18.0 Procedure for using GeneMapper ID-X Analysis Software Version 1.4 as an Expert System for Analysis of Convicted Offender, Arrestee and/or Known DNA Reference Samples

SCOPE

This procedure describes the methods by which GeneMapper ID-X, v1.4 may be used as an expert system for the analysis of convicted offender, arrestee and/or known DNA reference samples that have been amplified using the Globalfiler DNA amplification kit and separated using the AB 3500 or 3500xl Genetic Analyzer. Samples for which this expert system may be used are limited to **samples worked at TBI or by Bode Technology**. No other vendor laboratory sourced data may be analyzed using these expert system settings. Please note that the Expert System settings in GeneMapper ID-X are to be used ONLY for review of convicted offender, arrestee and/or known reference DNA samples. These settings may not be applied to unknown DNA samples. For unknown DNA samples, refer to *Forensic Biology STR Typing Manual, section 9*, regarding the use of GeneMapper ID-X, v1.4 for the analysis of AB 3500 data for Globalfiler or Yfiler Plus.

18.1 Starting the GeneMapper ID-X Software

1. Click on the GeneMapper ID-X icon located on the desktop or select **Start>All Programs>Applied Biosystems>GeneMapper ID-X**.

2. Login using the appropriate username and password.

18.2 Creating a Project

1. Add samples to the project either by going to **Edit>Add Samples to Project** or by selecting Ctrl+K.

2. Select the location of your data runs. Highlight plate name or data folder and click **Add to List>>**. The data from the plate or run folder will be added to the right hand side. Next click **Add** on the bottom right of the screen.

3. Once the samples have been imported, edit and fill down the following columns as follows:

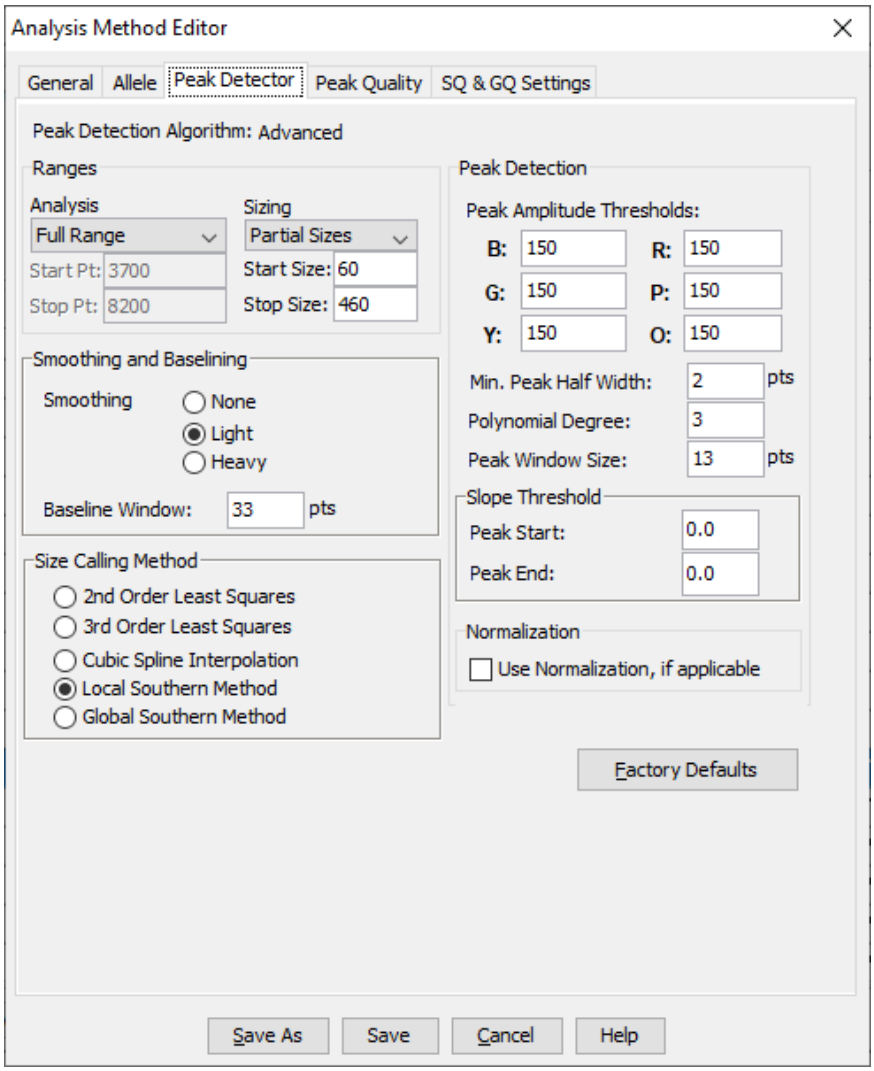
Analysis Method:	GF Expert Settings
Panel:	Expert_System_GlobalFiler_Panels_v1
Size Standard:	GS600_LIZ_(60-460)
Sample Type:	Label Allelic Ladder, Positive and Negative Control appropriately

The columns may be filled down by highlighting the column name, then clicking **Edit>Fill Down** or Ctrl+D to fill in the information for each sample.

