

Forensic Biology Database Procedures Manual

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Section 1 Preparation for Direct Amplification of buccal swabs

Reference:

- *GlobalFiler™ Express PCR Amplification Kit User Guide – Applied Biosystems October 2012*

1.1 Sample Preparation

- Prepare sample tubes by labeling ninety-six 1.5mL tubes.

Note: Sample tubes 1, 25, 49, & 73 will be used as place holders for the allelic ladder. DO NOT place samples in these tubes. Sample tubes 95 and 96 will be place holders for the positive and negative amplification controls. DO NOT place samples in these tubes.

- For serrated buccal swabs, cut a portion of the swab into the appropriate tube. For cotton swabs, cut a portion of the swab tip into the appropriate tube. If two swabs were collected, the entire cotton tip of one swab may be snapped off and used.
- Each batch of extractions must include a minimum of five randomly placed internal control samples. These may be previously typed samples, staff duplicates, or offender duplicates for samples in the current run.

1.2 Amplification Preparation

Preparation of the amplification reagents takes place in the PCR prep room. Once the reagents have been aliquotted into the plate, the plate, covered with Press and Seal, is taken to the extraction lab for loading the sample lysates.

1.2.1 Reagent preparation

- Thaw the Master Mix, Master Mix Additive, and the Primer Set. Vortex for 3 seconds and centrifuge briefly. NOTE: Thawing is only required for the first use of the kit. After first use, reagents are stored at 2 to 8° C and therefore, do not require subsequent thawing.
- For the first use of the kit only: Add the following volumes of Master Mix Additive to the master mix tube:
 - 200 reaction kit – 80 uL Master Mix Additive

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- 1000 reaction kit – 390 uL Master Mix Additive
- Gently invert the Master Mix tube 10 times and centrifuge briefly.
- Mark the cap of the Master Mix tube with a (+) to indicate that Master Mix Additive has been added.
- Discard the Master Mix Additive tube.
- Note the thaw date on each reagent tube – the expiration date for the reagent is now one month from the date of the first thaw (i.e. the same date on the following month).
- Calculate the volume of each component needed to prepare the reactions:
 - Master Mix: 6.0 uL per reaction
 - Primer Set: 6.0 uL per reaction
 - NOTE: Include additional reactions in your calculations to provide extra volume for loss in pipetting transfers.
- Pipet 12 uL of master mix into each well of a MicroAmp Optical 96-well Reaction Plate. NOTE: wells A1, A4, A7, A10 and any unused wells do not require master mix.
- To the positive amplification control well (typically well G12): add 3 uL of DNA Control 007.
- Cover the plate with Glad Press and Seal.

1.3 Sample Lysis and Loading to the Amplification Plate

- Add 400 uL of Prep-n-Go buffer to each sample and let stand for at least 20 minutes but ideally no more than 60 minutes at room temperature (about 20 to 25° C). NOTE: Incubation time can have a significant impact on the profile ultimately obtained for the sample. It may be necessary to process the plate in sections in order to minimize the length of time a sample incubates at room temperature.
- After incubation, transfer 3 uL of lysate to the prepared amplification plate.
- To the negative amplification control well (typically well H12): add 3 uL of Prep-n-Go buffer.
- Seal the plate with adhesive film.
- Transfer the tubes with lysates to short-term (less than 2 weeks) storage at 2 to 8° C.

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Section 2 PCR Amplification with GlobalFiler™ Express

Reference: *GeneAmp® PCR System 9700 96-Well Sample Block Module User's Manual*

2.1 Amplification Cycling Parameters

- Ensure AB GeneAmp® PCR System 9700 thermal cycler has been turned approximately one hour in advance to allow the instrument time to properly warm up.
- Confirm the cycling parameters for **user: alaska, program: gfe26c**
- Confirm that ramping mode is set to **Max**, and reaction volume is set to **15 uL**.

HOLD
95°C for 11 minutes, then:

26 CYCLES
94°C for 3 seconds
60°C for 30 seconds

HOLD
60°C for 8 minutes

HOLD
4°C for up to 24 hours

2.2 Amplification on the thermal cycler

- Load the amplification plate into the thermal cycler.
- Place a MicroAmp compression pad on top of the plate to further prevent evaporation during thermal cycling. Close the heated cover.
Start the run.
- On completion of the run, the amplified DNA is stored at 2 to 8° C.

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Section 3 Capillary Electrophoresis on the AB 3500xl Genetic Analyzer

References: Applied Biosystems 3500/3500xL Genetic Analyzer User Guide

3.1 Start the Software and Instrument

- Turn the computer on and log into the computer
- Turn on the 3500xl Genetic Analyzer. Wait for the green status light to turn on.
- Ensure both the Daemon and Service Monitor has started by observing a green checkmark icon in the lower right hand corner. This indicates that all 3500 services have loaded. This may take several minutes.
- Launch the Data Collection Software:
Start > Programs > Applied Biosystems > 3500
- Log in to the 3500 Series Data Collection Software

3.2 Preparing the Instrument

- Navigate to the Dashboard of the software
- Check consumables by clicking **Refresh** to update consumable status.
- Refer to the **Forensic Biology General Lab Maintenance** manual if any maintenance or instrument preparations are required prior to running samples on the instrument.
- Click **Start Pre-heat 60 °C** to warm the oven.
- Check the pump assembly for bubbles and run the Remove Bubble wizard if necessary.

3.3 Sample Preparation for the 3500xl

Reference: QIAgility® User Manual, 11/2011

3.3.1 QIAgility (Note: the QIAgility plate set-up protocol is for full plates only)

3.3.1.1 Reagent Preparation

Prepare the 3500 xL master mix in 1.5mL tube for position E in M1 (Master Mix block):

- 988 µL deionized formamide
- 52 µL GeneScan™ 600 LIZ® Size Standard v2.0

3.3.1.2 QIAgility Set-up

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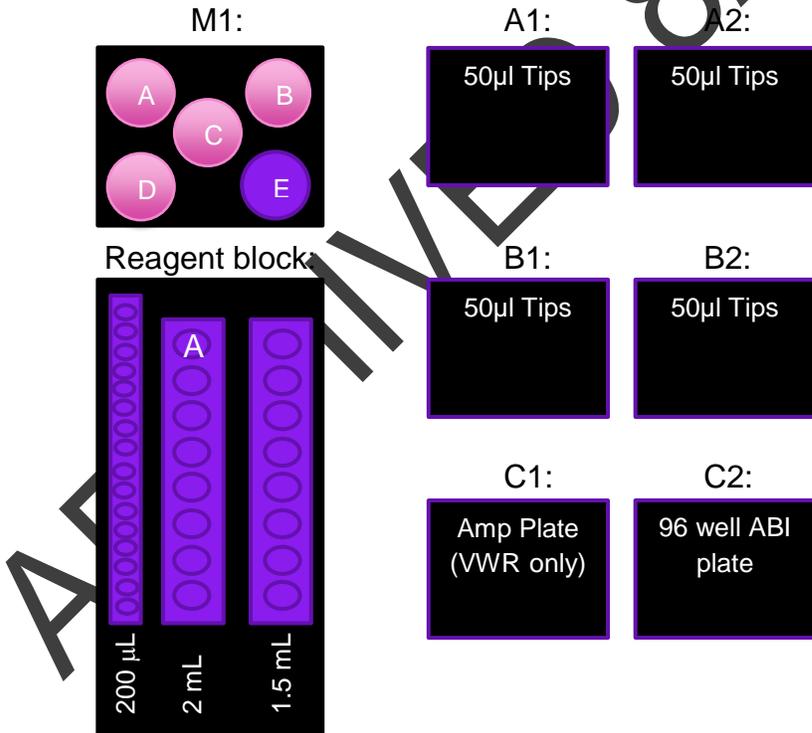
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- Ensure that the power for the QIAgility has been switched on. The power switch can be located on the rear left of the instrument. A blue light on the front of the instrument will indicate the instrument is powered on.
- Click on the QIAgility icon to launch the software; it may take a minute to initialize.
- Navigate to the database amplification protocol by clicking **Recent** → **Browse** → **Alaska** → **GFE_CE_fullplate**
- Obtain a 96-well genetic analyzer plate.
- Centrifuge the amplified product plate briefly and carefully remove adhesive film, preventing splatter of PCR amplicon.
- Open the QIAgility and load the deck as follows:

