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Section 1 DNA Amplification

References:

- *PowerPlex® 16 System Technical Manual*
- *GeneAmp® PCR System 9700 96-Well Sample Block Module User's Manual*

1.1 Amplification Cycling Parameters

- Ensure AB GeneAmp® PCR System 9700 thermal cycler has been turned on to allow the instrument time to properly warm up.
- Confirm the cycling parameters for **user: alaska, program: pp16-32cyc**

  95°C for 11 minutes, then:

  96°C for 1 minute, then:

  ramp 100% to 94°C for 30 seconds
  ramp 29% to 60°C for 30 seconds
  ramp 23% to 70°C for 45 seconds
  for 10 cycles, then:

  ramp 100% to 90°C for 30 seconds
  ramp 29% to 60°C for 30 seconds
  ramp 23% to 70°C for 45 seconds
  for 22 cycles, then:

  60°C for 30 minutes
  4°C hold

1.2 Amplification Set-up of Forensic Casework Samples

- If samples have been stored at 2-8°C, allow them to warm to room temperature, then vortex and spin briefly using a centrifuge.
- Transfer the amplification reagents to the designated PCR set-up area.

Note: Do not expose reagents to light for extended periods of time.

- Ensure that all kit components have thawed completely before use. Vortex reagents and centrifuge briefly to ensure uniform mixing and collection of tube contents.

Note: Centrifugation of the primer mix should be minimal to avoid primer collecting at the bottom of the tube.

- Prepare a PCR master mix by adding the following volumes of reagents to an appropriately sized tube:
    - # of samples x 2.5µL Gold STR 10X Buffer
    - # of samples x 2.5µL PowerPlex® 16 10X Primer Pair Mix
    - # of samples x 0.8µL AmpliTaq Gold™ DNA Polymerase (5U/µL)

Note: Prepare enough for a few extra reactions to allow for loss during pipetting. This is calculated automatically on the DNA amplification/electrophoresis worksheet.

- Vortex the master mix and spin briefly. Transfer 5.8µL of master mix to each sample well of a 96-well amplification plate. Cover the entire well plate with Glad® Press 'n Seal (or equivalent).

- Prepare the samples to be amplified as follows (in individual 0.5mL tubes):

  Forensic Case Samples
  Add approximately 0.5ng to 1ng template DNA. The amount of template DNA may be higher or lower depending on the sample (i.e. low-level samples that are likely to be mixtures may be targeted higher, while bloodstains likely to be single-source may be targeted lower). Add sterile de-ionized water to bring the sample to a final volume of 19.2µL.

  Quantification Value <0.05ng/µl
  Samples with a quantification value <0.05ng/µl shall be routinely amplified in duplicate to ascertain reproducibility of alleles.

  Quantification Value >0.05ng/ul
  Samples with a quantification value >0.05ng/µl need not be routinely amplified in duplicate.

  Negative Quantification Value
  Samples with no detectable DNA, including reagent blanks and negative controls, may be amplified one time (i.e. duplicate amplification not required).

  Reagent Blank Sample(s)
  Add 19.2µL of the extraction reagent blank.

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Positive Control Sample
Vortex and spin the tube briefly and add diluted positive control DNA to a 0.5mL tube. Typically, 1-2uL will yield a full profile with minimal artifacts. Bring the volume up to 19.2μL with sterile, de-ionized water.

Negative Control Sample
Add 19.2μL of sterile de-ionized water.

- Transfer the entire 19.2μL of the above prepared samples to the appropriate sample well, already containing the PCR master mix. During sample addition, the pipette tip is inserted through the Press 'n Seal.
- Once all samples have been added, remove the Press 'n Seal and cover the plate with a sheet of amplification tape.
- Transfer the plate to the PCR room and place directly into the thermal cycler. Start the run.
- Store amplified products at 2-8°C. All amplified products will be disposed of upon completion of the technical and administrative reviews of the case.

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Section 2 Data Collection by 3500xl Genetic Analyzer

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

2.1 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 have 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

2.2 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** to warm the oven.
- Check the pump assembly for bubbles and run the Remove Bubble wizard if necessary.

2.3 Sample Preparation for the 3500xl

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

Note: Prepare enough for a few extra reactions to allow for loss during pipetting.

- 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 and 1µl of each amplified sample to the appropriate wells. When all samples have been added, cover with plate septa.
- 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.

2.4 Creating a Plate Record

2.4.1 Create a Plate Record from the Data Collection Software

- In the dashboard click **Create New Plate**
- In the Define Plate Properties screen:
  - Enter a plate name (i.e. 10-1222KAL_Q_101224)
  - 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:
  - PPlex16_15sec
- 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.

2.4.2 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. 10-1222KAL_Q_101224)
  - 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.

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- Click **Save Plate**.
- Click **Assign Plate Contents**.
- 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.

2.5 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.

2.6 Viewing Data During a Run

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

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Section 3 Data Analysis with GeneMapper™ ID-X

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

The settings for the Analysis Methods are viewed by selecting GeneMapper ID-X Manager under the Tools drop-down menu, then clicking on the Analysis Methods tab then double clicking to select a particular Analysis Method. A range of Analysis Methods are available to analyze casework data. Options for Analysis Methods vary by analysis range. These methods shall not be modified. New methods shall only be created or modified with permission from the DNA Technical Manager.

The panel for casework on the 3500xl is: 3500_PowerPlex_16_Casework.

3.1 Casework Analysis Methods

3500CW-2400
3500CW-2800
3500CW-3200
3500CW-Blank

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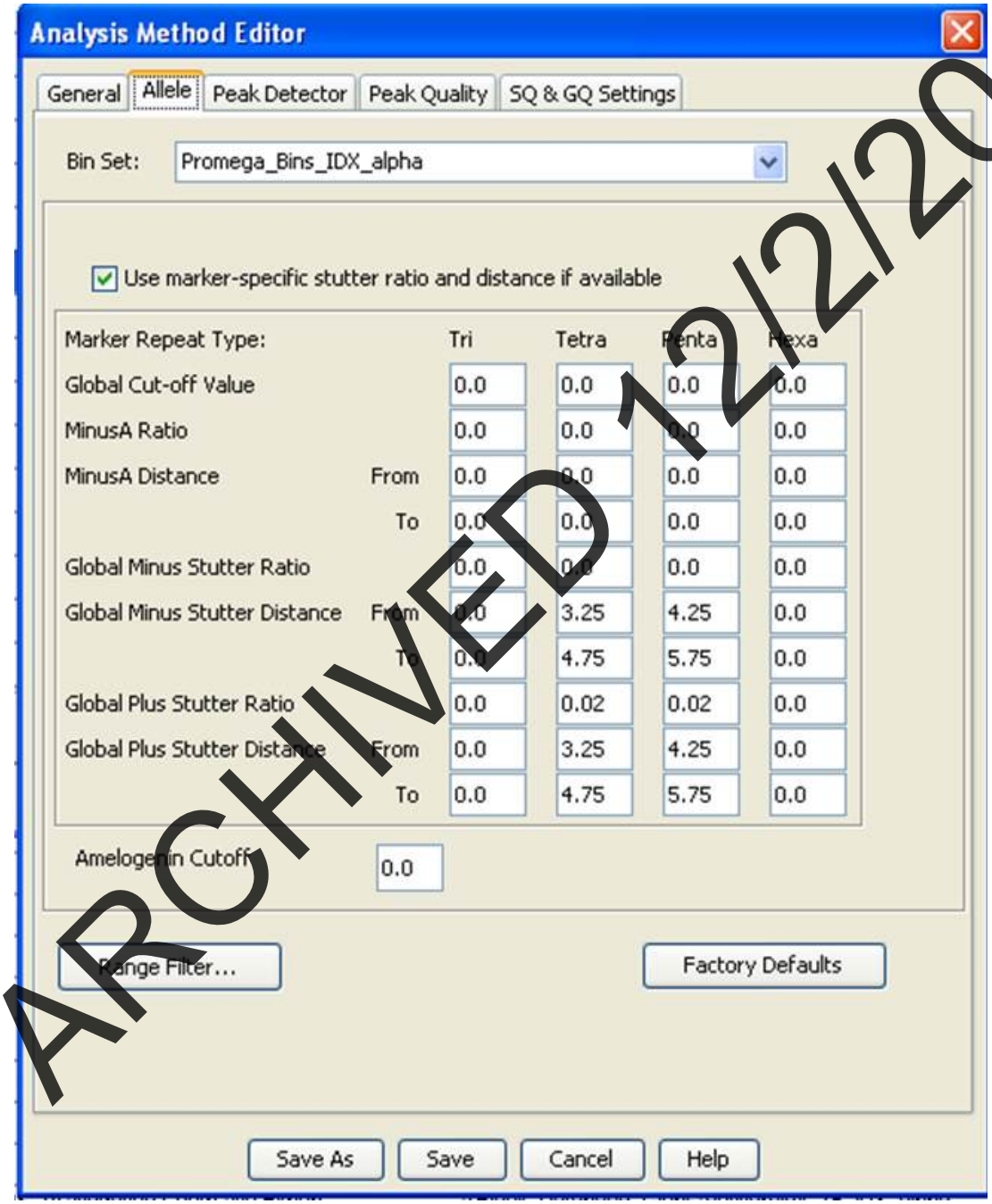
3.2 Casework Allele Tab Settings

These settings are consistent within all casework methods.



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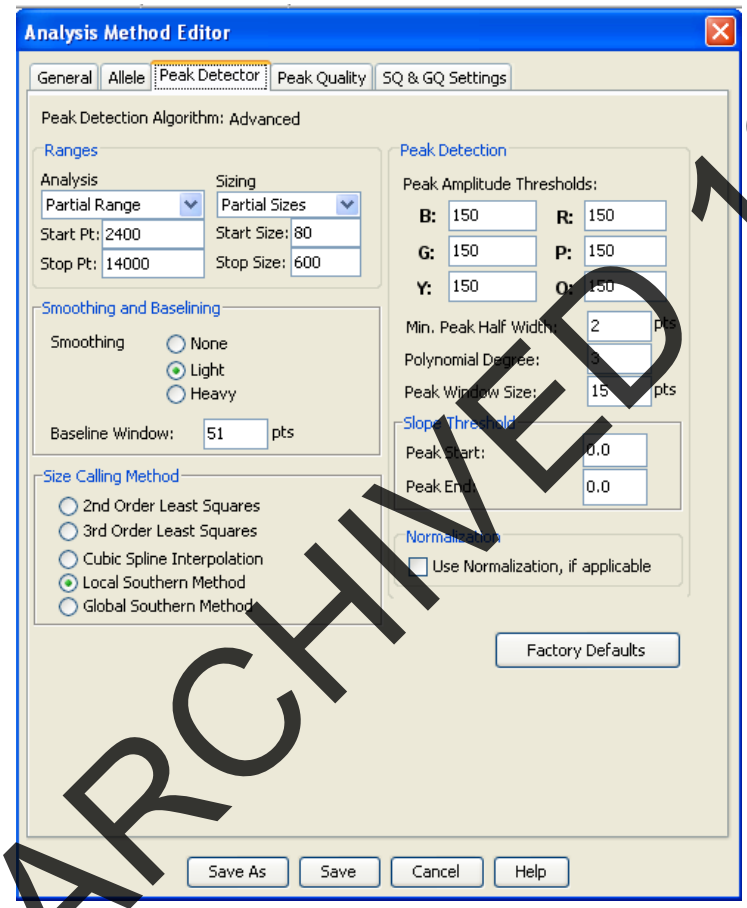
3.3 Casework Peak Detector Tab Settings

The Analysis Range is the only setting that varies between the alternative Analysis Methods. The Analysis Range is selected to ensure capture of the 80bp ILS peak for all allelic ladders, positive controls, and questioned and known samples in the project. The Analysis Range is selected to capture the primer peaks for reagent blanks, negative amplification controls, and samples that do not yield at least a partial profile.