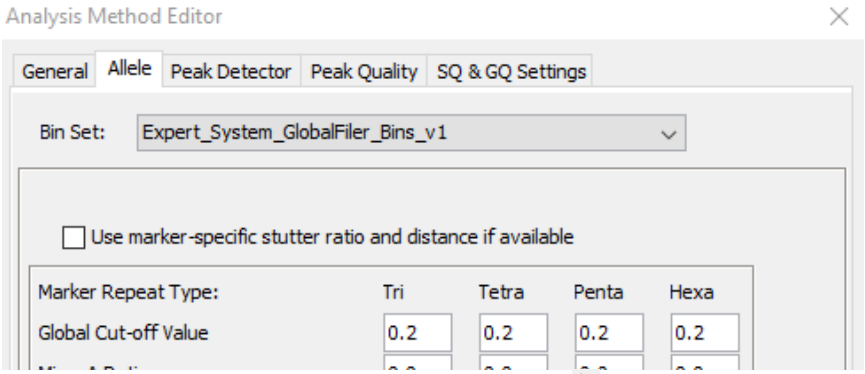
Note: The expert settings are for use with TBI sourced data and any first pass Bode data. Any further processed or reworked samples shall be manually reviewed for acceptance or rejection.

For all dye channels, the thresholds for analysis are set at 150 RFU.

The screenshot below provides the appropriate settings for the expert system use in conjunction with the Globalfiler amplification kit:



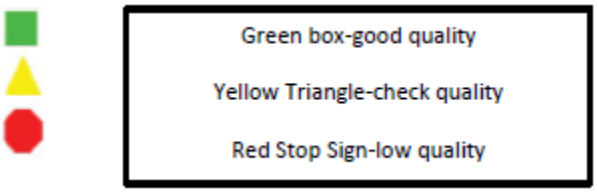
The marker specific stutter filter for GlobalFiler will be unchecked and a global 20% stutter filter applied as shown below.



4. On the toolbar, press the green arrow key to analyze the samples. A new window will open requesting the GeneMapper ID-X project to be named and saved. Analysis automatically begins after saving the project.

Note: The name given at this step will be displayed on all printouts. The project name may incorporate the analyst's initials, but is not required.

