Arizona Department of Public Safety

Scientific Analysis Bureau

DNA Database Analytical Protocols

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Prior to using this manual, it is necessary to read and understand the DNA Database Quality Manual, the Scientific Analysis Bureau General Procedures Manual and the Scientific Analysis Bureau Quality Assurance Manual.

Follow all requirements in the above manuals when using this manual.

Certain database samples may require minor adjustments to these procedures. The procedures are meant to be guidelines with the analyst's experience being the final judge. All changes to the published manual will be subject to the DNA Database Technical Leader and QA Manager's approval and may be noted in the analyst's batch file.

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1 Database Laboratory Information Management System

1.1 STaCS™ (Sample Tracking and Control System™) is the system that is used by the database unit to track all submissions and analyses.

1.2 The following are some of the features tracked in STaCS™:

1.2.1 Submission Check In

1.2.1.1 Date received

1.2.1.2 STaCS™ barcode

1.2.1.3 Database personnel performing the check in

1.2.1.4 Offender/arrestee identifying information

1.2.1.5 Scanned images of databank forms

1.2.2 Data Processing and Analysis/Hit Confirmation Processing

1.2.2.1 Analyst performing processing

1.2.2.2 Instrument used for processing step

1.2.2.3 Reagents and lot numbers used in processing step

1.2.2.4 Plate comments entered by database analyst

1.2.2.5 Database sample profiles

1.2.2.6 Positive control and in-house known sample profiles

1.2.2.7 CODIS upload date, if applicable

1.2.3 Quality Assurance and Control

1.2.3.1 Reagent information (including dates received, lot numbers, expiration dates, amount received)

1.2.3.2 QC tracking/release of critical reagents and prepared reagents

Periodically, certain steps may be run outside of STaCS, such as reinjections and dilutions. When such steps are performed, the analyst will note the step performed, the lot numbers for the reagents used for the instrument and dilutions (or a note referencing the lot numbers previously used), the barcodes for the

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instrument and pipettes used and any other significant information (e.g., date performed, injection time used, etc.). If performing reinjections or dilutions, it is not necessary to note any reagent and/or instrumentation that have been previously documented. Unless otherwise noted, it is expected that those same reagents and/or instrumentation were utilized in all subsequent processes.

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2 BSD Punching Procedures for Database Samples

2.1 Label a new 96-well microplate with the assigned plate barcode.

2.2 Plate Preparation:

2.2.1 For plates that will be amplified with Identifiler® Direct or GlobalFiler® Express, add 2.0 µL of Prep-n-Go™ Buffer to all sample wells and the reagent blank well. Additional Prep-n-Go™ Buffer may be added and noted in the batch file if multiple punches are used.

2.2.2 For plates that will be amplified with MiniFiler™, add 10.0 µL of Punch Solution™ to all sample wells and the reagent blank well.

2.3 Log into the BSD program and select the appropriate punching program (programs are listed in Appendix D). Turn on the vacuum pump (if applicable). Verify the humidifier is on and has enough water. Make sure the cleaning reservoir is empty and in the proper position.

2.4 Load the 96 well microplate onto the puncher, verifying the correct orientation of the plate.

2.5 Blood samples will be punched in a blood-stained area. Buccal samples will be punched in an area expected to yield the optimal amount of DNA. Scan the barcode and punch into the appropriate well. Between sample punching, a minimum of two waste disks must be punched into the waste container.

NOTE: The maximum number of samples that can be run at a time is 88 samples and two controls (one in-house known sample and one negative control). The remaining six empty (unused) wells will be used for PCR amplification controls and ladders.

2.6 Once the plate is complete, check that all punches are present and select "All spots present". Exit out of the program to end the run. If the BSD program was launched through STaCS, the analyst will need to select "All spots present" even if punches are missing from the plate (samples missing from the plate will need to be punched manually). For STaCS, if an analyst needs to abort the punching process, close completely out of the BSD software.

2.7 To clean the puncher, empty the waste reservoir and disassemble the punching mechanism. Thoroughly clean with either canned air or a swab with alcohol on it only. Then reassemble the mechanism and verify no paper remnants are found on the punching apparatus.

2.8 Incubation procedure for samples that will be amplified with MiniFiler™:

2.8.1 Place the unsealed plate onto a 70°C heat block for 30 minutes or until dryness.

3 AmpFlSTR® Identifiler® Direct, GlobalFiler® Express and MiniFiler™ PCR Amplification Kits

The Identifiler® Direct kit gives a method for direct amplification and typing at 15 short tandem repeat (STR) loci plus Amelogenin. The GlobalFiler® Express kit provides a method for the direct amplification and typing of 21 autosomal STR loci, one Y STR locus and one Y insertion/deletion locus plus Amelogenin. The MiniFiler™ kit has been validated for use with a method for direct amplification and typing at eight STR loci plus Amelogenin. The STR loci included consist of repeat units four base pairs in length with the exception of the SE33 locus whose repeats are three base pairs in length. The number of repeat units at any one locus may differ, so alleles of different lengths are possible. The loci are amplified using the polymerase chain reaction (PCR) process. Identifiler® Direct includes the loci D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amelogenin, D5S818 and FGA. GlobalFiler® Express includes the loci D3S1358, vWA, D16S539, CSF1PO, TPOX, Y indel, Amelogenin, D8S1179, D21S11, D18S51, DYS391, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, and D2S1338. MiniFiler™ includes the loci D13S317, D7S820, Amelogenin, D2S1338, D21S11, D16S539, D18S51, CSF1PO and FGA. For each locus, one primer carries a fluorescent tag. The amplified alleles are detected using capillary electrophoresis with laser excitation and CCD camera detection. An internal size standard is run with each injection and the sizes are compared to an allelic ladder for allele calls.

All three kits may be used on database samples, with the intention that samples will be run with GlobalFiler® Express and, if necessary, Identifiler® Direct and/or MiniFiler™ for challenging samples. The goal is to obtain the minimum number of core loci to meet CODIS upload requirements.

3.1 Amplification

3.1.1 Use the master mix, primer set and control DNA from the same lot of amplification kit. Do not intermix these components from kits with different lot numbers.

3.1.2 The fluorescent dyes attached to the primers are light sensitive. Store the primer sets and amplified DNA protected from light.

3.1.3 In the designated hood assemble the PCR amplification reagents as outlined below. Vortex or gently mix PCR reagents and tap excess liquid down from cap.

3.1.4 Identifiler® Direct Full Reactions

3.1.4.1 Prepare a PCR Reaction Mix by combining the following volumes of reagents (it may be necessary to use two microcentrifuge tubes):

Number of samples x 13.5 µL of Identifiler® Direct Master Mix

Number of samples x 13.5 µL of Identifiler® Direct Primer Set

Vortex briefly and quick spin.

3.1.4.2 Pipette 25 µL of PCR Reaction Mix into the negative control well and into each reaction well of the 96-well microplate containing previously punched samples in Prep-n-Go™ Buffer. The amplification blank well will only contain the PCR Reaction Mix.

3.1.4.3 Pipette 2.0 µL of Control DNA 9947A into the positive control well.

3.1.5 Identifiler® Direct Half Reactions

3.1.5.1 Prepare a PCR Reaction Mix by combining the following volumes of reagents:

Number of samples x 6.75 µL of Identifiler® Direct Master Mix

Number of samples x 6.75 µL of Identifiler® Direct Primer Set

Vortex briefly and quick spin.

3.1.5.2 Pipette 12.5 µL of PCR Reaction Mix into the negative control well and into each reaction well of the 96-well microplate containing previously punched samples in Prep-n-Go™ Buffer. The amplification blank well will only contain the PCR Reaction Mix.

3.1.5.3 Pipette 1.5 µL of Control DNA 9947A into the positive control well for 25 cycle reactions and 1.0 µL of Control DNA 9947A into the positive control well for 26 cycle reactions.

3.1.6 GlobalFiler® Express Reactions

3.1.6.1 Initial use only: Pipette appropriate volume of GlobalFiler® Express Master Mix Additive into the GlobalFiler® Express Master Mix and date/mark the Master Mix container/the outer box to indicate completion of this step.

For 1000 reaction kits, use 390 µL of Master Mix Additive.

For 200 reaction kits, use 80 µL of Master Mix Additive.

3.1.6.2 Prepare a PCR Reaction Mix by combining the following volumes of reagents:

Number of samples x 6.0 µL of GlobalFiler® Express Master Mix (with Additive)

Number of samples x 6.0 µL of GlobalFiler® Express Primer Set

Number of samples x 2.5 µL of PCR Enhancer

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Vortex briefly and quick spin.

3.1.6.3 Pipette 14.5 µL of PCR Reaction Mix into the negative control well and into each reaction well of the 96-well microplate containing previously punched samples in Prep-n-Go™ Buffer. The amplification blank well will only contain the PCR Reaction Mix.

3.1.6.4 Pipette 2.0 µL of Control DNA 007 into the positive control well.

3.1.7 MiniFiler™ Half Reactions

3.1.7.1 Prepare a PCR Reaction Mix by combining the following volumes of reagents:

Number of samples x 5.5 µL of MiniFiler™ Master Mix

Number of samples x 2.75 µL of MiniFiler™ Primer Set

Number of samples x 2.75 µL of AmpSolution™

Vortex briefly and quick spin.

3.1.7.2 Pipette 2.5 µL of TE buffer into each well except the positive control.

3.1.7.3 Pipette 10.0 µL of PCR Reaction Mix into the negative control well and into each reaction well of the 96-well microplate containing previously punched samples incubated in Punch Solution™. The amplification blank well will only contain the PCR Reaction Mix and TE buffer.

3.1.7.4 Pipette 2.5 µL of Control DNA 007 into the positive control well.

3.1.8 Seal the 96-well microplate with 8-well cap strips.

3.1.9 Centrifuge the microplate to dislodge any bubbles and to ensure the punches are immersed in solution.

3.1.10 Make sure the cap strips are on tightly, then place the microplate into the GeneAmp® PCR System 9700 thermal cycler. Close the lid and select the applicable program with the appropriate sample volume. The temperatures and times for the amplification programs are outlined in Appendix E.

3.1.11 Verify that the program has started.

3.1.12 After samples have been analyzed and the data reviewed for the plate and all re-runs associated to the plate, discard the plate containing the amplified DNA in the hazardous waste container.

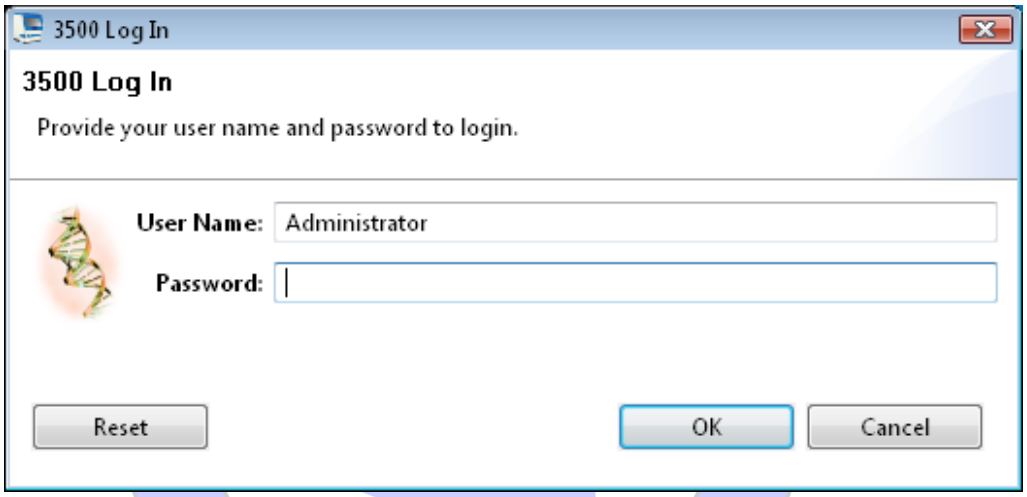
4 AB 3500xL Genetic Analyzer Software and Maintenance

4.1 Data Collection Software

4.1.1 Turn on the computer.

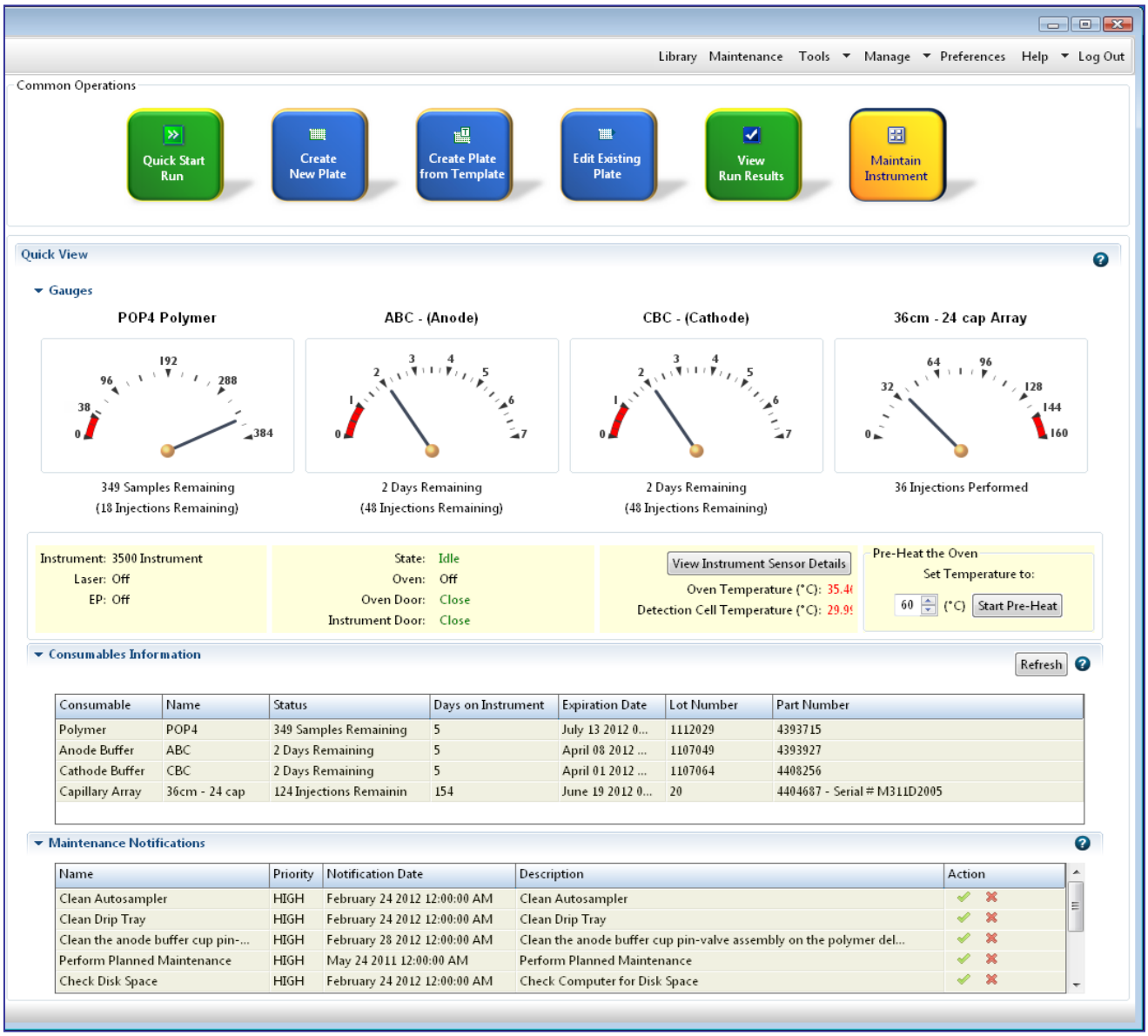
4.1.2 Turn on the 3500xL instrument. Wait for the green light to come on and for all 3500 services to load.