The Analytical Threshold for all analyses is 150RFU.

<u>Example: 2400 Casework Analysis Method</u>



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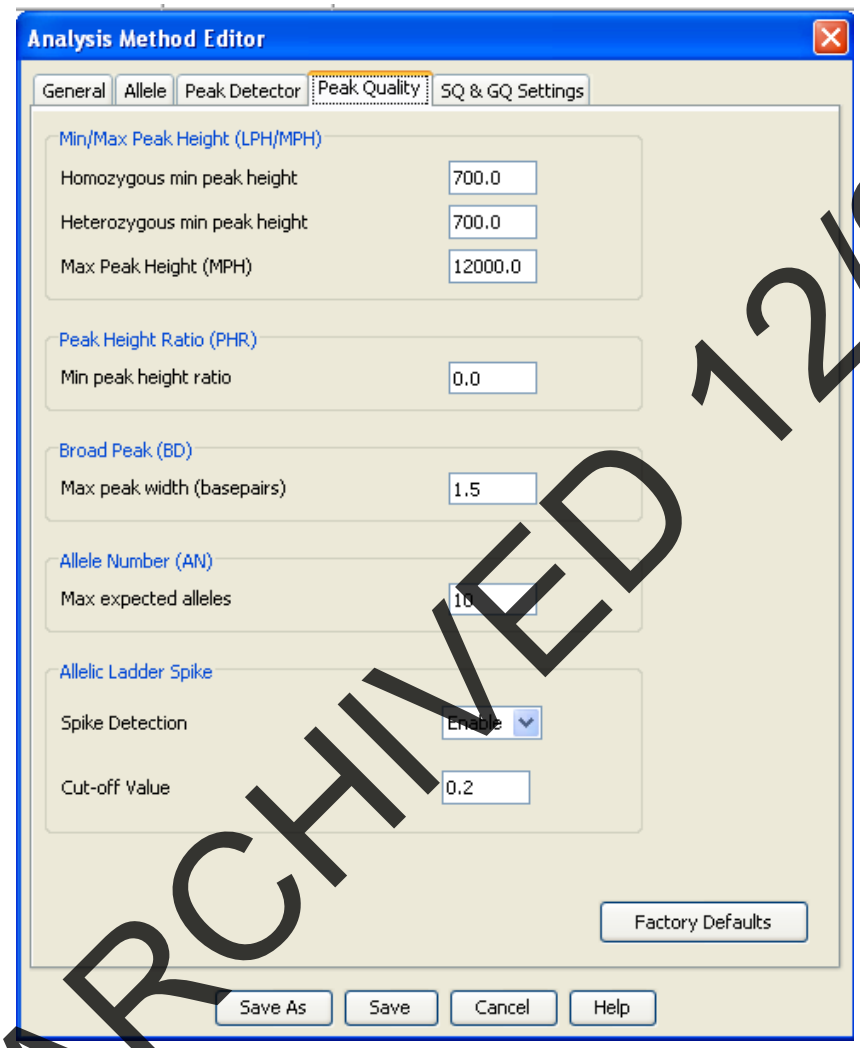
3.4 Casework Peak Quality Tab Settings

These settings do not vary among the casework methods and they are not relevant in analysis of any samples where the sample type is set to Negative Control.

Example: 2400 Casework Analysis Method



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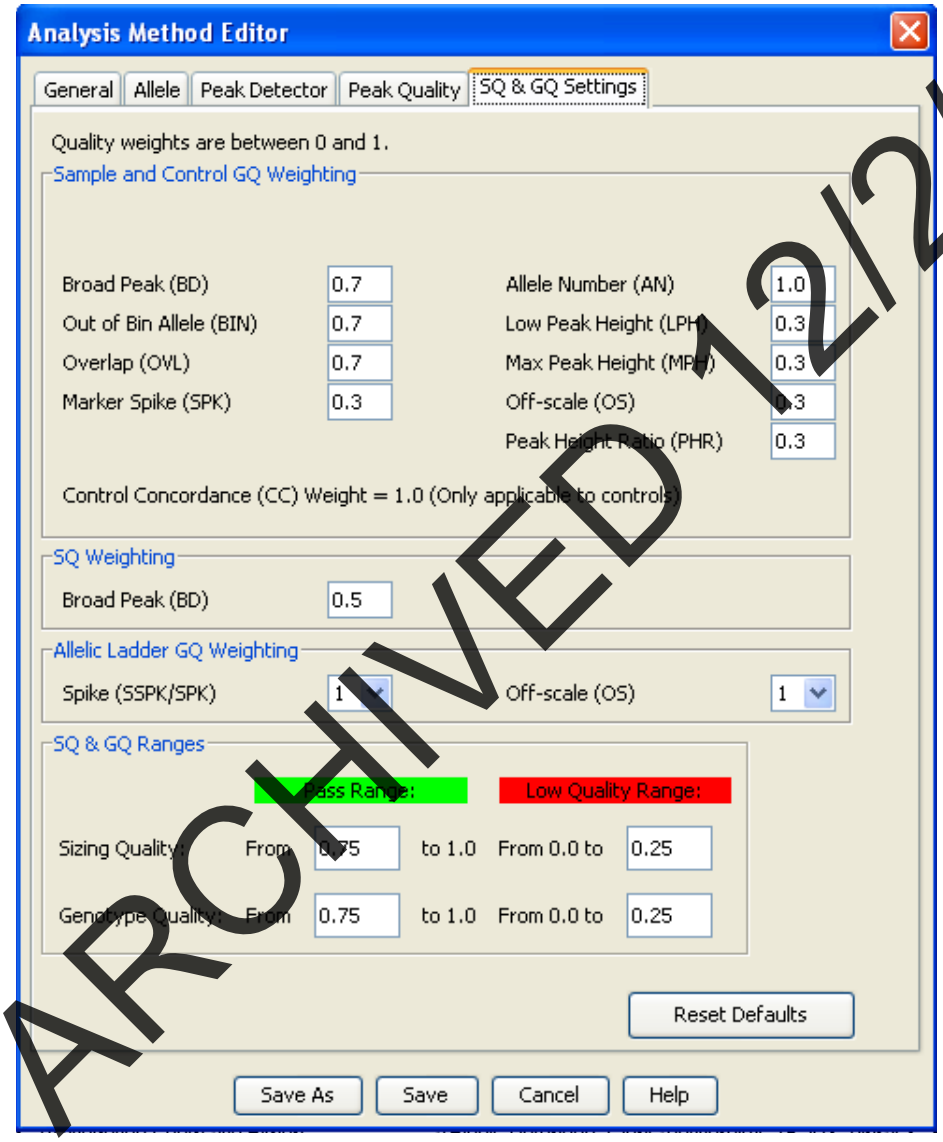
3.5 Casework SQ and GQ Tab Settings

These settings do not vary among any of the analysis methods, and they are not relevant, as all samples are currently manually reviewed and interpreted, regardless of flagging.

<u>All Analysis Methods</u>



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Section 4 STR Data Interpretation

Interpretation of casework samples is based on the guidelines contained in this section, as well as the qualified DNA analyst's training and experience, experimental results, and case specifics. 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 following abbreviations are commonly used in bench notes and on other documents generated during DNA analysis.

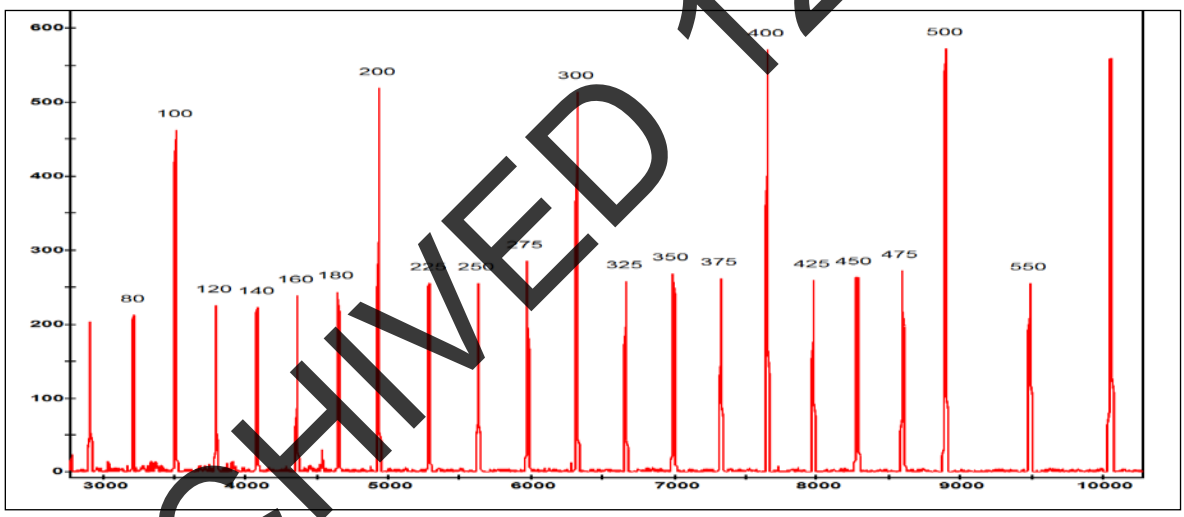
DNA Abbreviations and Definitions	
BL	Noisy baseline
PHR	Peak Height Ratio
PU	Pull-Up
DO	Complete Drop-Out at a Locus
PDO	Partial Drop Out at a Locus
D5Mut	D5 Mutation
OL	Off Ladder
OMR	Outside Marker Range
OS	Off Scale/Saturation
NP	No genetic profile obtained
NR	Data not reported
NS	Data not used for 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

4.1 Interpretation of Batch Controls

Prior to assessment of casework 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 and negative amplification controls, and extraction reagent blanks. 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 casework samples. Unresolved failure of a batch control requires the Technical Manager, or designated individual, to approve a course of action.

