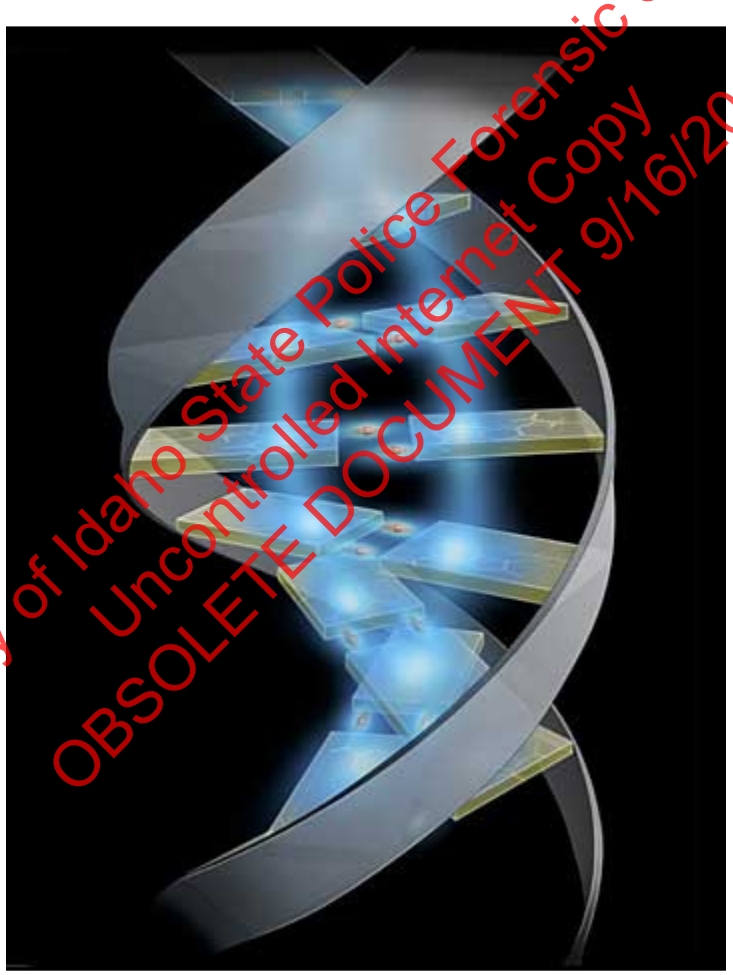
ISP FORENSIC BIOLOGY

DATABASE ANALYTICAL METHODS MANUAL



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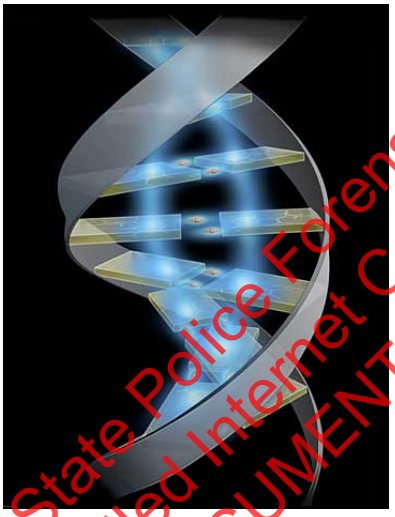
Forensic Biology Database Analytical Methods Manual

Revision #15



APPROVED
September 10, 2014

Cynthia Hall
DNA Technical Leader

Forensic Biology Database Analytical Methods Manual

REVISION RECORD

The following table must be filled out when revisions to the Biology Quality/Procedure Manual are made.

Date: The effective date of the revision(s).
Revision #: The manual revision number.
Description: A brief description of the changes made to the manual.
Addition: This column is checked if the revision reflects an addition (e.g. new SOP or form) to the manual.
Deletion: This column is checked if the revision reflects a deletion (e.g. SOP or form no longer in use) from the manual.
Initials: Initials of the Technical Leader making the revisions.

Date	Revision #	Description	Addition	Deletion	Initials
8/10/09	9 (Rev. 1-8 part of original single document. See Quality Manual, Appendix E)	Separated quality/casework methods/database methods into three separate manuals, added BSD/DNA IQ/Biomek 3000/PP16HS , and Driftcon procedures and forms, fixed clerical errors throughout	X		CRH
11/29/10	10	Updated methods to reflect 3130 upgrade to 3130xl, clarified wording to agree with NDIS procedures			CRC
8/29/11	11	Added offender thumbprint verification stamp info and date in Tracker for criminal history flag, updated offender sample storage location, allowed for ILS at 50 RFU, removed print instructions for Driftcon, fixed clerical errors			CRC
12/7/11	12	Separated out CODIS methods, removed Quantifiler Human, added Plexor HY and quant/normalization/amp setup automation, clarified contamination definition, clerical	X	X	CRC
5/23/12	13	Replaced 9947A with 2800M, clerical errors			CRC
10/15/13	14	Removed collection report from BI-301, removed use of non-filter tips for FTA extraction, added clear amp seals, clerical errors			CRC
9/10/14	15	Removed FTA extraction, added FSS i^3 expert system and modified interpretation guidelines, corrected clerical errors	X	X	CRH

Forensic Biology Database Analytical Methods Manual
Table of Contents

INDIVIDUALIZATION OF DNA SOURCES BY STR ANALYSIS

1.0 BACKGROUND:

Once a DNA source has been detected, and identified as to 'source type' where applicable and feasible, it is often important to attribute the DNA sample to a particular individual inasmuch as possible. Current DNA technology, in the analysis of STR loci, offers individualization potential. However, the individualization of a particular sample occurs through a comparative process. This process requires a DNA profile from a 'known' sample to which the evidence sample profile can be evaluated. Often times, this 'known' sample is identified through a search of the CODIS (either SDIS, or NDIS) database in cases where the perpetrator of the particular crime is originally unknown. DNA samples are collected from individuals convicted of qualifying crimes under Idaho code and are then analyzed at STR loci for entry into and subsequent searching against the DNA database.

2.0 SCOPE:

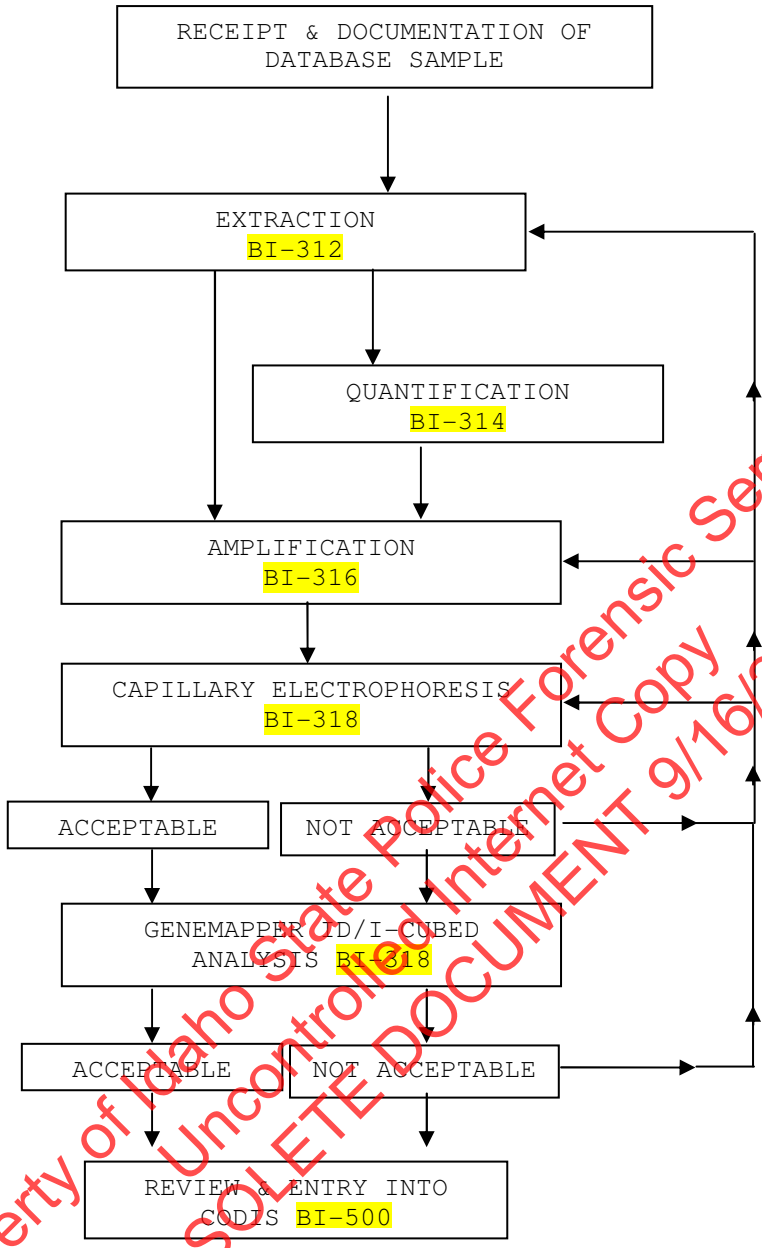
To provide uniform processing of DNA samples to achieve high quality data and consistent interpretation.