4.1.3 Double click on the 3500 icon on the desktop to open the 3500xL Data Collection software. Enter the appropriate username and password in the pop-up window shown below.



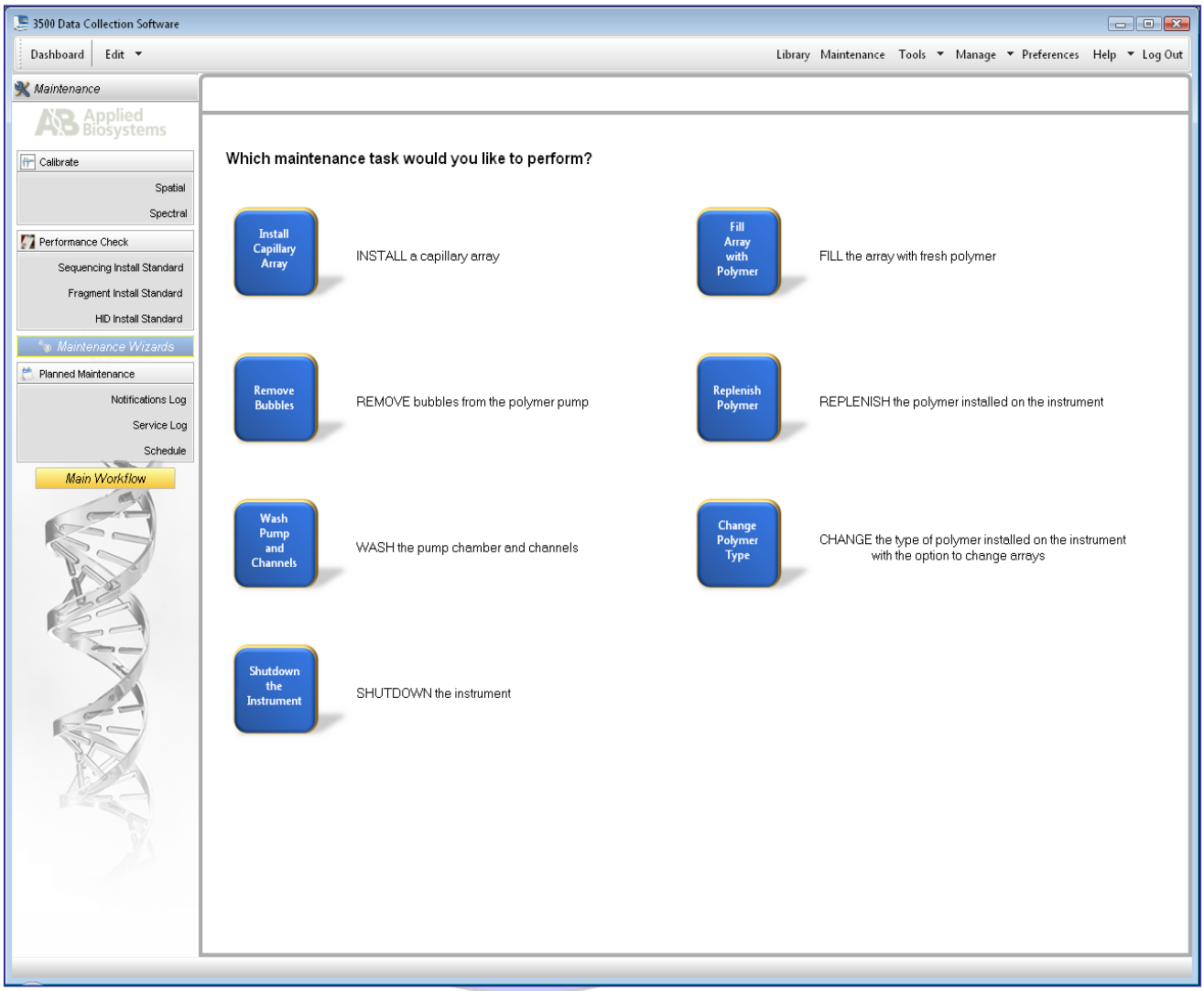
4.1.4 When the Data Collection Software opens, the Dashboard screen will be displayed. The Dashboard provides lot number and expiration dates of all consumables, instrument status and sensor details, maintenance notifications, and buttons to access common user operations.

4.1.5 Following is a screen shot of the dashboard:



4.1.6 The 3500xL Data Collection Software comes equipped with wizards to assist with various maintenance tasks, including replenishing the polymer, performing water washes, removing bubbles, installing the capillary array, etc. To view the wizards, select the button "Maintain Instrument" from the Dashboard Common Operations, or by selecting Maintenance from the tool bar. Once the maintenance screen is displayed select Maintenance Wizards from the navigation pane on the left side of the screen.

4.1.7 Following is a screen shot of the maintenance wizards:



4.2 Instrument Maintenance

The *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* outlines all the required maintenance on the instrument. Following are some of the procedures to be performed on a regular basis.

4.2.1 Polymer Addition

4.2.1.1 Remove the POP-4™ from the refrigerator and allow it to acclimate to room temperature.

4.2.1.2 The polymer on the instrument should be changed out on a regular basis. To do this, select the Wash Pump and Channels wizard and follow the instructions given.

4.2.1.3 Periodically, the instrument may run low on polymer prior to its regular cleaning. If this occurs, the same lot of polymer may be added to the instrument following the instructions given in the Replenish Polymer wizard.

4.2.1.4 Both the Wash Pump and Channels wizard and the Replenish Polymer wizard contain steps for removing bubbles from the instrument. If at any other time bubbles are noted, they may also be removed using the Bubble Remove wizard.

NOTE: Smaller bubbles (less than one millimeter in diameter) in the pump chamber may be removed at the analyst's discretion.

4.2.2 Water Trap Flush

4.2.2.1 The water trap located at the top of the pump block should be flushed with DI water on a regular basis to wash out any diluted polymer and to prolong the life of the pump. There is no wizard for this procedure.

4.2.2.2 Loosen the forward-facing Luer syringe fitting at the top of the pump block by turning it approximately one-half turn counterclockwise.

4.2.2.3 Fill a 20 mL Luer lock syringe with about 5 mL of DI water. Remove any bubbles from the syringe. Attach the syringe to the Luer fitting.

4.2.2.4 Flush the trap by pushing slowly and steadily on the syringe plunger. Take approximately 30 seconds to flush the 5 mL of DI water. Do not use excessive force as this may damage the trap seals.

4.2.2.5 Remove the syringe and close the Luer fitting by turning it clockwise until the fitting seals against the block. Do not over-tighten.

4.2.2.6 Remove the water trap waste container to the right of the pump block. Dispose of the flushed water collected inside and return the emptied container.

4.2.2.7 Once completed, note the water trap was flushed in the instrument logbook.

4.2.3 Capillary Array Removal/Installation

4.2.3.1 The capillary array will be changed on an as needed basis. Poor sizing precision/allele calling, poor resolution and/or decreased signal intensity may be indications that a new capillary array is required.

4.2.3.2 To remove or replace the capillary array, open the instrument and oven doors. Then follow the instructions outlined in the Install Capillary Array wizard.

4.2.3.3 When finished, close and lock the oven door, then close the instrument doors.

4.2.4 Buffer Installation

4.2.4.1 Allow the anode and cathode buffer containers to acclimate to room temperature prior to use.

4.2.4.2 Before unsealing the anode buffer container, ensure that all buffer has been poured out of the overflow space into the main buffer reservoir space. Verify that the buffer is at the fill line. Remove the seal on the anode buffer container and place it on the lower polymer block.

4.2.4.3 Tilt the cathode buffer container back and forth gently and carefully to ensure that the buffer is evenly distributed across the top of the baffles. Verify that the buffer is at or above the fill line. Unseal the cathode buffer container and cover both sides with the appropriate septa, verifying the septa are seated properly. Install the cathode buffer container on the autosampler tray.

4.2.5 Spatial Calibration

4.2.5.1 A spatial calibration must be done after an optical service procedure such as realigning or replacing the laser or CCD camera or mirrors has been performed, the capillary array has been removed or replaced, the instrument has been moved, the detector door has been opened or the detection cell has been moved.

4.2.5.2 From the Maintenance window, select Spatial under Calibrate in the navigation pane on the left side of the screen.

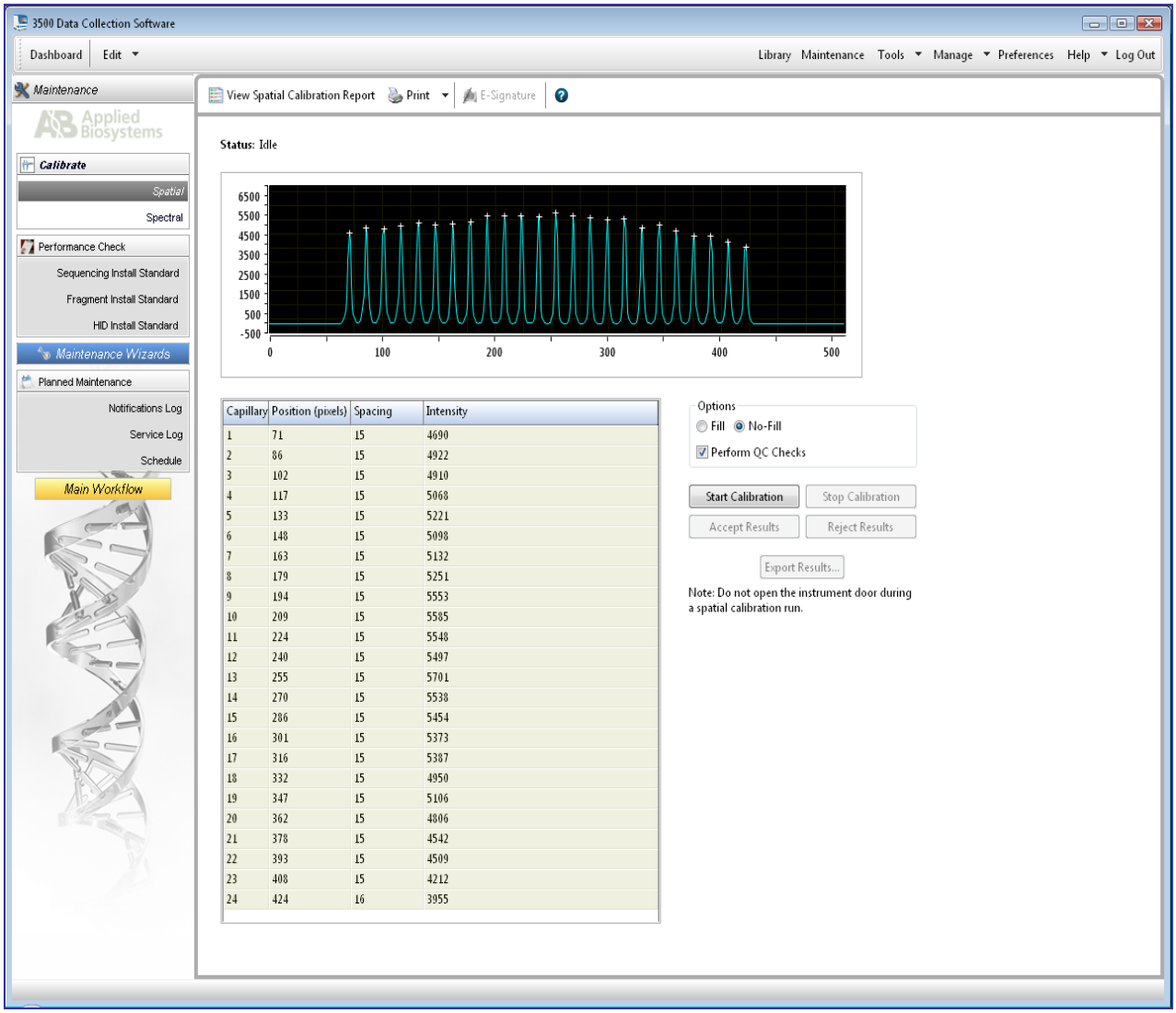
4.2.5.3 To fill the capillaries prior to the spatial run, select the “Fill” radio button in the options box. To perform a spatial run without filling the capillaries, select the “No-Fill” radio button. Then select “Start Calibration”.

4.2.5.4 When the spatial run is complete, verify that the spatial calibration profile has the following: one sharp peak for each capillary, one marker (+) at the apex of every peak and all peaks are about the same height. If there are any off-apex markers, large shoulders, split peaks, peak heights lower than usual, or the peak heights are significantly sloped up or down, the calibration should be done again.

4.2.5.5 If the spatial calibration is acceptable, select “Accept Results”. If not, select “Reject Results” and run again. The capillaries do not need to be filled again. If the spatial continues to fail, clean the peak detection window on the array and try again.

4.2.5.6 Select "View Spatial Calibration Report" to view report. Then select Print and print to the PDF printer. Save this report in the Spatial Calibration Folder.