4.1.1 Internal Lane Standard (ILS)

The Promega PowerPlex16® System uses ILS 600. The analyst should verify that all peaks from 80-550 base pairs are present and labeled as shown.



The peak heights for the ILS peaks must be at least 150RFU (relative fluorescence units) to be identified and labeled by the GeneMapper™ ID-X software with a casework analysis 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 the size standard.

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4.1.2 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 allele calls for the Promega PowerPlex16® System allelic ladder are shown below. 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 13 FBI STR core loci, the Penta D and Penta E loci, and Amelogenin. 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. In such instances, re-injection is usually appropriate.

Additional information about the allelic ladder can be found in the Promega PowerPlex16® System Technical Manual.

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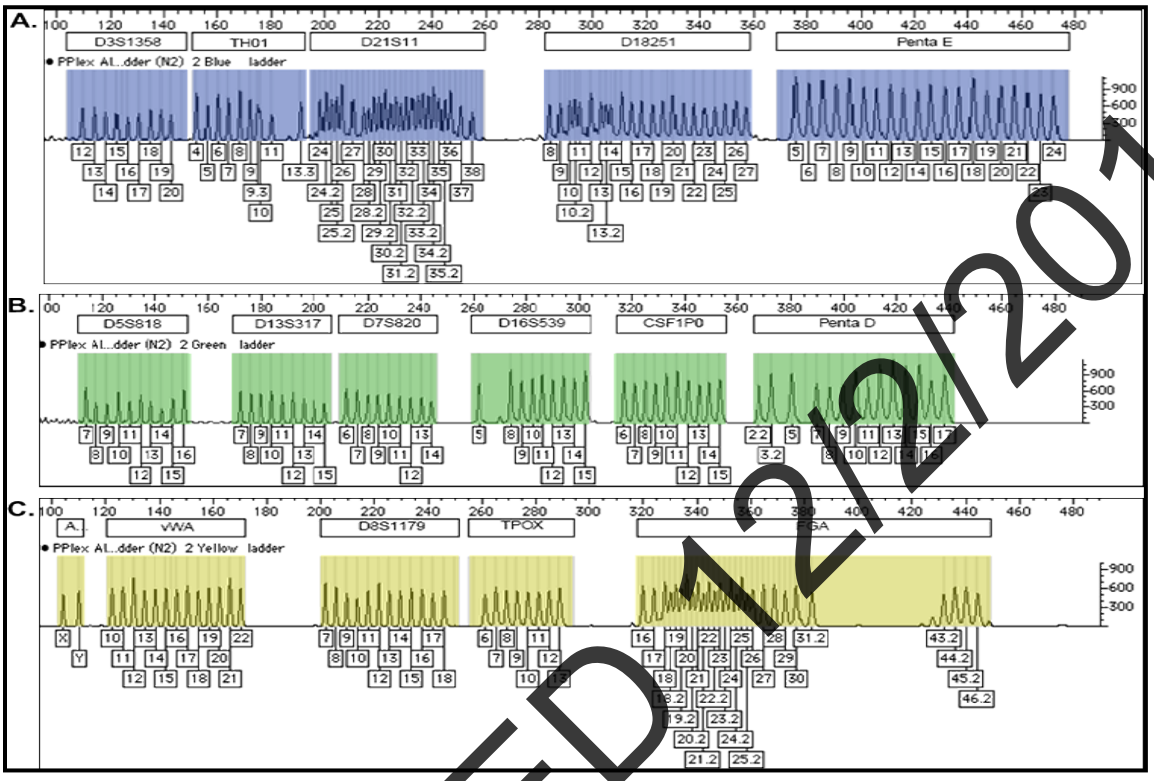
PowerPlex16® Allelic Ladder



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4.1.3 Internal Control Specimen (ICS)

An internal control specimen (ICS) is a designated, predetermined quality control sample whose expected profile is already known. The ICS is processed with an extraction batch and carried through the batch concurrently with the other samples. The purpose of this control is to demonstrate that all analytical processes are working correctly. An ICS may include any known sample, including those obtained from laboratory staff, where the individual has been previously typed to generate a genetic profile.

At least one ICS shall be processed with each casework batch, and will typically be extracted with the known reference samples. An electropherogram of the successfully typed ICS shall be included in the central log folder.

An ICS with a partial profile or no interpretable profile does not invalidate the batch, provided other positive controls in the batch yield the correct results. The Technical Manager, or other designated individual, shall determine whether or not to approve the ICS when only a partial profile or no profile is obtained. The data quality of other positive control samples in the batch and/or the quality of known /reference samples will be reviewed to determine the approval process.

If an incorrect STR profile is obtained for the ICS in a casework batch, the analyst will attempt to determine the cause of the discrepancy. The discrepancy will be documented and corrective and future preventive actions may be taken as deemed necessary by the Technical Manager.

NOTE: Casework batches often contain multiple samples from previously typed individuals. Any one of these may be designated as the ICS for the batch. As good QA practice, the analyst confirms that all profiles from previously typed individuals yield expected profiles. This review is typically documented on the DNA worksheet for the appropriate case.

4.1.4 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. Either standard DNA template 9947A or 2800M, included with the Promega PowerPlex16® System, may be used as a positive amplification control. A minimum of one positive amplification control must be amplified and processed concurrently with each casework amplification.

It may be necessary to re-prep 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.

Alternatively, other samples in an amplification may serve as a positive control (e.g. duplicate offender/staff sample, offender confirmation sample, casework known sample with a verified profile, or a buccal swab freshly obtained from laboratory personnel whose genetic profile(s) was/were previously determined and documented). Use of an alternate positive control must have documented approval of the Technical Manager.

All casework samples co-amplified will be re-analyzed if no positive control was successfully typed for that amplification. The laboratory will routinely include more than one positive control with every casework amplification reaction, in order to prevent reanalysis of samples that are limited in DNA content. If at least one of these positive control samples produces acceptable and expected results, the batch will not be reanalyzed.

If all interpretable results agree for the samples between the first and second amplification/run, and the positive amplification control yields a correct STR profile for the second amplification/run, then the STR data obtained in either amplification/run may be used.

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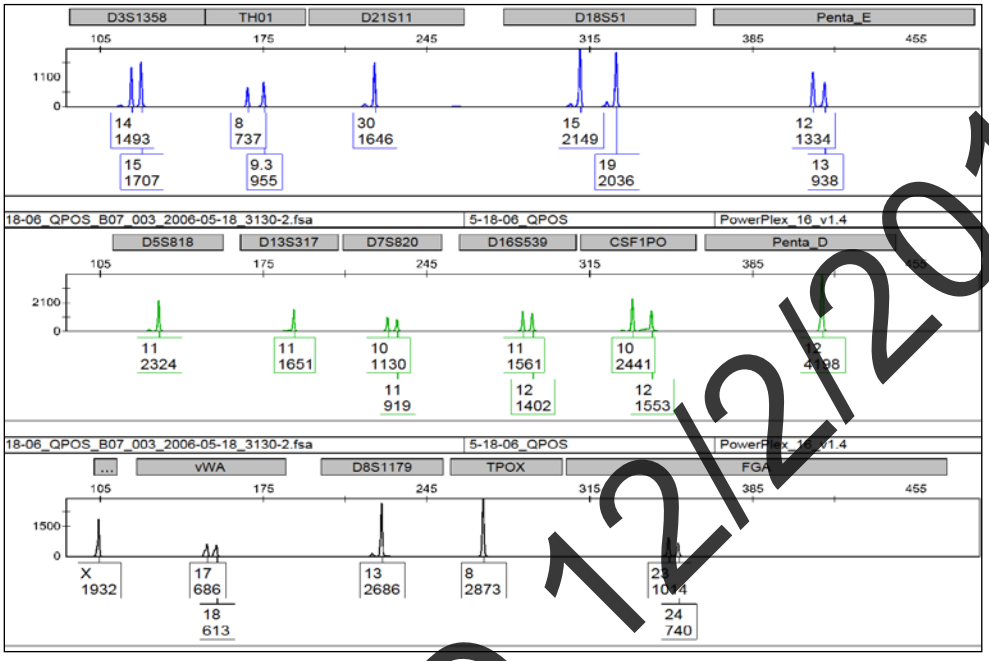
STR Profile of 9947A



STR Profile of 2800M

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4.1.5 Negative Amplification Control and Extraction Reagent Blanks
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.

The negative amplification control contains only the PCR master mix (reagents used to prepare the PCR amplification mixture) for each batch of samples and the water used to dilute the DNA samples.

The purpose of the negative controls is to detect systemic 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 controls must be run at the most stringent set of conditions for the batch.

Verification of the presence of amplicon in the negative controls is performed by viewing the presence of unincorporated primer peaks. The negative controls should not yield any true STR allelic peaks above the analytical threshold. When peaks greater than or equal to the reporting threshold are present in the range between 80-550 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.

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 then 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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4.2 Distinguishing Alleles from Artifacts

The initial assessment of a casework sample electropherogram separates interpretable alleles from artifacts. Artifacts can occur in data and much of their nature and origin have been determined and documented. Peaks that are determined to be artifacts are documented by printing a zoomed in view of the artifact that includes base pair sizing. Peaks determined to be artifacts are struck on the full view electropherogram. Peaks struck manually are also initialed by the analyst. When it is not possible to distinguish between an artifact and a true allele, this is indicated on the STR results table and the electropherogram as Artifact/True allele (A/TA). If the presence of a definite or possible artifact at a locus impacts the suitability of that locus for either comparisons or statistical analysis, that is documented on the STR table and the electropherogram. Artifacts are often associated with an overabundance of template DNA. If the presence of many artifacts complicates data analysis, it may be appropriate to re-amplify the sample with less template DNA.

4.2.1 Baseline noise

The analytical threshold (AT) for analysis of casework samples is 150 RFU. Data below the AT is considered indistinguishable from baseline noise and not suitable for interpretation. However, if data below the AT exhibits consistent peak morphology and alignment to bins, the analyst may note the possible presence of data below the reporting threshold (DBRT) on the electropherogram. No further interpretation is suitable for DBRT.

Ideally, true allele peak heights should fall between 3000 and 12,000 RFU. Samples with peak heights near or above 12,000 RFU may include baseline noise that exceeds 150 RFU. Such baseline noise may be struck. However, if baseline noise is difficult to distinguish from possible lowlevel DNA, it may be more appropriate to re-amplify the sample with less template DNA.