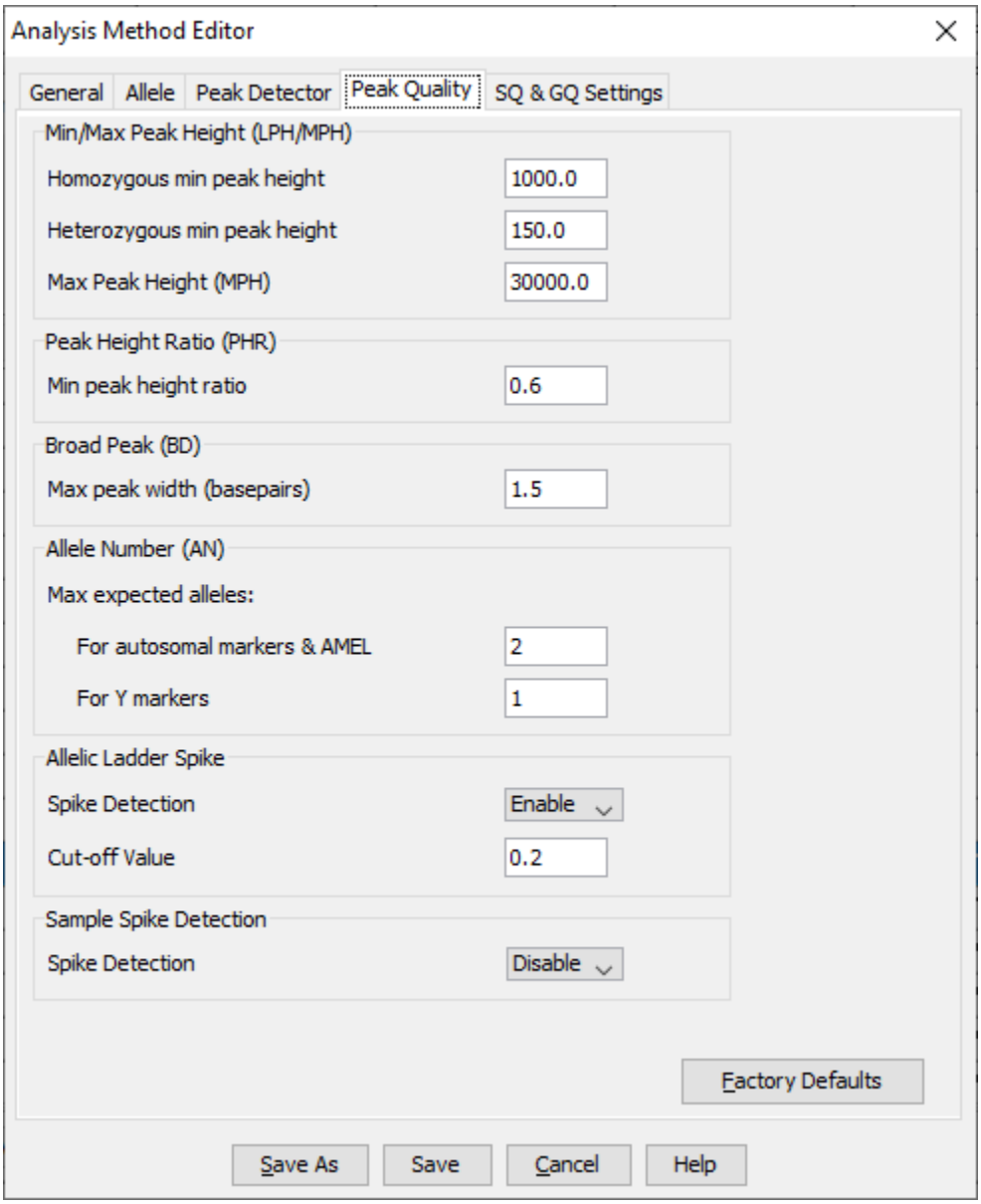
5. The quality value flags must be checked to provide the analyst a view of samples that may have issues. The green quality flag only is a replacement for analyst interpretation of each sample, ladder, control or standard. Any samples with a yellow or red quality flag must be assessed by the analyst. The quality flags assess the following items: SQ (Size Quality), SOS (Sample Off-Scale), SSPK (Sample Spike), MIX (Mixed Source), OMR (Outside Marker Range), CGQ (Composite GQ).



Note: Flags on a sample do not automatically indicate a failed sample. Manual review of a sample may result in a passing profile. Examples of flagged samples with passing profiles:

- Samples that have been flagged with a CGQ yellow triangle due to peak height imbalance or homozygous peak height issues. These issues may not lead to a failing sample, but must be reviewed by the analyst.
- Samples that have been flagged with a CGQ red stop sign in which review reveals that the sample was flagged due to peak heights reaching the set maximum height but no associated artifacts (pull up, stutter, etc.) exist in the profile.

The following screenshot provides the recommended Peak Quality settings for the expert system use in conjunction with the Globalfiler amplification kit:



Note: The validated TBI homozygous min peak height is set at 700 and is consistent with the validated stochastic threshold. The min peak height used by Bode Technology is set at 1000. The setting used by Bode Technology is more conservative and was incorporated into the expert system settings. Additionally, Bode Technology uses a Min peak height ratio of 0.5 (50%). TBI uses a more conservative setting of 0.6 (60%). The more conservative setting used by TBI was incorporated into the expert system settings.

6. Verify that the peaks for the allelic ladders are labeled with the correct allele designations. To view the ladder, see **18.4 Viewing Samples in the Project**.

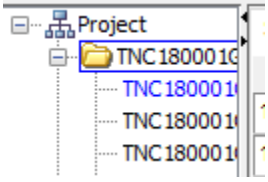
7. Verify that the positive control is the known profile from the kit and that the negative control/reagent blanks are free of a profile or contamination by ensuring that the profile is labeled with a green box. If a positive or negative/reagent blank is flagged for manual review, view the sample to determine the source of the issue.

   Note: While the TBI uses the positive control DNA included with the GlobalFiler amplification kit, Control DNA 007, Bode Technology uses an internal positive control and an additional employee profile. These differing profiles for positive can lead to the profile being flagged during the control cross check.

