1. Scope

This protocol is used to analyze the raw data generated by the Applied Biosystems™ (AB) 3130 Genetic Analyzer. The data collected by the AB™ 3130 Genetic Analyzer for each sample is displayed graphically as an electropherogram. These samples include questioned and known specimens, positive controls, negative controls, and allelic ladders. The intensity, color, and scan number of each peak detected for the sample are recorded in the electropherogram. The intensity of the peak corresponds to the amount of amplified DNA present; the scan number corresponds to the size of the fragment. AB™ GeneMapper® ID is an automated genotyping software program which converts the information contained in the electropherograms ("raw data") to a more readily usable format ("analyzed data").

The size of each fragment of amplified DNA is determined by comparison to the internal size standard, GS500. GS500 fragments, which contain a range of sizing fragments between 35 and 500 base pairs, are labeled with the LIZ dye and are displayed as orange peaks. The 75 – 450 base pair range is required for sizing. GeneMapper® ID generates allelic designations from the sized data. This is accomplished by comparison to the allelic ladder. The GeneMapper® ID software recognizes the first allele of the ladder and creates approximately one base pair bins (+/- 0.5 base pair) around each allele in the ladder. Sample peaks are labeled by comparison of their size to the size of the ladder bins. The ladders are composed of the more common alleles in the general population. They also contain virtual bins for some less common alleles. If a sample peak falls into a bin or a virtual bin, an allelic designation will be assigned to the peak. However, if the peak is not represented by either a bin or a virtual bin created by the ladder, the

GeneMapper® ID software will designate this peak as "OL" for "off-ladder" allele.

2. References

The procedures described here are derived from a variety of sources. Portions of the protocol come directly from some of the references cited below.

2.1. Applied Biosystems™ GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide, 2004.

2.2. Prince George's County (MD) Police, Forensic Services Division, Serology / DNA Laboratory Short Tandem Repeat (STR) Analysis Protocol, 2006.

2.3. J.M. Butler, DNA Separation Methods, DNA Detection Methods, Instrumentation For STR Typing, and STR Genotyping Issues in: Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers, second ed., Elsevier Academic Press, 2005, pp 313-388.

2.4. S.B. Klein, J.M. Wallin, M.R. Buoncristiani, Addressing the Ambient Temperature Variation Effects on Sizing Precision of AmpFlSTR® Profiler Plus™ Alleles Detected on the AB Prism® 310 Genetic Analyzer, Forensic Sci. Comm. 5 (2003) 1.

3. Equipment

3.1. Computer with GeneMapper® ID software.

4. Safety/Quality Assurance

4.1. The positive control (9947A) for each amplification set-up must type correctly in order to use the associated samples.

4.2. Negative controls (negative amplification controls, reagent blanks, and formamide blanks) must be run and analyzed along with the associated samples. Refer to the "STR Interpretation Guidelines – Identifiler™" protocol for a discussion of negative controls.

4.3. At least one positive control and one formamide blank must be included in each GeneMapper® ID project.

4.4. Minor deviations from the protocol may be made at the analyst's discretion based on the analyst's training and experience. Deviations shall be indicated in the analyst's notes. Significant deviations from the protocol must be approved by the DNA Technical Leader.

5. Procedure

5.1. Turn on the computer station and login.

5.2. If not performing data analysis on a computer directly attached to a 3130 Genetic Analyzer, then connect the selected computer to the network and copy the run folder of interest to the selected computer.

5.3. Launch the GeneMapper® ID software and login.

5.4. Select *File > Add Samples to Project* from the menu. Find the run folder of interest, highlight that folder (or the sample files in the folder) and click *Add to List*. When all samples have been selected for the project, click *Add*.

5.5. In the tree pane of the GeneMapper® ID window, highlight the samples. Select *View > Raw Data* from the menu. Viewing the raw data may be useful in determining the start and stop

points for the analysis range, the overall quality of the injection and electrophoresis, and whether or not artifacts are present.

5.6. Select *View > Samples* from the menu. Under the *Samples* tab, make sure that the correct *Sample Type* ("sample, negative control, positive control, or allelic ladder"), *Analysis Method, Panel* ("Identifiler_v1"), and *Size Standard* ("CE_G5_HID_GS500") are selected. See the Appendix for further information.

5.7. Select *Analysis > Analyze* from the menu or click on the green triangle icon. A "Save Project" window will pop up. Type in the name of the project. The project name shall include at a minimum the date the project was created, the analyst's initials, and the instrument name. Be sure to save your project frequently when working with it.

5.8. After analyzing the data, look at the "Sizing Quality (SQ)" column at the far right hand side of the GeneMapper® ID window. If the icon in this column is yellow or red for any samples, verify that all of the GS500 peaks for that sample fall within the range of analysis.
- In the tree pane, select the sample that has a yellow/red "SQ" icon.
- Select *View>Raw Data* from the menu.
- Place the pointer at the 75 bp peak and 450 bp peak, and note the number listed at each "Data Point".
- Select *View>Samples* from the menu.
- Select *Analysis>Analysis Method Editor* from the menu (or use the bell curve icon).
- Make sure that the "Start Point" number is less than the "Data Point" number for the 75 bp peak.
- Make sure that the "Stop Point" number is greater than the "Data Point" number for the 450 bp peak.