4.2.2 Stutter

The PCR amplification of tetranucleotide STR loci typically produces a minor product peak four bases shorter (N-4), two bases shorter (N-2, infrequently observed), or four base pairs larger (N+4) than the corresponding base peak. This is the result of slippage of the Taq polymerase and is referred to as the stutter peak or stutter product. In the penta-nucleotide loci, the stutter peak is typically five bases shorter than the corresponding base peak.

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 following table lists the values of the maximum expected percentage of N-4 (or N-5) stutter for the loci in the

PowerPlex® 16 System when run on the Applied Biosystems 3500xl. The stutter values are based on data obtained by internal validation studies. These values, along with a universal N+4 stutter of 2%, are set as stutter filters used by the GeneMapper ID-X software.

Maximum Expected N-4 (N-5) Stutter Percentages for PowerPlex® 16 loci on the 3500xl (in single source samples)

D3S1358 14%	TH01 5%	D21S11 13%	D18S51 14%	Penta E 7%	
D5S818 13%	D13S317 10%	D7S820 11%	D16S539 11%	CSF1PO 11%	Penta D 8%
Amelogenin NA	vWA 14%	D8S1179 11%	TPOX 6%	FGA 17%	

Peaks in the stutter position greater than the listed values may indicate the presence of DNA from more than one individual. However, elevated stutter is also associated with both excess template DNA and very low amounts of template DNA. When there is no indication of a mixture other than elevated stutter, the analyst will document the stutter as such on the electropherogram (including the calculated % versus the expected, and a notation that it was determined to be an elevated stutter peak).

4.2.3 Amplification and Injection Artifacts

The following are examples of artifacts which may be struck and initialed on electropherograms. Re-prep and/or re-injection is not required if the artifact in question is clearly distinguishable and thus does not interfere with interpretation.

4.2.3.1 Pull-Up

Multi-component analysis is the process that separates the four different fluorescent dye colors into distinct spectral components. The four dyes (Fluorescein, JOE, TMR and CXR) 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.

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.

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4.2.3.2 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. Spikes usually appear in more than one color at the same base size and are not reproducible by re-injection.

4.2.3.3 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.

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

While –A peaks are a known artifact, none of these were observed in the 3500xl validation studies. As such, any instances of –A require approval by the Technical Manager before the sample injection may be used for casework.

4.2.3.5 Persistent Kit Artifacts

Occasionally, one or more lots of PCR kits may exhibit persistent artifacts. These artifacts may or may not appear as true alleles. Sometimes, these artifacts are so common that they are observed in a high number of routine samples. Such artifacts will be noted in the verification process and acknowledged by the Technical Manager. Once documented in verification, these artifacts should be struck and initialed, and do not require TM acknowledgement when observed in casework.

4.2.4 Off-Ladder (OL) Alleles

The allelic ladder contains the most commonly observed alleles for the STR loci. 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.

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.

OL alleles considered to be true alleles may require re-amplification to confirm, particularly when they are only observed in a mixture. If the OL allele is observed in a double amplification of a single-source sample, or in multiple single source samples each amplified once and injected simultaneously, further amplifications are not necessary to verify the OL allele. The DNA Technical Manager must approve reporting an OL allele without re-amplification (e.g. limited amount of sample available for analysis).

Documentation of the OL allele must include an electropherogram with both the sample and the associated allelic ladder for the locus, showing the OL allele(s) in the locus, with the bins and base pair sizes. The OL allele designation is to be handwritten on the full view electropherogram.

4.2.5 Tri-alleles

Occasionally, a single-source sample may be observed to have three alleles at one (rarely more) locus/loci. Tri-allelic patterns have been observed 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 potential tri-alleles may be re-amplified to confirm the genotype observed at the locus (or loci). Statistical calculations, however, will not be performed at these loci.

If the potential tri-allele containing sample is re-amplified and found to be reproducible, the analyst may include the locus with the tri-allele in the STR results table. If the potential tri-allele is not reproducible, it will be reported as an Artifact/True allele (A/TA).

If the same tri-allelic pattern is also observed in multiple samples (from the same case) but from different tissue sources – for example, blood / semen / saliva / hair, with the same STR profile, the potential tri-allele may be considered a reproducible observation and may be included on the STR results table without the A/TA designation. No re-amplification of samples would be required in such instances.

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4.3 Assessing interpretable alleles

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, and they are clearly distinguishable from artifacts. All alleles are examined and evaluated in the process of data interpretation.

In addition to the Analytical Threshold (AT) of 150 RFU, a Stochastic Threshold (ST) of 700 RFU is also used for casework analysis. When replicate amplifications are performed, only reproducible alleles above the AT will be relied up for casework interpretation. All allelic peaks greater than or equal to the ST are reportable when only a single amplification is performed.

4.3.1 Alleles below the Stochastic Threshold

Stochastic effects result when limited DNA molecules are used to initiate PCR, and unequal sampling of the two alleles present from a heterozygous individual leads to peak height imbalance, elevated stutter, and/or allelic dropout. The ST of 700 RFU is based on validation studies and represents the RFU value below which stochastic effects are commonly observed. Alleles which fall between the AT and the ST are subjected to a higher degree of scrutiny due to the possibility of stochastic effects. Alleles in this range are noted with an asterisk on the electropherogram.

4.3.2 Reproducing Alleles Below ST for Interpretation

In most instances, allelic peaks below the ST must be demonstrated to be reproducible by re-amplification in order to be included on the STR table and deemed suitable for use in comparisons and statistical calculations. Non-reproducible alleles are struck and initialed by the analyst. These are not included on the STR table.

Questioned samples with a quantification value of <0.05ng/µl and greater than zero, shall be routinely amplified in duplicate to ascertain reproducibility of alleles below the ST.

Samples with a quantitation result of zero do not require duplicate amplification.

Questioned samples with a quantification value >0.05ng/µl need not be amplified in duplicate. When a sample that was not initially amplified in duplicate yields a DNA mixture profile with the probative fraction being the minor or low-level component and/or contributor, re-amplification may be appropriate to assess allele reproducibility of the minor component and/or contributor.

Note: If a sample is amplified more than twice, the reproducible alleles in at least two amplifications shall be reported on the STR results table and considered suitable for comparison.

4.3.3 Using Alleles Below ST Without Demonstrating Reproducibility

Ideally, any STR alleles below the ST should be demonstrated to be reproducible in order to be reported in the STR table and used for statistic calculations. However, in a few specific instances (ex. Amelogenin peaks), alleles below the ST may be included on the STR table and/or used in statistical calculations without demonstrating reproducibility.

Non-probative / intimate samples: If all the alleles below ST can be attributed to a non-probative contributor (such as the owner on a body swab, the consent partner on a vaginal swab, etc.), then those alleles may be included in the STR table and used for interpretation. Since they are attributable to a non-probative source, statistical considerations do not apply.

Single-source profiles with heterozygous alleles below ST: In single source samples, the primary concern with using alleles below ST is the possibility of allelic dropout. An apparent homozygous allele below ST may in fact be a heterozygote with dropout of the sister allele. If both sister alleles of a heterozygote are present below the ST, dropout is not a possibility and therefore the alleles may be included in the STR table and used for statistical calculations.

Any other instances of using alleles below ST without demonstrating reproducibility, including compositing alleles from multiple amplifications, require documented approval by the Technical Manager.

4.4 Assessing for Genotypes

Genetic profiles from questioned samples may be from a single individual (single source) or from multiple individuals (mixtures). Assessment of whether a profile is a single source or mixture and whether a mixture can be separated out into single source components impacts subsequent interpretation. Documentation of interpretation, including assumptions, must be included in the bench notes. This documentation may be written on the electropherogram or on a Mixture Interpretation Worksheet.

Assessment of a questioned profile includes the determination of which loci are suitable for comparison and/or for the calculation of population statistics. Loci considered to be complete – that is, with no indication of possible drop-out, either for the entire locus or just for a resolved component of a mixture – are suitable for exclusions as well as for population statistics. Loci where possible drop-out is evident may either be suitable for

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exclusions only or not suitable for comparison, depending on the number of sources and the extent of the possible drop-out.

The assessment of a questioned sample profile takes place with 'sequential unmasking': initial assessment occurs before any probative reference samples are compared to that profile. However, an evidence item taken directly from an identified anatomical location (i.e., vaginal swab, penile swab, fingernail scrapings, etc.) and / or a piece of intimate apparel (i.e., undershirts, panties, bra, etc.) typically will yield DNA from the individual from which the evidence item was taken. In such circumstances, it is common to recover a DNA mixture which includes DNA consistent with that individual. In such cases it is useful to use the "owner's" (or any other non-probative) reference profile to assist in the interpretation of the questioned sample.

4.4.1 Single Source Samples

Typically, each locus is characterized by one or two labeled peaks or alleles. If two peaks/alleles (heterozygous locus) are detected at a locus, they should ideally be of equal intensity – that is, the peak height ratio (PHR) is approximately 1:1.

Peak height ratios 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. Based on validation studies, the minimum expected PHR for single-source samples, where there is no indication of a mixture and 0.5 to 1 ng of template DNA is used, is 60%. This ratio, however, may be lower with lower amounts of DNA. Peak-height-ratio imbalance may also result from degraded DNA or the presence of PCR inhibitors. Allelic dropout may occur in degraded/inhibited samples and the possibility of a second allele should be considered.

A single source profile must have complete information for at least 4 of the 13 core loci to be deemed suitable for all comparisons. This applies to profiles with a single source of DNA as well as to deduced contributors and major or minor components from mixtures. A profile with complete information at three or fewer loci may be suitable for exclusions only.

4.4.2 DNA Mixtures

Indications of a DNA mixture sample can include:
(1) more than two alleles at two or more loci,
(2) a peak in a stutter position that is unusually high
(3) significant peak height ratio imbalance for a heterozygous genotype
(4) Observation of low-level DNA/data below reporting threshold

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In general, a profile with more than two alleles at two or more loci is deemed to be a mixture. The minimum number of contributors may be assessed by evaluating the loci that exhibit the most allelic peaks (i.e., if at most five alleles are detected at a locus, the DNA results are consistent with having arisen from at least three individuals, provided that none of those peaks are consistent with being elevated stutter). However, phenomena such as tri-alleles, primer binding site mutations, or allele sharing between close relatives can complicate the determination of number of contributors.

4.4.2.1 DNA Mixture Type Categorization

It is not possible to anticipate the nature of all potential DNA results, or the nature of the evidentiary samples from which they may be obtained. These categories do not exhaust the possibilities that may be encountered by the forensic scientist, nor the conclusions that may be rendered from his/her interpretation of the results.