M1 position E = 3500xl Master Mix

Reagent block position A = GlobalFiler™ Express Allelic Ladder (at least 15 µL in tube)



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Note: When loading tips, there is only one correct orientation. Ensure tips are loaded correctly, with the notch aligned in the frame.

- To reset the tips, hover over the block of tips to reset. Using the mouse, right click and select **“Set all tips on current plate to available”**.

Note: Tips designated in blue are available and white are unavailable

- Ensure tip waste box is attached and there is ample room for waste.
- Click the green arrow at the top of the screen. The “Save As” window appears. Enter the batch name under “File name:” and click **“Save”**.
- The “Checklist” screen appears. Review the messages and follow the prompts.

Note: A “Pre-Run Report” may be previewed by clicking **“Pre-Run Report”**. Click **“Close”** to return to the “Checklist” screen.

- Click **“OK”**.
- The run will begin and will take approximately 30 minutes to process. Upon completion, the samples will be ready for analysis using the 3500xL Genetic Analyzer.
- Remove the VWR plate containing the amplified product and cover with an adhesive seal. Store at 4°C. All amplification products will be disposed of upon completion of the reviews and upload of the batch.
- Remove CE plate and cover with septa.
- Unload the deck of the QIAgility by removing and discarding empty tip racks and tubes located in the M1 and Reagent block.

3.3.1.3 QIAgility Maintenance

Daily maintenance is performed each day the instrument is in use and is described below. Additional maintenance procedures are detailed in the Forensic Biology General Lab Maintenance manual. All maintenance is recorded on the QIAgility Maintenance Log provided at the back of the Forensic Biology General Lab Maintenance manual.

Daily maintenance is required after each run on the QIAgility and at the end of the day.

- Wipe down the deck with ethanol.
- Wipe with water and wipe dry with paper towels.

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Note: Do not wipe the rails. These rails support the pipetting head and allow it to slide backwards and forwards easily. Wiping the rails will remove the grease and make them more susceptible to rust.

- You may now run another protocol or switch off the QIAgility instrument.

3.3.2 Sample Preparation for the 3500xl - Manual

- Prepare a loading master mix by adding the following volumes of reagents to an appropriately sized tube:
 - # of samples x 0.5µl GeneScan 600 LIZ Size Standard
 - # of samples x 9.5µl of Hi-Di Formamide

Note: Prepare enough for a few extra reactions to allow for loss during pipetting. This is calculated automatically on the Database Batch Worksheet.

- Vortex the master mix and spin briefly. Transfer 10µl of master mix to appropriate sample wells of a 96 well plate.
- Add 10µl of Hi-Di Formamide to unused wells of a set of 24 (i.e. A1-H3).
- Add 1µl of allelic ladder or 1µl of each amplified sample to the appropriate wells. When all samples have been added, cover with plate septa.

3.3.3 Final sample preparations following either QIAgility or Manual prep

- Briefly centrifuge the 96 well plate to remove any bubbles. Denature samples at 95°C for 3 minutes and then snap chill for 3 minutes.
- Place the 96-well plate into the plate base provided with the instrument and snap the plate cover onto the plate, septa, and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- Place the plate in the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler. Close the instrument doors.

3.4 Creating a Plate Record

3.4.1 Create a Plate Record from the Data Collection Software

- In the dashboard click **Create New Plate**
- In the Define Plate Properties screen:

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- Enter a plate name (i.e. DB10-1222AB_KAL), this will vary depending on analyst
- Select 96 for the number of wells
- Select HID for plate type
- Select 36cm for capillary length
- Choose POP4 for polymer
- Owner, barcode and description are optional fields.
- Click **Save Plate**
- Click **Assign Plate Contents**
- Using either plate view or table view enter sample names in the corresponding wells.
- Under the heading Assays, File Name Conventions, and Results Groups click **Add from Library** select the following Assay:
 - GFE_POP4_xl_24s
- Select a naming convention, and a results group of your choice. Click **add to plate** and then click **close**.
- Select the wells for which to specify the assay file name conventions and results groups and enable the checkbox next to the name to assign it to the selected wells.
- Click **Save Plate** and save.

3.4.2 Create a Plate Record from an Export File

- Open a plate record template export file (i.e. .xls exported from instrument).
- Copy and paste sample name info into the export file. (Rows not in use may be deleted but do not delete any columns) Save the record.
- Using the 3500xl Data Collection Software, click on **Create New Plate** on the dashboard.
- In the Define Plate Properties screen:
 - Enter a plate name (i.e. DB10-1222AB_KAL)
 - Select 96 for the number of wells
 - Select HID for plate type
 - Select 36cm for capillary length
 - Choose POP4 for polymer
 - Owner, barcode and description are optional fields.
- Click **Save Plate**.
- Click **Assign Plate Contents**.

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- Click **Import** at the top of the screen and navigate to the saved plate record (make sure .xls files are searched).
- Click **Save Plate** and save.

3.5 Linking a Plate and Starting a Run

- Click **Link Plate for Run**. A message will appear saying Plate loaded successfully. Click **OK**.
- Ensure plate is linked to proper position on 3500xl.
- Select **Create Injection List**.
- Click **Start Run**.

Note: Ensure all prompts have cleared and the run begins before walking away.

3.6 Viewing Data During a Run

Refer to Applied Biosystems 3500/3500xl Genetic Analyzer User Guide

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Section 4 Data Analysis with GeneMapper™ ID-X

References: *GeneMapper™ ID-X Software User Guide*
GeneMapper™ ID-X Software Tutorial
GeneMapper™ ID-X Software User Bulletin

4.1 Logging in to GeneMapper™ ID-X

- Open GeneMapper™ ID-X
- Select the User Name and Database Host from the drop-down list and enter the appropriate password.
- Click OK.
- The main project window will open.

4.2 Creating a Project

- To add samples from the collection software at a workstation, go to the edit menu and select **Add Samples to Project**. Navigate to **My Computer > Uncontrolled Documents > Section Shares > DNA Share > CE Data** on the laboratory "I Drive" as per current designation(s).

Note: Location is dependent on network mapping and may vary slightly.

- Select the raw data folder to be imported or select individual samples and click **Add to list**.
- When all samples have been selected, click **Add**.
- Select the appropriate Sample Type, Analysis Method (GFE), Panel (GlobalFiler_Express_v1.2X) and Size Standard (GS600_LIZ_(60-460)) for each sample and click the green arrow on the tool bar to analyze the samples. NOTE: these methods shall not be modified. New methods shall only be created or modified with permission from the DNA Technical Manager.
- The user will be prompted to name and save the project to the appropriate Security Group (GeneMapper ID-X Security Group). Name the project with the Batch name. Click OK.
 - Example: DB13-0319A_KAH
- Analysis is complete when the green arrows in the Status column on the left are gone, and an Analysis Summary Screen appears.