4.2.5.7 Following is a screen shot of the spatial calibration:



Capillary	Position (pixels)	Spacing	Intensity
1	71	15	4690
2	86	15	4922
3	102	15	4910
4	117	15	5068
5	133	15	5221
6	148	15	5098
7	163	15	5132
8	179	15	5251
9	194	15	5553
10	209	15	5585
11	224	15	5548
12	240	15	5497
13	255	15	5701
14	270	15	5538
15	286	15	5454
16	301	15	5373
17	316	15	5387
18	332	15	4950
19	347	15	5106
20	362	15	4806
21	378	15	4542
22	393	15	4509
23	408	15	4212
24	424	16	3955

4.2.6 Spectral Calibration

4.2.6.1 A spectral calibration is performed when a new dye set is used that hasn't been previously calibrated, the laser or CCD camera has been realigned, the capillary array has been changed or when a decrease in spectral separation is noted in the raw or analyzed data.

4.2.6.2 Vortex and spin the appropriate Matrix Standard. Use DS-33 for Identifiler® Direct and Minifiler™ spectral calibrations. Use DS-36 for GlobalFiler® Express spectral calibrations.

4.2.6.3 For DS-36 spectral add 3.0 µL of G6 Matrix Standard to 297 µL of formamide. For DS-33 spectral add 3.0 – 8.0 µL of G5 Matrix Standard. Vortex and spin.

4.2.6.4 Aliquot 10 µL of the formamide/matrix mixture into each of the 24 wells in the first injection of a 96-well plate and cover with a septum.

4.2.6.5 Briefly centrifuge plate to dislodge any bubbles. Denature the matrix samples for three minutes at 95°C. Snap cool the samples for three minutes.

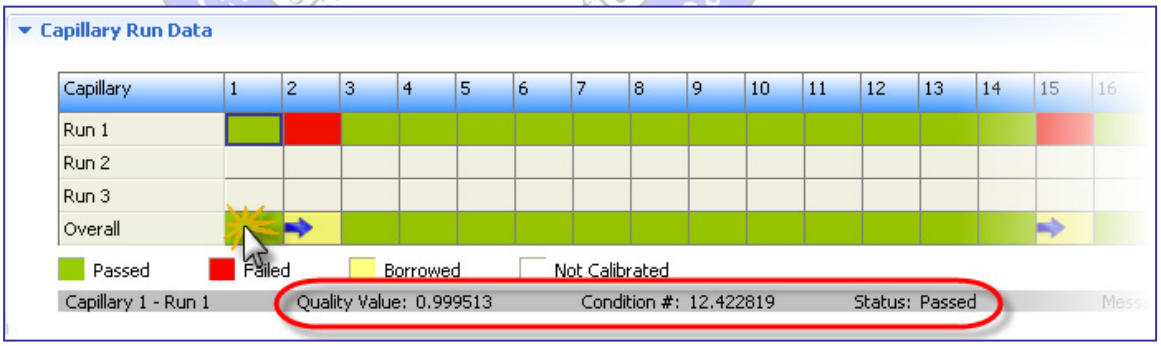
4.2.6.6 Place the sample plate on the plate base and attach the plate retainer.

4.2.6.7 Place the plate in the autosampler and ensure the instrument door is closed.

4.2.6.8 In the Maintenance window, select Spectral under Calibrate in the navigation pane on the left side of the screen.

4.2.6.9 Select the number of wells, chemistry standard, dye set, and plate position on the instrument under Calibration Settings. Ensure that "Allow Borrowing" is selected. Then select "Start Run".

4.2.6.10 When the spectral calibration is complete, the results can be viewed in the Capillary Run Data section. Information for each capillary is available by selecting the wells in the table.



4.2.6.11 The figure above from the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* shows the Capillary Run Data for a spectral calibration. Capillaries that fail are shown in red and passing capillaries are shown in green. Borrowed capillaries are shown in yellow with an arrow indicating the adjacent capillary from which results were borrowed. For dye set G5, the software will pass a capillary if the Quality Value is greater than 0.95 and the Condition Number is

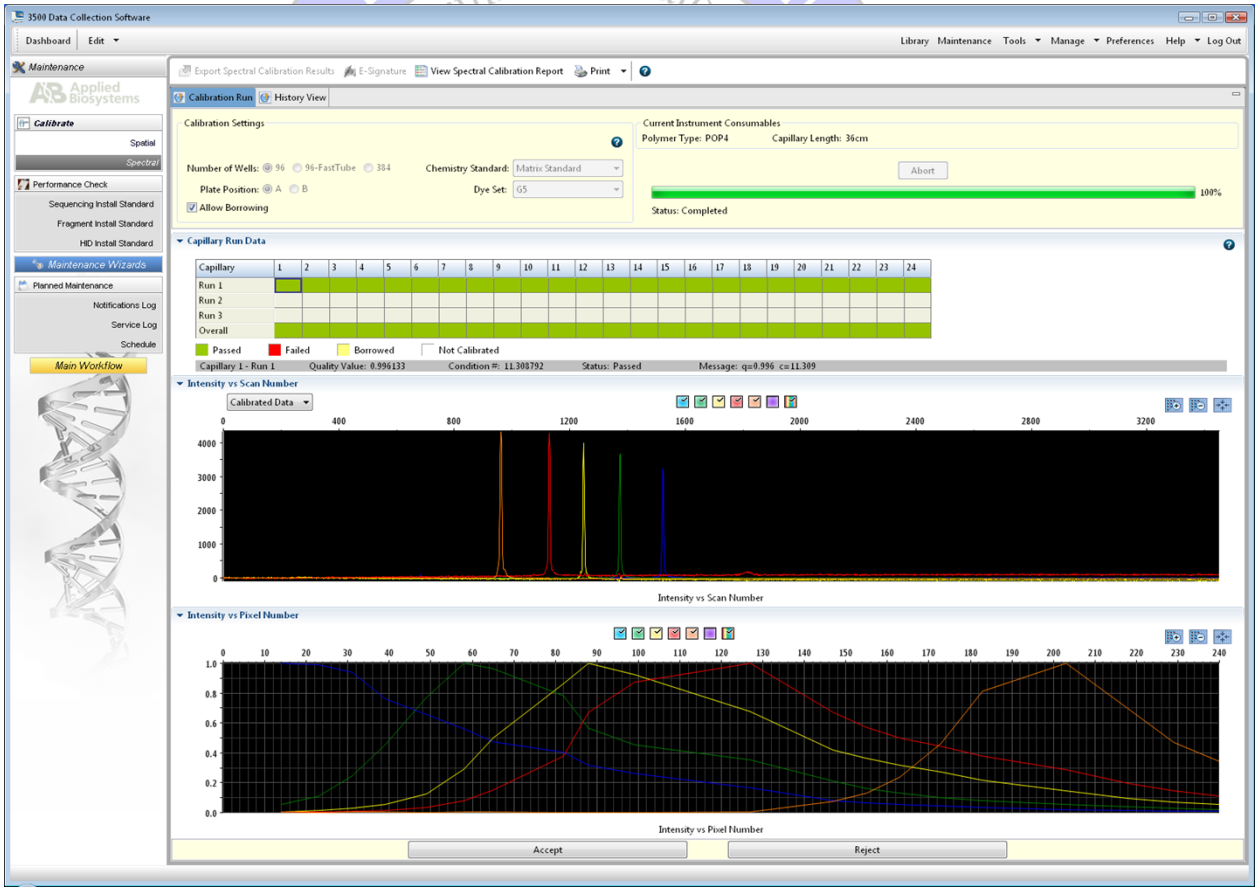
less than 13.5. For dye set J6, the software will pass a capillary if the Quality Value is greater than 0.95 and the Condition Number is less than 8.0. An array needs at least 21 of 24 capillaries to pass for the spectral to be successful. If 21 passing capillaries are not achieved, the Spectral Calibration is automatically re-injected up to two more times.

4.2.6.12 If the array fails the Spectral Calibration after all three injections, select "Reject" and re-run the Calibration. If the array fails again, it may be that the array is bad and needs replacement.

4.2.6.13 If the Spectral Calibration data is acceptable, select "Accept".

4.2.6.14 Select "View Spectral Calibration Report" to view report. Then select Print and print to the PDF printer. Save this report in the Spectral Calibration Folder.

4.2.6.15 Following is a screen shot of a five-dye spectral calibration:



4.2.7 Computer Maintenance

4.2.7.1 To ensure there is sufficient drive space, regularly archive data, delete unnecessary files, empty trash, and defragment the drives (this should be done before fragmentation reaches 10%).

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5 AB 3500xL Genetic Analyzer Sample/Instrument Set-Up

5.1 Sample Preparation

5.1.1 Label a 96-well microplate with the assigned barcode and assemble the 3500xL sample tray by placing the 96-well sample plate onto the plate base. This will become the daughter plate.

5.1.2 Add an appropriate amount of GeneScan™ 600 LIZ® to a previously aliquoted formamide tube.

5.1.2.1 For Identifiler® Direct full reactions, keep the manufacturer recommended ratio of 0.5 µL GeneScan™ 600 LIZ® to 8.5 µL formamide. Vortex and quick spin.

5.1.2.2 For Identifiler® Direct half reactions, use the ratio 0.25 µL GeneScan™ 600 LIZ® to 8.75 µL formamide. Vortex and quick spin.

5.1.2.3 For GlobalFiler® Express reactions, use the ratio 0.25 µL GeneScan™ 600 LIZ® to 9.5 µL Formamide. Vortex and quick spin.

5.1.2.4 For MiniFiler™ half reactions, use the ratio 0.25 µL GeneScan™ 600 LIZ® to 8.75 µL formamide. Vortex and quick spin.

5.1.3 Pipette appropriate amount of Formamide/LIZ® into each well.

5.1.3.1 For Identifiler® Direct full reactions, Identifiler® Direct half reactions and MiniFiler™ half reactions, pipette 9.0 µL into each well.

5.1.3.2 For GlobalFiler® Express reactions, pipette 10.0 µL into each well.

5.1.4 Add no more than 1.0 µl of amplified DNA into the corresponding wells. Follow the amplification and/or run plate layouts for sample designations.

5.1.5 Pipette no more than 1.0 µl of ladder into appropriate wells.

5.1.6 Place the 96-well septum on the 3500xL sample plate. Briefly centrifuge plate to dislodge any bubbles.

5.1.7 Denature the sample plate for three minutes at 95°C.

5.1.8 Snap cool the sample plate for three minutes. Check wells of plate for bubbles and dislodge, if necessary. Attach a plate retainer to the base.

5.1.9 In the 3500xL Data Collection Software, proceed to the Main Workflow by selecting the blue arrow in the top left corner of the Dashboard or by selecting the "Main Workflow" button in the navigation pane to the left of the Maintenance screen.

5.1.10 Unlink and remove any plates still residing on the autosampler. To unlink plates select Load Plates for Run in the navigation pane on the left. Then select the "Unlink" button in the Plates on Instrument section.

5.1.11 Place the new plate onto the 3500xL autosampler.

5.2 Creating a New Plate Record

5.2.1 In the Main Workflow navigation pane select Define Plate Properties.

5.2.2 Enter a plate name in the name field. Select 96 for the number of wells, HID for the plate type, 36 cm for the capillary length, and POP4 for the polymer type.

5.2.3 Click "Assign Plate Contents" at the bottom of the Define Plate Properties screen.

5.2.4 In Plate View define the Assay, File Name Convention, and Results Group.

5.2.4.1 Select Add from Library under Assays. In the window that opens select the appropriate assay (see Appendix F) and click "Add to Plate". Then click "Close".

5.2.4.2 Select Add from Library under File Name Conventions. In the window that opens select the file name convention named "DPS File Name Convention" and click "Add to Plate". Then click "Close".

5.2.4.3 Select Add from Library under Results Groups. In the window that opens select the results group named "DPS Results Group" and click "Add to Plate". Then click "Close".

5.2.5 While still in Plate View, select all reaction wells. Once all wells are selected click the check box next to the Assay, File Name Convention, and Results Group chosen previously. This applies all three defined parameters to the selected wells.

5.2.6 Select Table View to add plate content.

5.2.7 Enter all sample names and define the sample type for each. Use Positive Control for the amplification positive control and Negative Control for both the reagent and amplification blanks. All ladders must be set to Allelic Ladder.

5.2.8 Click "Link Plate for Run" at the bottom of the Assign Plate Contents screen. Alternatively, plates may be linked by selecting Load Plates for Run from the navigation pane.

5.2.9 Click "Create Injection List" at the bottom of the Load Plates for Run screen. Create Injection List creates a list for all linked plates.

5.2.10 Click "Start Run" at the bottom of the Preview Run screen.

5.3 Importing a Plate Record

5.3.1 In the Main Workflow navigation pane select "Assign Plate Contents".

5.3.2 Select Import in the Assign Plate Contents toolbar.

5.3.3 From the Import pop-up window, navigate to and select the file to be imported. Click "Open".

5.3.4 After applicable changes have been made, save the file with the plate barcode name.

5.4 Linking and Running an Existing Plate

5.4.1 In the Main Workflow navigation pane select Assign Plate Contents.

5.4.2 Select Open Plate in the Assign Plate Contents toolbar. In the resultant drop down menu select Edit Existing Plate. From the Open Plate pop-up window select the plate name desired and click "Open".

5.4.3 Update/edit existing content as needed. Then click "Link Plate for Run" at the bottom of the Assign Plate Contents screen.

5.4.4 To begin processing samples, click "Create Injection List" at the bottom of the Load Plates for Run screen. Click "Start Run" at the bottom of the Preview Run screen.

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6 GeneMapper® *ID-X* Data Analysis

6.1 The GeneMapper® *ID-X* software is used to determine allele calls from sized data. Sign in to the GeneMapper® *ID-X* software under analyst's name (this should be set up to be the same as the analyst's CODIS User ID), making sure the appropriate Database Host is selected.

6.2 Under the Edit drop down menu, select "Add Samples to Project." Navigate to the run folder and click on "Add to List" at the bottom of the screen. Click "Add" (or "Add and Analyze") to import the files into the project window and close the dialogue box.

6.3 After the files have been imported into the project window make sure that the required fields were populated correctly (e.g., Sample Type, Analysis Method, Panel, and Size Standard). If not, the appropriate information can be added with the use of drop-down boxes within the columns and the Fill Down feature. The more frequently used analysis methods are listed in Appendix G.

6.4 All samples will be analyzed at the analytical threshold. The GeneMapper® *ID-X* method in use is set to flag all loci with alleles that fall below the stochastic threshold.