Three categories of mixed samples have been designated to guide general decision making as illustrated in the Interpretation Flowchart. Mixtures with a known contributor (i.e. intimate samples) are possible in each category:

- **TYPE A**: mixtures with indistinguishable contributors, including multiple major contributors
- **TYPE B**: mixtures with major/minor contributors
- **TYPE C**: indistinguishable mixtures containing DNA from at least three sources and/or mixtures that exhibit potential stochastic effects

TYPE A:

Description: If the amounts of biological material from multiple contributors are similar, it may not be possible to further refine the mixture profile. When major or minor contributors cannot be distinguished because of similarity in signal intensities (i.e. peak heights), the sample is considered to be an indistinguishable mixture. In two source mixtures where the "owner" can be assumed to be one contributor, the "owner" reference sample may be used to deduce a single source unknown contributor.

The classification of indistinguishable does not imply that the profile is not interpretable. Individuals may still be included or excluded as possible contributors to an indistinguishable mixture. However, a minimum of 4 complete loci (i.e. loci suitable for calculating population statistics) are required in order for an unresolved mixture to be suitable for comparisons.

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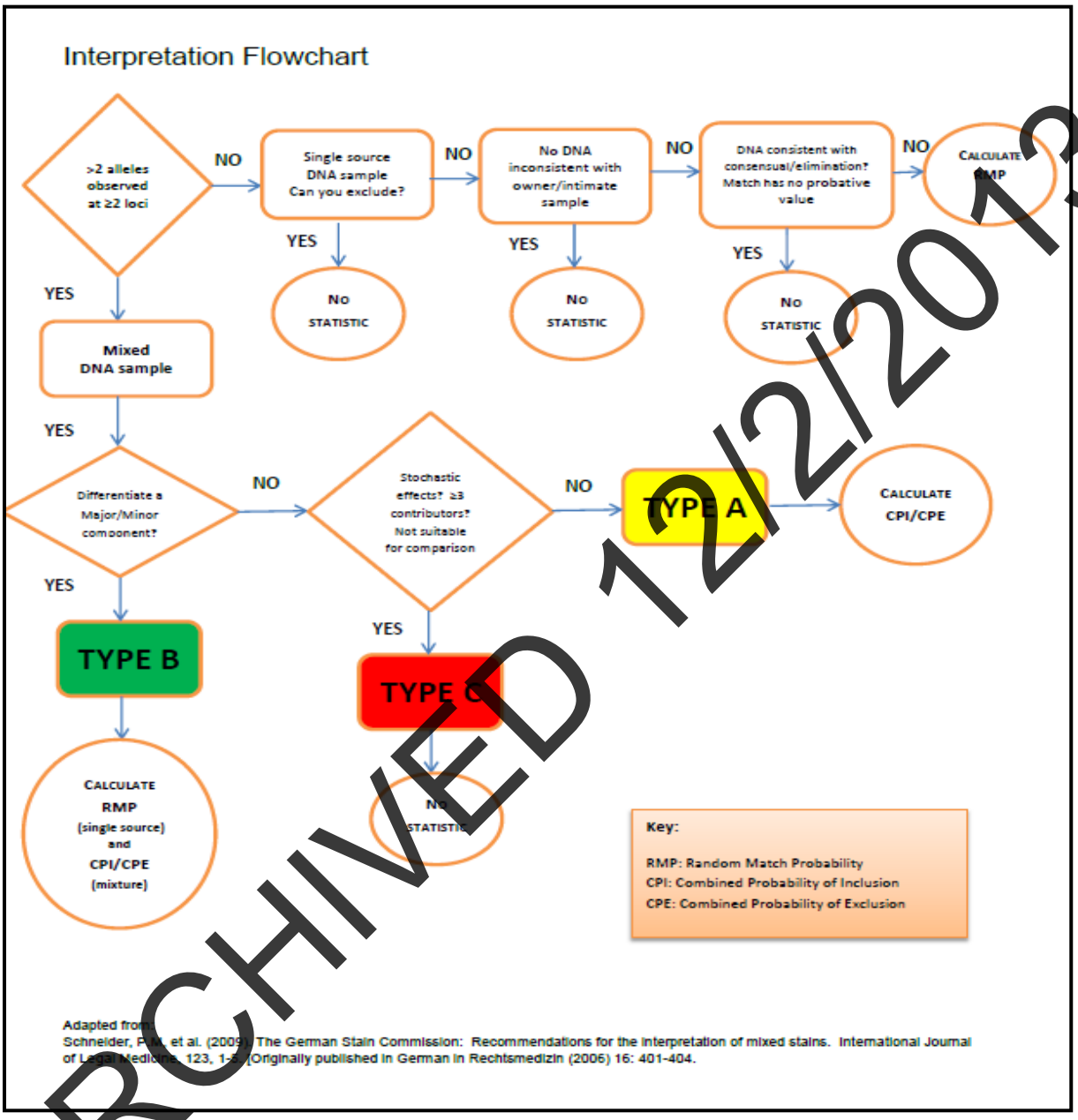

Adapted from:
Schneider, P.M. et al. (2009) The German Stain Commission: Recommendations for the interpretation of mixed stains. International Journal of Legal Medicine 123, 1-8. [Originally published in German in Rechtsmedizin (2006) 16: 401-404.

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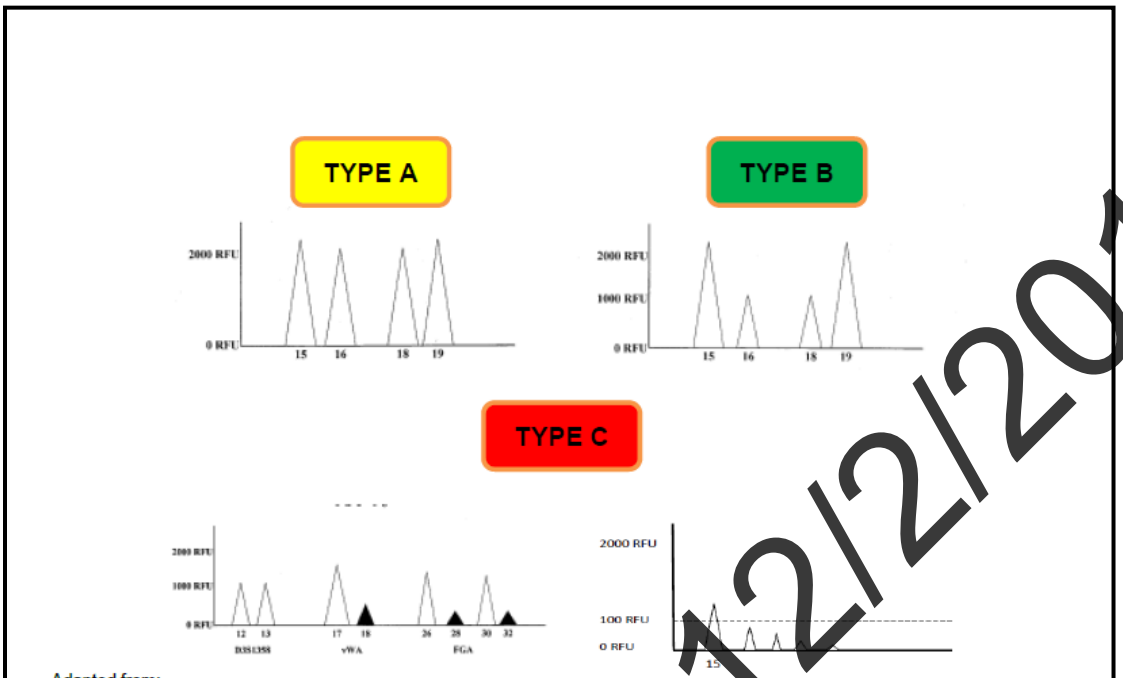

Adapted from:
Budowle, B., et al. (2009). Mixture Interpretation: Defining the Relevant Features for Guidelines for the Assessment of Mixed DNA Profiles in Forensic Casework. *J Forensic Sci, Vol. 54, No. 4, 810-821*

TYPE B:
Description: If the amounts of biological material from multiple contributors are dissimilar, it may be possible to further refine the mixture profile. When major or minor contributors can be distinguished because of differences in signal intensities (i.e. peak heights), the sample is considered to be a distinguishable mixture. The difference is evaluated on a case-by-case basis.

If the sample demonstrates a clear reproducible major component at a minimum of 4 of 13 core STR loci a conclusion as to the source of the major contributor DNA may be reported and will be included in statistical analysis. This may also encompass mixture profiles that exhibit more than one major contributor.

TYPE C:
Description: In general, type C mixtures contain DNA from at least three (3) individuals and/or exhibit stochastic effects.

If no clear major component is observed this type of genetic profile may be deemed unsuitable for comparison. As such, this profile will not be included on the STR results table and no statistical analysis will be performed.

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A sample may be deemed not suitable for comparison if the majority of alleles in a sample are not reproducible and/or insufficient DNA is detected (i.e. partial profile). Additionally, if a mixture contains DNA from known close relatives a comparison may not be appropriate.

4.4.2.2 Separating Major and Minor Contributors in DNA Mixtures

Separating a two-source mixture into major and minor components is easier when the number of loci exhibiting four alleles increases and the ratio of the major contributor to the minor contributor increases above a 5:1 ratio. Since peak height ratios as low as 60% have been observed in single source samples with ideal concentrations of DNA, and even lower peak height ratios have been observed with lower concentrations of DNA, peak height ratio information should be used very conservatively in separation of major and minor components. Quantitative assessment of peak height ratios is not appropriate with the current platform; and qualitative information (e.g. assessment of which peaks at a given locus are consistently higher or lower) should be used very conservatively. Duplicate amplifications are recommended in order to allow an analyst to assess reproducibility of peak height ratios, as well as reproducibility of alleles.

When applicable, an “owner” reference may also be used to assist in separating the major and minor components.

If a mixture can be successfully separated into major and minor components, the minor component alleles are reported within parentheses. Due to the possibility that the minor contributor’s alleles may be shared by the major contributor and that such alleles may be below reporting threshold, determination of the minor contributor profile may be possible at only some loci.

An analyst may partially dissect a DNA mixture profile into major and minor components and report the loci on the STR results table in brackets (e.g. [10,11,12]) where an unambiguous major or minor component is not distinguishable. Peak height ratios may be noted on the electropherogram or mixture interpretation worksheet to document possible genotypes, if appropriate.

When evaluating if a minor component is suitable for comparison the number of potential contributors and whether the peak falls into stutter position should be considered. A minor component is suitable for comparison if a complete minor profile is determined for at least 4 of 13 core loci. This is rarely possible when there is more than one contributor in the minor component. Minor component alleles often are comparable in peak height to stutter. Because of the possibility that a potential minor allele may be masked by being in a stutter position to a major allele, a minor component whose alleles are close in height to stutter peaks (i.e. about 14% of the heights of the major

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component alleles) can only be considered complete when heterozygote minor alleles are detected outside of stutter positions.