18.3 Viewing Raw Data in the Project

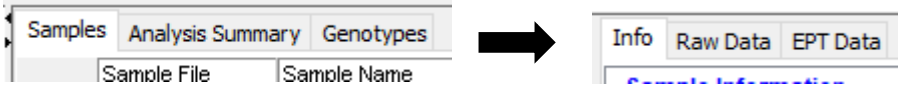
Note: *Raw data may be viewed at any time before or after analyzing the samples.*

1. In the left hand window, double click on the run folder (or click the plus sign next to the folder name) listed under the project folder icon to show a list of all the samples in the project.



2. Single click to highlight any sample name from the list to view raw data.

3. With any sample name highlighted, the *Samples/Analysis Summary/Genotypes* tab in the right hand window will change to the *Info/Raw Data/EPT* Data tab.



4. From here, one may view the general information, raw data or the run conditions of the selected sample.

5. Use the up and down arrow keys to scroll through the list of samples or select files individually with the mouse.

6. Clicking on the run folder or the project icons will return the project to the *Samples/Analysis Summary/Genotypes* tab screen.

18.4 Viewing Samples in the Project

1. From the Samples tab, to view a sample individually, highlight it and click on the Display plots icon ( ), use **View>Display Plots** or use Ctrl+L.

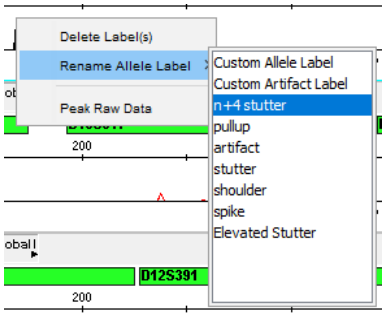
2. To zoom in, hold the cursor above the numbered X-axis and a magnifying glass will appear. Left click, hold and drag to the desired area. To zoom back out, double click when the magnifying glass is visible.

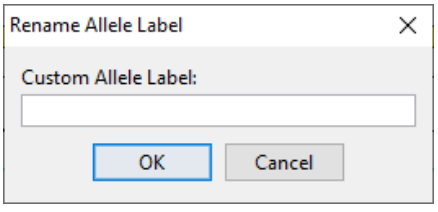
18.5 Labeling Artifacts

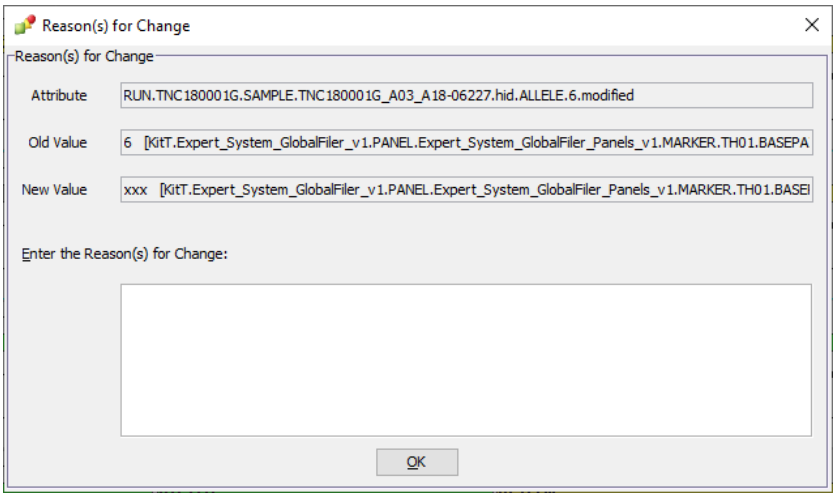
1. From the Samples Plot Screen, display the sample plot to be edited.

2. Select the peak that requires editing by left clicking the peak. Then, right click and select the appropriate editing option (e.g. Rename Allele Label).

3. If renaming an allele, click on the Rename Allele Label option, then choose the appropriate label or use the Custom Artifact Label. When *Custom Allele Label* is chosen, fill in the appropriate label name, and click OK. When the *Reason for Change* window opens, fill in reason for allele label change, and then click OK.







4. To undo any changes to an allele, click “Edit” on the toolbar and select “Undo.”
   Note: *Artifacts may also be labeled by hand on the printed electropherogram*.

5. To save changes, click on the “Save Project” button ( ) on the toolbar or **File>Save Project**.

18.6 Deleting Projects

1. Once a project has been named, analyzed, and saved, the same project name cannot be used again. To delete project, click on the GeneMapper ID-X Manager icon ( ), use **Tools>GeneMapper ID-X Manager** or Ctrl+M to open the GeneMapper ID-X Manager screen.

2. Click on the project name to highlight it.

3. Click on the delete button in the lower, right of the window. A *GeneMapper Deletion Alert* screen will open up.

4. Click on the “Yes” button to permanently delete the project.
   Note: Once a project is deleted, the object is removed from the database and the action cannot be undone.