4.2.1 Analysis Methods

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These methods shall not be modified. New methods shall only be created or modified with permission from the DNA Technical Manager.

4.2.1.1 Allele Tab Settings



Analysis Method Editor

General | **Allele** | Peak Detector | Peak Quality | SQ & GQ Settings

Bin Set: AmpFLSTR_Bins_v4X

Use marker-specific stutter ratio and distance if available

Marker Repeat Type:		Tri	Tetra	Penta	Hexa
Global Cut-off Value		0.2	0.2	0.2	0.2
MinusA Ratio		0.0	0.0	0.0	0.0
MinusA Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0
Global Minus Stutter Ratio		0.0	0.0	0.0	0.0
Global Minus Stutter Distance	From	0.0	3.25	0.0	0.0
	To	0.0	4.75	0.0	0.0
Global Plus Stutter Ratio		0.0	0.0	0.0	0.0
Global Plus Stutter Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0

Amelogenin Cutoff: 0.0

Range Filter... Factory Defaults

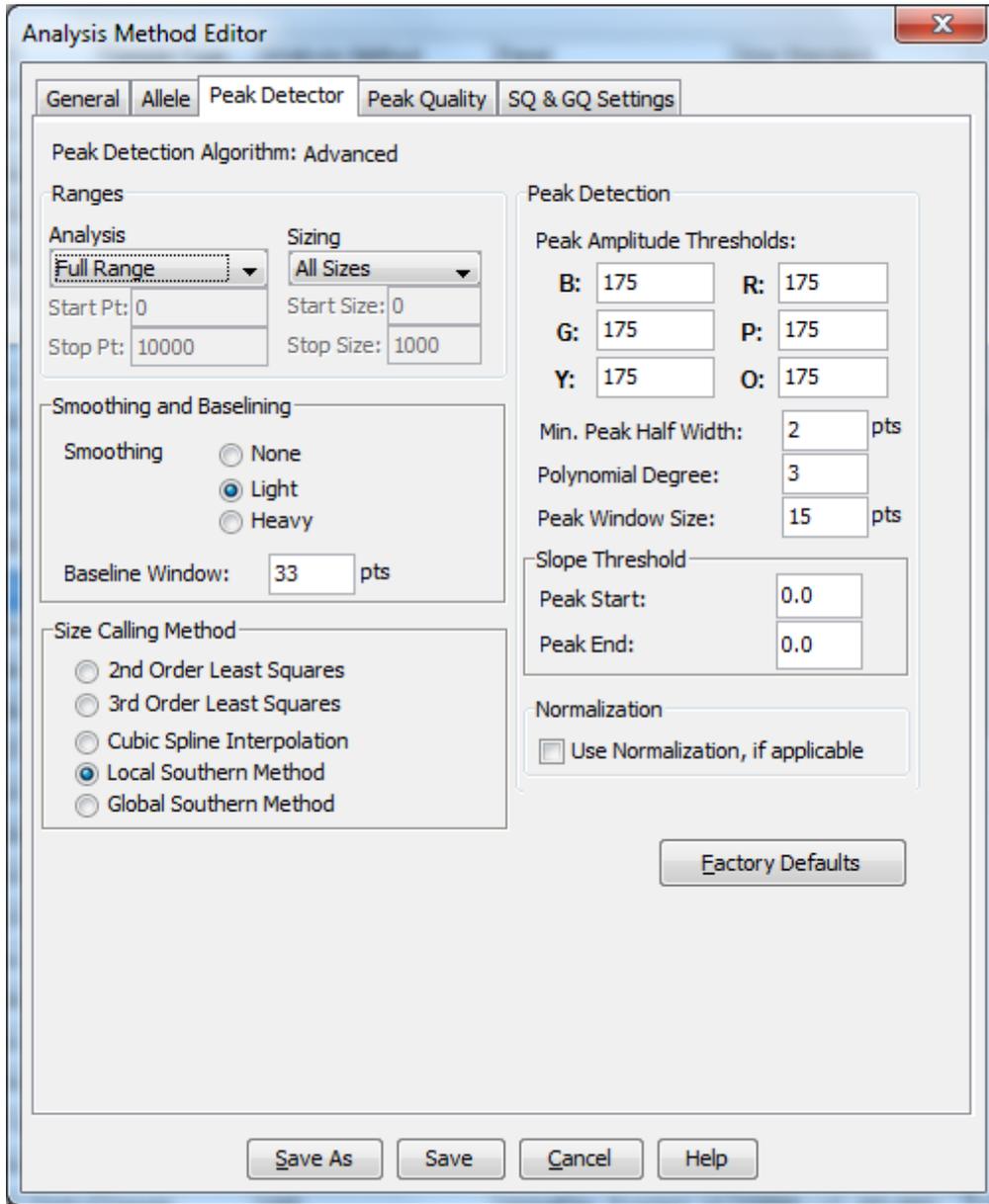
Save As Save Cancel Help

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4.2.1.2 Peak Detector Tab Settings



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4.2.1.3 Peak Quality Tab Settings

Analysis Method Editor

General | Allele | Peak Detector | **Peak Quality** | SQ & GQ Settings

Min/Max Peak Height (LPH/MPH)

Homozygous min peak height: 350.0

Heterozygous min peak height: 175.0

Max Peak Height (MPH): 20000.0

Peak Height Ratio (PHR)

Min peak height ratio: 0.5

Broad Peak (BD)

Max peak width (basepairs): 1.5

Allele Number (AN)

Max expected alleles:

For autosomal markers & AMEL: 2

For Y markers: 1

Allelic Ladder Spike

Spike Detection: Enable

Cut-off Value: 0.2

Sample Spike Detection

Spike Detection: Enable

Factory Defaults

Save As | Save | Cancel | Help

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4.2.1.4 SQ and GQ Tab Settings

These settings do not vary and are not relevant, as all samples are currently manually reviewed and interpreted, regardless of flagging.



The screenshot shows the 'Analysis Method Editor' dialog box with the 'SQ & GQ Settings' tab selected. The dialog contains several sections for configuring quality control parameters:

- Quality weights are between 0 and 1.**
- Sample and Control GQ Weighting:** A table of settings for various peak types and metrics.
- SQ Weighting:** A single setting for Broad Peak (BD).
- Allelic Ladder GQ Weighting:** Two dropdown menus for Spike (SSPK/SPK) and Off-scale (OS).
- SQ & GQ Ranges:** Two rows of range settings for Sizing Quality and Genotype Quality, with highlighted 'Pass Range' (0.75 to 1.0) and 'Low Quality Range' (0.0 to 0.25).
- Buttons:** 'Reset Defaults', 'Save As', 'Save', 'Cancel', and 'Help' are located at the bottom of the dialog.