3.0 EQUIPMENT/REAGENTS:

As listed in individual analytical procedures.

4.0 PROCEDURE:

See Flow Chart on following page.



5.0 COMMENTS:

5.1 Careful scrutiny at each step will ensure insufficiencies are identified, and compensated for where feasible, at the earliest possible point (see BI-318 for specifics).

OFFENDER SAMPLE RECEIPT AND DNA TRACKER ENTRY

1.0 BACKGROUND:

The implementation of the Combined DNA Index System (CODIS) in forensic DNA laboratories has provided an additional tool in assisting law enforcement agencies in solving or linking crimes that otherwise may not have resulted in the identification of a suspect. It is important; however, that samples entered into the database be given a unique identifier, which does not include any personal or identifying information, in order to maintain the confidentiality of the individual. Each laboratory must develop a method of identifier assignment so that each sample may be tracked and identified at a later time, if the need arises.

Idaho Statutes: Title 19, Criminal Procedure, Chapter 55 "The Idaho DNA Database Act of 1996"

ISP Forensic Biology Quality/Procedure Manual, Appendix C

2.0 SCOPE:

To provide a method for tracking offender database samples submitted for STR testing and CODIS entry, while ensuring individual confidentiality.

3.0 EQUIPMENT/REAGENTS:

Computer Workstation with ISP Intranet Access
Barcode Equipment
Court Orders, Database Samples, and Report Forms

4.0 PROCEDURE:

4.1 SAMPLE RECEIPT:

4.1.1 Offender DNA samples received by the laboratory

are to be marked with the date of receipt and the initials of the scientist who received them.

4.1.2 An IDOC# is not necessary for data entry into DNA Tracker, but it is preferred. Contact necessary IDOC personnel or search the corrections website (www.idoc.idaho.gov/content/prisons/offender_search) for a number if one has not been recorded.

4.1.3 The offense listed on the DNA sample card must be a qualifying offense under Idaho Code 19-5506 in order for the sample to be entered into DNA Tracker and subsequently CODIS/NDIS. The corrections website listed above, Idaho Court Repository, and/or ILETS may be consulted for more information if the offense listed is non-qualifying or unclear. The scientist relies, in good faith, on the information provided by the submitting agency for entry into Tracker. It is not necessary, nor is it the scientist's responsibility, to verify the offense(s) of every Offender if the information provided by the submitting agency qualifies as listed. The ISP Attorney General's Office will be consulted on a case by case basis regarding the release of information if a non-qualifying offender is involved in a candidate match.

4.1.4 Samples received in the laboratory that do not have an associated qualifying offense will not be entered into DNA Tracker. A copy of the sample card will be made, marked as a non-qualifying offense, and returned to the submitting agency. The sample(s) will then be destroyed.

4.1.5 A thumbprint verification stamp will be placed near the thumbprint on the DNA sample card for all samples to be retained and entered into DNA Tracker. The stamp will be used to record the thumbprint verification for hit confirmations and in order to enter a DNA flag in the offender's criminal history. If no thumbprint is present, the submitting agency will be contacted and a new sample with print will be requested. It is

preferable to have a state identification number (SID) to facilitate the thumbprint verification. If no SID is listed on the card or report, the scientist should consult ILETS and record the SID, if found. The latent fingerprint section should be consulted, as necessary, to determine if a new sample and print needs to be requested due to insufficient print quality.

4.2 COURT ORDER RECEIPT:

4.2.1 Court order forms received by the laboratory are to be marked with the date of receipt and the initials of the scientist who received them.

4.2.2 The offense listed on the court order must be a qualifying offense under Idaho Code 19-5506 in order for the court order information to be entered into DNA Tracker.

4.2.3 If the offense on the court order is not a qualifying offense, no further action will be taken. The court order will be marked to indicate it is a non-qualifying offense and returned to the submitting agency.

4.3 DNA TRACKER PRE-ENTRY SEARCH:

4.3.1 Prior to data entry for any new sample or court order, a database search is performed to eliminate duplicate offender entry. Log on to the DNA Tracker database program, located under Forensics on the ISP Intranet.

4.3.2 A duplicate offender search will be performed using the 'Name' field, followed by at least one of the 'ID' fields to maximize the potential for locating an offender. Note: the 'DOB' field may only be searched in combination with a name.

4.3.3 If all of the searches return 'No matches found', the data for the new sample or court order may be entered as a 'New Offender' (see **4.4**).

4.3.4 If a record(s) is returned that meets the criteria, the data is examined and compared with the new sample or court order received. If it is determined that the Offender already exists in DNA Tracker, the new sample or court order and any additional Offender information will be entered under the appropriate tabs for the already existing Offender. Each sample received for an offender will be assigned a unique barcode number and will be retained in the laboratory (see **4.4**).

4.4 DNA TRACKER ENTRY:

4.4.1 Enter basic Offender information from the court order, and/or sample card as follows:

1) For Offenders not currently in Tracker, click 'New Offender' at the top of the screen and fill in each of the appropriate fields with the Offender's primary information. Additional information for Offenders already in Tracker may be entered by clicking the 'Edit Basic Details' tab for that Offender.
2) Verify all of the information is correct and press the 'Save' button in the top right corner of the screen.
3) Add any additional alias names, DOB's, SSN's, and State Identification Numbers on the appropriate alias tab(s) that become available after saving the new offender. The SID verification date will be filled in following thumbprint/SID confirmation. Click the 'Save' button after each entry.

4.4.2 Enter the offense and its associated information from the court order, and/or sample card as follows:

1) Click the 'Offenses' tab for the saved offender.
2) Choose the appropriate offense from the pull down menu and enter the corresponding information into the remaining fields.
3) Verify the information is correct and press the 'Save' button in the top right corner of the window. Multiple offenses for the same Offender may be entered and saved individually.
4) Offense information may be updated/edited if additional data is received at a later time (e.g. when a sample arrives, fulfilling a court order or an additional sample for the same offense is received). Under the 'Offenses' tab, click on the appropriate offense code listed in the table of offenses. Enter the appropriate information and click the 'Save' button.

4.4.3 Enter Court Orders for an Offender as follows:

1) Click the 'Court Orders' tab for the saved offender.
2) Enter the court order issue and received dates. Note: the order received date will automatically populate in the sample history, once a sample has been received fulfilling that court order.
3) Click the 'Add Offense' link and choose the appropriate offense/court case from the pull down menu. Note: the offenses in the pull down list are those previously entered in the 'Offenses' tab. A court order must have an associated offense in order to be entered; therefore, the offense information must be entered prior to the court order.
4) Verify the information is correct and click the 'Save' button.
5) Once the court order has been entered it is retained in the laboratory.

