**One-Time GeneMarker-HID (GM-HID) Setup**

User Management

1. Within the GM-HID software on each analyst's computer, at least two Administrator accounts and one Analyst account are created by going to Help→ User Management. Additional accounts may be created if appropriate.
   a. An Administrator account has full privileges. Only designated staff will have Administrator access. Administrator accounts require a password.
   b. An Analyst account has the default set of privileges for this account type, with the addition of access to "Edit – View Preferences".
   c. Users designated as Administrators still need an Analyst account as well.
2. Log in as an Administrator.
3. Within the User Manager, on the "User Manager" tab, enter an Organization (ex. "CT Forensic Lab"). Check the box for "Run User Protection".
4. On the "Settings" tab, check the box for "Record Data Edit History". Click Ok.

Analysis Templates

1. Open the software and click on the folder icon in the upper left of the menu to open data. When analyzing GlobalFiler (GF) data, click on the 'Dyes' button in the lower left and make sure 6 dyes is selected. Click on 'Change Chemistry' and select 'AB'. Channel 1 should read 'Blue, 6-FAM', channel 2 should read 'Green, VIC', channel 3 should read 'Yellow, NED', channel 4 should read 'Red, TAZ', channel 5 should read 'Purple, SID', and channel 6 should read 'Orange, LIZ'. Click 'Ok'. Click on the 'Add' button in the upper right and navigate to the location of the data folder you want to import. Double click on the folder to access the .hid files. Select all or some of the files to import and click 'Open'. The files will be added to the 'Data File List'. The 'Add' button can be clicked again to add more data from other folders. When done adding data, click 'Ok' and the raw data will be added to the project.
2. Log in as an Administrator (if not already).
3. Click on the green arrow button in the toolbar menu to open the 'Run Wizard' window. Click on 'Select an existing template or create one'. Name the template 'GF_CTDSS' or 'GF_CTDSS_STRmix' depending on whether the stutter filters are applied. For known samples amplified by the GlobalFiler Express (GFE) Kit, name the template 'GFE_CTDSS_20%'. Select the panel with the same name. Set the size standard to GS600 and the standard color to 'LIZ'. The GS600 size standard should be edited using the 'Size Template Editor' to disable the following sized peaks of the standard: 20, 40, 514, 520, 540, 560, 580, and 600. After opening the editor and selecting the size standard, change the value in the 'Enabled" column from '1' to '0' for the sizes listed above. Save the changes. The analysis type should be 'HID'. Click on the 'Next' button to display the 'Data Process Options' window.
   a. As a note, the three panels contain the same settings except that the GF_CTDSS_STRmix panel has no thresholds for stutter, and the GFE_CTDSS_20% panel is programmed for diploid results.
4. For 'Raw Data Analysis' select 'Auto Range', 'Smooth', 'Superior', 'Spike Removal', 'Saturation Detection', and 'Saturation Repair'. For 'Size Call' select 'Local Southern'. For 'Allele Call' deselect 'Auto Range' and set the 'Start' to 60 and the 'End' to 502. For 'Peak Detection Threshold', leave the default

parameters of '50', '32500', and '20' for 'Min Intensity', 'Max Intensity', and 'Percentage >, Global Max', respectively. Click on the 'Next' button to display the 'Additional Settings...' window.

5. Select 'Auto Select Best Ladder' and set 'Allow Match # Variance' to '0', 'Max Average Size Diff' to '0.40' and deselect 'Use Ladder Library'. Select 'Auto Panel Adjustment'. For the 'Positive Control Template' select 'GF'. If it is not part of the positive control list, it can be added using the 'Positive Control Template Editor' under the 'Tools' menu. For 'Allele Evaluation' set both 'Peak Scores' to zero. Leave 'Mixture Evaluation' deselected. If this is the first time you are running this 'Run Wizard' template click on the 'Save' button. Click on the 'Ok' button. The raw data will be analyzed and allele calls will be generated. The 'Data processing...' window remains open until you click 'Ok'.
6. To confirm the analysis thresholds for allele peak detection, go to the 'tools' menu and select 'Panel Editor'. In the upper left window of the editor select each of the three panel templates used for analysis in turn. The '[Ploidy]' number should be set to '0' for the two templates used for evidence and '2' for the template used only for knowns. If not, right click on the panel name and select 'edit' and change the ploidy to the appropriate value and click 'Ok'. To check peak detection thresholds and type of stutter filters used, right click on any marker name of the panel at the top center of the window and select 'Edit Marker'. The minimum 'Homozygote', 'Heterozygote', and both 'Inconclusive' intensities are dye specific and should be set to '40' RFU for Blue, '55' RFU for Green, '35' RFU for Yellow, '50' RFU for Red, and '55' RFU for Purple. The 'Max Heterozygote Imbalance (%)' should be 1% for forensic unknown profiles and 21% for knowns. The 'Min Heterozygote Imbalance (%)' should be 0% for forensic unknown profiles and 20% for knowns. The imbalance settings for knowns effectively creates a 20% marker filter in lieu of stutter filters. If changes needed to be made, select the 'Apply Homo/Hetero Settings to All Markers in this dye' option. For the stutter filter, 'Use Allele-Specific Values (From Panel)' should be selected for the GF_CTDSS and GF_CTDSS_STRmix panels and 'Use Marker-Specific Values' for the GFE_CTDSS_20% panel. If changes needed to be made, select the 'Apply Stutter Settings to All Markers' option. Click 'Ok'. If changes were applied, click the 'Save Changes' icon in the toolbar menu (**not** the 'Save Changes with Signal Info' icon).