Parameter	Value	Parameter	Value
Broad Peak (BD)	0.8	Allele Number (AN)	1.0
Out of Bin Allele (BIN)	0.8	Low Peak Height (LPH)	0.3
Overlap (OVL)	0.8	Max Peak Height (MPH)	0.3
Marker Spike (SPK)	0.3	Off-scale (OS)	0.8
AMEL Cross Check (ACC)	0.0	Peak Height Ratio (PHR)	0.3

Control Concordance (CC) Weight = 1.0 (Only applicable to controls)

SQ Weighting
Broad Peak (BD) 0.5

Allelic Ladder GQ Weighting
Spike (SSPK/SPK) 1 Off-scale (OS) 1

SQ & GQ Ranges
Pass Range: Low Quality Range:
Sizing Quality: From 0.75 to 1.0 From 0.0 to 0.25
Genotype Quality: From 0.75 to 1.0 From 0.0 to 0.25

Reset Defaults

Save As Save Cancel Help

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4.3 Viewing the Data

- The ILS is viewed by highlighting all samples and clicking on the Size Match Editor, the icon with the red peaks on the toolbar.
- To view each sample, highlight the sample and click on the icon with the colored peaks to display plots. (This can also be done by View > display plots or Ctrl + L).
- Electropherograms can be printed from the sample's plot window. Various plots have been created for different sample types. Commonly used plot displays include: Traditional Genotype Plot, Casework Blank, Casework artifacts and Casework zoom view.
- To view information on a sample's injection time and other run information, highlight the sample of interest and click **View** on the toolbar > Sample Info (Ctrl + F1).
- To view raw data for a sample, click **View > Raw Data**.
- To view allele calls by sample and locus click the Genotypes tab from the main project window.

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Section 5 STR Data Interpretation

Interpretation of database samples is based on the guidelines contained in this section, as well as the qualified DNA analyst's training and experience. In all cases, interpretations must be independently reviewed and verified based on established peer review procedures. The DNA Technical Manager may authorize the interpretation/reporting of data outside of the criteria defined herein and will document this approval by initialing and dating comments on the relevant paperwork.

The minimum peak height acceptable for database sample STR loci alleles is 175RFU. Lower threshold limits for hit confirmations or ILS (Internal Lane Standard) may be authorized by the DNA Technical Manager.

The following abbreviations are commonly used in bench notes and on other documents generated during DNA analysis.

DNA Abbreviations and Definitions	
BL	Noisy baseline
PHR	Peak Height Ratio
PU	Pull-Up
DO	Complete Drop-Out at a Locus
PDO	Partial Drop Out at a Locus
D5Mut	D5 Mutation
OL	Off Ladder
OMR	Outside Marker Range
OS	Off Scale/Saturation
NP	No genetic profile obtained
NR	Data not reported
NS	Data not used for statistics
A/TA	Artifact (i.e. stutter) or true allele
IF	Instrument failure
ILS	ILS failure
DBRT	Data below reporting threshold
ART	Artifact
TRI	Tri-allele
()	Minor component allele
[]	Major/Minor components not separated

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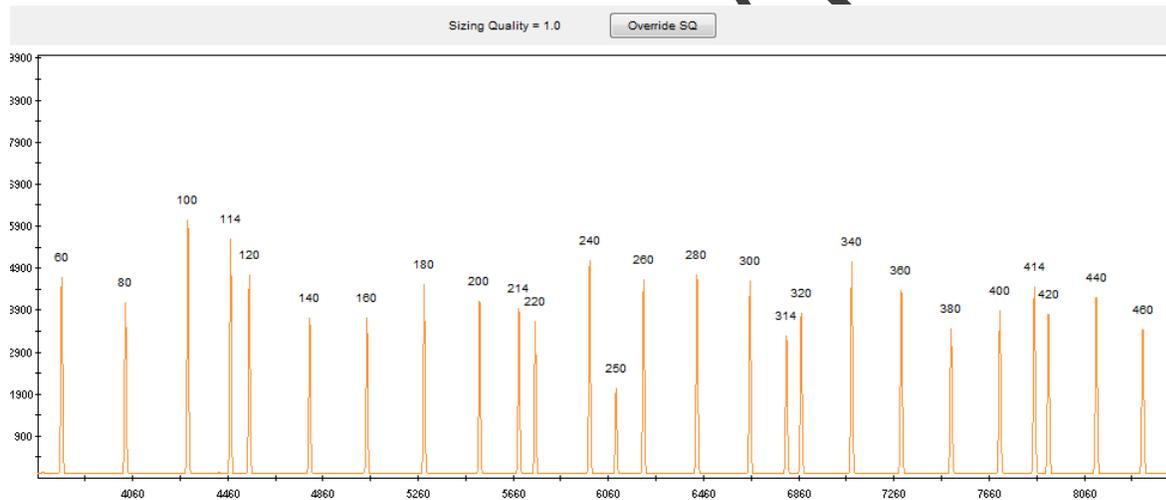
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5.1 Interpretation of Batch Controls

Prior to assessment of data, the analyst must first examine the batch controls to ensure that the extraction, amplification and genetic analysis processes are functioning correctly. These include: Internal Lane Standard (ILS), allelic ladder(s), internal control specimen (ICS), positive amplification control, and negative amplification control (also serves as reagent blank). All or part of an extraction, amplification and/or run may need to be repeated depending on the results of the batch controls. Any issues raised by the performance of a batch control must be addressed prior to the release of affected database samples. Unresolved failure of a batch control requires the Technical Manager, or designated individual, to approve a course of action.

5.1.1 Internal Lane Standard (ILS)

GlobalFiler Express uses GeneScan 600 LIZ as an internal lane standard. The analyst should verify that all peaks from 60-460 base pairs are present and labeled as shown.



The peak heights for the ILS peaks must be at least 175RFU (relative fluorescence units) to be identified and labeled by the GeneMapper™ ID-X software with the database method.

Failure of an ILS can often be resolved by re-prepping and/or re-injecting the affected sample(s). If the ILS continues to fail after re-prepping and re-injecting, the analyst should consult the Technical manager for a course of action.

Refer to the GeneMapper™ ID-X Software User Guide for details on manually labeling / overriding the size standard.

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5.1.2 Allelic Ladder

The GeneMapper™ ID-X software uses the allele calls of the ladder to assign allele calls to all the other samples in the project. The analyst should verify that all peaks from the allelic ladder(s) are present and labeled as shown.

The allelic ladder contains the most common alleles determined for each of the 18 FBI STR core loci, Amelogenin, and several other loci. In addition, alleles not labeled in the allelic ladder (virtual alleles), may be detected and labeled in some of the samples analyzed.

At least one allelic ladder must be included in a GeneMapper™ ID-X project with the sample type designated as “allelic ladder”. All the peaks in the allelic ladder must be labeled correctly for the software to assign the correct allele calls to samples in the run. When more than one ladder in a project has the sample type designated as “allelic ladder”, the software will average the allelic ladders to make the allele calls for the samples in the project.

Artifacts such as spikes or “pull-up” peaks may be present that are labeled as off-ladder (OL) peaks in the allelic ladder. If all the true allele peaks are labeled correctly, such OL peaks will not affect the sample allele calls. If a spurious peak (not a true allelic peak) is labeled as a true allele (i.e. it falls within an allele size range and is designated and labeled as an allele) at any particular locus, the remaining allele designations / labels at the locus shift and will impact the correct allele call / labeling of the samples in the project. Allelic ladders with such artifacts cannot be used to analyze data in the project. If other allelic ladders in the run are successful, it may be possible to change the sample type for the failed ladder from Allelic Ladder to Sample. In that case, the failed ladder is not averaged in with the other ladders. If omitting the failed allelic ladder does not lead to successful analysis, re-injection is usually appropriate.