4.4.4 Enter samples for an Offender and print sample barcodes as follows:

1) Click on the 'Samples' tab for the saved Offender.
2) Enter the sample information into the appropriate fields, leaving the barcode field blank (Tracker will automatically generate a unique barcode number if the field is not filled in).
3) If the submitting agency is not listed in the agency pull down menu, it may be added by clicking on the 'edit agencies' button to the right of the agency field.
4) If the sample received is pursuant to a previously entered court order, click on the 'Show Unfulfilled Court Orders' link. Click the box next to the appropriate court order to mark it as fulfilled by the sample being entered.
5) Enter the number of barcode labels to be printed. One label is to be placed on the DNA sample card/folder and one inserted behind the FTA card envelope for placement on the FTA card.
6) Verify the information is correct and click the 'Save' button. Barcode labels will automatically print upon saving the sample information.
7) The labeled DNA sample card will be stored in the DNA Database Laboratory.
8) Barcode labels may be printed at a later time for individual samples, by clicking on the appropriate sample from the 'Samples' tab or for multiple samples, by using the 'Batch Samples' function at the top of the screen.

EXTRACTION PROTOCOLS FOR PCR DNA TYPING TESTS

1.0 BACKGROUND:

Many methods exist to obtain DNA, suitable for amplification, from a variety of sources. It has become increasingly important to streamline the extraction process through automation. A variety of extraction methods and robotics systems have become available for the successful extraction and downstream processing of known database DNA samples, using both blood and buccal collection methods. The Promega DNA IQ Extraction System and the Beckman Coulter Biomek 3000 robotic workstation are both examples of systems that have proven successful in the forensic community.

Comey, CT et al. "DNA Extraction Strategies for Amplified Fragment Length Polymorphism Analysis." J For Sci, Vol. 39, 1994, pp. 1254-1269.

McLaren, B et al. "Automating the DNA IQ System on the Biomek 3000 Laboratory Automation Workstation." Profiles in DNA, 2006 Vol. 9(1): 11-13

Tereba, A et al. "High-Throughput Processing of Samples on Solid Supports Using the SlicPrep 96 Device." Profiles in DNA, 2005 Vol. 8(2): 3-5.

DNA IQ System-Database Protocol, Technical Bulletin (revision date 4/06), Promega.

Automated DNA IQ System Protocol for the Beckman Coulter Biomek 3000, Technical Bulletin (revision 1/11), Promega.

2.0 SCOPE:

To provide appropriate protocols for the extraction of database sample DNA, both manual and automated, suitable for PCR amplification and subsequent analyses.

3.0 EQUIPMENT:

Pipettes and tips
Harris Punch
BSD600-Duet Semi-Automated Sample Punch Instrument w/ Scanner

96-well Reaction Plate and Base
MicroAmp Tubes
1.5mℓ microcentrifuge Tubes (1.5mℓ tubes)
SlicPrep Device
Adhesive Foil Seal or Clear Amplification Seal
70°C Water Bath or Heat Block with SlicPrep Adaptor
Centrifuge with Swinging Plate Rotor
Biomek 3000 Robotics System with MagnaBot 96 Magnetic Separation Device, 1/4 Inch Foam Spacer, Variomag Teleshake, Heating Block, and Tip Disposal Container
Reagent Reservoirs and Frame
1.2 mℓ Round-Well, Deep Well Plate

4.0 REAGENTS:

PCR TE (TE, 10mM Tris-HCl; 0.1mM EDTA, pH 8.0)
DNA IQ System Reagents
99% Isopropyl Alcohol
95-100% Ethanol
1M Dithiothreitol (DTT)

5.0 PROCEDURE:

5.1 AUTOMATED DNA IQ EXTRACTION FROM OFFENDER DATABASE SAMPLES

5.1.1 SAMPLE PUNCHING USING THE BSD600-DUET

5.1.1.1 Fill out the BSD/Biomek Load Sheet on the 'BSD Load Sheet' tab of the Database worksheets template(Form 312-BI). Choose the 'BSD Input File' tab and ensure the information is correct and corresponds to the Load Sheet information entered. Delete any unused wells, making sure to keep 'End File' as the last cell. Perform a 'Save As' of the worksheet to disc (e.g. USB drive) for subsequent transfer to the BSD600 puncher. The document must be saved as a text tab delimited file. Save the template as samples are automatically populated on the other tabs for subsequent processing steps. A copy of the completed Load Sheet will be printed for the batch record/file.

Note: The last two columns of the plate should be left empty for control placement in

subsequent steps. This allows for 78 samples, an extraction control, and a reagent blank to be tested per plate.

5.1.1.2 Ensure the BSD tray table is adjusted properly by placing the appropriate plates in both positions and adjusting the height, as necessary. The bottom of the chute should be ~2mm from the top of the plates, measured at each corner.

5.1.1.3 Turn on the BSD puncher and computer and plug in the air/pressure pump. Make sure water is present in both filter bottles (~50-100ml). Double click the 'BSD Duet Main Menu' icon and login using the appropriate user name and password.

5.1.1.4 Choose **Configure System** and open the **Files** tab. Ensure that **File Input** is selected from the **Sample Number Mode** dropdown list. Click the **Browse** button and locate your saved input file to fill in the **Input File Name** box. Click **Save and Exit**.

Note: Using the input file option only allows for a single plate to be punched at a time. To punch multiple plates, the process must be repeated for each new set of samples.

5.1.1.5 Choose **Distribute Spots** and click **Continue**. When prompted to select test group to punch, highlight **All Available Tests** and click **Continue**. Check the box next to **Input File.tst** and check the samples box on the right. Press **Continue**. This test file will punch 3 of the 3.2mm spots (verify before beginning). If punching for FTA extraction, the number and size of punches will need to be changed under the Edit Test Sequences option prior to beginning.

5.1.1.6 Place the appropriate plate into position 1 of the BSD tray table with well A1 located in the upper left corner. Click **Continue** to begin punching. When using the SlicPrep 96

Device, remove the white collar, push the basket down into the plate, and set the collar aside before processing.

5.1.1.7 Scan the sample barcode using the scanner attached to the BSD and place the card under the card clamps on top of the instrument. Use the light guide dots to properly position the card for punching. Press the foot switch when ready to punch or use the delay switch for automatic punching. Repeat until all samples have been punched.

Note: The scanned barcode must match that on the input file or the instrument will not punch the card.

5.1.1.8 Remove the plate and proceed to SlicPrep pre-processing (see **5.2.2**) or FTA extraction (see **5.1**).

5.1.2 SLICPREP PRE-PROCESSING

5.1.2.1 Add 400ul DNA IQ Lysis Buffer with DTT (see **5.2.3.1**) to each well of the device plate. Seal the top of the plate with a foil seal and place into a 70°C water bath or heat block with SlicPrep adaptor for 15-60 min.

5.1.2.2 Without removing the foil, raise the filter basket and place the white collar back onto the device by sliding it under the basket and aligning the detents on the device.

5.1.2.3 Centrifuge the device at 1500 x g (~3000 RPM)for 5 min. in a swinging plate rotor. Remove and discard the collar and filter basket upon completion of centrifugation.

5.1.2.4 Proceed to DNA IQ Extraction on the Biomek 3000 (see **5.2.4**).

5.1.3 DNA IQ REAGENT PREPARATION

5.1.3.1 When opening a new DNA IQ kit, add 1.5ml 1M DTT to the bottle of Lysis Buffer (1ul DTT/100ul lysis buffer) and mix. Mark the

bottle with initials, the date the DTT was added, and the DTT lot number. The 'activated' Lysis buffer may be used for up to 2 months following DTT addition. Alternatively, the lysis buffer may be prepared as needed based on the number of samples to be processed.

5.1.3.2 Prepare the new wash buffer (70ml 2X bottle) by diluting with 35ml Isopropyl Alcohol and 35ml Ethanol for a final 1X concentration. Mark the bottle with initials, the date of alcohol addition, and the alcohol lot numbers. The 1X wash buffer may be used for a period of 1 month after 'activation'. Alternatively, the wash buffer may be prepared as needed based on the number of samples to be processed.