6.5 To analyze the data (if not already done) click on the green arrow on the toolbar. If there are any conditions present that prevent analysis, the Analysis Requirements Summary dialog box will open. The analyst can choose to either view the samples that do not meet requirements or continue analysis.

6.6 The Save Project dialog box will then open. Enter the name of the project and verify the appropriate security group is selected. Select OK.

6.7 Once analysis is complete, the Analysis Summary tab is displayed. This tab provides a summary of the analysis status for the samples in the project as well as an overview of the ladder, control and sample quality for the project. Samples that do not meet all the quality thresholds may then be viewed separately from samples that pass by clicking on the applicable link, which will open up the Samples tab in a filtered mode. To revert to the unfiltered mode to view all samples, click on the project name in the left navigation pane.

6.8 The analyzed samples will display colored flags (green, yellow or red) for the PQVs (process quality values) and the SQ (sizing quality). These flags may be used to aid in reviewing the data but may not substitute for a full review of the data.

6.9 Select the samples to be reviewed in the Project window and click on Display Plots on the toolbar to examine the electropherograms.

6.10 Examine all allelic ladders and verify that the alleles were called correctly. If more than one ladder is imported into the project, the data from all the ladders will be averaged to produce a bin which is used in sizing the controls and samples. Allelic Ladders with bad or otherwise unsuccessful

injections may be removed from the project or the ladder's Sample Type may be changed from "Allelic Ladder" to "Sample". At least one ladder must remain in the project.

6.11 Examine the positive and negative controls for expected results.

6.12 Examine samples following the interpretation guidelines defined in Section 7.

6.13 Examine the GeneScan™ 600 LIZ® data, verifying the presence and appearance of required peaks.

6.14 Use the UD1, UD2, and UD3 columns to add notes regarding the sample and applicable rework points for entry into STaCS™.

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7 Interpretation

The interpretation of convicted offender/arrestee database results is a matter of professional judgment and expertise. Not every situation can or should be covered by a pre-determined rule. The following criteria are considered a minimum for the interpretation of analytical results. They are based on validation studies, published literature, population studies and database experience. It is expected that these guidelines will continue to evolve as the collective experience of the laboratory grows. The guidelines ensure that conclusions are scientifically supported by analytical data, including appropriate standards and controls. Interpretations are made as objectively and consistently as possible from analyst to analyst. Any variations to these interpretation guidelines should be approved and documented accordingly.

7.1 During the interpretation process, it may be necessary to perform re-analyses on database samples. These re-analyses may require sending the database sample back to various steps in the process. For the purposes of this protocol, the following terms are defined below:

7.1.1 Re-punch – Samples will be punched and analyzed on a new plate.

7.1.2 Re-run – Samples on the original amplification plate are set up on a new post PCR plate prior to being analyzed on the 3500xL.

7.1.3 Re-injection – Samples on the original post PCR plate are reanalyzed on the 3500xL without setting up a new plate.

7.1.4 Re-work – This is a general term implying that the analyst may either re-punch, re-run, or re-inject at their discretion.

7.2 Peak Heights

Based on validation studies, the analytical and stochastic thresholds for Identifiler® Direct full reactions, Identifiler® Direct half reactions, GlobalFiler® Express reactions and MiniFiler™ half reactions are listed in the table below. Therefore, heterozygous peak heights used for database inclusion purposes should be greater than or equal to the analytical threshold and homozygous peak heights used for database inclusion purposes should be greater than or equal to the stochastic threshold.

Method	Analytical Threshold	Stochastic Threshold
Identifiler® Direct full reactions	300	600
Identifiler® Direct half reactions	250	600
GlobalFiler® Express reactions	250	600
MiniFiler™ half reactions	300	700

7.2.1 If heterozygous peak heights are less than the analytical threshold or homozygous peak heights are less than stochastic threshold, the sample will be re-run or re-injected using a

higher validated injection time or re-punched using one or more sample punches in an area with more DNA saturation, if possible.

7.2.2 The amplification blank and the negative control associated with the samples must be analyzed at the analytical threshold to show that no DNA pattern is present above the analytical threshold.

7.2.3 If a sample is analyzed using a higher injection time, the amplification blank and the negative control will also need to be analyzed with the same or more stringent parameters.

7.2.4 No set number is defined for the maximum signal intensity, as the data analysis software will flag any such samples so the analyst can then determine data acceptance. Peak heights may be accepted if the criteria outlined in Section 7.8 are met. Samples that exceed the maximum signal intensity should have a peak shape consistent with a single allele. That is, there should be no evidence of peak broadening or shouldering that could mask another allele. Samples in which some loci are flagged as exceeding the maximum signal intensity may be accepted, but samples with excessive overload should be re-worked with less DNA.

7.3 Allelic Ladders

Sample allele calls are made by comparing the sample allele fragment size to the corresponding allelic ladder fragment size. The GeneMapper® *ID-X* software will assign allele types to sample peaks based on this comparison.

The Identifiler® Direct/Identifiler Plus ladders consist of the following alleles:

Blue:
D8S1179: 8-19
D21S11: 24, 24.2, 25-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36-38
D7S820: 6-15
CSF1PO: 6-15

Green:
D3S1358: 12-19
TH01: 4-9, 9.3, 10, 11, 13.3
D13S317: 8-15
D16S539: 5, 8-15
D2S1338: 15-28

Yellow:
D19S433: 9-12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2
vWA: 11-24
TPOX: 6-13
D18S51: 7, 9, 10, 10.2, 11-13, 13.2, 14, 14.2, 15-27

Red:
Amelogenin: X and Y

D5S818: 7-16
FGA: 17-26, 26.2, 27-30, 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

The GlobalFiler® Express ladders consists of the following alleles:

Blue:
D3S1358: 9-20
vWA: 11-24
D16S539: 5, 8-15
CSF1PO: 6-15
TPOX: 5-15

Green:
Y indel: 1, 2
Amelogenin: X, Y
D8S1179: 5-19
D21S11: 24, 24.2, 25-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36-38
D18S51: 7, 9, 10, 10.2, 11-13, 13.2, 14, 14.2, 15-27
DYS391: 7-13

Yellow:
D2S441: 8-11, 11.3, 12-17
D19S433: 6-12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, 19.2
TH01: 4-9, 9.3, 10, 11, 13.3
FGA: 13-26, 26.2, 27-30, 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

Red:
D22S1045: 8-19
D5S818: 7-18
D13S317: 5-16
D7S820: 6-15
SE33: 4.2, 6.3, 8, 9, 11-20, 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

Purple:
D10S1248: 8-19
D1S1656: 9-14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18.3, 19.3, 20.3
D12S391: 14-19, 19.3, 20-27
D2S1338: 11-28

The MiniFiler™ ladders consist of the following alleles:

Blue:
D13S317: 8-15
D7S820: 6-15

Green:
Amelogenin: X and Y

D2S1338: 15-28
D21S11: 24, 24.2, 25-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36-38

Yellow: **D16S539**: 5, 8-15
D18S51: 7, 9, 10, 10.2, 11-13, 13.2, 14, 14.2, 15-27

Red: **CSF1PO**: 6-15
FGA: 17-26, 26.2, 27-30, 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

In order to be used, these allelic values should be greater than or equal to the analytical threshold. Occasionally an anomalous peak may be labeled by GeneMapper® *ID-X* that falls outside of the usable range of the ladder. These peaks may be accepted at analyst's discretion.

7.4 Internal Size Standard

Sample alleles are sized utilizing the GeneScan™ 600 LIZ® Size Standard v2.0, which has defined size standard peaks from 20 to 600 bp, of which the following are used for sizing (in bp): 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440 and 460. These peaks are used to determine base pair lengths of sample alleles. The GeneScan™ 600 LIZ® Size Standard v2.0 peaks should be greater than or equal to the analytical threshold and should all be of similar shape and fit the expected pattern. Identifiler® Direct, GlobalFiler® Express and MiniFiler™ were validated for use incorporating the Local Southern sizing method.

7.5 Blank Controls

A negative control and an amplification blank are run with each set of samples and typed with the samples from that amplification. A negative control is a well that has had Prep-n-Go™ Buffer or Punch Solution™, as applicable, added to it at the punch set up stage as well as the amplification reagents added during amplification set-up. An amplification blank will only have the amplification reagents added to it during amplification set-up. The following should be taken into account when interpreting blank controls:

- **Spikes**: If multi-color or single color spikes are labeled by GeneMapper® *ID-X* at the analytical threshold in a blank control, then the blank may be re-injected and noted in the batch file.

- **Anomalous Artifacts**: Any laboratory documented artifacts (e.g., dye artifacts, degradation artifacts, etc.) that are labeled by GeneMapper® *ID-X* may be accepted at analyst's discretion.

- **Extraneous DNA**: If there is evidence of a DNA pattern greater than the analytical threshold in a blank control, re-inject the blank. If the same peaks are still seen, the entire plate is

inconclusive and all samples associated with the blank must be re-punched, with the event being documented in the batch file and the Extraneous DNA Log. A DNA pattern is present if there are heterozygote peaks at three or more loci and/or allelic data at four or more loci above the analytical threshold. This may be a single well event indicating contamination in the reagents and supplies or it may result from the set-up process itself.

- **Spectral Pull-Up**: If there are labeled peaks that appear to be from pull-up, it may be necessary to re-run the spectral calibration and then re-work the samples with the new matrix. Spectral pull-up labeled by GeneMapper® *ID-X* may be accepted at analyst's discretion.

7.6 Positive Controls

Two positive controls, the Control DNA 9947A and an in-house known sample, are run with each Identifiler® Direct batch. Two positive controls, the Control DNA 007 and an in-house known sample, are run with each GlobalFiler® Express and MiniFiler™ batch.

7.6.1 Control DNA 9947A is a pristine DNA sample which serves as the national standard. It is amplified with each Identifiler® Direct batch of samples and must show the proper allele calls. The results for the 9947A control are as follows:

D8S1179 – 13, 13	D2S1338 – 19, 23
D21S11 – 30, 30	D19S433 – 14, 15
D7S820 – 10, 11	vWA – 17, 18
CSF1PO – 10, 12	TPOX – 8, 8
D3S1358 – 14, 15	D18S51 – 15, 19
TH01 – 8, 9.3	Amelogenin – X, X
D13S317 – 11, 11	D5S818 – 11, 11
D16S539 – 11, 12	FGA – 23, 24

7.6.2 Control DNA 007 is a pristine DNA sample which serves as the national standard. It is amplified with each GlobalFiler® Express or MiniFiler™ batch of samples and must show the proper allele calls. The results for the 007 control are as follows:

7.6.2.1 GlobalFiler® Express DNA 007 allele calls

D3S1358 – 15, 16	Amelogenin – X, Y
vWA – 14, 16	D8S1179 – 12, 13
D16S539 – 9, 10	D21S11 – 28, 31
CSF1PO – 11, 12	D18S51 – 12, 15
TPOX – 8, 8	DYS391 – 11
Y indel – 2	D2S441 – 14, 15
D19S433 – 14, 15	TH01 – 7, 9.3
FGA – 24, 26	D7S820 – 7, 12
D22S1045 – 11, 16	SE33 – 17, 25.2
D5S818 – 11, 11	D10S1248 – 12, 15

D13S317 – 11, 11
D12S391 – 18, 19
D1S1656 – 13, 16
D2S1338 – 20, 23

7.6.2.2 MiniFiler™ DNA 007 allele calls

D13S317 – 11, 11
D7S820 – 7, 12
Amelogenin – X, Y
D2S1338 – 20, 23
D21S11 – 28, 31
D16S539 – 9, 10
D18S51 – 12, 15
CSF1PO – 11, 12
FGA – 24, 26

7.6.3 An in-house known sample is a laboratory known. It is punched and amplified with each batch of samples and must show the proper allele calls.

7.6.4 Positive control results should be greater than or equal to the analytical threshold. If no profile or a profile less than the analytical threshold is seen for the control, this may indicate a problem within the analytical process. This may be a single well event or may indicate a problem with reagents or equipment. Check the GeneScan™ 600 LIZ® size standard. If there is a problem with the GeneScan™ 600 LIZ® size standard, it indicates an injection problem. Re-inject the control. If the re-injection results in no profile or a profile less than the analytical threshold, then all samples associated with that plate must be re-punched. For re-run plates, the positive controls may be accepted as outlined in Section 7.13. On rare occasions, a positive control may have a few loci with peaks below the analytical threshold. These controls may be accepted at analyst's discretion provided that the below threshold peaks are concordant with expected allele types for the control. Analysts should take into account the overall batch results when making this determination. In addition, the in-house positive control may also take the place of the amplification control if it fails.

7.6.5 If an incorrect profile is seen in either the Control DNA or the in-house known sample, the entire plate associated with these controls is inconclusive. All samples must be re-punched.

7.6.6 If extra peaks greater than the analytical threshold are seen and labeled by GeneMapper® *ID-X* (e.g., spikes), the positive control is inconclusive and must be re-injected or re-run (with the exception of pull-up peaks as discussed in Section 7.8.6 or Incomplete 3' A nucleotide addition (minus A) as discussed in Section 7.8.4 of this manual). If no extra peaks are seen in the positive control on the reanalysis, the samples from that run may be reported. If the extra peaks are still seen (indicating the presence of extraneous DNA), then all samples associated with that positive control must be re-punched.

7.7 Off-Ladder Alleles

7.7.1 If multiple OLs are noted in a sample this may be due to temperature variations during the run resulting in migration differences between the ladders. If this occurs, the data may need to be reanalyzed using different ladders or the plate may need to be re-injected.

7.7.2 Samples that have alleles falling within the allelic calling range but less than or greater than the allelic ladder must be re-worked for verification if they are tagged "OL" by GeneMapper® *ID-X*. Sample peaks that fall in virtual bins as defined by GeneMapper® *ID-X* are accepted as designated. Alleles that are less than the lowest allele on a specific ladder are designated "less than". Alleles that are greater than the largest allele on a specific ladder are designated "greater than".

7.7.3 Samples that have alleles within the range of the allelic ladder that are tagged "OL" by GeneMapper® *ID-X* must be re-worked for verification. Verified alleles will be reported in accordance with the recommendations of the DNA Commission of the International Society of Forensic Haemogenetics with the number of complete repeat units represented by an integer and any partial repeat designated by a decimal followed by the number of bases in the partial repeat. Sample peaks that fall in virtual bins as defined by GeneMapper® *ID-X* are accepted as designated.