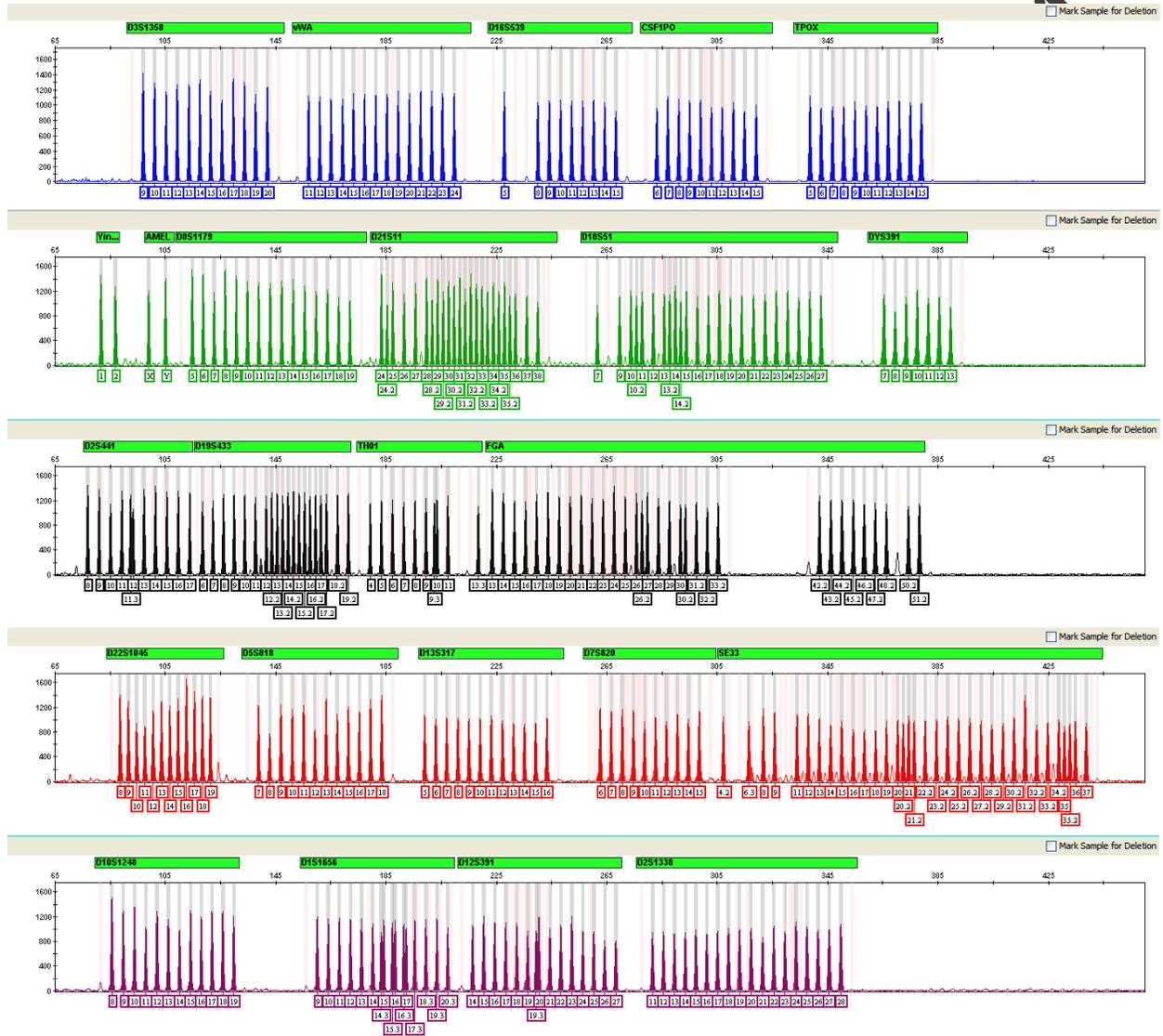
Additional information about the allelic ladder can be found in the GlobalFiler Express PCR Amplification Kit User Guide.

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GlobalFiler Express Allelic Ladder:



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5.1.3 Positive Amplification Control

A positive control is defined as a single source sample whose genetic profile was previously determined and from which a full profile was developed; it is used to evaluate the performance of the amplification and typing procedures. The standard DNA template 007 is currently used as the positive amplification control for the GlobalFiler Express Amplification Kit.

Generally, approximately 5% of the samples in a database batch will be quality control samples such as a duplicate offender sample or a staff duplicate sample. One of these samples may also serve as the positive amplification control when the kit control fails. Failure to obtain the expected profile for at least one positive control sample requires re-amplification of the entire plate.

Another option for a failed positive control is to re-prepare and/or re-inject the positive control if the cause of failure appears to be a failed electrophoretic injection. As long as the re-prepped and/or re-injected positive control types successfully, the positive control is considered successful.

When an incorrect STR profile is obtained for one or more of the positive control samples in a batch, other profiles in the batch will not be entered into CODIS until the issue is resolved. The CODIS Administrator, or a designated individual, may approve the entry of select profiles on a case by case basis.

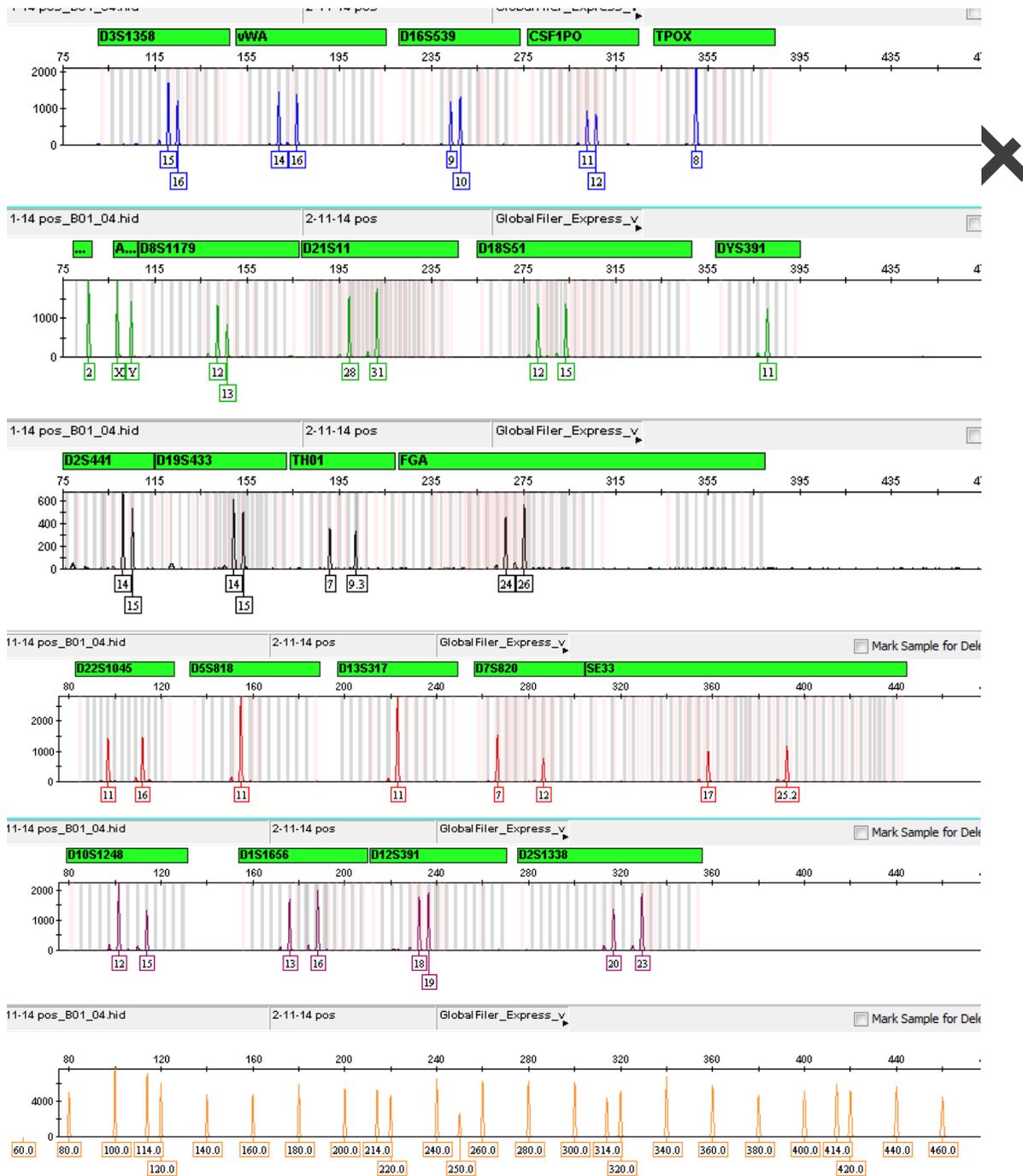
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STR profile of 007