5.1.3.3 Prepare/aliquot each of the DNA IQ reagents at the time of use, based on the following calculations and reservoir positions (instructions listed in the 'Reagent Preparation' comments window):

1: Elution Buffer = 2.5ml + (#samples x 100ul) 80 samples = 10.5ml
Note: be careful not to contaminate the elution buffer with lysis buffer or other reagents
2: Lysis Buffer w/ DTT + Resin = [860ul + (#sample columns x 344ul)lysis] + [140ul + (#sample columns x 56ul)resin]
80 samples = 4.3ml lysis + 0.7ml resin
3: 1X Wash Buffer = 1.5ml + (#sample columns x 2.4ml) 80 samples = 25.5ml
4: Lysis Buffer w/ DTT = 2.0ml + (#sample columns x 0.8ml) 80 samples = 10.0ml

5.1.4 DNA IQ EXTRACTION USING THE BIOMEK 3000 ROBOTICS SYSTEM

5.1.4.1 Turn on the Biomek 3000, computer, and Watlow heater. Make sure that the heater is set to 85°C and that the shaker electrical box dial is turned to the red at the far left stop.

5.1.4.2 Open the Biomek Software and choose the 'B3K_DNAIQV2.0.1.ISP' method.

5.1.4.3 Set up the Biomek 3000 deck as follows (outlined in the 'Beckman Hardware and Deck Setup' comments window):

Rack 1 (A1): Tool rack with MP200 (1)and gripper (3)
ML1 (A2): P250 barrier pipette tips
ML2 (A3): P250 barrier pipette tips (dependent on sample number)
ML3 (A4): P250 barrier pipette tips (dependent on sample number)
ML4 (A5): empty
P6 (A6): Heating system
P1 (B1): PCR plate and base
P2 (B2): Deep round-well purification plate
P3 (B3): Reservoir frame and reservoirs with reagents
P4 (B4): Deep round-well purification plate
P5 (B5): Magnabot
P7 (B6): Pre-processed deep square-well plate stacked on the Variomag Teleshaker

Note: The number of tip boxes will depend on the number of samples to be extracted. The instrument will only extract full columns on the plate.

5.1.4.4 Click the green run instrument arrow button to begin the run. When prompted, enter the number of columns to be extracted (end column), the first tip column, and either 50ul, or 100ul for elution volume. The 50ul volume may be preferable for lower concentration samples. Verify the reagent and deck layout and click ok.

Note: It is recommended that a 'home all axes' be performed prior to the first instrument run for the day.

5.1.4.5 When the run has finished remove the PCR/elution plate. A brief spin may be necessary to remove any bubbles present in the wells.

5.1.4.6 Proceed to realtime PCR (BI-314) for quantification of the purified DNA obtained in 5.2.4.5.

5.1.4.7 Clean the reservoirs between runs. At the completion of all runs for the day, wipe down the deck with 10% bleach or Dispatch solution. Tools may also be cleaned with 70% Ethanol if necessary.

6.0 DNA EXTRACTS:

6.1 After a plate of samples has been extracted and during subsequent analyses (e.g. quantification and amplification), the DNA extracts may be stored sealed with foil at approximately 4°C. For longer storage periods, the extracts should be frozen at approximately -20°C. These extracts are in-progress work product during this stage(s).

6.2 Any extract remaining, following the successful completion of analysis and data review, will be discarded.

7.0 Comments:

7.1 A reagent blank shall be carried through all extraction steps to check the purity of the reagents being used. There need only be one reagent blank per extraction plate, but the analyst may choose to run additional blanks on a given plate.

7.2 The analyst may optionally incorporate cleaning punches during BSD sample punching. At a minimum, the puncher is cleaned bi-weekly but may be cleaned more often at the analyst's discretion.

7.3 These procedures may be used for blood or buccal samples on FTA paper.

7.4 The DNA IQ resin must be thoroughly mixed/resuspended prior to aliquoting.

7.5 The final elution volume attained on the Biomek 3000 will be ~8-10ul less than the volume chosen, due to evaporation during the processing.

7.6 The DNA IQ extraction procedure may be performed manually, if necessary, following the procedure outlined in the DNA IQ

System-Database Protocol, Technical Bulletin (revision date 4/06). This procedure follows the same steps as performed on the Biomek 3000.

DNA QUANTIFICATION: REAL-TIME PCR

1.0 BACKGROUND:

DNA methodologies that employ the PCR, such as STR analysis, necessitate consistent quantification of human DNA to obtain optimum data.

"Developmental Validation of a Real-Time PCR Assay for the simultaneous Quantification of Total Human and Male DNA," Krenke, B.E., et al, Forensic Science International: Genetics 3 (2008), pp 14-21 Vol. 3.

"Improving Efficiency of a Small Forensic DNA Laboratory: Validation of Robotic Assays and Evaluation of Microcapillary Array Device," Crouse, C., et al, Croat Med J 2005, Vol. 46, No. 4, pp. 563-577.

Plexor® HY System for the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems, Technical Manual (revision 11/07), Promega Corporation.

Automated Plexor® HY System Setup for the Biomek 3000, Technical Bulletin (revision 1/10), Promega Corporation.

7500/7500 Fast Real-Time PCR Systems Maintenance Guide, Applied Biosystems.

2.0 SCOPE:

To provide a reliable method for the consistent quantification of variable amounts of human DNA isolated from database samples.

3.0 EQUIPMENT/REAGENTS:

ABI 7500/Computer
ABI 7500 SDS Software
Plexor® Analysis Software
Pipettes and Tips
Plate Clamp
Automated Tube Holders
96-well Reaction Plate/Base
Optical Adhesive Cover
Microcentrifuge Tubes
Strip Tubes/Caps
Reservoir frame
Centrifuge

Plexor® HY Kit PCR-TE
Biomek 3000 Robotics System with MagnaBot 96 Magnetic Separation Device, 1/4 Inch Foam Spacer, Variomag Teleshake, Heating Block, and Tip Disposal Container

4.0 PROCEDURE:

4.1 MANUAL PREPARATION OF DNA STANDARDS:

4.1.1 Label 8 sterile microcentrifuge tubes, or strip tubes A through H or 1-8.

4.1.2 Dispense 40µℓ of PCR-TE into tubes B-H (Std. 2-8).

4.1.3 Mix the Plexor HY Male Genomic DNA Standard thoroughly by vortexing approximately 5 seconds. Transfer 10µℓ to tube A (Std. 1/undiluted) and to tube B (Std. 2). Mix the dilution thoroughly.

4.1.4 Prepare Std. 3-7 via a serial dilution by mixing and subsequent 10µℓ transfers starting with tube B and ending with G. The dilution series consists of 50, 10, 2, 0.4, 0.08, 0.016, 0.0032ng/µℓ (or adjusted per kit QC), and a no-template control, respectively.

4.2 MANUAL REACTION PREPARATION:

4.2.1 Determine the number of samples to be quantified (including, at minimum, 2 sets of DNA standards).

4.2.2 Finish filling out the 7500 Load Sheet on the Database worksheets template (Form 312-BI). Print a copy for the batch record/file.

4.2.3 Calculate the volume of reaction components needed, based upon the number of samples to be quantified and adding 2 or 3 reactions to compensate for loss and variability due to pipetting. The following are the volumes needed per reaction.

Plexor HY 2X Master Mix	10µℓ
Plexor HY 20X Primer/IPC Mix	1µℓ
Water	7µℓ

Note: The volume of reaction components necessary to prepare the Master Mix will be automatically

calculated upon Load Sheet data entry and choosing the manual option.

4.2.4 Thaw the master mix and primer mix and vortex 3-5 seconds. Pulse-spin prior to opening the tube (do not overspin).

4.2.5 Place a 96-well reaction plate into a base, being careful not to touch the top or individual wells. Do not place the plate directly onto the counter or any surface other than its base or the ABI 7500 thermal block.

4.2.6 Prepare the Master Mix by pipetting the required volumes of water, primer and master mixes into an appropriately sized microcentrifuge tube or reservoir. Mix thoroughly and pulse spin if mixing in a tube.