7.7.4 Samples that have an allele falling outside the allelic calling range and between two loci must be re-worked for verification. If it can be determined which locus the allele should be assigned to (e.g., one of the two defined loci is heterozygous while the other is homozygous), then the analyst may assign the allele to its appropriate locus. If it cannot be determined which locus the allele should be assigned to, then the sample should be re-punched and amplified with a different amplification kit to determine the locus to which the allele belongs, if possible.

7.8 Extra Peaks

If extra peaks are seen in a sample, this may indicate a mixture, spikes, stutter, incomplete 3' A nucleotide addition, matrix failure, overloading, a trisomy or extraneous DNA.

7.8.1 Assess the likelihood of a mixture. The presence of greater than two alleles per locus across multiple loci, the presence of higher than normal stutter percentages or the presence of imbalanced heterozygotes may indicate the presence of extraneous DNA or a chimeric individual. If extraneous DNA is labelled by GeneMapper® *ID-X*, assess all other samples and blank controls for indications of contamination. The affected sample will need to be re-worked. If extraneous DNA is still observed in the sample after the second analysis, a new sample will need to be requested for the individual. If the source of the extraneous DNA cannot be determined, then the mixed profile may be uploaded to CODIS as a multi-allelic offender. If possible, a second submission from the individual should be worked to verify the chimeric profile. If the results from the second analysis demonstrate no mixed profile, then the mixed profile must be removed from CODIS. The DNA Database Technical Leader will be notified and the event will be logged and maintained as a record.

7.8.2 If electronic spikes are labeled by GeneMapper® *ID-X*, re-inject or re-run the sample. Pull-up peaks that are labeled "spike" by GeneMapper® *ID-X* may be accepted at the analyst's discretion as outlined in Section 7.8.6.

7.8.3 Stutter peaks can occur as a minor product one repeat segment shorter than the principle peak. On rare occasions, stutter may also be observed as a minor product one repeat segment greater than the principle peak. Peaks in the stutter position labeled by GeneMapper® *ID-X* may be an indication of a mixture, elevated stutter, a trisomy, or a primer binding site mutation. The labeled stutter peak shall be assessed for signs of a mixture. If no mixture is present and no trisomy is suspected, then the sample may be accepted and documented in the batch file.

7.8.4 Incomplete 3' A nucleotide addition (minus A) results in a split peak separated in size by only one base pair. This indicates too much DNA is present in the amplification. Incomplete 3' A nucleotide addition labeled by GeneMapper® *ID-X* may be accepted at analyst's discretion provided that only a few alleles exhibit minus A and the minus A is not masking another peak. This is not intended to pertain to samples in which the vast majority of alleles demonstrate this phenomenon or a plate in which many samples exhibit this phenomenon. It may be possible to reduce incomplete 3' A nucleotide addition by re-extending the amplified DNA in the thermal cycler at 60°C for 25 minutes. The re-extended sample may also be diluted in Formamide/LIZ prior to preparing it for injection (referred to as an in-well dilution). If many samples exhibit minus A, then the analyst should send those samples back for re-punching.

7.8.5 On rare occasions samples may exhibit split peaks separated in size by only one base pair, where the shoulder peak is one base pair larger than the main peak. This appears to be a result of analyzing amplified product that has been retained for an extended amount of time. Samples in which this shoulder peak is labeled by GeneMapper® *ID-X* will not be accepted and may need to be re-punched.

7.8.6 A poor matrix can result in spectral pull-up where the major peak in one color is seen as a minor peak in another color. These minor peaks are due to the process by which the instrument filters out spectral overlap between the dyes. To correct this problem in batches where the vast majority of samples demonstrate pull-up, run a new spectral calibration and re-work the samples using this new matrix. Spectral pull-up may be accepted at analyst's discretion provided the electropherogram demonstrates that the extra labeled peaks in one color are due to pull-up from another color and the pull-up peak is below the stochastic threshold. It should be noted that this exception is intended for a few samples within a run and not meant to pertain to runs in which the vast majority of samples demonstrate this phenomenon. On rare occasions an analyst may accept pull-up peaks above the stochastic threshold if several attempts have been made to remedy the situation.

7.8.7 Overloading can result in peaks greater than maximum signal intensity or larger peaks at small loci and progressively smaller peaks as the loci get larger. To try to correct this problem, an in-well dilution may be performed and the sample re-injected. An in-well dilution is performed by removing a specified amount of well content (sample + Formamide/LIZ) and replacing it with the same volume of Formamide/LIZ. Alternatively, the sample may be diluted and rerun on a new plate set up. Overloading may also result in

stutter being labeled. Labeled stutter may be accepted as outlined in section 7.8.3. If the sample is still too strong, it may be necessary to re-punch the sample in a region that would be expected to yield less DNA.

7.8.8 On rare occasions, an individual may possess a three peak pattern (trisomy) at a locus. The third peak in the triad is often smaller in height than the other two and sometimes may fall below the analytical threshold. When the third peak is labeled by GeneMapper® *ID-X*, the sample shall be re-worked to verify the presence of the triad if the peak is to be entered into CODIS. It may be necessary to re-punch and amplify the sample with a different amplification kit to verify the locus to which the peak belongs. If the third peak is significantly lower in peak height than the other two peaks, then that peak may be declared inconclusive and only the two acceptable peaks should be entered into CODIS. The presence of the third peak shall be noted in the batch file. If the labelled third peak is considered to be stutter, then see section 7.8.3.

7.8.9 Some amplification kits may demonstrate anomalous dye artifacts that may be kit specific or lot specific. Dye artifacts that are known to have been seen in the laboratory and are documented may be accepted at analyst's discretion provided it does not appear to be masking an allelic peak. Any samples exhibiting dye artifacts that haven't been documented will need to be re-worked.

7.8.10 On rare occasion, high level artifacts may be observed at the Y indel locus. These peaks are typically the result of spectral pullup as outlined in section 7.8.6 and are usually smaller in height than peaks at other loci. To try to correct this problem, the sample may be diluted and re-run. Artifact peaks may be labeled as off-ladder by GeneMapper® *ID-X*; however, unlike STR loci, the Y indel insertion / deletion locus is not susceptible to true off-ladder alleles. Additionally, Y indel will not exhibit stutter peaks that are typical at STR loci. Artifact peaks may be discerned from true peaks by reviewing allele calls at other gender specific loci. If Y peaks do not exist at Amelogenin and DYS391, it is likely that any called peaks at Y indel can be attributed to artifacts. Y indel artifacts (pull up, spikes, etc.) may be accepted at analyst's discretion in accordance with the above considerations and section 7.8.6.

7.8.11 Labelled artifacts (pull up, multi-color spike, etc.) at the Amelogen and DYS391 loci may be accepted at analyst's discretion based upon the artifact and consideration of the gender of the sample being tested. In these situations, the analyst shall document the artifact as inconclusive and may accept the locus results.

7.9 Degradation

At certain levels of degradation only the smaller loci may be detected. If degradation is suspected, the sample should be re-punched with multiple punches to obtain more DNA if possible. It may be necessary to re-punch the sample and amplify using the MiniFiler™ kit to create a combined profile. If a CODIS eligible profile still cannot be developed, it may be necessary to obtain a new sample

from the individual. Peaks at CODIS non-core loci may have inconclusive peak heights due to degradation. This would not preclude the sample from being uploaded into CODIS

7.10 Heterozygosity

In sole source samples, heterozygote pairs are generally well balanced resulting in values above 70% for heterozygote ratios. Unbalanced heterozygote pairs at ratios lower than 70% may be observed. The analyst may take this into account and accept data from unbalanced heterozygote pairs if no other evidence of a mixed profile exists (more than two alleles at multiple loci and the presence of higher than normal stutter percentages). In any event, the database analyst is the one responsible for the declaration of profiles containing unbalanced heterozygotes and entry of the profiles into CODIS. Based on peak morphology and overall profile heterozygosity, an analyst may re-work the sample if an unbalanced heterozygote is in question. Duplication of the unbalanced heterozygote pair increases the confidence that it is a true unbalanced heterozygote pair. Data will be accepted if the database analyst is confident with the heterozygote call.

7.11 Inhibition

The Applied Biosystems' kits have been optimized to overcome PCR inhibition. Instances of inhibition are not expected; however, inhibition may be occurring if no DNA peaks or weak DNA peaks are observed. If inhibition is suspected, the sample may be re-punched in a less saturated area to reduce the amount of template DNA and the possible inhibitors.

7.12 Combined Profiles

A DNA profile may be obtained by combining the results from multiple runs (from the same or different amplification kits) as long as all of the alleles shared between the multiple runs show complete concordance with each other. On occasion, there may be a discordant allele between MiniFiler™ and Identifiler® Direct or GlobalFiler® Express, which does not necessarily preclude the creation of a combined profile. If this occurs, the sample shall be re-punched and amplified. If the discordance is duplicated, it may be accepted at analyst's discretion and shall be documented in the batch. These are not composite profiles as defined by NDIS and are therefore acceptable for CODIS upload.

7.13 Re-injections and Re-runs

At times it will be necessary to re-inject a plate or re-run samples. Re-injections and re-runs of samples from previously run plates are considered extensions of their previous run. Therefore, positive control results from previous runs may be used if the positive control on the reanalyzed plate does not work, provided those controls were determined to be acceptable. Negative control results from previous runs may be used if the negative control on the reanalyzed plate does not work, provided those controls were determined to be acceptable and provided the negative controls are run with the most stringent parameters used for that plate as outlined in Section 7.2.3. In these instances,

the allelic ladder may act as the positive control and the GeneScan™ 600 LIZ® internal lane size standard may be used to demonstrate that the reanalyzed data is acceptable.

7.14 Amelogenin, Y indel, DYS391 and SE33

If peak data from any of these four loci do not meet the above written guidelines, the analyst may document the loci inconclusive because they are not part of the 20 CODIS core loci and are not required to be entered into CODIS. If this is the case, any peak data from these loci will not be entered into CODIS. Amelogenin, Y indel are not STR loci. DYS391 is a haplotype locus. Therefore, peaks at these loci are not subject to stochastic threshold requirements.

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8 Hit Confirmations

Database samples (from offender and arrestee collections) are uploaded into the Combined DNA Index System (CODIS) and searched on a regular basis as outlined in the CODIS Procedures Manual and the CODIS Standard Operating Procedures Manual. When a database hit occurs, the confirmation procedure begins. Once the sample has been confirmed, database information will be released to the casework laboratory.

8.1 Hit Confirmation Procedure

8.1.1 The detailed procedure for confirming hits is outlined in the *CODIS Procedures Manual*.

8.1.2 The fingerprints of the matching offender/arrestee are compared to the fingerprints on file with DPS for that offender/arrestee, if possible.

8.1.3 The database sample will be re-analyzed. The sample may be batched with other database samples and/or other database hits. Once the sample has been re-analyzed, the newly obtained profile is compared to the previously obtained profile for confirmation. The database confirmation batch will be assembled similarly to database batches and will go through technical and administrative reviews.

8.1.4 The appropriate CODIS paperwork will be completed and released to the casework laboratory.

8.2 Interpretation

Interpretation of database samples that have been analyzed for confirmatory purposes will follow the guidelines listed in Section 7 with the following exceptions:

8.2.1 Sometimes amplified confirmation samples will have peaks below threshold. If this is the case, the analyst may re-analyze the data at a lower threshold in order to obtain at least 6 core GFE loci for comparison to the original analysis. The analyst may accept the confirmation provided there is no exclusionary data when compared to the original analysis. Matching homozygote peaks below the stochastic threshold may be accepted at analyst's discretion. Those loci that fall below the analytical threshold will be documented as inconclusive.

8.2.2 If a confirmation sample has an off-ladder allele that matches the original database profile uploaded into CODIS, the off-ladder allele in the confirmation sample will not need to be re-worked, as it has already been confirmed.

8.2.3 If a confirmation sample has a multi-color spike, the sample may still be accepted, provided the allele calls match the original database profile uploaded into CODIS.

8.2.4 If the positive control has a couple of loci below the analytical threshold, the control may still be accepted at analyst's discretion provided the allelic information below the analytical threshold is consistent with the expected results. The same holds true for matching homozygote peaks below the stochastic threshold. If one of the positive or negative controls fails (excluding EDNA), the other control (positive or negative) may be used as the batch positive/negative control and the batch may be accepted at analyst's discretion. This is acceptable since the hit confirmation sample profiles have previously been determined.

8.3 Matches Not Confirmed

The expectation is that offender confirmation sample will match with the original database profile uploaded into CODIS. However, in the unlikely event a sample is not confirmed as matching the previously uploaded sample, further investigation must occur before any paperwork is released to the casework laboratory, which may include, but is not limited to, the following:

8.3.1 In the event the sample matches a different offender/arrestee than expected, the analyst will need to investigate the original database analysis batch containing the sample in question to try to deduce the source of the mix-up.

8.3.2 It is possible that the confirmation sample will need to be re-worked.

8.3.3 It may be necessary to remove the original sample from CODIS until the source of the mix-up is established. It may also be necessary to communicate the issue to the casework agency as there may be a delay in the release of the database information.

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9 RapidHit™ 200 DNA Analysis

Database samples may be analyzed and uploaded into CODIS using the RapidHit™ 200 analysis system and Modified Rapid DNA Analysis. The Rapid DNA instrument is an all-in-one DNA analysis system designed to detect STR DNA profiles from biological samples within approximately two hours. All of the steps of DNA analysis (extraction, purification, amplification, capillary electrophoresis separation and profile detection) are performed in this single instrument.

9.1 Sample Preparation

9.1.1 Database samples must be checked into STaCS prior to analysis.

9.1.2 Fill out analysis worksheet with samples that are being analyzed.

9.1.3 Place the cotton swab into the corresponding well of the sample cartridge. Samples should be opened and placed in the cartridge one at a time. Alternately, if a swab is not available then a 6 mm size sample punch from the curved end of the collector may be used. If this is done, the samples should be punched one at a time and an indicator (cotton swab, lancet, etc.) should be used to cover the sample prior to punching the next sample.

9.1.4 An in-house control along with a blank control will be analyzed on the first cartridge run for the specific lot number in use. This will be performed before database samples are analyzed. The in-house positive control will be a freshly prepared buccal swab from an analyst with a known profile.