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5.1.4 Negative Controls

A reagent blank is carried through the entire analytical process as part of each extraction or type of extraction. It contains all the reagents - except DNA template - used during extraction, amplification and typing.

For GlobalFiler Express, the negative amplification control contains only the PCR master mix (reagents used to prepare the PCR amplification mixture) for each batch of samples and Prep-n-Go Buffer. For GlobalFiler Express, the negative amplification control also contains all the reagents used for extraction and therefore serves as a reagent blank as well.

The purpose of the negative control is to detect DNA contamination that might occur from the reagents, the laboratory environment, between the samples being processed, and/or due to improper handling of the samples by the analyst. The negative control must be run at the most stringent set of conditions for the batch.

Verification of the presence of amplicon in the negative control is performed by viewing the presence of unincorporated primer peaks.

The negative control should not yield any true STR allelic peaks above the reporting threshold. When peaks greater than or equal to the reporting threshold are present in the range between 60-460 base pairs, the analyst will determine if the peaks are artifacts (e.g. spike, pull-up) or true allele peaks.

The presence of peaks above the reporting threshold will not invalidate the sample as long as the peaks can be shown to be artifacts. Artifacts will be documented on the electropherogram or the database batch worksheet.

When probable true allele peaks are detected above the reporting threshold, at several or all STR loci, the analyst will investigate possible causes. The Technical Manager will be consulted to determine the appropriate course of action.

Since the failure of a negative control may indicate a problem at the extraction or amplification level, a Quality Review Form may need to be completed based on the nature of the discrepancy.

Note: Although appropriate quality assurance practices are stringently applied and enforced, it is not unexpected that low-levels of adventitious DNA (i.e., levels that result in peak heights for allelic data that are below the Laboratory's reporting threshold) attributable to staff scientists and/or consumable products directly involved in the analytical steps of such a highly sensitive technique may be detected

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5.1.5 Quality Control Check

Offender profiles are compared against the profiles of relevant staff members to ensure that contamination by laboratory staff is not included in uploaded profiles. This can be accomplished using the Profile Comparison tool in GeneMapper ID-X or by manual comparison. Staff member buccal swabs may be used intentionally as quality control samples; and these are expected to yield profiles concordant with the profiles on record. However, the unexpected presence of a laboratory staff profile indicates a problem at the extraction or amplification stage and requires a Quality Review Form.

5.2 Interpretation of Samples

Typically, each locus is characterized by one or two labeled peaks or alleles of approximately equal intensity.

Allele peak heights should typically fall between 1000 and 6000 RFU. Peak heights outside this range may be acceptable; however, it is possible that these samples will require careful interpretation. Alleles with peak heights less than 350 RFU may occasionally exhibit peak height imbalance due to stochastic effects caused by low template copy number.

Batches of database samples are uploaded into SDIS by creating an Export Table for CODIS. Prior to creating this table, the analyst must confirm that only eligible profiles are marked for export (as Convicted Offender, CO Duplicate, Arrestee, Staff Duplicate). This is done by selecting the appropriate Specimen Category while in GeneMapper ID-X. Samples not exported to CODIS are marked "no export".

Peak height ratios (PHR) are calculated by dividing the peak height (in RFUs) of the lower RFU allele by the peak height of the higher RFU allele, and then multiplying this dividend by 100 to express the PHR as a percentage. The GeneMapper™ ID-X software is set to flag samples with an intralocus PHR below the expected minimum of 50%. PHR imbalance (in a single source sample) may occur with low quantities of template DNA, degraded template DNA or in the presence of PCR inhibitors. Severe imbalance may result in allelic dropout, where one or more alleles occur below the detection threshold or are not visible at all.

The presence of more than two peaks or severe PHR imbalance at a locus may be explained by a tri-allele, primer binding site mutation, variant allele, or other biological or procedural artifact. The presence of these at several loci is indicative of a mixed sample.

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5.2.1 Tri-alleles

Tri-allelic patterns (three alleles at a single locus) are known to occur in single source samples and there are documented instances where different tissues from the same individual may or may not exhibit the tri-allelic pattern.

Samples exhibiting tri-allelic patterns with all alleles of approximately equal intensity, or with the 3rd allele having a peak height greater than or equal to 50% of the second largest peak, are entered as such in CODIS.

For samples exhibiting possible tri-allelic patterns where the 3rd allele is less than 50% the height of the second-largest peak, only the two largest alleles will be entered into CODIS. The analyst will note that there is a possible tri-allele at the locus in question on both the batch worksheet and the comments section in the CODIS entry.

5.2.2 Off-Ladder (OL) Alleles

The allelic ladder contains the most commonly observed alleles for the STR loci. True alleles are peaks that are detected and labeled by the GeneMapper™ ID-X software with a number and should lie within the range of the alleles in the ladder. Alleles which are not assigned a numeric designation in the standard ladder will be designated as off-ladder (OL) alleles.

Peaks that are labeled as OL (off ladder) at a particular locus have a base size that is different from any of the alleles in the ladder and any of the virtual alleles. These peaks may be true alleles but additional analysis is required to verify this. Before determining whether an OL is a true allele or an artifact, the analyst must consider all other possible causes of OL occurrence.

True OL alleles will exhibit peak morphology similar to the other alleles in the sample and the PHR should be appropriate relative to the other allelic peaks in the sample. When the overall sample quality is poor, the analyst should consider re-amplification prior to designating an OL peak as a true allele.

When a true OL allele lies within the range of alleles for a particular locus, the allele will be reported as a variant of the integer (i.e. X.1, X.2, etc.). The appropriate allele call is determined by simultaneously examining the base sizes for the sample allele peak and the associated allelic ladder peaks in the relevant locus.

When the OL allele lies either above or below the largest or smallest allele in the ladder, the OL allele will be designated as either greater than (>) or less than (<) the allele from the ladder that is closest in proximity to the OL allele.

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5.2.3 Low-Level DNA Samples

Stochastic effects, including extreme peak height imbalance, are typical of samples with very low levels of DNA. One risk associated with such samples is the possibility that a true heterozygote will appear to be a homozygote due to a sister allele below the analytical threshold. To minimize this risk, a homozygote threshold of 350 RFU must be used for interpretation of database sample. The homozygote threshold is analogous to the stochastic threshold employed in casework analysis, but specifically addresses the concern of partial drop out in a low-level database sample. A homozygous locus may only be considered complete if the peak height of the allele is equal to or greater than 350 RFU. Any homozygous locus with an allele peak height below 350 RFU must be considered as potentially a partial profile at that locus.