4.2.7 Carefully pipette 18µℓ of the PCR Reaction Mix into the bottom of each reaction well to be used. 'Blowing-out' the pipette is not recommended to avoid splashing and/or bubbles in the well.

4.2.8 Add 2µℓ of sample or standard to the appropriate reaction well, being careful to avoid bubbles as much as possible.

4.2.9 Seal the reaction plate with an Optical Adhesive Cover. Proceed to **4.4**.

4.3 AUTOMATED REAL-TIME PCR SETUP USING THE BIOMEK 3000 ROBOTICS SYSTEM:

4.3.1 Finish filling out the 7500 Load Sheet on the Database worksheets template (Form 312-BI). Choose the automated option and print a copy for the batch record/file.

4.3.2 Thaw the master mix and primer mix and vortex 3-5 seconds. Pulse-spin prior to opening the tube (do not overspin).

4.3.3 Turn on the Biomek 3000 and computer. Open the Biomek Software and choose the 'B3KPlexorHY_V1.0.1.ISP' method.

4.3.4 Set up the Biomek 3000 deck as follows (outlined in the 'Labware Requirements' comments window):

Rack 1 (A1): Tool rack with MP200 (1), P200L (2), and gripper (3)
ML1 (A2): P250 barrier pipette tips
ML2 (A3): P50 barrier pipette tips
ML3 (A4): P50 barrier pipette tips (dependent on tip quantity)
ML4 (A5): empty
P6 (A6): Heating system (not used)
P1 (B1): PCR plate and base with extracted DNA
P2 (B2): Plate clamp and base with strip tubes in positions 1 and 12
P3 (B3): Reservoir frame with automated tube holders at left-most and right-most positions
P4 (B4): PCR plate and base
P5 (B5): Magnabot (not used)
P7 (B6): Variomag Teleshaker (not used)

Note: The position 1 strip tubes will be used for preparation of the standard dilution series. If preparing standards manually or using previously prepared standards, these will be placed in position 1.

4.3.5 Place reagent tubes in the position P4 tube holders as follows (outlined in the 'Tube Holder Positions and Reagents' comments window):

Left 1: Plexor HY 2X Master Mix
Left 2: Plexor HY 20X Primer/IPC Mix
Left 3: Empty
Left 4: Empty 1.5ml microcentrifuge tube
Right 1: Amplification Grade Water
Right 2: PCR-TE Buffer in 1.5ml tube
Right 3: Plexor HY Male Genomic DNA Standard
Right 4: Empty

Note: The empty microcentrifuge tube will be used for the master/reaction mix preparation. If preparing the reaction mix manually, it will be placed in this position. The left positions 1 and 2 will be empty if using manually prepared reaction mix. Right positions 2 and 3 will be empty if using manually or already prepared standards.

4.3.6 Click the green run instrument arrow button to begin the run. When prompted, enter Y or N for standards and master mix preparation, the number of samples to be

quantified, the first available P250 tip, and the first available p50 tip. Verify the reagent and deck layout and click ok.

4.3.7 When the run has finished remove the PCR plate and seal with an Optical Adhesive Cover. A brief spin may be necessary to remove any bubbles present in the wells. Proceed to **4.4.**

4.3.8 At the completion of all runs for the day, wipe down the deck with 10% bleach or Dispatch solution. Tools may also be cleaned with 70% Ethanol if necessary.

4.4 RUNNING THE REACTION:

4.4.1 Turn on the 7500 computer and login with the appropriate user name and password. After the computer has completely started up, power on the 7500 instrument, allowing it to warm up at least 30 seconds. Launch the ABI 7500 SDS Software.

4.4.2 Open the instrument tray by pushing on the tray door. Place the plate into the tray holder so that well A1 is in the upper-left corner and the notched corner of the plate is in the upper-right corner.

4.4.3 Close the instrument tray by gently pushing the right side of the tray door.

4.4.4 In the SDS software, select **File>New** and choose **Absolute Quantitation** for Assay, **96-Well Clear** for Container, and **Plexor HY** for Template.

4.4.5 Highlight the wells that contain samples or standards and apply the autosomal, Y, and IPC detectors. Do not include any unused wells. It is not necessary to name the samples.

Note: Detectors are created during the initial instrument set-up and/or kit usage. Refer to the Quantifiler Kits User's Manual (page 2-11) for instructions on creating detectors if needed.

4.4.6 Select the **Instrument** tab and review the thermal cycler conditions [Stage 1: 1 cycle, 95°C, 2:00 min.; Stage 2: 38 cycles, 95°C, 00:05 min, 60°C, 0:35 min.; Stage 3: 1 cycle, 95°C, 00:15 min, 60°C, 1:00 min, 95°C, 00:15 min;

20µℓ sample volume; 9600 emulation unchecked; Data collection: Stage 2, Step 2 (60.0@0:35)]

4.4.7 Save the plate document as a .sds file with the appropriate plate name.

4.4.8 Under the **Instrument** tab, click **Start** to begin the run. When the run has completed, proceed to **4.5.**

Note: Choose 'yes' at the SYBR Green message prompts.

4.5 ANALYSIS AND RESULTS:

4.5.1 Open the plate document to be analyzed.

4.5.2 Select **Analysis>Analyze.**

4.5.3 Export the amplification data as a .csv file (e.g. to USB drive) by selecting **File>Export>Delta RN.**

4.5.4 Export the melt/dissociation data as a .csv file (e.g. to a USB drive) by selecting **File >Export >Dissociation >Raw and Derivative Data.** Select 'Yes' at the SYBR Green message prompt.

4.5.5 Launch the Plexor Analysis Software and deselect "Set Passive Reference On Import" in the file menu.

4.5.6 Import the .csv files by selecting **File>Import New Run.** Enter the plate (assay) name, choose the Applied Biosystems 7500 instrument and assign dyes to the autosomal (FLR), Y (CO560), and IPC (CR610) targets. Check the amplification and melt boxes for each and click 'next'.

4.5.7 Enter the analyst's initials under Operator Name and select 'next'. Select the appropriate amplification and melt files using the 'browse' buttons and click 'finish'.

4.5.8 Select the 'Sample IDs' tab and enter the names for each well. Alternatively, the 7500 Load Sheet may be copied and pasted into the Sample IDs window using the ctrl-T function.

Note: sample names (including standard and no-template controls) must be unique or the software will report

concentration averages and an error will occur when importing data into the worksheets.

4.5.9 Define the unknown samples, no-template controls, and standards by highlighting the appropriate wells in the well selector pane of the 'PCR Curves' tab and clicking the corresponding icon in the toolbar.

4.5.10 Highlight the standard wells and click the 'Create Dilution Series' icon in the tool bar. Select 'vertical series' and 'decreasing'. Enter 50 (or adjusted value from the kit QC) as the starting concentration and 5 as the dilution factor. Click 'apply'.

4.5.11 Leave the standard wells highlighted and in the melt curves window, drag the target melt temperature line to the midpoint of the melt curves. Do this for the autosomal, Y, and IPC curves. The melt temperature (T_m) for each sample will be displayed in the table on the right.

A 'yes' or 'no' in the T_m column indicates whether a sample has a melt temperature within the target range. A 'no call' indicates that the sample has a melt curve within the range but there is insufficient sample to cross the threshold. An increase in the T_m for unknowns may indicate the presence of impurities.

4.5.12 With the standard wells still highlighted, select the autosomal channel and click the 'Add Standard Curve' icon in the tool bar. Repeat for the Y channel. Select the 'Standard Curves' tab to view the standard curves and print the autosomal screen for the batch record. Review the autosomal data for inconsistencies from the following:

An R^2 value of >0.990 indicates a close fit between the standard curve regression line and the individual C_T data points of quantification standard reactions.

An R^2 value of <0.98 may be due to variability of the 0.0032ng/ul dilution. These may be omitted as necessary. If the R^2 value does not improve with removal of these standards, the Plexor HY System Technical Manual (section X) may be referred to for troubleshooting guidelines.

The slope should fall within the typical slope range of -3.2 to -4.0. A slope of -3.3 indicates 100% amplification efficiency.

