txt).

7.9.9. Navigate to where the file should be saved

    7.9.9.1. Select Export

7.10. Exporting the Project:

7.10.1. Click on Tools > GeneMapper® ID-X Manager.

7.10.2. Select (highlight) project to export.

7.10.3. Select **Export**.

7.10.4. Save to an appropriate location.

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7.10.4.1. Note: Separate projects must be saved after initial analysis and again after STRmix reanalysis.

7.11. Report Manager:

7.11.1. **OPTIONAL** – To aid with completion of the FBU Database Entry Form (Document Control Number: 3880), a report table may be generated using the Report Manager:

7.11.1.1. In the Project window, select View > Samples.

7.11.1.2. Select the rows in the Samples table to report.

7.11.1.3. Click the Report Manager icon.

7.11.1.4. Select Edit > View Table by Marker.

7.11.1.5. Copy/Paste sample profiles to the appropriate cells in the FBU Database Entry form. Edit the profiles as needed for CODIS and/or FBU QA database entry (See FBQ45). For homozygous loci, the alleles will need to be manually edited to duplicate the allele.

8. Sampling

8.1. Not applicable

9. Calculations

9.1. Not applicable

10. Uncertainty of Measurement

10.1. Not applicable

11. Limitations

11.1. HID analysis requires the presence of at least one allelic ladder per project. When multiple ladders are present in a run folder the average of all ladders is used to calculate allelic bin offsets.   It is recommended to include at least one ladder per injection on the 3500xL and one ladder per 3 injections on the 3500.

11.2. Allelic ladder samples must be identified as "allelic ladder" in the sample type column in a project. Failure to do so will result in failed analysis.

11.3. Allelic bin definitions are stored in the panel manager.

11.4. Injections containing the allelic ladder should be analyzed with the same analysis method and parameters used for all other samples.

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11.5. Any GMID-X project can be thought of as a program shortcut, meaning that the sample data files (*.hid) are stored as a link to that project. If the sample data files are moved or the folder containing those files is renamed, GMID-X will not be able to locate the files for data processing.

12. Documentation

12.1. Applicable STACS documentation

12.2. Electropherograms

13. References

13.1. GeneMapper® *ID-X* Software Version 1.5 Reference Guide

13.2. Applied Biosystems GlobalFiler™ PCR Amplification Kit User's Manual

13.3. GlobalFiler™ Interpretation (FBS31)

13.4. GlobalFiler™ Data Analysis Using STRmix™ (FBS32)

13.5. Use and Maintenance of the FBU Quality Assurance DNA Database (Version 2.1) (FBQ45)

13.6. GeneMapper™ ID-X Software v1.6 User Bulletin

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14. Appendix

14.1. GeneScan™ 600 LIZ® Internal Lane Size Standard

14.2. GlobalFiler™ DNA Positive Control (007)

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14.3. GlobalFiler™ Ladder

14.4. Validated Analysis Method Settings - GlobalFiler_v1.6

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14.5. Validated Analysis Method Settings - Globalfiler_STRmix_v1.6

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