5. Projects should be removed from the database as needed.

6. When deleting projects associated with data review (i.e. Bode Technology data review), ensure that the project has been exported for permanent storage to another location prior to deletion.

18.7 Printing Samples

Note: *Electropherograms will need to be printed only in certain circumstances, such as when the expert system settings are used to analyze verification packets or in-house sample analysis.*

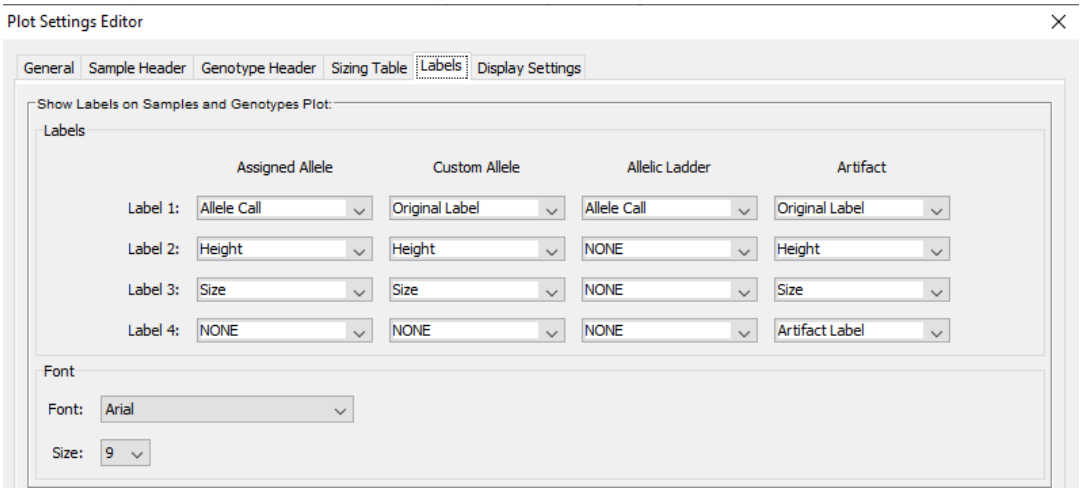
1. From the "Samples" tab, highlight all samples to print. Click on the Display plots icon ( ) or use Ctrl+L.

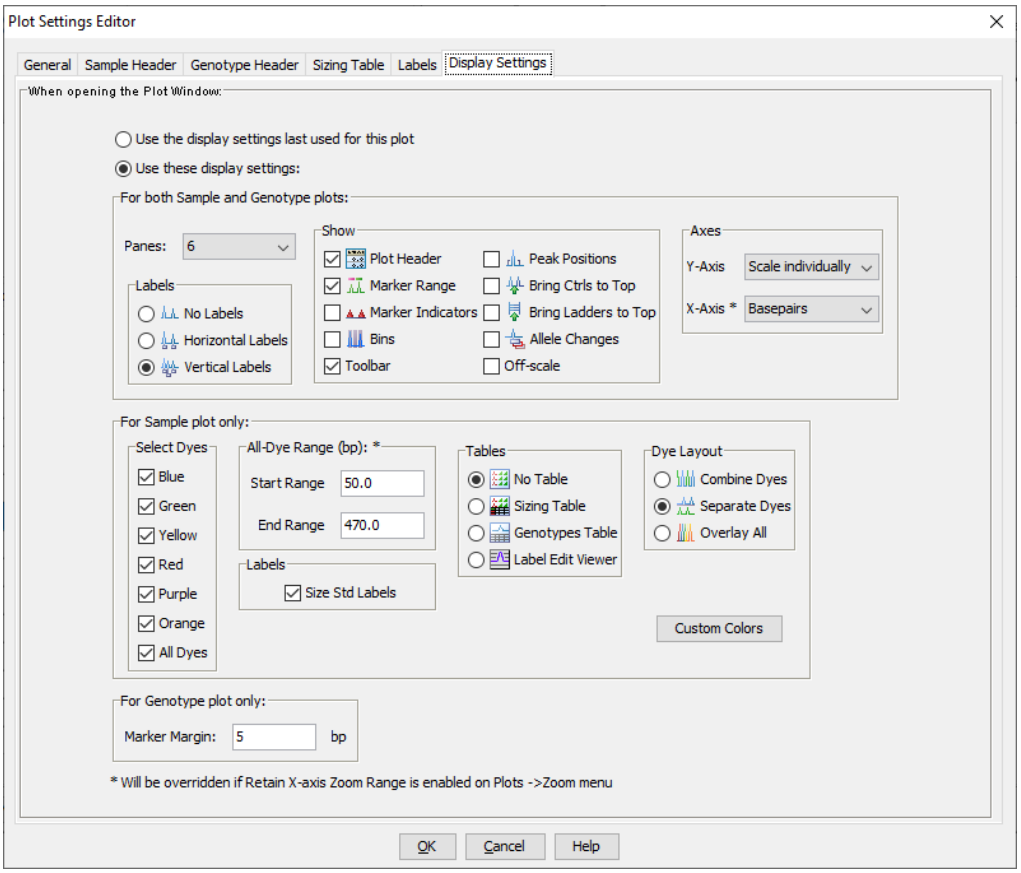
2. Zoom to the desired region, if needed.