5.8.1. If necessary, adjust the range of analysis to encompass all of these peaks, 75 bp to 450 bp.
- In the "Analysis Method Editor", make the "Start Point" number lower than the "Data Point" number for the 75 bp peak.

5.8.2. Alternatively, a different "Analysis Method" can be chosen that encompasses the necessary range.
NOTE: All samples in a project shall be analyzed with the same method. To choose a different analysis method:
- Select *View>Samples* from the menu.
- For the first sample, choose a method in the "Analysis Method" drop-down menu.
- Click on the column heading "Analysis Method" to highlight the whole column.
- Type *Ctrl-D* to copy down the Analysis Method to all samples.
- Select *Analysis > Analyze* from the menu or click on the green triangle icon to re-analyze the samples.

5.9. Select *Analysis>Display Plots* from the menu. Choose the appropriate plot from the "Plot Setting" drop-down menu. See the appendix for the plot requirements. Also reference Chapter 8 of the "Applied Biosystems™ GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide" for more detailed information on plot windows. Verify that all of the peaks in the GS500 size standard have been correctly assigned for each sample.

5.10. The 250 base pair peak in the GS500 standard, which is not automatically assigned a size, can be used to gauge whether or not the sample(s) will type correctly based on any given allelic ladder. The actual size of the 250 base pair peak depends on the electrophoresis conditions at the time of when the sample(s) ran on the 3130 Genetic Analyzer. If the 250 base pair peak for a sample is greater than one base pair away (+/- 0.5 base pairs) from the same peak in the

allelic ladder, it will probably not type correctly. Removing ladders (changing Sample Type from "Allelic Ladder" to "Sample") and/or creating multiple GeneMapper® ID projects for one run may be necessary in order for all of the samples to type correctly.

5.11. Each project created from one run must contain, at a minimum, one allelic ladder, a correctly typed positive control, and a negative control in addition to the samples of interest. It may be necessary to re-inject samples along with an allelic ladder, positive control, and a negative control if these criteria cannot be met.

5.12. Ensure that the peaks of the allelic ladder are labeled with the correct allele designations. For guidance, see "STR Interpretation Guidelines - Identifiler™" protocol.

5.13. While in the "Samples Plot" window, view each sample and examine the peak labels. It may be necessary to edit peak labels to account for things such as stutter, spectral pull-up, or spikes. For guidance, see "STR Interpretation Guidelines – Identifiler™" protocol.

    5.13.1. To edit allele calls, peak labels must be displayed. In the "Samples Plot" window, select *View>Labels>Vertical Labels* to display peak labels. To edit a peak label, select the peak and then right-click the mouse.

- If the peak is already labeled, the options are:
    - "Delete the allele call"
    - "Rename the allele"
    - "View the History"
- If the peak is not labeled, the options are:
    - "Add an allele call"
    - "View the History"

Choose the appropriate option by clicking on it.

- When renaming or adding an allele call, choose the allele call or enter a custom name.

    5.13.2. After making the necessary changes, enter an "Allele Edit Comment", and Click *Ok*. The following are common "Allele Edit Comments":

- Stutter – used for stutter peaks.
- Spike – used for spikes.
- Artifact – used for non-specific artifact peaks.
- Pull-up – used for pull-up peaks.
- Minus A – used for minus A peaks.

NOTE: All allele edits (computer-generated or by hand) must be displayed on the electropherogram.

5.14. Print the analyzed data and place it in each associated case file. See the Appendix for specifics on printing plots. In the "Samples Plot" window, select *File>Print***.** The following data must be printed:

- The electropherogram for each case sample.
- The electropherograms for ALL allelic ladders, positive controls, and negative controls (reagent blanks, negative amplification controls, formamide blanks) associated with the case samples.
- The GS500 Size Standard Plot for ALL of the associated samples and controls within the project.
- The raw data for ALL negative amplification controls and reagent blanks.

NOTE: If multiple cases are in the same GeneMapper® ID project, the data for the associated allelic ladders, positive controls, negative controls, GS500 plots, and raw data will have to be printed FOR EACH CASE.

5.15. Next, print a table showing the list of samples within the project. In the GeneMapper® ID window, select the appropriate table from the "Table Setting" drop-down menu. Select *File>Print* from the menu. On the "Page Setup" tab in the "Print" screen, select the *Landscape* button. See Appendix 1 for specifics on printing the table.

5.16. Run information, including instrument settings and data collection settings, will be printed for each associated case.

- Highlight a positive control sample in the project and select *View>Sample Info* from the menu.
- Print the "Sample Info" (two pages).