4.5.13 Under the 'PCR Curves' tab, highlight all of the samples, standards, and no-template controls (if any standards were omitted in 4.5.12, do not include them here). Select the 'Add Standard Curve' icon for the autosomal and Y channels. Click 'ok' to replace the existing standard curves.

4.5.14 Open the forensics report by selecting **Forensics >Set Normalization and IPC Parameters >OK.** Check the IPC and Curves Status columns for potential problems and troubleshoot as necessary (viewing other report tabs if desired). Unknown samples with IPC C_t values several cycles higher than those of DNA standards with similar concentrations, is an indication that inhibition may have occurred.

4.5.15 Highlight and copy the forensics report. Paste the data into the 'PHY Raw Data' tab of the database worksheets. Open the 'PHY 7500 Results' tab and review the imported data. Delete any unused wells from the sheet.

If performing automated normalization and amplification set-up follow the instruction on the sheet, delete the standards and no-template controls and print the results sheet for the batch record. A .tab file will automatically be saved for Biomek 3000 import.

If performing manual normalization and amplification set-up choose the check mark from the manual dilution scheme drop down menu and un-gray the associated columns. Delete the standards and no-template controls and adjust values in the dilution scheme columns as needed. Print a copy of the results sheet for the batch record.

4.5.16 Proceed to STR amplification (BI-316).

5.0 COMMENTS:

5.1 After initial thawing, the Plexor HY Male Genomic DNA Standard will be stored at approximately 4°C.

5.2 Refer to the Plexor HY System Technical Manual for additional user information and troubleshooting guidelines.

5.3 A template may also be created in the Plexor Analysis Software when running multiple plates with the same layout.

5.4 The Halogen Lamp may be checked manually to determine if replacement is needed. Place the Green Calibration Tray in the block. Select **Instrument > Calibrate** and set the exposure time to **4096ms**, lamp control to **Max**, and select **Filter A**. Click Snapshot and observe results. Expected results should consist of red fluorescence displayed in all wells. Lack of fluorescence indicates the need for lamp replacement. The lamp status should be checked as well by selecting **Instrument > Lamp Status/Replacement** and viewing the condition.

5.5 In order to extend the life of the Halogen Lamp, the instrument should be turned off anytime it is not in use. Lamp life is approximately 2,000 hours.

STR AMPLIFICATION: PP16 HS

1.0 BACKGROUND:

DNA analyses have revolutionized forensic biology. The advent of PCR allowed scientists to analyze evidentiary material present in minute quantities and degraded states. The identification of forensically significant STR loci has allowed scientists to combine the discrimination attainable with the older RFLP technology with the speed and sampling capabilities of other PCR-based methodologies. The PowerPlex™ 16 HS allows the co-amplification of the core CODIS 13 loci, as well as, Amelogenin, and two pentanucleotide-repeat loci, Penta D and Penta E.

Butler, J. *Forensic DNA Typing: Biology and Technology Behind STR Markers.* (2001) Academic Press.

PowerPlex™ 16 HS System Technical Manual, Promega Corporation

2.0 SCOPE:

To provide a reliable method for consistent, high quality amplification of DNA from offender database samples ensuring the generation of suitable PCR product for capillary electrophoresis and analyses of these STR loci.

3.0 EQUIPMENT/REAGENTS:

Thermalcycler	Half reservoir and base
Centrifuge	Automated tube holders
Microcentrifuge tubes	PCR plate and base
1.1ml square-well, v-bottom plates	Pipettes and tips
Foil Seal/Clear Amplification Seal	Compression pad

PowerPlex™ 16 HS Kit

Biomek 3000 Robotics System with MagnaBot 96 Magnetic Separation Device, 1/4 Inch Foam Spacer, Variomag Teleshake, Heating Block, and Tip Disposal Container

4.0 PROCEDURE:

4.1 DNA TEMPLATE:

4.1.1 Based upon the quantity of DNA isolated and its initial concentration, samples should be normalized to an optimal concentration for amplification (e.g., 0.25ng/µℓ for manual normalization). It is also convenient to have all samples in a batch at the same concentration, if possible, for ease in the preparation of PCR Master Mix and reaction additions. The maximum amplification volume for low level or undetected samples is 4.4µℓ for manually prepared PowerPlex 16 HS, quarter volume reactions and 4.0µℓ for automated reactions.

4.1.2 The amount of DNA template and 2800M added to a quarter volume amplification reaction should be targeted at ~0.50ng, but may be adjusted as necessary.

4.2 MANUAL AMPLIFICATION SET-UP:

4.2.1 Thaw the PowerPlex 16 HS 5X Master Mix, PowerPlex 16 HS 10X Primer Pair Mix, and Amplification Grade Water (optional) contained in the HotStart kit.

4.2.2 Calculate the volume of reaction components needed based upon the number of samples (including extraction and amplification controls) to be amplified and adding 1 or 2 reactions to compensate for loss and variability due to pipetting. Use the 'Amp. Worksheet' tab of the Database Worksheets Template (Form 312-BI) and the manual setup option to automatically calculate necessary volumes. Print a copy of the completed worksheet for the batch record/file. The following is a list of the 'fixed' amounts to be added for a 6.25µℓ reaction.

PowerPlex 16 HS 5X Master Mix	1.25µℓ
PowerPlex 16 HS 10X Primer Pair Mix	0.6µℓ
DNA Template + dH_2O	4.4µℓ

Note: The amount of Reaction Mix added to each sample is dependent on the volume needed to add the DNA template.

4.2.3 Prepare the reaction mix by pipetting the required volumes of 5X master mix, 10X primer mix, and water (if needed) into an appropriately sized microcentrifuge tube or reservoir. Mix thoroughly and pulse-spin if using a tube.

4.2.4 Pipette the appropriate amount of reaction mix into each well of a labeled 96-well reaction plate. The negative amplification control should be the last sample processed.

4.2.5 If DNA concentrations were not the same, add appropriate volume of dH_2O as necessary.

4.2.6 Pipette each DNA sample into the appropriate well. Use 2800M control DNA for the positive amplification control and dH_2O for the negative amplification control.

4.2.7 Thoroughly seal the plate with an adhesive seal. Use a seal applicator to tightly seal between and over each well.

4.2.8 Remove lab coat and, touching only the plate, transport the samples to the thermal cycler in the Amp/PostAmp room, using the other hand on the door knob.

4.2.9 Place a compression pad over the plate and put it into the thermal cycler. Remove/discard gloves before exiting the Amp/PostAmp room. Proceed to 4.4.

4.3 AUTOMATED AMPLIFICATION SET-UP USING THE BIOMEK 3000 ROBOTICS SYSTEM:

4.3.1 Thaw the PowerPlex 16 HS 5X Master Mix and PowerPlex 16 HS 10X Primer Pair Mix contained in the HotStart kit.

4.3.2 Finish filling out the Amp Worksheet on the database worksheets template (Form 312-BI). Choose the automated option and print a copy for the batch record/file.

4.3.3 Turn on the Biomek 3000 and computer. Open the Biomek Software and choose the 'B3KNormSTR_V1.0.0.ISP' method.

4.3.4 Set up the Biomek 3000 deck as follows:

Rack 1 (A1): Tool rack with P20 (1), P200L (2), and gripper (3)
ML1 (A2): P250 barrier pipette tips
ML2 (A3): P50 barrier pipette tips
ML3 (A4): P50 barrier pipette tips
ML4 (A5): P50 barrier pipette tips (dependant on tip count)
P6 (A6): Heating system (not used)
P1 (B1): PCR plate and base with extracted DNA
P2 (B2): Dilution plate (square-well, v-bottom)
P3 (B3): Reservoir frame with automated tube holders at left-most and right-most positions and half reservoir in the center
P4 (B4): Dilution plate (square-well, v-bottom)
P5 (B5): PCR plate and base (magnabot removed)
P7 (B6): Variomag Teleshaker (not used)

4.3.5 Place reagents in the position P3 tube holders and reservoir as follows:

Left 1: PP16HS 5X Master Mix
Left 2: PP16HS 10X Primer Pair Mix
Left 3: Empty
Left 4: Empty 1.5ml microcentrifuge tube
Half Reservoir: autoclaved nanopure water
Right 1: 1ng/µl 2800M DNA Standard
Right 2: Empty
Right 3: Empty
Right 4: Empty

Note: The empty microcentrifuge tube will be used for the master/reaction mix preparation. If preparing the reaction mix manually, it will be placed in this position. The left positions 1 and 2 will be empty if using manually prepared reaction mix.