3. Click the print icon ( ), use **File>Print**, or use Ctrl+P.

Note: When electropherograms are printed, the electropherograms will be printed with the allele calls. The electropherograms must also include peak heights except for the allelic ladders. Peak sizes may also be included at the analyst's discretion.

The following two screenshots provide the recommended plot settings:





18.8 Assessment of Data

Assessment of data from samples worked at TBI generally follows the guidelines set forth in the Forensic Biology STR Typing Manual, section 10.0 *Assessment and Reporting of GlobalFiler Data*. When using the expert system for analysis of data received from the vendor lab, Bode Technology, the following must be taken into consideration.

Bode Technology is the approved vendor laboratory for processing of TBI convicted offender, arrestee, and quality control samples. Per guidelines set forth by the FBI, 100% of all data returned from Bode Technology must be reviewed prior to upload to SDIS. The expert system settings in GeneMapper ID-X may be used to analyze first pass runs (TxF run data) received from Bode. Run folders containing re-amplified samples (TxA), re-cut samples (TxC), re-loaded samples (TxL), and dilutions (TxD) will be analyzed manually by the analyst. It must be noted that while some of the secondary data listed above is analyzed manually using the same analysis settings as the first pass data, the expert settings are intended only for analysis of the first pass data.

When examining data from Bode Technology, it should be noted that the following constitute reasons for a first pass failure:

- More than one locus exhibiting peak height imbalance (PHI) below 50% and any locus exhibiting PHI below 40%

- Off ladder alleles not found on the *Bode GlobalFiler Confirmed Off-Ladders* list (provided by Bode). Listed alleles do not have to be repeated, as they have been confirmed by Bode to be accurate.
- Homozygous peaks below 1000 RFU, except for Y-Indel and DYS391
- Tri-alleles
- N+4 stutter greater than or equal to 5% and/or N-4 stutter greater than or equal to 18% (it should be noted that Bode applies a 20% global filter, but will further review samples based on the listed percentages)
- Heterozygous DYS391
- Dropout – any uncalled alleles in a profile ruled as failed sample
- Out of Bin (BIN) alleles and Out of Marker Range (OMR) alleles
- Un-sizeable alleles
- Sizing quality (SQ) issues with broad peaks – loss of resolution
- Large spikes if peaks appear to be sister alleles
- Mixtures

When assessing data, the analyst should keep in mind the following differences that exist in the analysis settings between those set in the Forensic Biology STR Typing Manual, those used by Bode Technology and those in the Expert System settings (GF Expert Settings). Below are the differences between the three sets of settings:

	TBI	**Bode**	**Expert System**
Peak Amplitude Thresholds (Note: TBI uses separate settings for stochastic and analytical analysis)	(Stochastic): 700 (Analytical): 150	125	150
Stutter	Use marker specific	Global 20%	Global 20%
Homozygous min peak height	1000	700	1000
Heterozygous min peak height	700	125	150
Max peak height	20000	30000	30000
Peak Height Ratio	0.6	0.5	0.6

18.9 Training and Competency Testing

To show competency in the use of the GeneMapper ID-X Expert System settings, the analyst will need to use the Expert System settings to analyze data created at both TBI and Bode and evaluate the results. These results will be reported on an answer sheet to indicate pass/fail status of each sample, as well as listing any artifacts or peaks not attributed to the intended profile that are present or may prevent the profile from being uploaded into the CODIS database.

Be sure to treat the analysis of the data for the competency test as with any samples run in the lab. All pull ups, stutter, spikes, etc. should be labeled accordingly.

Complete Competency Test Answer Sheet (Expert Settings Analysis Results Only)

1. Two sets of raw data to be analyzed can be found in the Expert System Competency Test folder located on the G drive. These folders contain data generated at TBI and at Bode and both must be analyzed in GeneMapperID-X using the Expert System settings:

    a. Analysis method: GF Expert Settings

    b. Panel: Expert_System_Globalfiler_Panels_v1

Be sure to use two separate GMID-X projects for the TBI and Bode data.