The assessment of the major/minor components must be documented on either the electropherogram or a Mixture Interpretation Worksheet and included in the bench notes. Documentation includes a statement of any assumptions made, such as number of contributors, as well as a brief explanation of the rationale used to separate major and minor, such as subtracting an owner's alleles or peak height ratio information.

4.4.2.3 Deducing an Unknown Contributor in a DNA Mixture

In instances of samples collected directly from an individual's body or clothing, where DNA from no more than two individuals appears to be detected, it may be possible to use the "owner's" profile to deduce the genetic profile for an unknown contributor. It may not be possible to deduce an unknown contributor at all loci; those loci which cannot be deduced must be labeled as such. The unknown contributor profile must be deduced at a minimum of four loci in order to be deemed suitable for comparison. A deduced profile (or partial profile) is treated as a single source profile for the purposes of drawing conclusions and issuing statistics. When fewer than 9 loci can be deduced for a mixture, both a mixture statistic for the profile as a whole (including all complete loci), and a single source statistic for the deduced contributor should be issued.

The rationale for deduction of the genetic profile for an unknown contributor must be documented in the bench notes, either on the electropherogram or on a mixture interpretation worksheet.

4.5 Comparison of DNA Results

The interpretation process generally results in one or more of the following conclusions:

- Exclusion
- Cannot exclude
- Inconclusive
- Insufficient DNA / Data
- No genetic profile
- Complex mixture

Note: Comparisons and conclusions for Forensic Parentage cases are addressed specifically in the Forensic Parentage Manual.

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Exclusion:
An exclusion is declared when the known sample has alleles that are not observed in the questioned sample, and there is no scientific explanation for the absence.

Scientific explanations may include, but are not limited to, factors such as inhibition, degradation, low template DNA and/or primer binding site variations or mutations.

Cannot Exclude:
For single source samples (including resolved mixtures): The donor of the known sample cannot be excluded as a source of the questioned sample when there are no significant differences between the allele designations obtained from these samples.

For unresolved mixtures: When alleles detected in a known/reference sample are also detected in at least 75% of the detected core STR loci in a DNA mixture, and when there is a scientific explanation for any inconsistencies, an individual may be considered a potential contributor to the sample.

If alleles that could not have been contributed by the individuals for whom known/ reference sample profiles were submitted for comparison with the questioned samples, the conclusions for this sample may include a statement indicating that DNA that could not have been contributed by X/Y/Z was detected in the sample.

Inconclusive:
If the STR results support neither inclusion nor exclusion, the comparison will be deemed inconclusive and reported as such. When a profile is deemed inconclusive, an explanation must be included for the reason.

Insufficient DNA/Data:
Low amounts of DNA can result in allelic drop out, elevated stutter, and non-reproducibility of results. In many instances, incomplete loci cannot be appropriately used to make exclusions or inclusions. When fewer than four loci can be considered complete, this profile will be considered insufficient and will not be utilized for comparison. As such, this data will not be included on the STR results table.

If a genetic profile obtained from a sample does not satisfy the laboratory's inclusionary reporting criteria it is possible it may be utilized for exclusionary purposes only. The Technical Manager will be consulted to determine the appropriate course of action.

No Genetic Profile:
No genetic profile is declared when there is no reproducible data observed. This also may include low-level data observed at less than 4 of 13 core STR loci.

Complex Mixture:
A complex mixture may be declared when DNA from multiple sources is detected in a sample, there is evidence of stochastic effects (i.e. drop out) at several or all loci (from limited DNA), a clear major component cannot be deduced, and/or a majority of the alleles are not reproducible when amplified in duplicate. Accordingly, the sample will be deemed not suitable for comparison. This is typically the case for mixtures with four or more contributors, but may also be true for mixtures with three contributors.

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Section 5 Statistical Analysis of STR Data

References: Budowle, B. et al., Population data on the thirteen CODIS Core Short Tandem Repeat Loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians. (1999) Journal of Forensic Science, 44(6): 1277-1286.
Budowle, B. et al., Population studies on three Native Alaska population groups using STR loci. (2002) Forensic Science International 129: 51-57.

Statistical analyses will be performed when the genetic typing results fail to exclude an individual(s) as a source of the DNA in a questioned sample and the result is probative. A statistical analysis may not be necessary when an individual is identified as a possible source of DNA in a sample that is intimate to that individual or the result is not germane to the case (i.e. DNA consistent with consent partner detected).

Statistical analyses are calculated with the U.S. Department of Justice, FBI Popstats program, using either the Random Match Probability Formula for single source samples or the Mixture Formula for mixed samples. The resultant values may be truncated for reporting, but should never be rounded up (i.e. 34,675,000 may be reported as 34 million or 34.6 million, but not 34.7 million).

The Alaska Scientific Crime Detection Laboratory routinely reports the frequency/probability for the following populations: Caucasian, African-American, Athabaskan, Inupiat and Yupik.

5.1 Random Match Probability Formula

To calculate the frequency of a locus genotype, the following formulae are used:

Heterozygotes	$2pq$
Homozygotes	$p^2 + p(1-p)\theta$

The p and q represent the frequencies of two different alleles. θ (theta) is an empirical measure of population subdivision/substructure or “relatedness”.

For the Caucasian and African-American populations, θ=0.01
For the Alaskan Native populations, θ=0.03

The multi-locus genotype frequency is estimated by multiplying together the genotype frequencies from the different loci. The expected frequency of a DNA profile in a population is the inverse of the multiple locus genotype frequency.

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5.2 Combined Probability of Inclusion (CPI; Mixture Formula)

To calculate the probability of a random individual in the population being a contributor to a mixture for a locus, the following formula is used:

$$(p_1 + p_2 + \ldots p_n)^2 = P_{LOCUS}$$

The p_1, p_2, and p_n are the frequencies of occurrence of the alleles at the locus.

The combined mixture profile probability is calculated by taking the product of the individual locus probabilities. To estimate the number of individuals in a population that could have contributed to the mixture, the inverse of the combined mixture profile frequency is calculated.

5.3 Calculating Frequencies/Probabilities Using Popstats

- Open Popstats and double click to select either Forensic Single Sample or Forensic Mixture from the menu.
- In the Specimen ID field, enter the lab #_Item#
- In the Comments field, add any additional info (i.e. sperm fraction, major profile)
- Enter the alleles deemed appropriate for statistical analysis (see the section on Data Interpretation Guidelines in the Forensic Biology Procedures Manual).

Note: It is not necessary to enter alleles for Amelogenin or the Penta loci as population data for these loci are not contained in the database.

- Click the Calculate button or select Calculate from the Popstats pull-down menu.
- Click the 1/f button.
- Click the printer icon or select Print from the File pull-down menu. Select Broward Report and print.

Note: The above steps must be performed for both the Alaska Native database and the FBI STR database.

- To select a database, choose Options from the Tools pull-down menu.
- Click on Population Database and then Browse to navigate to the desired database.
- Select either the Alaska or STR folder. Click OK, Apply and OK.

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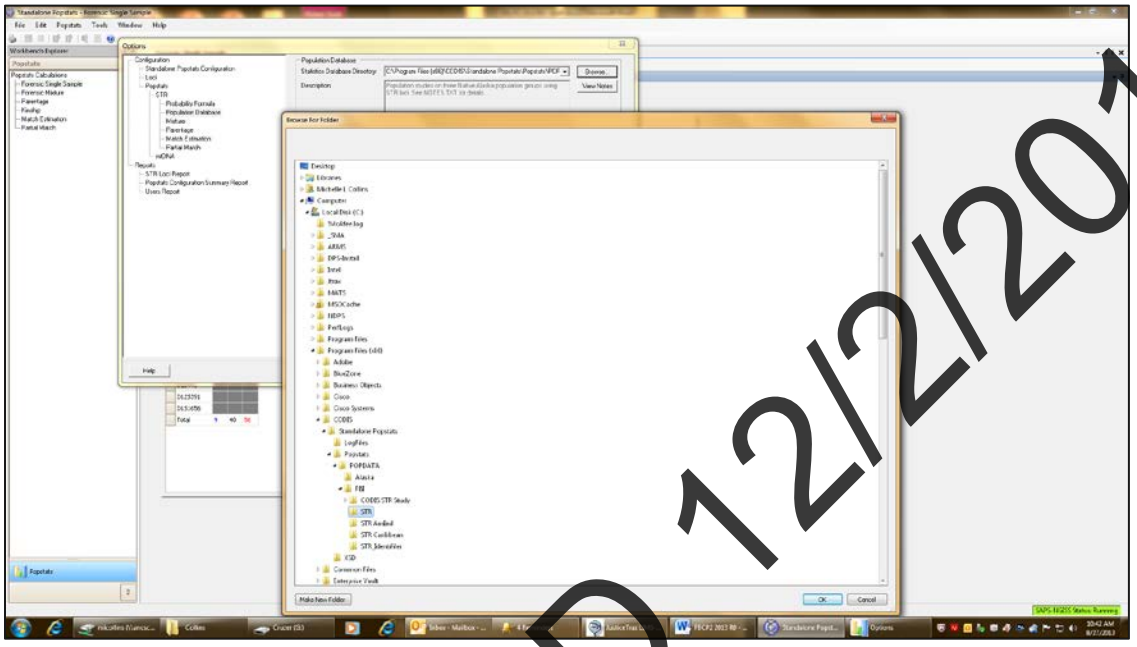
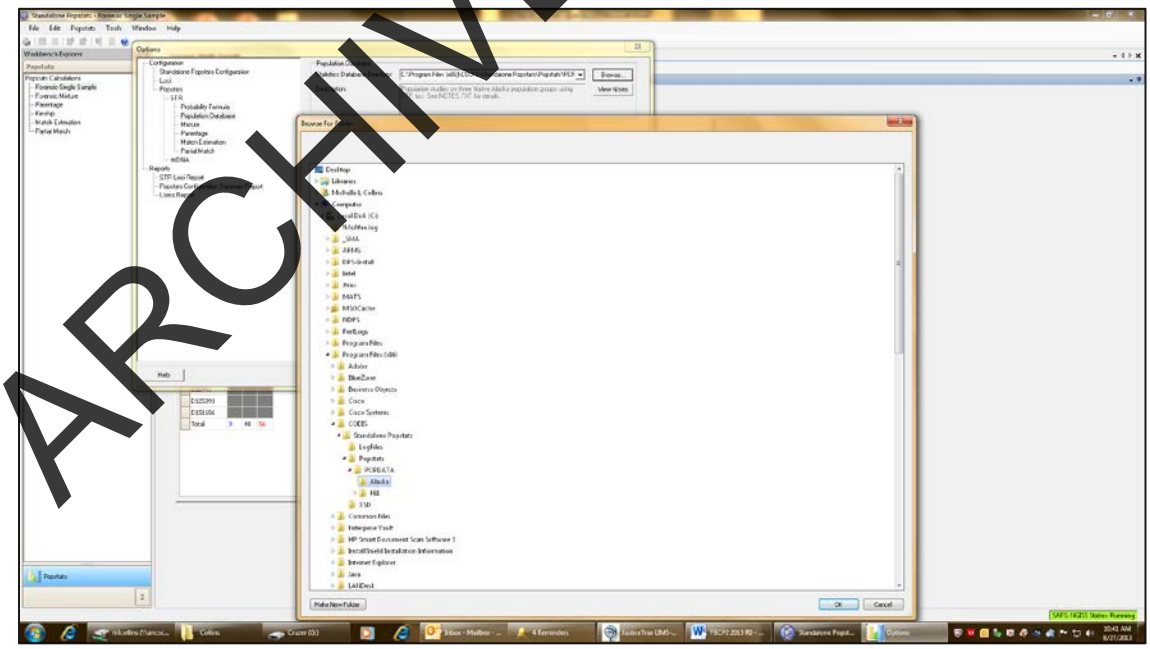
Selecting the Caucasian and African-American Population Databases