Viewing Preferences

1. Select 'View' from the toolbar menu then select 'Preference...'.
2. Under the 'Start up Settings' tab, select 'Wizard' and 'Show Navigator'. The default network path (C:\) for importing/opening raw data and projects and exporting reports can be modified if desired by checking the appropriate box and selecting the browse button on the right, e.g., U:\3130 NUCLEAR DNA CASEWORK & QC\To Be Analyzed\Analyst Initials.
3. Under the 'Display Settings' tab 'Allele Label', the 'Decimal Precision' should be '1'. Select 'Mark Off-Marker as 'OL'', 'Mark Off-Bin as 'OB'', and 'Set Missed Allele as a Single Line in Ladder'. Under 'Chart settings', select 'Max # of Open Charts and set to 96, Max Chart # in Page' (set to '4'), and 'Max Allele Label Layers' (set to '8'). Under 'Peak Label', select 'Height' and 'Vertical' and set the 'Position' to 'Allele Label'.
4. Under the 'Forensic' tab, set the Ladder, Positive Control, and Negative Control Identifiers to 'LADDER', 'POS', and 'NEG', respectively. Select 'Show Ladder Samples in Report', Show Control Samples in Report', and 'Mark Deleted/Edited Peaks with Symbols'. When done, click 'Ok'.

Edit Record

1. View any sample in the All Color Browser. Right click on any peak. Select "Edit Comments."
2. Add template comments for common edits. See SOP 1 Appendix 2 for designated abbreviations. Click Ok.
    a. Tip: Make the top comment template one that is not used that frequently. Entering a comment requires the analyst to select it by clicking, and as this comment is selected by default an extra click is needed to select it.
3. Right click on any peak. Select "Show Columns". Edit this list so that the following columns (and no others) are checked: Size, Height, Marker, Allele, and Allele Comments.

Print Templates

1. Select the 'Print Report' icon in the toolbar menu. Click on 'Select an Existing Template or Create One' and name the template.
2. Under the 'Standard' tab, select 'Normal', 'Selected Samples', 'Electropherogram', '6-FAM', 'VIC', 'NED', 'TAZ', 'SID', 'Hide Bins', 'Peak Table', and 'Start on Separate Page'. Under the 'Advanced' tab, select 'Print Report Header', 'Each Page', 'File Name', 'Print Markers', 'Print Alleles', 'Print Edited Peak Only', 'New Page for Each Sample' and 'Implement Y Axis Settings'. Set the 'Chart Height' to 64. Under the 'Page' tab, select 'Portrait' and set all four Margins to '0.0'. Click 'Save'. Click 'Preview'.
3. If the analyst routinely processes known samples, create a separate print template for known egrams. (Other analysts may add this template as well). Use the same settings as above with these exceptions: de-select 'Peak Table' on the 'Standard' tab, de-select 'File Name' on the 'Advanced' tab, and set the Chart Height to 37-40 depending on whether peak heights will be displayed. Ideally known egrams should be a single page; the Chart Height may be adjusted further.
4. Optionally, analysts may create additional print templates. For example, a template for controls could use the same settings as the known template but have 'Peak Table' selected with the option 'Start on Separate Page'.

Report Settings

1. Click on the "Report Settings" icon.
2. Select the following options: 'Forensics', 'Show File Name', 'Vertical', 'Exclude Sample Index', 'Exclude Report Header', 'Split Items', 'Show Allele Name', 'Show Size', 'Show Height', and 'Show Rejected Low Score Alleles'. De-select all other options. Click Ok.

**Analysis in GM-HID**

*Note that all settings selected remain in effect until changed. If any of the above default settings are changed during analysis of a project, be sure to change them back again.*

Creating a Project

1. Open GM-HID and log in using the appropriate Analyst (not Administrator) account.
2. Import all of the pertinent data files as in Step 1 under "Analysis Templates".