2. When analyzing the data, an overall quality flag approach will be taken when assessing the status of the sample. If a red flag is indicated in any column (SOS, SQ, SSPK, etc.) that sample can be considered to have a red quality flag. If only a yellow triangle is seen in any column, that sample can be considered to have a yellow quality flag. Both red and yellow quality flags indicate that the analyst must look at the profile and asses the sample to determine what artifacts or other issues exist that require addressing. If only green squares are seen throughout that sample is considered to have a green quality flag and may be passed without the need of human interaction.

3. During analysis of the data (using the expert system settings), ensure that all pull ups, stutter, spikes, peak height imbalances, etc. are addressed such that the profile would be able to upload to CODIS.

4. Open the “Competency Test Answer Sheet.xlsx” workbook located in the Expert System Competency Test folder. Save this file to your computer. This file can be named something similar to “Competency Test Answer Sheet– *Analyst name – Date*” (e.g. *Competency Test Answer Sheet – Hardy – 051619*).

5. Fill in the sheet by recording the color of the overall quality flag for the sample (red>yellow>green). Recall that if a sample has a red stop sign and yellow triangle, record that as red.

6. If this sample would pass analysis for upload to CODIS, indicate that in the *Pass (Y/N)* column.

7. Regardless of pass/fail status for the sample, if a quality flag of red or yellow was indicated, record the reason and associated locus on the sheet (e.g. PU: D2S441, PHI: D22S1045 (51.81%), weak amp, etc.)

8. This answer sheet must be completed and printed for evaluation by the Technical Leader.

18.10 Quarterly Recertification

Per the *NDIS Operational Procedures Manual,* once an expert system is approved for use in the review of offender, arrestee and/or known reference DNA samples and implemented in the NDIS participating laboratory, the laboratory shall monitor the use of the expert system in accordance with the recertification requirements below.

1. A recertification shall be performed using a defined quality control data set of a minimum of 200 unique samples. The quality control data set may consist of data from the calibration set used for validation, in addition to recently analyzed data. In order to be recertified for continuous use, the expert system shall demonstrate complete concordance of the defined data set with the known DNA typing results.

2. This quality control set shall be run through the NDIS approved expert system on a quarterly basis (regular intervals of three months).

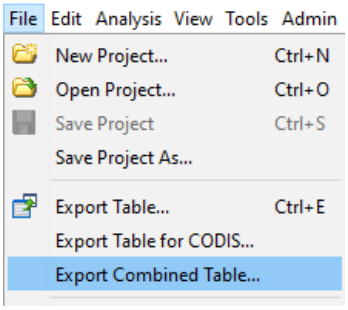
3. Following the repair, service or calibration of an NDIS approved expert system, the above-described recertification shall be conducted in accordance with Standard 10 of the FBI's QAS for DNA Databasing Laboratories.

4. Documentation of the concordance results shall be retained and available for review.

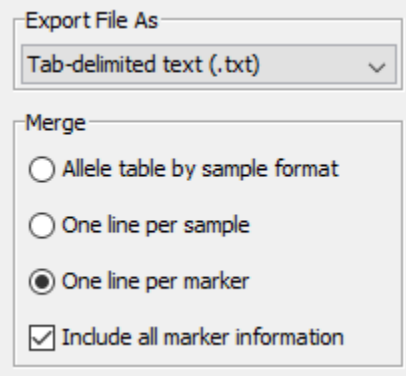
Quarterly recertification will be conducted using the raw data found on the G drive in the Expert System Quarterly Recertification folder. This data will be imported into GeneMapper and analyzed using the expert system settings (**Note: combine <u>all</u> the raw data into one GeneMapper project**). These results will then be exported *as is* and copied into the recertification Excel spreadsheet. A template file named "Quarterly Recertification MMYYYY.xlsx" is also located in the Expert System Quarterly Recertification folder.

Recertification process:

1. Once the analysis has been completed, export the combined table using **File>Export Combined Table…**.

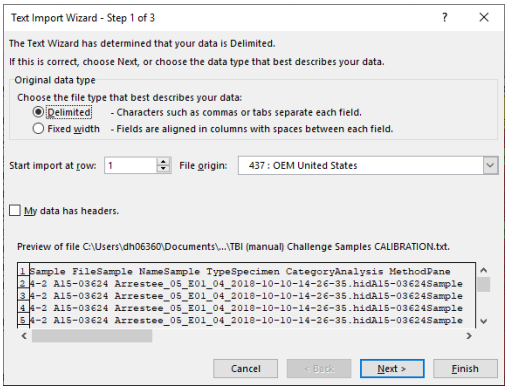


2. At the next pop up window, be certain that the radio button next to “One line per marker” is selected before hitting the Export button. Save the created .txt file to an easily remembered location on your computer.