9.1.5 Cover the sample cartridge with the plastic seal when completed.

9.2 RapidHit™ 200 Run

9.2.1 Turn on the instrument by pressing the power switch on the front of the instrument.

9.2.2 Log into the instrument software by using the fingerprint reader or known password. Be sure that the computer cursor is in the log-in box prior to fingerprint scanning.

9.2.3 In the home screen, select the green start button to initiate a run.

9.2.4 Follow the steps on the software screen to set up a run. If at any time you need to get back to the previous menu/screen, select the blue back arrow button.

9.2.5 The software will prompt for Cartridge A and Cartridge B to be inserted into their respective positions. Follow the on-screen instructions. Remove the used Cartridge A and discard it appropriately prior to inserting the new cartridge. Do the same for Cartridge B. Make sure the doors for both cartridges are closed. Once the changes are successful, a green checkmark will appear by the cartridge icons on the instrument screen.

9.2.6 Insert the two sample cartridges. Green arrows will appear by the cartridge icons on the instrument screen when the sample cartridges are inserted correctly.

NOTE: The two plastic strips on the front side of the sample cartridge face out (towards the user).

9.2.7 When all four cartridges are correctly inserted into the instrument, the Rapid software will prompt the user to select the analysis protocol. Select the icon for the Buccal program. Alternately, if a punch is used select the icon for the Other program.

9.2.8 Enter sample identification numbers (STaCS numbers) in the display fields on the sample ID screen using the on-screen keyboard and the run analysis worksheet.

NOTE: Well six is reserved for the allelic ladder. Therefore, sample information cannot be typed in well six.

9.2.9 Close the sample cartridge door. This will automatically start the run.

9.2.10 The progress bar on the middle of the instrument screen displays the remaining run time.

9.2.11 When the run is complete, select the blue arrow button to continue. This step releases the sample cartridges so they can be removed. Remove the sample cartridges when the instrument software prompts. This is indicated by red arrows pointing upwards towards the cartridge icons. When the sample cartridges are removed, the run data will be processed.

NOTE: Do not turn off the instrument prior to removing the sample cartridges. This will cause the run data to be erased.

9.2.12 View the sample profiles when the results screen is displayed. Select the Quality button associated with the sample. The three quality boxes are: a green box with a "check", a yellow box with a "flag" and a red box with an "X". These boxes are used to assist basic users in determining whether sample data is of requisite quality.

9.2.13 Use the slide bar on the right side of the instrument screen to scroll down through the electropherogram profile results.

9.2.14 To view run data from a previous run, select the View Results button (multi-page icon with an eye found on the lower left bottom of the screen) from the home screen. The displayed screen will show all of the previous runs sorted by run date. Select the green box associated with the desired sample to view the run. See next section to print run electropherogram data.

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9.2.15 When finished, select the log out button (icon with lock and arrow found on the lower right bottom of screen). This will bring the instrument to the login screen. Turn off the instrument when finished.

9.3 Review of Sample Profiles

9.3.1 While on the home screen, select the Export Run Folder button (multi-page icon with arrow found on left bottom of the screen).

9.3.2 Select the desired sample run to export by touching the box to the right of the sample.

9.3.3 Insert a flash drive into the USB port. The run data will transfer automatically to the flash drive.

9.3.4 When the transfer is completed, take the flash drive to a computer and insert it into the computer USB port. Find the desired run on the flash drive and click on the run folder to be reviewed. Double click the GM_Analysis.sgf file to open the project in GeneMarker®.

9.3.5 Review the sample data and make appropriate edits.

9.3.6 Verify the ILS score is at least 90 for each sample and ladder by clicking on the Size Calibration button. If the ILS score is below this value, then the results for that sample will not be used.

9.3.7 Once the data is reviewed, the project will be saved into a shared folder for technical review.

9.4 Viewing the PDF Report

9.4.1 Find the desired run on the flash drive and click on the PDF Report. This report is a multi-page document. One of the pages displays the dynamic analytical threshold for each locus. This information may be used to help determine whether single peak profiles have reached the stochastic threshold.

9.5 Data Interpretation Guidelines

9.5.1 Peak Heights

9.5.1.1 Heterozygous peaks used for database inclusion purposes must be equal to or greater than the analytical threshold calculated for that locus (average instrument noise plus 15 times the standard deviation). Homozygous peaks used for database inclusions must be greater than or equal to the stochastic threshold calculated for that locus (5 times the locus specific analytical threshold for the Buccal program or 18 times the locus specific analytical threshold for the Other program). See 9.5.1.2 for the exception.

9.5.1.2 If heterozygous peaks are below the analytical threshold and homozygous peaks are below the stochastic threshold, the loci will be declared inconclusive and the sample will be re-worked using the 3500xL genetic analyzer. For proficiency test samples, an analyst may accept single peaks below the stochastic threshold (and above the analytical threshold) as a homozygote if the same results are previously obtained from another conventional amplification kit. This will help cut down on excessive use of Rapid kits. SE33 peaks that fall out of these criteria may be declared as inconclusive.

9.5.1.3 No set number is defined for the maximum signal intensity. DNA peaks should have shapes consistent with single alleles. That is, there should be no evidence of peak broadening or shouldering that could mask another allele. Labeled pull-up peaks are an indication of strong sample data. However, labeled pull-up peaks may be accepted and edited at analyst's discretion. Sample data with excessive labeled pull-up peaks should be rejected.

9.5.2 Allelic Ladder

The GlobalFiler® Express allelic ladder is present in the sample cartridge and is run with each analysis. The results from the allelic ladder run may not be used by the instrument software. The instrument may pull from a database of previously analyzed allelic ladders and use the ladder that best fits the migration for each sample in the current analysis.

The alleles documented in the ladder represent the most commonly encountered alleles within the population. On occasion, minor peaks within the ladder may be labeled by GeneMarker®. This should not preclude the interpretation of the sample runs.

9.5.3 Internal Size Standard

An Internal Size Standard (ILS) is used in each sample run. The following size standard peaks are used in the sample analyses: 80bp, 90bp, 100bp, 120bp, 140bp, 160bp, 180bp, 190bp, 200bp, 220bp, 240bp, 260bp, 280bp, 300bp, 320bp, 340bp, 360bp, 380bp, 400bp, 425bp, 450bp, 475bp, 490bp and 500bp. On occasion, some of the size standard peaks may not be labeled by the instrument software. This should not preclude the interpretation of samples if this occurs provided the ILS score is at least 90 coupled with the correct placement of the ILS markers.

9.5.4 Blank Control

A blank control will be analyzed once on the first cartridge set run for the specific lot number in use. This will be performed prior to the analysis of any database samples run with that cartridge set lot number. This control should show no DNA profile results. The following labeled peaks are permitted at analyst's discretion: pull-up peaks from the ILS, single and multi-color spikes and anomalous artifacts (dye, raised baseline and primer).

If a DNA pattern is present above the analytical threshold, the profile results from the run are deemed inconclusive and the controls must be analyzed again. A DNA pattern is present if there are heterozygous peaks at three or more loci and/or allelic data at four or more loci above the analytical threshold.

9.5.5 Positive Control

An in-house positive control will be analyzed on the first cartridge run for the specific lot number in use. This will be performed prior to the analysis of any database samples run with that cartridge set lot number. This control is a freshly prepared buccal sample collected from an analyst with a known profile. The control should show a DNA profile consistent with the expected results (see in-house positive control allele table). On occasion, a few loci may not amplify successfully or homozygous peaks may fall below the stochastic threshold. This should not preclude the acceptance of the positive control since this is a known sample. The following additional labeled peaks that are permitted at analyst's discretion include: labeled pull-up peaks, multi-color spikes or other electronic noise issues and anomalous artifacts.

If the profile is not sized correctly due to migration issues, and there are no other positive controls run on that cartridge set then an in-house positive control must be run on another cartridge set from that specific lot number. If the positive control is not sized correctly on any of the cartridge sets in the batch, then the lot number will be rejected.

If an incorrect profile is observed in the control then an in-house positive control must be run on another cartridge set from that specific lot number.

If there is evidence of a mixture in the positive control above the analytical threshold, which may indicate the presence of extraneous DNA, then an in-house positive control must be run on another cartridge set from that specific lot number.

9.5.6 Off Ladder Alleles

Any alleles labeled by the instrument software as "OL" must be examined to determine if these peaks are associated with the sample or not. If they are, then the sample must be re-analyzed to verify the "OL" if they are to be entered into CODIS. If they are not true DNA peaks associated with the sample (e.g., pull-up, spikes etc.), then they may be disregarded and edited at the discretion of the Rapid analyst. A sample may contain "OL" alleles at the SE33 locus. This locus will be deemed as inconclusive.

Any rare micro-variants that fall into virtual bins and are labeled by the software will be accepted.

9.5.7 Extra Peaks

If extra peaks are observed in a sample, this may indicate a mixture, spikes, stutter, incomplete 3' A nucleotide addition, matrix failure, trisomy or anomalous artifacts.

9.5.7.1 Assess the likelihood of a mixture. The presence of greater than two alleles per locus across multiple loci, the presence of higher than normal stutter percentages or the presence of imbalanced heterozygotes may indicate the presence of extraneous DNA or a chimeric individual. If extraneous DNA is observed above the analytical threshold, assess all other samples for indications of contamination. The affected sample will need to be re-worked. If extraneous DNA is still observed in the sample after the second analysis, a new sample will need to be requested for the individual. If the source of the extraneous DNA cannot be determined, then the mixed profile may be uploaded to CODIS as a multi-allelic offender. If possible, a second submission from the individual should be worked to verify the chimeric profile. If the results from the second analysis demonstrate no mixed profile, then the mixed profile must be removed from CODIS. The DNA technical leader will be notified and the event will be logged and maintained as a record.

9.5.7.2 Labeled peaks due to electronic spikes (single color and multi-color) may be accepted at analyst's discretion. These peaks will be documented as such by the Rapid analyst in the case file.

9.5.7.3 Stutter peaks can occur as a minor product one repeat segment shorter than the principle peak. On rare occasions, stutter may also be observed as a minor product one repeat segment greater than the principle peak. Peaks in the stutter position labeled by the instrument software may be an indication of a mixture, elevated stutter, a trisomy, or a primer binding site mutation. The labeled stutter peak shall be assessed for signs of a mixture. If no mixture is present and no trisomy is suspected, then the sample may be accepted and documented as such in the case file.

9.5.7.4 Incomplete 3' A nucleotide addition (minus A) results in a split peak separated in size by only one base pair. This indicates too much DNA is present in the amplification. Incomplete 3' A nucleotide addition labeled by the instrument software may be accepted at analyst's discretion provided that only a few alleles exhibit minus A and the minus A is not masking another peak. It is not intended to pertain to samples in which the vast majority of alleles demonstrate this phenomenon. This will be documented by the Rapid analyst in the case file.

9.5.7.5 A poor matrix can result in spectral pull-up where the major peak in one color is seen as a minor peak in another color. These minor peaks are due to the process by which the instrument filters out spectral overlap between the dyes. Spectral pull-up may be accepted at analyst's discretion provided the electropherogram demonstrates that the extra labeled peaks in one color are due to pull-up from

another color. It should be noted that this exception is intended for a few pull-up peaks within a cartridge run and not meant to pertain to runs in which there is an excessive amount of pull-up.

NOTE: All homozygous loci should be examined to determine the likelihood of pull-up peaks being labeled as alleles in other loci. Accepted pull-up peaks must be documented as such by the Rapid analyst in the case file.

9.5.7.6 On rare occasions, an individual may possess a three peak pattern (trisomy) at a locus. The third peak in the triad is often smaller in height than the other two and sometimes may fall below the analytical threshold. When the third peak is labeled by the instrument software, the sample shall be re-analyzed to verify the presence of the triad if the peak is to be entered into CODIS. It may be necessary to re-analyze the sample with a different amplification kit to verify the locus to which the peak belongs. If the third peak falls below the analytical threshold, then the analyst may declare the peak as inconclusive and only the two acceptable peaks should be entered into CODIS. The presence of the third peak shall be noted by the Rapid analyst in the case file. If the labelled third peak is considered to be stutter, then see section 9.5.7.3.

9.5.7.7 Labeled anomalous artifacts that are known to have been seen in the laboratory and are documented may be accepted at analyst's discretion. These documented artifacts will be noted by the Rapid analyst in the case file. Any samples exhibiting artifacts that haven't been documented will need to be re-analyzed.

9.5.8 **Heterozygosity**

In sole source samples, heterozygote pairs are generally well balanced resulting in average values above 70% for heterozygote ratios. However, unbalanced heterozygote pairs at ratios lower than 70% will be observed. The analyst may take this into account and accept data from unbalanced heterozygote pairs if no other evidence of a mixed profile exists (more than two alleles at multiple loci and the presence of higher than normal stutter percentages). In any event, the database analyst is the one responsible for the declaration of profiles containing unbalanced heterozygotes and entry of the profiles into CODIS. Based on peak morphology and overall profile heterozygosity, an analyst may re-analyze the sample if an unbalanced heterozygote is in question. Duplication of the unbalanced heterozygote pair increases the confidence that it is a true unbalanced heterozygote pair. Data will be accepted if the database analyst is confident with the heterozygote call.

9.5.9 Amelogenin, Y indel, DYS391 and SE33

If peak data from any of these four loci do not meet the above written guidelines, the analyst may document the loci inconclusive because they are not part of the 20 CODIS core loci and are not required to be entered into CODIS. If this is the case, any peak data from these

loci will not be entered into CODIS. Amelogenin and Y indel are not STR loci. DYS391 is a haplotype locus. Therefore, peaks at these loci are not subject to stochastic threshold requirements.

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Appendix A Reagents Supplied in Kits

A.1 AmpFlSTR® Identifiler® Direct Kit, 1000 reactions [*200 reactions*]

Upon receipt, separate the Master Mix, Primer Set, and Control DNA from the rest of the kit and store at -15° to -25°C away from amplified DNA product. Reagents may be stored at 2° to 8°C after initial use. The Allelic Ladders are stored at -15° to -25°C in the post-amplification lab. After initial use, they may be stored at 2° to 8°C in the post-amplification lab.

A.1.1 AmpFlSTR® Identifiler® Direct Master Mix, 1 bottle, 12.5 mL [*2 tubes, 1.25 mL/tube*], contains $MgCl_2$, deoxynucleotide triphosphates (dATP, dCTP, dGTP, dTTP), enzyme, bovine serum albumin (BSA) and 0.04% sodium azide (NaN_3) in buffer and salt.