Selecting the Alaska Native Population Databases



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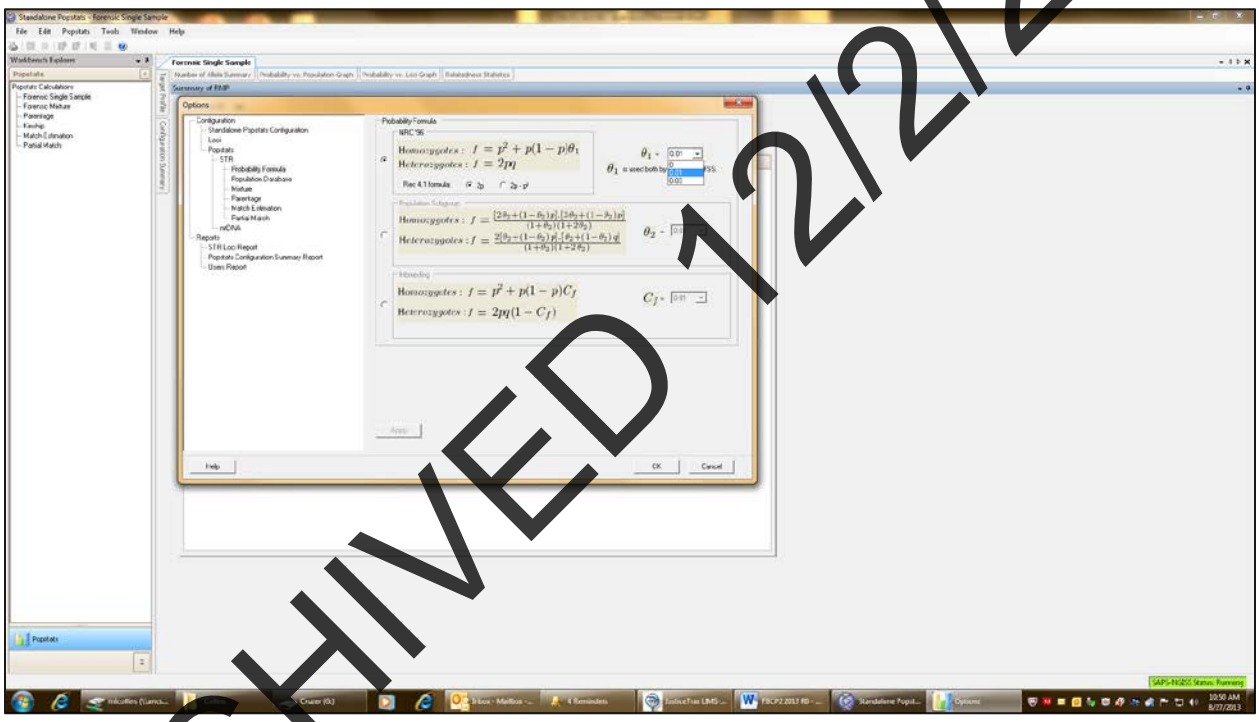
Note: The theta correction factor (0.01 for the STR database and 0.03 for the Alaska database) must be specifically selected when toggling between databases.

- To select a theta value, choose Options from the Tools pull-down menu.
- Click on Probability Formula and select the correct theta value from the pull down menu shown in the image below.
- Click Apply and OK.



- Once the theta value is changed, it is necessary to close out the calculation window for the change to take effect. This is done by clicking the black X button near the top right of the window. Clicking the red X in the top right will close out the Popstats program entirely.
- Select either Forensic Single Sample or Forensic Mixture from the menu to repeat the process for the second population database.

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Section 6 DNA Report Writing and Review

Proficiency test reports will be written in a manner similar to casework reports. Proficiency tests for DNA reports will not require the STR results table used for casework reports. Instead, the completed STR table from the test provider's form will be included as the first page in the bench notes.

6.1 Report content

All DNA discipline reports will contain the following:

- Date report was issued (automatically populates)
- Laboratory and agency case numbers (automatically populates)
- Name of submitting agency and case officer (automatically populates)
- List of all items analyzed
    - include the agency item # in ( ) if the item was re-numbered at the laboratory
    - this is not necessary if the laboratory number contains the original agency item #
- When DNA analysis is reported for samples retained by another discipline in the laboratory, or comparisons are made to profiles reported by another laboratory, the report should reference the previously issued report
    - i.e. "Reference **biological screening** report dated **Month Day, Year** by **Analyst**."
    - include the issuing laboratory, if other than the AK SCDL.
- Results of presumptive/confirmatory tests, if not previously reported in a biological screening report
- Results, conclusions and opinions, for all tested items, based on the DNA typing results
- Results of any statistical analyses performed
- Explanation of why testing was stopped, if appropriate
- Description of analyses performed, including a list of STR loci amplified (this is contained in the Methods section of the report
- Description of statistical analyses performed including the methods and loci used in the calculations (this is contained in the Methods section of the report)
- The disposition of the evidence (this is contained in the Methods section of the report)
- Signatures of the reporting analyst and the technical reviewer (the analyst will electronically sign the report by setting the milestone to Draft Complete prior to submitting for technical review; only the analyst is required to scan their barcode and enter a pin when signing the report in LIMS)

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6.2 Report Wording

The following tables contain samples of results, conclusion and opinions appropriate for reporting various case results. These are not all inclusive and may be modified slightly on a case by case basis.

For single source samples	Report
Single Source: exclusion	XXX was excluded as the source of DNA detected in this sample.
Single Source: fail to exclude	XXX cannot be excluded as the source of DNA detected in this sample.
Same profile (for 2+ items)	The genetic profiles obtained from these samples were the same.
Single source statistic	**Single Source formula** The estimated frequency (13 core loci) of the genetic profile from the above sample(s) is approximately 1 in XXX quadrillion (Caucasian population), 1 in XXX quadrillion (African-American population), 1 in XXX quadrillion (Athabaskan population), 1 in XXX quadrillion (Inupiat population) and 1 in XXX quadrillion (Yupik population).

For single source and/or mixtures	Report
Unknown Male DNA	A genetic profile from an unknown male individual was obtained from this sample.
Female present	The genetic profile obtained from this sample was consistent with being from an unknown female individual.
Male DNA present	DNA from (a OR at least one) male individual was observed in this sample.
No DNA inconsistent	No DNA inconsistent with XXX was detected in this sample.
DNA consistent	DNA consistent with XXX was detected in this sample
When cannot exclude but don't have all 13 core loci	DNA consistent with XXX was detected at XX of 13 core loci in this sample. Therefore, XXX cannot be excluded as a source of DNA detected in this sample. (change 13 if not all loci yielded data above reporting threshold)

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For mixtures	Report
More than 1 individual	DNA from more than one individual was observed in this sample.
To indicate # of individuals	DNA from at least XX individuals was observed in this sample. OR DNA consistent with being from two individuals was observed in this sample.
Mixture: exclusion	XXX was excluded as a source of DNA detected in this sample.
Mixture: fail to exclude	XXX cannot be excluded as a source of DNA detected in this sample.
Foreign DNA present	DNA inconsistent with XXX was also detected in this sample.
Complex Mixture	Due to the complexity of the genetic profile obtained from this sample, no meaningful comparisons can be made to known reference samples.
Mixture statistic	Alleles below the reporting threshold and/or non-reproducible alleles may be present at the following loci: XXX. Therefore, population statistics are not reported for these loci.
Mixture statistic	**Mixture formula** The estimated probability (13 core loci) of an individual from each of the following population groups contributing to the DNA detected in the above sample(s) is as follows: approximately 1 in XXX quadrillion (Caucasian population), 1 in XXX quadrillion (African-American population), 1 in XXX quadrillion (Athabaskan population), 1 in XXX quadrillion (Inupiat population) and 1 in XXX quadrillion (Yupik population).

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Deduced Profile with Single Source statistics	DNA from more than one individual was observed in this sample. A single source genetic profile for the unknown (male) contributor was deduced from the DNA mixture obtained from this sample. XXX cannot be excluded as the source of the deduced genetic profile. No DNA inconsistent with XXX and YYY was detected in this sample. A single source genetic profile for the unknown contributor could not be deduced at the following loci: XXX, XXX and XXX. Therefore, these loci were not used to calculate population frequency statistics. **Single Source Formula** …….
Deduced Profile with Mixture and Single Source statistics	DNA from more than one individual was observed in this sample. XXX and YYY cannot be excluded as sources of DNA detected in this sample. Alleles below the reporting threshold and/or non-reproducible alleles may be present at the following loci: XXX. Therefore, population statistics are not reported for these loci. **Mixture formula** …… A single source genetic profile for the unknown (male) contributor was deduced from the DNA mixture obtained from this sample. XXX cannot be excluded as the source of the deduced genetic profile. A single source genetic profile for the unknown contributor could not be deduced at the following loci: XXX, XXX and XXX. Therefore, these loci were not used to calculate population frequency statistics. **Single Source Formula** …….

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Not Suitable for comparison	Report
Not suitable	The data obtained from this sample was not suitable for comparison (due to XXXXXX).
No profile	No genetic profile was obtained from this sample.
Inconclusive	No conclusions can be made as to whether XXX contributed DNA to this sample due to insufficient reportable DNA.
Insufficient	No (other) conclusions are reported for this sample due to insufficient reportable DNA.

6.3 Review of Casework Analyzed In-House

All case reports issued by the Forensic Biology discipline, and all supporting documentation that is part of the case record or the central log, will be subjected to a technical review and an administrative review.