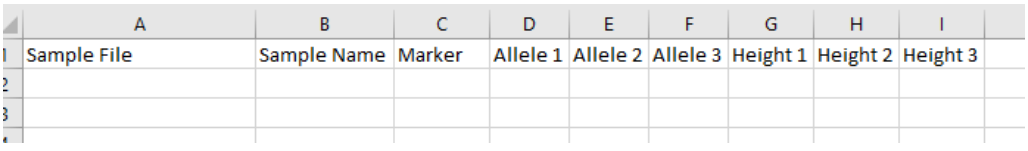


3. Open this exported .txt file in Excel using **File>Open**, and then browse to where you saved the exported file. Be sure that you select “All Files (*.*)” or “Text Files (*.prn;*.txt;*.csv)” to be able to see the .txt file.

4. When the *Text Import Wizard* window opens, click on “Finish” to complete opening the file.

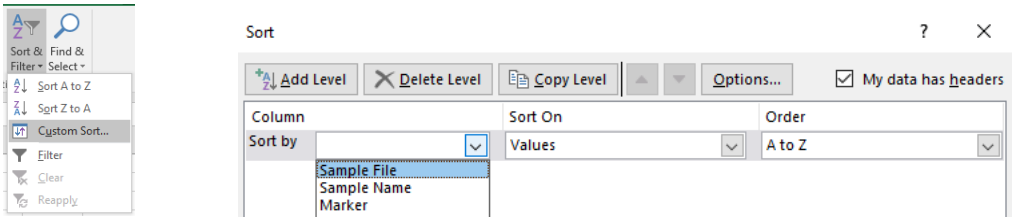


5. Once the file has opened, delete all columns but the following nine:
Sample File, Sample Name, Marker, Allele 1, Allele 2, Allele 3, Height 1, Height 2, Height 3



	A	B	C	D	E	F	G	H	I
1	Sample File	Sample Name	Marker	Allele 1	Allele 2	Allele 3	Height 1	Height 2	Height 3
2									
3									

6. Sort the data: Highlight all columns, then use “Custom Sort” under “Sort & Filter” on the menu bar. In the drop down for *Sort by* use “Sample File”. If Sample File is not a choice in the *Sort by* column, ensure that the box next to *My data has headers* is checked.



7. Once sorted, all the data should be highlighted, including the column headers. Copy all the data using **Ctrl+C** or right click and select “Copy”. Open the concordance Excel file (*Quarterly Recertification MMYYY* found in the Expert System Quarterly Recertification folder on the G drive) and click on the “Recertification Data” tab to bring that worksheet to the top. Click on cell A1 and paste the data you previously copied using **Ctrl+V** or right click and select “Paste”.

8. Click on the *Details* tab to bring forward the data comparison page.

9. No errors in cell J1 indicates no errors/discrepancies exist between the original and newly analyzed data.

10. Save this file with the month and year the recertification occurred and initials of the analyst in the *Recertification Concordance files* folder (e.g. “Quarterly Recertification 052019 CSH.xlsx”)

11. Any errors detected is a failing result. Should this occur, check the data on the Recertification tab to ensure the marker listed in cell C1 is D3S1358 and the final marker in column C is D2S1338. If these are not correct, the data may not have properly exported from GeneMapper ID-X. Return to GeneMapper, close and re-open the project, then export the data once again.

12. Should errors still exist, contact the CODIS Supervisor or DNA Technical Leader.

18.11 Proficiency Testing

Per the *NDIS Operational Procedures Manual,* the expert system shall be included in the NDIS laboratory's routine, external proficiency testing program, for at least one (1) annual proficiency test. To satisfy this requirement, the submitted known standards and associated controls will be analyzed using the Expert System settings in GeneMapper ID-X.

1. During proficiency testing, the analyst will use the Expert System settings to analyze the submitted known standards, as well as the negative and positive controls used during amplification of those samples. All unknown samples will be analyzed using TBI's approved analysis settings.

Note: The panel used for analysis in GMID-X is printed on each electropherogram. This ensures that the standards will be shown to have been analyzed with the Expert System settings.

2. All DNA results for the knowns will be recorded on the accompanying test data sheets for technical and administrative review and submission to the proficiency test provider.

3. Receipt of an error-free External Proficiency Test Result Sheet for each analyst will indicate a passing grade.