4.3.6 Click the green run instrument arrow button to begin the run. When prompted, enter the first available P250 tip, the first available p50 tip, and true/false for manual master mix preparation.

4.3.7 The STR Normalization Software will open automatically. Login with the appropriate user name and password.

4.3.8 Enter the plate name and select 'Idaho State Police Qtr Rxn 16HS 0.5ng Plexor' as the template and click 'next'.

4.3.9 Select use an existing extraction control template (Idaho State Police Database Ext Control Template) and choose 'next'.

4.3.10 Choose use existing injection control template and select 'Idaho State Police Inj Control Template'. Click 'next'.

4.3.11 Click 'browse' to locate the .tab input data file created in step 4.5.15 of BI-314. Select 'next' and review the analysis breakdown table.

4.3.12 Click 'review extraction plate' and make any necessary changes (e.g. manually add or remove samples for amplification). Select 'done' and 'next'.

4.3.13 The amplification plate may be reviewed by selecting 'review amplification plate'. Samples will be re-ordered on the plate to fill any blank spaces created by removing samples. Select 'done' and 'next'.

4.3.14 Select the appropriate CE results group and instrument protocol, ensure open report is checked, enter the appropriate user name and password, and click 'finish'. Select 'ok' for the P20 tool message.

4.3.15 Verify the deck layout and reagent setup and click 'ok'.

4.3.16 When the run has finished remove the PCR plate and thoroughly seal the plate with an adhesive seal. Use a seal applicator to tightly seal between and over each well.

4.3.17 Remove lab coat and, touching only the plate, transport the samples to the thermal cycler in the Amp/PostAmp room, using the other hand on the door knob.

4.3.18 Place a compression pad over the plate and put it into the thermal cycler. Remove/discard gloves before exiting the Amp/PostAmp room. Proceed to **4.4**.

4.3.19 At the completion of all runs for the day, wipe down the deck with 10% bleach or Dispatch solution. Tools may also be cleaned with 70% Ethanol if necessary.

4.4 THERMAL CYCLING PARAMETERS:

4.4.1 After the samples have been placed in the thermal cycler, turn on the power and select the pre-programmed 'pp16HS' cycling profile.

96°C for 2 minutes, 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 **20 cycles**, then

60°C for 30 minutes, then:

4°C soak

5.0 AMPLIFIED DNA PRODUCT:

5.1 After cycling has concluded remove samples from thermal cycler. Samples should be run on the Genetic Analyzer as soon as possible after amplification. Prior to capillary electrophoresis and/or before analysis is completed the samples may be stored at approximately 4°C for minimal time periods. To minimize evaporation and for longer storage periods, samples should be frozen at approximately -20°C. Amplified product is **ONLY** stored in the Amp/PostAmp room.

5.2 At a point in time after STR analysis is completed (i.e., data has been reviewed and approved for upload), the amplified product will be disposed of in a biohazard container in the amp/post-amp room. As needed, this container will be sealed and transported directly out of the

laboratory. The container will be placed into a second biohazard bag, sealed and disposed of with other biohazardous material.

6.0 COMMENTS:

6.1 Clean surfaces with freshly made 10% bleach solution or Dispatch® prior to set-up.

6.2 Wear gloves at all times during amplification set-up.

6.3 Mix all reagents thoroughly (e.g., vortex) and pulse-spin them in a microcentrifuge prior to dispensing.

6.4 Excessive evaporation may be observed following amplification when using a foil seal. To minimize the occurrence, a clear amplification seal may be used to seal the plate rather than the foil seal. Additionally, 2-5 ul sterile water may be added to the evaporated sample and mixed thoroughly prior to running on the genetic analyzer.

6.5 A second manually pipette positive amplification control may optionally be added to Biomek 3000 amplification plate prior to performing PCR.

6.6 After initial thawing the 2800M DNA standard and any dilutions will be stored at approximately 4°C.

6.7 If amplified product is stored in the refrigerator, an increased amount of condensation may form on the foil seal. Punching through the foil, following a brief spin, may be preferable to removing the seal in order to reduce the potential for contamination.

STR TYPING: CAPILLARY ELECTROPHORESIS AND DATA ANALYSIS

1.0 BACKGROUND:

Any eukaryotic genome is interspersed with repeated DNA sequences that are typically classified by the length of the core repeat sequence, and the range of contiguous repeats typically seen or the overall length of the repeat region. STR (Short Tandem Repeat) loci are scattered throughout the genome occurring every 10,000 nucleotides or so, and have core repeat units of 2-6bp in length with overall lengths of less than 400 bp.

STR loci examined for human identification purposes were selected for the following characteristics: 1) high discriminating power (generally >0.9) with observed heterozygosity of >70%, 2) loci on separate chromosomes to avoid linkage, 3) ability to obtain robust, quality, reproducible data when multiplex amplification is performed, 4) low stutter, 5) low mutation rate and 6) small allele sizes (<500 bp) for enhancement of analysis of degraded samples.

By 1997, as the result of a community-wide forensic science effort, the following 13 STR loci, all tetranucleotide repeats, were selected as the basis for NDIS, the CODIS (COmbined DNA Index System) National Database: D3S1358, TH01, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, CSF1PO, vWA, D8S1179, TPOX, FGA. When all 13 CODIS core loci were examined, the average random match probability was found to be <1 in $1x10^{12}$ among unrelated individuals, offering the promise of individualization.

In addition to the 13 core CODIS loci, the PowerPlex 16 HS multiplex includes Amelogenin, a gender identification locus, and two pentanucleotide repeat STR loci, Penta D and Penta E. STR typing, with amplified products generated from this kit, separated by capillary electrophoresis on the 3130xl Genetic Analyzer with data collection and analysis software employed in developing the genetic profiles, will be used to produce STR profiles from convicted offender samples for entry into CODIS.

Butler, J. *Forensic DNA Typing: Biology and Technology Behind STR Markers.* (2001) Academic Press.

PowerPlex™ 16 HS System Technical Manual

PowerPlex® Matrix Standards, 3100/3130, Technical Bulletin

ABI 3130/3130xl Genetic Analyzer Getting Started Guide

ABI 3130/3130xl Genetic Analyzer Mainenance Troubleshooting and Reference Guide

GeneMapper™ ID Software User Guide

2.0 SCOPE:

To provide a reliable method for generating STR genetic profiles from offender DNA database samples.

3.0 EQUIPMENT/REAGENTS:

3130XL Genetic Analyzer with Data Collection Software
GeneMapper™ ID Software
Computers
9700 Thermal Cycler
Pipettes and Tips
Benchtop Cooler
Capillary Arrays
96 Well Reaction Plates and Septa
Buffer Reservoirs and Septa
POP4 Polymer
Genetic Analyzer Buffer
PowerPlex® 16 HS Kit Contents
PowerPlex® 16 Matrix Standards
Deionized Formamide
Nanopure Water

4.0 PROCEDURE:

4.1 AMPLIFIED FRAGMENT DETECTION USING THE 3130xl

Note: Prior to using the ABI 3130xl Genetic Analyzer for samples, a spectral calibration (matrix standards) must be run to achieve proper color separation of the dyes used for the amplification primers, allelic ladders and size standard. To prepare a matrix, four standards are run under the same capillary electrophoresis conditions that

will be used for samples and allelic ladders. Use the 3130 Matrix Standard set, which includes the Fluorescein Matrix, JOE Matrix, TMR Matrix and CXR Matrix for the blue, green, yellow and red matrix standards, respectively. This is performed when necessary due to performance, or after any instrument maintenance/repair that involves adjustment/replacement of the CCD camera or laser.