If allelic dropout, or possible drop-out based on the homozygous threshold, is observed in one or more core STR loci, the analyst will make a “good faith effort” (see Section 7) to re-process the sample in order to generate a complete genetic profile.

Note: The negative PCR control shall be processed under the most stringent conditions utilized for any associated sample.

If the “good faith effort” also fails to generate a complete genetic profile, a maximum of three core STR loci with elevated stutter and/or stochastic issues (e.g., partial dropout, heterozygous peak height ratio less than 50%) may be permissible in a sample. Samples that do not contain at least partial data at each of the 13 core STR loci are not eligible for upload to NDIS. The known allele 10 variant at the D5 locus in PowerPlex 16 is an accepted phenomenon and is not counted towards the maximum three core STR loci with stochastic issues. The presence of this variant, when observed, should be documented on the database batch worksheet.

When incomplete typing results are suspected at one or more STR loci, indicate this by selecting “Yes” in the Partial Profile field in CODIS for the applicable loci.

Data may be composited from multiple amplifications/injections of the same lysate/extract of a database sample in order to obtain a more complete profile. However, data cannot be composited between GFE and PowerPlex 16 amplifications.

5.2.4 Artifacts

Artifacts are data peaks resulting from the analytical process and are not attributable to an individual contributor to the sample.

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When artifacts are not pervasive throughout the sample and do not interfere with data interpretation, the sample does not require re-analysis. When an artifact is present within the size range of the alleles in a core locus and the presence of the artifact interferes with interpretation of the data in that locus, or when a sample exhibits artifacts in several loci, interpretation of the sample becomes more challenging and it may be necessary to reprocess the sample. Artifacts may be resolved by merely re-injecting a sample, or may require re-amplification or re-extraction to resolve. If the interfering artifact is located in one of the non-CODIS-core loci, the analyst may choose to enter the profile, omitting all data from the affected locus.

Samples uploaded into CODIS must not contain any labeled artifacts or OL alleles. The analyst may remove the artifact label. Artifact or OL peaks are either deleted or re-labeled by right clicking on the peak and adding a comment ('microvariant allele', 'artifact' etc.). The analyst should note any changes made to the allele calls in the appropriate column on the database batch worksheet.

5.2.4.1 Incomplete 'A' nucleotide addition (-A or split peaks)

AmpliTaq Gold™, like many other DNA polymerases, catalyzes the addition of a single nucleotide (predominantly adenosine) to the 3' ends of double-stranded PCR products. This non-template addition results in a PCR product that is one base longer than the actual template, and the PCR product with the extra nucleotide is referred to as the "+A" (base peak) form. The final step of the STR amplification process is a terminal extension step to promote complete non-template 'A' nucleotide addition. The "-A" form is the peak that represents the actual template length. The "+A" peak will be the predominant form, with the "-A" peak typically not being detected.

Failure to attain complete terminal nucleotide addition (typically due to an excess of template DNA) results in "split peaks", two peaks above threshold that are one base apart. If the labeled "-A" peaks are not pervasive throughout the sample and the sample appears to otherwise be a single source sample, the peaks may be documented as such on the paperwork and the OL labels removed. When split peaks occur at several loci, re-amplification with less template DNA may be appropriate.

5.2.4.2 Pull-up

Multi-component analysis is the process that separates the six different fluorescent dye colors into distinct spectral components. The six dyes (6-FAM, VIC, NED, TAZ, SID, and LIZ) emit their maximum fluorescence at different wavelengths, but there is some overlap of the emission spectra. A spectral calibration is performed for a specific dye set to create a matrix that corrects for the spectral overlap.

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Pull-up is the result of incomplete separation of the emission spectra and is typically observed as a non-allelic peak at the same base size as a peak in another dye. This typically occurs at high RFU values, when an excess of template DNA has been amplified.

Pull-up may also occur when the matrix file is not current. A new spectral calibration is performed whenever a new capillary array is placed on the instrument or whenever any part of the optics have been realigned and/ or replaced. A new spectral calibration may also need to be performed when an overall decrease in spectral separation is observed.

If the pull-up is prevalent enough to interfere with data interpretation, the sample may need to be re-injected or re-amplified with less DNA template. Otherwise, it can be noted on the batch worksheet and the label(s) manually removed.

5.2.4.3 Stutter

The PCR amplification of tetranucleotide STR loci typically produces a minor product peak four bases shorter than the corresponding base peak. This artifact is the result of slippage of the Taq polymerase and is referred to as the stutter peak or stutter product. Stutter products four bases longer, or two bases shorter, than the corresponding base peak are less frequently observed. Two base pair stutter products are so rare that peaks in these positions are not filtered out in the analysis parameters.

The proportion of stutter product relative to the main allele peak is calculated by dividing the height of the stutter peak by the height of the main allele peak. The stutter percentage is fairly reproducible for a particular locus.

The GeneMapper™ ID-X database analysis methods have a global stutter filter of 20%. Peaks in the stutter position greater than 20% may indicate the presence of DNA from more than one individual. When there is no indication of a mixture, and the data is determined to be elevated stutter, the analyst will document the elevated stutter on the database batch worksheet and manually remove the stutter label.

It is unlikely that the presence of stutter peaks would interfere with the interpretation of database samples and therefore, re-amplification and/or re-injection of the sample may not be required.

5.2.4.4 Spikes

Spikes are non-allele peaks that may arise due to air bubbles, urea crystals or voltage spikes. Spikes are typically quite sharp and easy to distinguish from a true allele,

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usually appearing in more than one color at the same base size, and not reproducible by re-injection.

Peaks labeled as such by the GeneMapper™ ID software cannot be manually removed. However, they do not interfere with upload to CODIS and no re-analysis is required if the spike does not interfere with interpretation of the sample.

5.2.4.5 Dye Blobs

Dye blobs, like spikes, are non-allele peaks that are easily distinguished from true alleles. Dye blobs may be the result from residual dye molecules left over from the synthesis of the primers or they may be dye molecules that fell off the primer during the amplification.

If the artifact is labeled but does not interfere with data interpretation, the dye blob(s) shall be documented on the batch worksheet and the label manually removed.

5.2.4.6 Persistent Kit Artifacts

Occasionally, PCR kits may exhibit persistent artifacts that may or may not appear as true alleles. Typically, these artifacts are observed and documented during kit verification process. As with other artifacts, if the artifact does not interfere with data interpretation, it may be documented on the batch worksheet and the label manually removed, if necessary.

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Section 6 Review of Data

Database projects are routinely reviewed electronically, with the technical reviewer looking at the controls/samples in GeneMapper ID-X rather than printed electropherograms. The analyst may choose to submit printed electropherograms to facilitate documentation and review of artifacts, true OL alleles, tri-alleles, concordance checks on re-analyzed samples, etc.

6.1 Review of Offender Database Samples Analyzed In-House

Genetic profiles and all supporting documentation generated in the course of analysis will be subjected to a technical review. The DNA analyst will submit a packet containing the database batch worksheet and any printed electropherograms to another qualified database analyst for technical review. This review will be conducted in accordance with the FBI QAS Guidelines and includes the following, at a minimum, to ensure compliance with the interpretation guidelines contained within this document:

- Review of the ILS for all passing samples
- Review of all allelic ladders designated as such
- Review of all amplification positive controls and quality control samples
- Review of all negative amplification controls
- Review of all DNA typing data for all passing samples
- Verification of eligibility for all profiles marked for export, to include selection of an appropriate specimen category
- Concordance with previous results for re-processed samples

The analyst and the reviewer may consult a third qualified DNA analyst, if necessary, when there is a disagreement on how to report a result. If the analysts are unable to come to an agreement, the DNA Technical Manager will be consulted to make the final decision.