CAUTION: Sodium azide can react with lead and copper plumbing to form explosive metal azides. On disposal of the reagents, flush with a large volume of water to prevent azide build-up.

A.1.2 AmpFlSTR® Identifiler® Direct Primer Set, 1 bottle, 12.5 mL [*2 tubes, 1.25 mL/tube*], contains locus-specific 6-FAM™, VIC®, NED™, and PET® dye-labeled and unlabeled primers in buffer to amplify the STR loci D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA and the gender marker Amelogenin.

A.1.3 AmpFlSTR® Identifiler® Direct Control DNA 9947A, 1 tube, 50 µL, contains 2.0 ng/µL human cell line DNA in 0.05% NaN_3 and buffer.

A.1.4 AmpFlSTR® Identifiler® Direct/AmpFlSTR® Identifiler® Plus Allelic Ladder, 1 tube, 0.1 mL [*1 tube, 50 µL*]

A.2 GlobalFiler® Express Kit, 1000 reactions [*200 reactions*]

Upon receipt, separate the Master Mix, Primer Set and Control DNA from the rest of the kit and store at -15° to -25°C away from amplified DNA product. Reagents may be stored at 2° to 8°C after initial use. The Allelic Ladders are stored at -15° to -25°C in the post-amplification lab. After initial use, they may be stored at 2° to 8°C in the post-amplification lab.

A.2.1 AmpFlSTR® GlobalFiler® Express Master Mix, 1 bottle, 5.64 mL [*1 tube, 1.13 mL*], contains $MgCl_2$, deoxynucleotide triphosphates (dATP, dCTP, dGTP, dTTP), enzyme, bovine serum albumin (BSA) and 0.05% sodium azide (NaN_3) in buffer and salt.

CAUTION: Sodium azide can react with lead and copper plumbing to form explosive metal azides. On disposal of the reagents, flush with a large volume of water to prevent azide build-up.

A.2.2 AmpFlSTR® GlobalFiler® Express Master Mix Additive, 1 tube, 0.45mL [*1 tube, 0.1mL*]

A.2.3 AmpFlSTR® GlobalFiler® Express Primer Set, 1 bottle, 6.0 mL [*1 tube, 1.2 mL*], contains locus-specific 6-FAM™, VIC®, NED™, PET® and TAZ™ dye-labeled and unlabeled primers in buffer to amplify the STR loci D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, DYS391, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338, along with the Y indel insertion/ deletion locus and the gender marker Amelogenin.

A.2.4 AmpFlSTR® GlobalFiler® Express Control DNA 007, 1 tube, 0.1mL [*1 tube, 0.05mL*], contains 2.0 ng/µL human cell line DNA in 0.05% NaN_3 and buffer.

A.2.5 AmpFlSTR® GlobalFiler® Express Allelic Ladder, 1 tube, 0.1 mL [*1 tube, 50 µL*]

A.3 AmpFlSTR® MiniFiler™ Kit, 100 reactions

Upon receipt, separate the Master Mix, Primer Set, and Control DNA from the rest of the kit and store at -15° to -25°C away from amplified DNA product. Reagents may be stored at 2° to 8°C after initial use. The Allelic Ladders are stored at -15° to -25°C in the post-amplification lab. After initial use, they may be stored at 2° to 8°C in the post-amplification lab.

A.3.1 AmpFlSTR® MiniFiler™ Master Mix, 2 tubes, 1.25 mL/tube, contains enzyme, salts, dNTPs, carrier protein, and 0.05% sodium azide.

A.3.2 AmpFlSTR® MiniFiler™ Primer Set, 1 tube, 0.5 mL, contains locus-specific 6-FAM™, VIC®, NED™, and PET® dye-labeled and unlabeled primers in buffer to amplify the STR loci D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO, FGA and the gender marker Amelogenin.

A.3.3 AmpFlSTR® MiniFiler™ Control DNA 007, 1 tube, 0.3 mL, contains 0.10 ng/µL human male genomic DNA in 0.05% sodium azide and buffer.

A.3.4 AmpFlSTR® MiniFiler™ Allelic Ladder, 1 tube, 0.05 mL

A.4 Punch Solution™ Kit [*100 preparations*]

Upon arrival, the Punch Solution™ reagent should be thawed completely, mixed by gentle inversion and stored at 2° to 10°C.

A.4.1 Punch Solution™ Reagent, 1 mL

A.4.2 5X Amp Solution™ Reagent, 500 µL

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Appendix B Reagents not Supplied in Kits

B.1 AmpFlSTR® Identifiler® Direct, GlobalFiler® Express and MiniFiler™

B.1.1 Anode Buffer Container (ABC) 3500 Series

B.1.2 Cathode Buffer Container (CBC) 3500 Series

B.1.3 Hi-Di™ Formamide

B.1.4 GeneScan™ 600 LIZ® Size Standard v2.0

B.1.5 DS-33 Matrix Standard Kit (Dye Set G5)

B.1.6 DS-36 Matrix Standard Kit (Dye Set J6)

B.1.7 POP-4™ Polymer for 3500/3500xL Genetic Analyzers

B.1.8 Conditioning Reagent, 3500 Series

B.1.9 3500xL Genetic Analyzer Capillary Array, 36 cm

B.1.10 Prep-n-Go™ Buffer

B.1.11 TE Buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0)

B.1.12 PCR Enhancer (GlobalFiler® Express)

Appendix C Reagent Preparation

Record when reagents are prepared or received in the QC/Inventory log. Follow all required quality control guidelines. The following reagents may be available commercially. Follow manufacturer's specifications.

C.1 <u>Formamide/LIZ® for Identifiler® Direct full reactions</u>

The following ratio will be used:

1020 µL deionized formamide

60 µL GeneScan™ 600 LIZ®

Vortex and quick spin. Store refrigerated for up to 2 weeks.

C.2 <u>Formamide/LIZ® for Identifiler® Direct and MiniFiler™ half reactions</u>

The following ratio will be used:

1020 µL deionized formamide

29 µL GeneScan™ 600 LIZ®

Vortex and quick spin. Store refrigerated for up to 2 weeks.

C.3 Formamide/LIZ® for GlobalFiler® Express reactions

The following ratio will be used:

1020 µL deionized formamide

27 µL GeneScan™ 600 LIZ®

Vortex and quick spin. Store refrigerated for up to 2 weeks.

WARNING: Formamide is a teratogen and is harmful by inhalation, skin contact and ingestion. Use in a well-ventilated area. Use necessary precautions when handling.

C.4 TE Buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0)

10 mL 1 M Tris-HCl, pH 8.0

0.2 mL 0.5 M EDTA

990 mL DI water

Dispense into aliquots and autoclave. Store at room temperature or refrigerated.

C.5 0.39 M DTT

1.0 mL sterile water

60 mg DTT (Dithiothreitol)

Vortex and quick spin.

C.6 Proteinase K (20 mg/mL)

1.0 mL sterile water

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20 mg Proteinase K

Dissolve in a sterile microcentrifuge tube. Aliquot 100 µL into each of 10 sterile microcentrifuge tubes. Store frozen.

C.7 Stain Extraction Buffer Premix

1.51 g Tris base

4.63 g $Na_2EDTA \cdot 2H_2O$

7.3 g NaCl

500 mL DI water

Dissolve and adjust pH to approximately 8.0. Adjust volume to 2 liters with DI water. Filter, sterilize or autoclave. Store refrigerated.

C.8 Stain Extraction Buffer Working Solution

320 µL Stain Extraction Buffer Premix

40 µL 20% SDS

40 µL 0.39 M DTT

Store frozen for up to 2 weeks.

Appendix D BSD 600/600+ Punch Programs

Program Name	Size of Punch	# Sample Punches	Description
stacsplateidd_1.2mm	1.2 mm	1	STaCS™ Plate
stacsplatemf_1.2mm	1.2 mm	1	STaCS™ Plate
[BSDName] 1.2 mm QC	1.2 mm	1	QC Plate
[BSDName]_1.2 mm_1 punch	1.2 mm	1	IDD, GFE or MiniFiler™ Plate
[BSDName]_1.2 mm_3 punch	1.2 mm	3	IDD or GFE Plate

Note: These are the most frequently used punch programs. Other programs may be created as needed.

Appendix E GeneAmp PCR System 9700 User Files

Program Name	Program
iddirect25 (IDD half rxns)	HOLD 95°C / 11 minutes
	STR 3 TEMP PCR 25 CYCLES (94°C 20 sec, 59°C 2 min, 72 °C 1 min) VOLUME = 15 µL
	HOLD 60°C / 25 minutes
	HOLD 4°C / ∞
iddirect26 (IDD half rxns)	HOLD 95°C / 11 minutes
	STR 3 TEMP PCR 26 CYCLES (94°C 20 sec, 59°C 2 min, 72 °C 1 min) VOLUME = 15 µL
	HOLD 60°C / 25 minutes
	HOLD 4°C / ∞
iddirect27 (IDD full rxns)	HOLD 95°C / 11 minutes
	STR 3 TEMP PCR 27 CYCLES (94°C 20 sec, 59°C 2 min, 72 °C 1 min) VOLUME = 27µL
	HOLD 60°C / 25 minutes
	HOLD 4°C / ∞
mini27 (MF half rxns)	HOLD 95°C / 11 minutes
	STR 3 TEMP PCR 27 CYCLES (94°C 20 sec, 59°C 2 min, 72 °C 1 min) VOLUME = 13 µL
	HOLD 60°C / 45 minutes
	HOLD 4°C / ∞
gfe26 (GFE rxns)	HOLD 95°C / 1 minute
	STR 2 TEMP PCR 26 CYCLES (94°C 3 sec, 60°C 30 sec) VOLUME = 17 µL
	HOLD 60°C / 8 minutes
	HOLD 4°C / ∞
25minext	HOLD 60°C / 25 minutes
	HOLD 10°C / ∞
denature	HOLD 95°C / ∞
cold snap	HOLD 4°C / ∞

Appendix F 3500xL Assays

Assay Name	Description
DPS_IDD+Norm_POP4_xl (24 sec)	IDD Full Reactions - 24 sec injection
DPS_IDD+Norm_POP4_xl (12 sec)	IDD Full Reactions - 12 sec injection
DPS_IDD+Norm_POP4_xl (9 sec)	IDD Full Reactions - 9 sec injection
DPS 2_IDD+Norm_POP4_xl (9 & 24 sec)	IDD Full Reactions - 9 & 24 sec injection
HALF_DPS_IDD+Norm_POP4_xl (24 sec)	IDD Half Reactions - 24 sec injection
HALF_DPS_IDD+Norm_POP4_xl (12 sec)	IDD Half Reactions - 12 sec injection
HALF_DPS_IDD+Norm_POP4_xl (9 sec)	IDD Half Reactions - 9 sec injection
HALF_DPS 2_IDD+Norm_POP4_xl (9 & 24 sec)	IDD Half Reactions - 9 & 24 sec injection
DPS_GFE+Norm_POP4_xl (24 sec)	GFE Reactions – 24 sec injection
DPS_GFE+Norm_POP4_xl (15 sec)	GFE Reactions – 15 sec injection
DPS 2_GFE+Norm_POP4_xl (15 & 24 sec)	GFE Reactions – 15 & 24 sec injection
DPS_MF_HALF+Norm_POP4_xl (24 sec)	MF Half Reactions - 24 sec injection
DPS_MF_HALF+Norm_POP4_xl (12 sec)	MF Half Reactions - 12 sec injection
DPS_MF_HALF+Norm_POP4_xl (12 & 24 sec)	MF Half Reactions - 12 & 24 sec injection

Appendix G GMID-X Analysis Methods

Analysis Method	Description
IDD_300RFU_NORM	IDD Full Reactions, AT=300, ST=600, w/norm
IDD_250RFU_NORM_HALFRXN	IDD Half Reactions, AT=250, ST=600, w/norm
GFE_250RFU_NORM	GFE Reactions, AT=250, ST=600, w/ norm
MINI_300RFU_NORM	MF Half Reactions, AT=300, ST=700, w/norm

Appendix H STR Normal Values for Result Interpretation

Stutter Percentages

The stutter percentages for Identifiler® Direct documented in the *AmpFlSTR® Identifiler® Direct PCR Amplification Kit User Guide*, those for GlobalFiler® Express as documented in the *AmpFlSTR® GlobalFiler® Express PCR Amplification Kit User Guide* and those for MiniFiler™ documented in the *AmpFlSTR® MiniFiler™ PCR Amplification Kit User Guide* are assigned to the appropriate marker in the panels and bins of GeneMapper® *ID-X*. Database analysts use a 20% filter across all loci for data analysis in lieu of individual locus specific stutter ratios. Based on internal validation studies, it was deemed that the 20% filter is suitable for database samples.

Appendix I Review Guidelines for Outsource Data

The acceptance of database results is a matter of professional judgment and expertise. Not every situation can or should be covered by a pre-determined rule. The following criteria are guidelines for the acceptance of analytical results of database data from vendor laboratories. Anything outside of these guidelines must have the approval of the DNA Database Supervising Forensic Scientist and the DNA Technical Leader prior to acceptance for upload to CODIS.

I.1 Sample Review

Outsource data will need to be 100% reviewed. Analysts will review the electropherogram data from GeneMapper projects that have already been analyzed and reported by the outsource vendor laboratory. The interpretation guidelines in Section 7 will be followed with the following exceptions.

I.1.1 Peak Heights

I.1.1.1 All samples and controls will be analyzed at the analytical threshold set forth in the contract. Peak heights that fall below this threshold may not be accepted for upload into CODIS.

I.1.2 Internal Size Standard

I.1.2.1 The internal lane size standard should have the correct peaks labeled. The size standard peaks should be within the acceptable peak height range. All peaks should be of similar shape and fit the expected pattern. Sample alleles should be flanked by at least two standard peaks on each side in order to be accepted.

I.1.3 Positive Extraction Control

The positive extraction controls are the vendor laboratory's in-house controls. Known profiles associated with these controls will be compared to the GeneMapper® *ID-X* data to verify concordance. If an incorrect profile is observed, all samples associated with the control will be rejected and re-analyzed.

I.1.4 Off -Ladder Alleles

I.1.4.1 If the sample contains an off-ladder allele that is not listed on the lab's approved microvariant list, the analyst should verify that a second analysis was completed and that the values between the two runs are concordant. If a second analysis was not performed, the sample with the off-ladder allele will be re-analyzed in-house. Once evaluations

have been completed, samples with off-ladder alleles may then be accepted for CODIS import.