Technical review of DNA casework will be conducted by a second qualified analyst, in accordance with the FBI QAS Guidelines (current version) and includes the following, at a minimum, to ensure compliance with the interpretation guidelines contained within this document:

- Review of all worksheets contained within the bench notes and central log
- Review of the ILS for all passing samples
- Review of all allelic ladders designated as such
- Review of all amplification positive controls
- Review of all reagent blanks and negative amplification controls
- Review of DNA typing data (used to draw conclusions) for questioned and known samples
- Verification that all results/conclusions in report are supported by data
- Review of all statistical analyses
- Verification of CODIS eligibility and review of entry for all entered profiles
    - Eligibility review must occur prior to entry

Administrative review of DNA casework will be conducted by a second qualified analyst, in accordance with the FBI QAS Guidelines (current version) and includes the following, at a minimum, to ensure compliance with the interpretation guidelines contained within this document:

- Review of case file and final report
- Review of chain of custody and evidence disposition

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The analyst and the reviewer may consult a third qualified 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 discipline checklist is used to document completion of the individual components of the technical and administrative reviews. The completion of the final laboratory report, technical review and administrative review are tracked electronically in LIMS.
-
When a case has been through technical and administrative review, the report will be issued in accordance with laboratory policies and procedures. Genetic profiles suitable for CODIS will be uploaded in accordance with CODIS policies and procedures.

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DNA STR Results Table

Laboratory Case # **Analyst:**
Batch:

Item #					
Description/ Locus					
D3S1358					
TH01					
D21S11					
D18S51					
Penta E [NS]					
D5S818					
D13S317					
D7S820					
D16S539					
CSF1PO					
Penta D [NS]					
Amelogenin					
vWA					
D8S1179					
TPOX					
FGA					

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Notes:

1. The Amelogenin locus indicates gender: X = Female; XY = Male
2. “NS” indicates data not used in statistical analysis
3. “NR” indicates data not reported
4. “ [DBRT] ” indicates data below reporting threshold
5. ( ) indicates minor component alleles
6. [ ] indicates Major/Minor components not separated
7. "A/TA" indicates artifact (e.g. stutter) or true allele
8. Genetic data unsuitable for comparison may not be included in the table

STR Casework Review Checklist

Date:_____
Technical Review Started: _____
Administrative Review Started: _____
Lab Number: _____

DNA CASEWORK REPORT AND BENCH NOTES

	Analyst:	Tech. Review:	Admin. Review:
Pages are numbered correctly, case # and analyst initials are on each page, report dated			
Requesting agency, agency case #, crime lab case #, and officer's name are correct			
Chain of Custody for all tested items can be tracked through RLS and LIMS			
Item numbers and descriptions on report / DNA worksheet are consistent with RLS/LIMS			
Packaging / extraction procedure used for all items is documented on worksheets			
Q-PCR results documented on DNA worksheet			
Screening results on DNA worksheets are accounted for in the report, if appropriate			
Disposition of sample extracts/slides documented; item created in LIMS (if appropriate)			
Technical Reviewer checked DNA worksheet: initialed and dated			
Technical review of all electropherograms: initialed and dated			
PP16 printouts for all questioned and reference samples are present in bench notes			
Peak patterns on the electropherograms are consistent with the STR results table (if applicable)			
The correct DNA profile and descriptive information is entered into **Popstats**			
Popstats printouts are included in the case file			
Popstats DNA profile frequencies are consistent with those shown on the report			

Check Grammar/Spelling in DNA report			
Results/conclusions/opinions are given for each tested item			
Conclusions/opinions drawn from results comply with laboratory guidelines			
Initials and date of technical review(er) are on each page of the bench notes			
Verify content of Methods and Disposition sections of report			
Stats are recorded in LIMS			
SOPs are linked to request in LIMS			
Disposition of evidence in LIMS is appropriate (HOLD or not)			

CENTRAL LOG FOLDER LOCATION:

Central Log Folder location documented in LIMS			
Q-PCR plate set-up is documented			
Q-PCR standard curve Mx3000P printout: Results are acceptable.			
Q-PCR Initial Template Quantity is documented by Mx3000P printout.			
Amplification volumes are documented.			
ICS checked by Technical Reviewer			
Technical Reviewer checked Controls and Standards sheet: initialed and dated.			
Disposition of blank extracts documented; item created in LIMS (if appropriate)			
PP16 printouts for reagent blanks and negative control(s): Results are acceptable.			
PP16 printouts for positive control(s): correct DNA profile obtained.			
PP16 printouts for all appropriate Allelic Ladders.			
Worksheets contain all lot #s and expiration dates for all reagents used.			

List of eligible profiles to be entered into CODIS (include specimen category and SDIS or NDIS)			
Specimen eligibility verified prior to CODIS entry (including match estimation, if applicable)			
Correct profile(s) and agency information entered in CODIS			
Appropriate specimen category selected			
Source ID updated if applicable			
Offender/arrestee laboratory comments updated in LIMS, if applicable			

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STR Review Checklist for Vendor Lab

Date:_____

SCDL Lab Number: _____

Vendor Laboratory: _____

Vendor Lab Number: _____

Agency: _____

Agency #: _____

Technology:_____

Platform: _____

Amplification kit: _____

	Tech Review:	CODIS Review:
PP16 printouts for all appropriate Allelic Ladders: obtained expected ILS and allelic		
PP16 printouts for positive control: obtained expected ILS peaks and DNA profile		
PP16 printouts for negative control: results are acceptable (including ILS)		
PP16 printouts for reagent blanks: results are acceptable (including ILS)		
Screening results on DNA worksheets are accounted for in the report, if appropriate		
Raw or analyzed data provided for each tested item		
Technical review of all electropherograms (including ILS); initialed and dated by		
Peak patterns on the electropherograms are consistent with the table (if applicable)		
Results/conclusions/opinions are given for each tested item		
Conclusions/opinions drawn from results are supported by the genetic data		
Initials and date of technical review(er) are on each page		
List of eligible profiles to be entered into CODIS (include specimen category and SDIS or NDIS)		
Specimen eligibility verified prior to CODIS entry (including match estimation, if applicable)		
Correct profile(s) and agency information entered in CODIS		
Appropriate specimen category selected		
Source ID updated if applicable		

NOTES:

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STR Single Offender Review Checklist

Date:_____
Technical Review Started: _____
Administrative Review Started: _____
Lab Number: _____

OFFENDER FOR DNA DATABASE OR CODIS CONFIRMATION

	Analyst:	Tech. Reviewer:
Offender number(s) and analyst initials are on each page		
Offender number(s) and name(s) on DNA worksheet are consistent with LIMS		
Packaging / extraction procedure is documented on DNA worksheet		
Q-PCR results documented on DNA worksheet		
Disposition of offender extract(s) documented		
Technical Reviewer checked DNA worksheet: initialed and dated		
Technical review of all electropherograms: initialed and dated		

Results (i.e. profile verified) given for each tested item		
Initials and date of technical review(er) are on each page of the bench notes		
SOPs are linked to request in LIMS		

CENTRAL LOG FOLDER LOCATION:

Central Log Folder location documented in LIMS		
Q-PCR plate set-up is documented		
Q-PCR standard curve Mx3000P printout: Results are acceptable.		
Q-PCR Initial Template Quantity is documented by Mx3000P printout.		
Amplification volumes are documented.		
ICS checked by Technical Reviewer		
Technical Reviewer checked Controls and Standards sheet: initialed and dated.		
Disposition of blank extracts documented: item created in LIMS (if appropriate)		
PP16 printouts for reagent blanks and negative control(s): Results are acceptable.		
PP16 printouts for positive control(s): correct DNA profile obtained.		
PP16 printouts for all appropriate Allelic Ladders.		
Worksheets contain all lot #s and expiration dates for all reagents used.		

List of eligible profiles to be entered into CODIS (include specimen category and SDIS or NDIS)		
Specimen eligibility verified prior to CODIS entry (including match estimation, if applicable)		
Correct profile(s) and agency information entered in CODIS		
Appropriate specimen category selected		
Source ID updated if applicable		
Offender/arrestee laboratory comments updated in LIMS, if applicable		

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Appendix A Revision History

Sections of this manual were taken from the current versions of FBWI2012 R0, FBP2012 R0 and 3500 CW 2013 R0. The revision history contains the substantive changes to the sections contained within this document.

FBCP2 2013 R0 Page	Original Manual Page	Location	Revision made
n/a	n/a	Entire manual	Updated references to other discipline manuals. Added missing references to instrument/equipment user manuals. Updated formatting.
1	1	Document Structure	Updated as required.
2-4	FBWI2012 R0, page 38-40	Section 1.2	Removed redundant and non-procedural language from entire section
3	FBWI2012 R0, page 39	Section 1.2	Updated target DNA range for casework samples to 0.5ng – 1ng.
4	FBWI2012 R0, page 39	Section 1.2	Updated preparation instructions for Positive Control Sample.
4	FBWI2012 R0, page 40	Section 1.2	Removed reference to silicon spacer (originally in step 10)
6	3500CW 2013 R0, pages 3-4	Sections 2.4.1 and 2.4.2	Updated example of plate record naming convention
13	FBP2012 R0, page 63	Section 4	Removed the following abbreviations from the table as they are not used or are defined elsewhere: SH, SPK, LV, RBQ, RBE, RBS, RBK, CIDI.
13-33	3500CW 2013 R0, pages 11-32	Section 4	Clarified documentation requirements throughout sections
27	FBP2012 R0, page 57	Section 4.4.2.1	Removed examples in ( ) from Type C description.
29	3500CW 2013 R0, page 28	Section 4.4.2.1	Moved paragraph beginning “If the sample demonstrates….” from TYPE C to TYPE B.
31	n/a	Section 4.5	Added reference to Forensic Parentage Manual

34	FBP2012 R0, page 77	Section 5	Added reference to Budowle paper with Caucasian and African-American population data.
35-37	FBWI2012 R0, pages 53-55	Section 5.3	Updated instructions based on change to Standalone Popstats to perform statistical analysis
n/a	FBP2012 R0, pages 78-84	Section 5	Removed allele frequency tables
39-42	FBP2012 R0, pages 74-76	Section 6.2	Updated reporting guidelines based on Data Interpretation changes with 3500CW 2013 R0. Paternity is now addressed in a separate manual.
42-43	FBP2012 R0, page 85	Section 6.1	Merged previous sections 9.1 and 9.1.1. Added significant detail with regards to the scope of review.
44	FBP2012 R0, page 88	Forms	Updated Note 4 at bottom of table
45-47	FBP2012 R0, pages 90,91,94	Forms	Added a line to document technical reviewer verification of CODIS eligibility and modified to allow for documentation of appropriate CODIS level for upload.

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