A review checklist is used to document completion of the individual components of the technical and administrative review. Upon completion of the technical review, the analyst may import the passing samples to SDIS. The analyst or a CODIS Administrator will then complete an administrative review, to include performing a duplicate offender search in SDIS.

Upon completion of the technical and administrative reviews, NDIS eligible samples are uploaded by a CODIS Administrator. Additional information on CODIS procedures is contained in the CODIS Administrative Manual. Upon completion of the upload, the following paperwork is archived as a .pdf in the LIMS:

- Database batch worksheet
- Electropherograms requiring documented review

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- STR Offender Batch Review Checklist
- SDIS Import Reconciliation Report

6.2 Review of Outsourced Offender Database Samples

The laboratory does not currently use a vendor laboratory for DNA analysis. Should the laboratory enter into a contract for DNA analysis of database samples with a vendor laboratory, a procedure for the in-house review of outsourced samples will be created before analysis begins.

Section 7 “Good Faith Efforts” at recovery of low-level samples

As mentioned in section 5.2.3, if allelic dropout, or possible drop-out based on the homozygous threshold, is observed in one or more core STR loci, as per the NDIS Operation Procedure, the analyst will make a “good faith effort” to obtain a complete genetic profile. A “good faith effort” may include either just the first or both of the following options:

7.1 Re-amplification

The incubation time of less than 60 minutes at room temperature is optimized for a majority of whole-swab samples. Samples containing less DNA, or samples where only half a swab is available for analysis, may benefit from prolonged incubation time at 2 – 8 °C. As a second attempt to gain a full profile for CODIS entry, the lysate may be incubated at 2 – 8 °C for up to two weeks and amplification re-attempted. In this case, the only change to the amplification protocol is the length of the incubation time.

7.2 Extraction by EZ1-XL and Amplification by PowerPlex 16

If re-amplification is not successful, extraction should be attempted. In order to concentrate and purify a lysate with low levels of DNA, the remaining lysate may be extracted using the Large-Volume Protocol on the BioRobot EZ1-XL, eluting in 40 uL TE buffer. A corresponding reagent blank using 400 uL of Prep-n-Go Buffer should be also be extracted. (Refer to Qiagen BioRobot EZ1 Advanced-XL DNA Extraction protocol in FBCP) The resulting extracts should then be amplified using PowerPlex 16, using both 5 uL and 10 uL amplification volumes of the sample extract and 19.2 uL of the reagent blank. (Refer to DNA Amplification, Data Collection by 3500xl Genetic Analyzer, Data Analysis with GeneMapper ID-X and STR Data Interpretation sections in FBCP)

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Controls and offender reference samples will be analyzed according to the parameters set forth for casework reference samples. However, documentation will be performed electronically as described in Section 6 of this manual.

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STR Offender Batch Review Checklist

Date: _____
 Technical Review Started: _____
 Administrative Review Started: _____
 Database Batch Number: _____

Analyst:	Tech. Review:	Admin. Review:
[Signature]	[Signature]	[Signature]

Database batch # and analyst's initials on each page			
Reagent worksheet complete: analysis dates, instruments, # samples, reagent lot # and expiration dates, raw data folder(s) and project name(s)			
Batch worksheet complete: well #s, sample IDs, upload eligibility, specimen category and analysis range.			
Technical Reviewer checked batch worksheet (inc. reagents): initialed and dated each page			
ILS labeled correctly (60-460) for all passing samples			
Allelic Ladder(s): correct allele calls obtained ¹			
Positive control(s): correct DNA profile obtained ¹			
Negative control(s): results are acceptable ¹			
Reagent blank(s): results are acceptable ¹ (PP16 amps only)			
Check for consistency between NO EXPORT samples on paperwork and in project			
Confirm appropriateness of NO EXPORT status			
Technical review of passing samples: ≤3 problem core loci, RFU ≥175, appropriate allele edits, OL allele documentation			
Correct specimen categories are assigned			
Confirm specimen category is either convicted offender or arrestee for passing samples (check for agreement between paperwork and project)			
Samples for SDIS only are flagged on paperwork			
Profiles checked against relevant staff profiles			
Profiles checked for concordance with prior results for any previously typed samples			

¹ includes verification of ILS and controls for re-worked samples

Eligible profiles uploaded into SDIS (in the Offender Index)			
Reconciliation report: #samples uploaded consistent with batch worksheet			
Problem samples corrected and documentation attached			
Duplicate offender search performed and all high stringency matches reconciled			
AK new moderate stringency search performed (only after previous step completed) may be completed by analyst or administrative reviewer			

Performed by CODIS Administrator

Upload to NDIS performed			
Reconciliation report received; consistent with # samples uploaded			

NOTES:

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Appendix A Revision History

FBDB 2014 R0 Page	FBDB 2013 R1 Page	Location	Revision made
2	2	Section 1.1	Revised the internal control sample requirements in the bulleted statement beginning "Each batch of extractions....."
2-3	2-16	Section 1	Removed QIASymphony extraction protocol. Added protocol for setting up direct amplification by GlobalFiler Express.
4	17-20	Section 2	Removed QIAgility plate set-up and amplification protocol. Added protocol for PCR amplification by GlobalFiler Express.
5-8	n/a	Section 3.3.1	Added protocol for 3500 plate set-up using the QIAgility
8	21	Section 3.3.3	Replaced ILS 600 with GeneScan 600 LIZ
9	22	Section 3.4.1	Revised the bullet point starting with "Under the heading Assays"...
11	24	Section 4.2	Revised bullet point starting with "Select the appropriate Sample Type" to reflect use of GlobalFiler Express.
11-15	25-31	Section 4.2.1	Revised Analysis Methods to reflect use of GlobalFiler Express.
17	33	Section 5	Revised minimum acceptable peak height to 175 RFU to reflect use of GlobalFiler Express.
18-24	34-38	Section 5.1	Revised Interpretation of Batch Controls to reflect use of GlobalFiler Express.

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24	n/a	Section 5.1.5	Added section on quality control checks
24	39	Section 5.2	Revised sentence in second paragraph, starting with “Alleles with peak heights”...
25	39	Section 5.2.1	Replaced “neighboring peak” with “second-largest peak”. Revised policy for reporting and confirming tri-alleles.
26	40	Section 5.2.3	Revised first paragraph to incorporate a homozygote threshold for use with GlobalFiler Express.
26	41	Section 5.2.3	Removed reference to reagent blank in Note.
26	41	Section 5.2.3	Replaced “best attempt” with “good faith effort”. Revised to include reference to Section 7.
25	41	Section 5.2.3	Revised paragraph beginning “Data may be composited...”
27	42	Section 5.2.4.2	Replaced reference to matrix dyes from PowerPlex 16 with dyes from GlobalFiler Express
30	45	Section 6.1	Removed “reagent blanks and” from fourth bullet point
31-32	n/a	Section 7	Added section describing “good faith efforts” protocols
33	47	Review Checklist	Added “Profiles checked against relevant staff profiles”
33	47	Review checklist	Revised checklist to reflect usage of GlobalFiler Express interpretation guidelines