I.2 CODIS Import

I.2.1 A CMF file for all accepted samples will be generated for import into CODIS. Analysts will use the vendor analyzed data to generate the CMF files.

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Appendix J Database Sample Control

The Arizona Department of Public Safety accepts convicted offender and arrestee DNA samples from various law enforcement agencies for analysis and storage pursuant to ARS §13-610. DNA database samples submitted to the laboratory are treated as reference materials and are not considered evidence per the Scientific Analysis Bureau Quality Assurance Manual. The following procedures ensure database sample integrity including, but not limited to, minimizing contamination and maintaining proper documentation of records related to database samples.

J.1 Database Collection Kits

J.1.1 DNA samples submitted pursuant to ARS §13-610 are contained within individual kits collected by law enforcement agencies such as sheriff's offices, probation departments, or prison facilities. A typical collection kit contains the following items:

J.1.1.1 One buccal swab collection device in the form of the Bode DNA collector (arrestee kits will also contain a cotton swab)

J.1.1.2 One inner envelope with a desiccator pack

J.1.1.3 One self-sealing return envelope

J.1.1.4 One databank form for information about the donor of the sample

J.1.2 Alternate collection kits and/or devices must be approved by the DNA Database Supervisor prior to sample check-in.

J.1.3 The databank form has instructions for the proper and safe collection of a buccal swab specimen. The instructions include recommendations for the use of protective equipment such as gloves for the agency representative responsible for the collection.

J.2 Sample Check-in

Database collection kits are processed using the Bode archival cassette (arrestee collection kits may be processed using a cotton swab). The archival cassette is a heavy stock paper card designed to work with the Bode DNA collector.

Samples will be processed with adherence to the DNA Database Quality Manual.

J.2.1 Only one database collection kit shall be opened at a time.

J.2.2 The databank form is verified for completeness and concordance with the information on the removable label on the buccal collector.

J.2.3 The label from the buccal collector is transferred to the archival cassette. Alternate buccal collectors may require handwriting information on the archival cassette. The paper swab portion of the buccal collector is then secured to the cassette with an adhesive seal and stored in a paper envelope. These envelopes are stored at room temperature in a secured room.

J.2.4 Matching barcode labels are placed on the archival cassette, cotton swab envelope (if applicable) and the corresponding databank form. The applicable information from the databank form is entered into the laboratory information management system currently in use.

J.3 CODIS Eligibility and Specimen Categories

J.3.1 Each collection kit includes a databank form containing information about the donor of the sample. CODIS eligibility and specimen categories are determined based on the information on the form, if possible.

J.3.1.1 Eligibility

- The qualifying offense is listed on the databank form by the collecting agency.
- The qualifying offense is verified to meet the requirements of ARS §13-610.
- If the offense doesn't appear to meet ARS §13-610, it will be verified through the donor's criminal history and/or court records. It may be necessary to contact the submitting agency for verification.
- If the offense cannot be verified, the sample will be rejected.

J.3.1.2 Specimen Category

- The collecting agency will mark on the databank form if the sample is from a Convicted Offender or an Arrestee.
- If the databank form does not indicate which type of sample it is, it may be possible to determine based on the submitting agency (e.g., all samples submitted by Police Departments will be

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Arrestees, all samples submitted by Department of Corrections will be Convicted Offenders).

- If the sample type cannot be determined from the information on the form, it will be determined through the donor's criminal history and/or court records. It may be necessary to contact the submitting agency for determination.

J.4 Rejection of Database Collection Kits

J.4.1 Database collection kits will be rejected for inclusion in the Arizona DNA Identification System if any of the following are observed:

J.4.1.1 Illegible or no fingerprints on the databank form

J.4.1.2 Identifying information not listed on the buccal collector

J.4.1.3 Name or identifying information of person collecting the specimen not listed

J.4.1.4 Outer envelope not properly sealed

J.4.1.5 Swab paper detached from buccal collector

J.4.1.6 Other issues, such as discordant information between databank form and buccal collector

J.4.1.7 No qualifying offense can be determined

J.4.1.8 White out

Exceptions may be made on a case by case basis upon approval from the DNA Database Supervisor or the DNA Database Technical Leader.

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Appendix K Revision History

Section Revised	Date	Issuing Authority
DNA PCR Protocol for Convicted Offender Samples, Version August 2003	09/01/2003	Todd A. Griffith, Superintendent
DNA PCR Protocol for Convicted Offender Samples, Version March 2005	03/01/2005	Todd A. Griffith, Superintendent
DNA PCR Protocol for Database Samples, Version December 2006	12/01/2006	Todd A. Griffith, Superintendent
DNA PCR Protocol for Database Samples, Version 2007, update version name should have been "Version 2007" not Version "December 2006". Update effective date should have been 12/4/06 not 12/1/06. Change watermark to "Printed Copies Uncontrolled."	04/29/2008	Todd A. Griffith, Superintendent
DNA Database Procedures Manual – DDPM2008.1, Update to new header/footer style, Remove old style watermark, Assign SAB Document ID#, re-named document. Added wording "…for that ladder" to first parenthetical phrase in Section 3.4.3.1. Changed "base" to "base pair" in Section 3.4.8.4. Updated occurrences of "convicted offender" to "convicted offender/arrestee" or "database" as appropriate. Updated Appendix E to reflect new macro. Added Appendices I and J.	05/06/2008	Todd A. Griffith, Superintendent
DNA Database Procedures Manual – DDPM2010.1, Moved centrifuge step from 3.2.3.8 to 3.2.3.6. Made Interpretation its own section (Section 4). Moved section referring to Off Ladder Alleles from 4.1.3 to 4.1.7. In 4.1.7.2, changed wording from Profiler Plus and Cofiler to Profiler Plus and/or Cofiler when regarding re-amplifying alleles that fall between two loci. Reworded 4.1.8.1 for added clarification and added reference to contamination being reported to Technical Leader. Reworded 4.1.8.3 and 4.1.8.7 to address third peaks. Added section 4.1.8.8 on artifacts. Updated condition numbers under I.1.14 to reflect AB User Bulletin. Updated reagent information for Formamide/LIZ® in Appendix D. Updated Appendix E. Added Appendix K regarding database sample control and Appendix F regarding amplification with Profiler Plus/ Cofiler. Added 113method and 105method to 9700 table in Appendix G. Added lysis buffer to Appendix C.	07/27/2010	Todd A. Griffith, Superintendent
DNA Database Procedures Manual – DDPM2012.1, Total re-write to incorporate new procedures using Identifiler® Direct for amplification, Applied Biosystem's 3500xL Genetic Analyzer for electrophoresis, and GeneMapper® ID-X for analysis.	5-30-12	Vincent A. Figarelli, Superintendent

Section Revised	Date	Issuing Authority
DNA Database Procedures Manual – DDPM2013.1, Corrected all spelling, grammatical and formatting errors found; made changes throughout document to accommodate STaCS procedures; changed all references of "reagent blank" to "negative control" and all references of "PT sample" to "in-house known sample"; added clarification on interpretation for off-ladder alleles that fall between loci; added Section 1 regarding STaCS; added Section 3 on Maxwell procedures; added Appendix D on BSD Programs, Appendix F on 3500xL Assays, and Appendix G on GMID-X methods; added programs to Appendix E on 9700s; rewrote Appendix I on Outsource Data Review to remove review with an expert system and add the manual review process. Added procedures for Identifiler® Direct half reactions and MiniFiler throughout protocol as applicable.	9-3-13	Vincent A. Figarelli, Superintendent
DNA Database Procedures Manual – DDPM2015.1. First Qualtrax revision. Added paragraph to end of Section 1 regarding documentation of steps run outside of STaCS. Added BSD punch program for MiniFiler to Appendix D. Corrected 12 second MiniFiler assay name in Appendix F. Added RapidDNA modifications	June 2015	SAB Superintendent

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Section Revised	Date	Issuing Authority
DNA Database Procedures Manual – DDPM2015.1. Second Qualtrax revision. Corrected all spelling, grammatical and formatting errors found. Incorporated new procedures using *AmpFlSTR® GlobalFiler® Express PCR Amplification Kit.* Sections: 2.3 Add statement regarding vacuum pump, 2.6 add last 2 sentences, 4 add last sentence for minimum core loci, 5.2.4.3 add clarification for septa, 6.3 reword subcategories, 8 add clarification for approval for variation to interpretation guidelines, 8.4 add clarifications for LIZ and ILS and remove last sentence, 8.5 add MEB and change "shall" to "may" in first bullet (spikes), 8.7.4 change "shall" to "must", 8.8.1 add verbiage for chimeric individuals, 8.8.4 add clarification for –A, 8.8.6 add "and the pull up is below the ST" and add last sentence, 8.8.7 add clarification for dilutions, 8.8.8 add clarification for low third peak in trisomy, 8.8.9 add clarification for dye artifacts masking peaks and add last sentence. Add Section 8.8.10. Sections: 8.9 change D2 locus to "CODIS non-core loci", 8.11 add last sentence, 8.12reclarified how to obtain combined profile, 9.2.1 add clarification for use of Minifiler kit, and add Section 9.2.4. Sections: 10 add "and upload into CODIS" and "and modified rapid DNA analysis" and remove last sentence, 10.1.4 redefine use of in-house + control, 10.2.2 add "or known password", 10.2.8 change "6" to "six", 10.2.12 remove "profile data being looked at" and remove last sentence, 10.3 change "printing electropherograms" to "review", 10.3.4 add verbiage for using GeneMarker and remove last sentence. Sections: 10.3.5, 10.3.6 and 10.3.7 change to documented wording. Sections: 10.4 change "printing" to "viewing", 10.4.2 removed, 10.5.1.2 add clarification for reworking samples on 3500xL and for proficiency testing, 10.5.1.3 change "high peak heights" to "strong sample data" and added "and edited", 10.5.1.4 removed, 10.5.2 remove ladder alleles, add clarification for minor peaks and remove last 2 sentences, 10.5.3 add ILS score of 85 and remove last sentence, 10.5.4 re-clarify blank control and remove last sentence, 10.5.5 re-clarify + control and remove last sentence, 10.5.6 add "(e.g., pull up, spikes, etc.)", change documentation in case file to "edited at the discretion of", removed "until the "OL" alleles are verified" and add verbiage to accept alleles labeled in virtual bins, 10.5.7 remove 2nd and 3rd sentences, 10.5.7.1 change to documented wording, 10.5.7.3 change references of "4 base" to "1 repeat unit", and 10.5.7.6 change "re-punch and amplify" to "re-analyze". Appendix Sections: A.1.3 remove allele calls, A.1.4 remove IDD ladder and allele calls and last part of sentence, A.2.4 remove Minifiler ladder and allele calls, C (throughout) add "the following ratio will be used", D remove "Duet", G corrected names for analysis methods, I.1.3 add "ID-X", I.1.4.1 add 2nd sentence, and J (throughout) add reference to arrestee kits.	Dec 2016	SAB Superintendent

Section Revised	Date	Issuing Authority
DNA Database Procedures Manual: Remove Section 3. Update references to changed section numbers. Re-numbered Sections: 7.5 – remove reference to Maxwell, 7.7.4 – add space between "must" and "be" in 1st sentence, 7.8.4 – change "8" to "eight" and "TE Buffer" to "Formamide/LIZ", 7.8.8 – add clarification in 3rd sentence regarding entering the third peak into CODIS and remove last sentence, 7.8.9 – remove last sentence, 7.8.10 – add parenthetical in last sentence and add Sections 7.8.11, 7.11 – remove last sentence and 7.14. Re-numbered Sections: 8.2.1 – re-word to accept below threshold data and 8.2.4 – change "AT" to "analytical threshold", add apostrophy in 1st sentence and add last sentence. Re-numbered Sections: 9.3.6 – change "85" to "90", 9.5.1.1 – add stochastic threshold for Buccal program, 9.5.1.2 – add comma in 2nd sentence, change "of 18" to "program specific" and add last sentence, 9.5.3 – change "85" to "90" and add clarification regarding correct placement of ILS markers, 9.5.5 – remove ":once" in 1st sentence, 10.5.6 – re-word 1st paragraph for the SE33 locus, 9.5.7 – remove last 2 sentences, 9.5.7.6 – add clarification regarding third peak being entered into CODIS and add last sentence and add Section 9.5.9. Remove Appendix A.4. Add Appendix B.1.6. Remove Appendix B.2. Appendix I.1 - add 2nd sentence and Appendix I.2 – add last sentence.	Oct 2017	SAB Superintendent
DDAP Rev 4 Significant Changes: Removed AutoOpen macro No additional changes	Aug 2018	SAB Superintendent
DDAP Rev 5 Significant Changes: Sections: 3.1.6 add clarification that marking can also be placed on outer box; 5.1.2 corrected spelling for aliquoted; 7.1 add hyphens to "re-analyses"; 7.6.4 reclarified acceptance of positive control by adding last four sentences; 7.8.1 reclarified definition of EDNA as being peaks labelled by GeneMapper® *ID-X*; 7.8.4 remove kit specific re-extension times and define in-well dilution; 8.2.4 added last two sentences regarding batch acceptance and use of positive and negative controls; 9.5.1.2 clarify acceptance of single peaks below stochastic threshold in proficiency tests; change AT to analytical threshold; 9.5.6 add space between "the" and "SE33". Appendix B.1.12 remove underline; Appendix C.2 changed 875 and 25 to 1020 and 29 respectively; Appendix C.3 added portion of underline and changed 950 and 25 to 1020 and 27 respectively.	Feb 2019	SAB Superintendent

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Section Revised	Date	Issuing Authority
DDAP Rev. 6 Significant Changes: Section 1.2.3.2 updated regarding notes for reinjections and dilutions. Section 3 changed primary kit use GFE and IDD/MF for challenging samples. Section 4.2.6.3 updated G5 spectral to 3.0 - 8.0 µL Matrix Standard with 300 µL formamide. Section 7.3 included IDD+ Ladder. Section 7.6.6 added minus A as extra peak greater than AT and labeled. Section 8.2.1 changed 6 loci to 6 core GFE loci. A.1.4 added IDD+ Allelic Ladder. Appendix D added BSD 600+ puncher. Appendix F added duel assay for MF. Appendix J added white out for rejection of database collection kits.	June 2020	SAB Superintendent

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