3. Select 'File' and then 'Save Project' from the toolbar menu. Save the project to a network folder containing the .hid files analyzed. Type in a name for the project in the format: "Kit-3500#-BatchID-Modifier#-Any Other Details" (ex. "GF-2-MGR050317-2-JES") and navigate to the appropriate location, and click the 'Save' button. Modifier number and other details are optional. For descriptions of all options and tools for GeneMarker, please see the GeneMarker HID User Manual.
4. Click on the green arrow button in the toolbar menu to open the 'Run Wizard' window. Select the template GF_CTDSS, GF_CTDSS_STRmix, or GFE_CTDSS_20% as appropriate to your samples. Click 'Next' on the 'Template Selection' and 'Data Process' screen and 'Ok' on the 'Additional Settings' screen. If this is a re-Analysis of a previous analyzed project, select 'Call Size Again' and click 'Apply to All' when prompted
5. Note that even though allele calls have been generated, the changes to the project have not been saved. Re-save at any time during analysis.
6. Ensure that all required internal lane size standard peaks are called in all samples. Select the 'Size Calibration' icon in the toolbar menu. Select each sample in the list on the left to view the ILS trace for that sample. Verify that these GS600 standard peaks are present (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, and 500) and the sizing quality score is ≥ 88 (the software flags the sizing quality of the ILS when it is <88). If a sample's internal size standard fails due to a low sizing quality (e.g. pull-up peaks into the size standard), the size standard may be successfully edited to produce a sufficient sizing quality score (see GM-HID User Manual). Alternatively, the sample may be re-injected, re-prepped and re-injected, or re-amplified.
7. Ensure that passing allelic ladders have all allele peaks detected. Ladders are listed in the sample file tree in blue type. Passing ladders will appear in bold blue type. Double-click on the first ladder and select the 'Browse by All Color' icon in the toolbar menu. Verify that the passing ladders have all allele peaks detected. With the 'Set Missed Allele as a Single Line in Ladder' flag enabled (see Step 3 under "Viewing Preferences"), allele labels for missed ladder peaks will be placed one row above allele labels of peaks that were called and there will be a red vertical line for that allele bin. Failed ladders will not be used to assign alleles to profiles.
8. To view and edit electropherograms for the samples, double click the first sample in the sample tree list. Then select the 'Browse by All Color' icon in the toolbar menu. Maximize the egram window. To view electropherograms of other samples, click in the vertical view slider on the far right to toggle up or down one sample at a time.



<u>Editing a Project</u>

1. View each sample in the 'All Color Browser'. (See SOP 39 for details on data analysis.)
2. Select a peak by clicking on it. De-select by clicking a selected peak while holding "Ctrl", or de-select all peaks in a color at once by double-clicking elsewhere in the color.
    a. Note that once a peak has been selected, it remains selected until performing one of the actions to de-select it or closing the All Color Browser.
3. To delete a peak, select it, right-click and select 'Delete'. Multiple peaks can be deleted at once.

4. For each deleted peak in evidentiary (forensic unknown) profiles, add a comment as to the reason for deletion (for example, the designated abbreviation for the artifact causing it). Right-click the peak and select 'Edit Comments'. A list of Comment Templates appears, in which commonly used comments can be stored and selected. (See SOP 1 Appendix 2 for designated abbreviations.)
5. To confirm a red/yellow flagged peak, right click the peak and select 'Confirm'.
6. To change an allele call, right click the peak and select 'Edit Allele", and adjust the label as appropriate.
7. If it's appropriate to insert an allele, right click the peak and select 'Insert Allele'. Adjust the label if needed.
    a. Zoom in on the peak in order to insert at the precise size where the peak is highest.
8. For casework known and database samples, reasons for routine edits do not need to be recorded. Issues such as change in allele call or insertion of an allele are noted on the 3500xL analysis worksheet.
9. Disable any samples not being reported and note the reason in the Comments section on the 3500xL analysis worksheet. Use sufficient detail as to be clear to a reviewer; for example: "re-inject at max due to low peaks" or "re-prep sample- ILS failed."
10. Re-save the project when edits are complete.



Printing Electropherograms

1. After editing is complete, close the 'All Color Browser'. Select the 'Set Axis' icon in the toolbar menu and select 'Fixed X'. Set the range from 70 to 450. Click 'Ok'.
2. Select the samples to print (all samples in the project that will use the same print template) by double-clicking to check-mark their sample name(s) in the sample tree. Select the 'Print Report' icon in the toolbar menu. Print each set of samples using the appropriate template. See SOP 23 for sample types required to print.
    a. If appropriate for a sample, chart height may be lowered to better fit data on fewer pages or raised for better view of profile details (see Step 2 under "Print Templates").
3. Should an electropherogram need to be re-printed, be sure to manually note edit changes on the peak table, or cross out the edit information for that sample from the original table if printing a new table.

Exporting a Report

1. Click on the "Save" icon next to the "Report Settings" icon.
2. For "Save as Type:", choose "text file". Save in the designated network folder for Analysis Export Files.

Reviewing a Project

1. Open GM-HID and log in using the appropriate Analyst (not Administrator) account.
2. See electropherogram headers to check that the correct Analysis Template was used.
3. When checking edits, match edited peaks onscreen (marked with an "X" or "E" symbol) to edits listed in the printed peak tables using size by holding crosshairs above edited peak.
4. To confirm that reasons for not reporting samples, as listed in Comments on the 3500xL analysis worksheet, are appropriate, re-enable disabled samples. (Do not save project.)