Additionally, a Spatial Calibration must be performed prior to running any samples. The instrument uses images collected during the spatial calibration to establish a relationship between the signal emitted for each capillary, as well as the position where that signal falls and is detected by the CCD camera. This is performed any time a capillary is installed or replaced (including temporary removal of a capillary) or if the instrument is moved.

4.1.1 Turn on the computer, turn on the instrument, start Data Collection Software and wait for green squares to appear for all applications on the service console. Expand the necessary subfolders on the left tree pane of Data Collection. Refer to the ABI 3130/3130xl Genetic Analyzers Getting Started Guide for detailed instructions on instrument set-up (including creation of instrument protocols, results groups, and spatial calibration). Fill in appropriate information in the 3130/3130xl Injection Log (Form 422-QC).

Shut down is performed in the opposite order (Data Collection software, 3130xl, then computer). The Data Collection Software must be closed by choosing 'Stop All' and waiting for all red symbols to appear before closing. Never use the 'X' to close while green or yellow symbols are displayed.

4.1.2 Create a new plate record:

4.1.2.1 For a spectral calibration plate expand the tree pane of the Data Collection Software and click 'Plate Manager,' under 'ga3130xl'. Choose 'New', and fill in the dialog boxes, with 'Spectral Calibration' as the application. Fill in the applicable dialog boxes on the

Spectral Calibration Editor as follows (clicking 'OK' when complete to save):

Sample Name: *date_Spectral*

Priority: May optionally be changed to a number <100 for injection priority.

Instrument Protocol 1: Choose the Spectral instrument protocol from the drop down menu

PowerPlex 16 specific run module and protocol settings for Spectral Calibrations are as follows:

Module Type:	Spectral
Template:	Spect36_POP4
Inj. kV:	3
Data Delay Time:	100
Run Time (seconds):	800
Protocol Type:	Spectral
DyeSet:	F
Array Length:	36
Chemistry:	Matrix Standard
Lower condition bound:	4.0
Upper condition bound:	12.0

Inj.Secs: 5

4.1.2.2 For a sample plate finish filling out the 3130XL Load Sheet on the database worksheets template (Form 312-BI). Print a copy for the batch record/file. Choose the '3130xl Plate Document' tab and ensure the information corresponds to the Load Sheet information. Verify the information on the template is as follows:

Container Type: 96-Well

Application Type: regular

GeneMapper: GeneMapper_Generic_Instance

Sample Name:

Allelic Ladder: LADDER (or PP16_LADDER)

Controls: POS [or (+), etc.], NEG [or (-), etc.], RB (reagent blank)

Database samples: ID########## (e.g., ID2001001412).

Priority: May optionally be changed to a number <100 for injection priority.

Sample Type: Sample categories of 'Sample', 'Allelic Ladder', 'Positive Control', or 'Negative Control', may optionally be typed in.

Results Group 1: Enter the appropriate results group. These are typically denoted by the analyst initials and should automatically populate from the Load Sheet.

Instrument Protocol 1: Enter the appropriate instrument protocol (e.g., PP16_5 sec, PP16_3 sec, PP16_10 sec).

PowerPlex 16 specific run module and protocol settings for sample plates are as follows:

Module Type: Regular
Template: HIDFragmentAnalysis36_POP4
Inj. kV: 3
Run Time (seconds): 2000

Protocol Type: Regular
DyeSet: F

Inj.Secs: 3-10

3 secs – Generally produces good results for Allelic Ladders and

samples ≥.50ng (injection times may be adjusted [3-10 seconds per analyst's discretion).

5 secs.- Samples <.50ng generally produce good results.

4.1.2.3 Delete any unused wells. Perform a 'Save As' of the Plate Template Worksheet to disc (i.e. USB drive) for subsequent transfer to the ABI 3130xl. The document must be saved as a .txt file (tab delimited).

4.1.2.4 Import the previously saved plate record by selecting 'Import' on the 'Plate Manager' window. Browse to locate the saved .txt file and choose 'OK'.

4.1.2.5 Open the imported plate record by highlighting it and clicking 'Edit'. Review the information in the GeneMapper Plate Editor to ensure that it is correct or make changes as necessary. Click 'OK' when complete to save the plate record.

4.1.3 In the manual control window, the scientist may choose to set the oven to 60˚C so that it will be ready to run. Choose Oven in the 'Send Defined Command for' drop down menu box. In the 'Command Name' box, choose Turn On/Off oven, with a 'Value' of On, and click 'Send Command'. Next, in the 'Command Name' box, choose Set oven temperature, with a 'Value' of 60.0 and click 'Send command'. **Note:** once the oven has been turned on and the temperature set, the oven will only preheat for 45 minutes before shutting itself off.

4.1.4 Prepare samples for capillary electrophoresis:

4.1.4.1 For amplified products (including controls), typically 1µℓ-1.5µℓ rxn is added to 10µℓ of ILS Master Mix (made by adding 0.5µℓ ILS600 size standard/sample; 9.5µℓ deionized formamide/sample and adding quantities for N+2 in Master) that has been dispensed into the wells of a

pre-labeled plate. For Allelic Ladders add 1µℓ Ladder to 10µℓ Master Mix. **Note:** The master mix may be altered by adding 0.25µℓ, 0.5µℓ, or 0.75µℓ ILS600 size standard to 9.75µℓ 9.5µℓ, or 9.25µℓ deionized formamide respectively to account for ILS peak height variability.

4.1.4.2 Matrix samples are diluted 1:10 in Nanopure H_2O (a 1:5 dilution may be necessary). 5µℓ of each matrix dye fragment is then added to 480µℓ of deionized formamide (without size standard). Load 25 µℓ of the fragment mix into each of 16 wells on the pre-labeled plate, which will include each of the 16 capillaries (e.g. well's A1 through H1 and A2 through H2).

4.1.5 Following sample addition, place a plate septa on the plate and heat denature for ~3 minutes at 95˚C. Immediately chill in benchtop cooler (or on ice) for ≥3 minutes (perform on all sample types – ladders, matrix, controls and samples). **Note:** the plate septa may be cut to cover only those well columns being used on smaller plate runs.

4.1.6 Place the sample plate into the plate base and secure the plate retainer clip on top, making sure that no gray is visible through the holes.

4.1.7 Place the plate assembly in the instrument in either position A, or position B and close the doors. The plate map on the 'Plate View' window, under 'Run Scheduler' will turn yellow when the plate is in place and has been detected by the instrument. **Note:** the 3130xl will accommodate two plates per run.

4.1.8 Prior to running the plate, confirm that dye set F is selected and the correct active calibration for dye set F is set in spectral viewer.

4.1.9 Locate the plate record in the 'Plate View' window and highlight it by clicking on it once. With the plate record highlighted, click the

appropriate plate map (position A or position B) to link the plate to that specific record. The plate map will turn from yellow to green when it is successfully linked. Verify the correct scheduling of the run in the 'Run View' window. Select a run and confirm that the corresponding wells highlighted in the plate diagram are correct for that run. Make adjustments to the plate record if necessary.

4.1.10 Click the green Run Instrument arrow button in the toolbar to start the run. Monitor electrophoresis by observing the run, view, array, or capillaries viewer window. Each injection (set of sixteen samples) will take ~45 minutes. **Note:** to run a duplicate plate record, the plate may need to be unlinked prior to linking the duplicated record. This is done by highlighting the currently linked plate record and clicking 'unlink'.

4.1.11 After completion of the spectral calibration run, open the 'Spectral Viewer' window to evaluate the spectral and set the active calibration. Confirm that Dye Set F is selected. Click on individual wells in the plate diagram to see results for each of the sixteen capillaries. For each capillary, verify that four peaks are present in the spectral profile (upper pane), that the order of the peaks are, from left to right, blue-green-yellow-red, and that the peaks are regular in appearance. Next verify that four peaks are present in the raw data profile (lower pane), that the order of the peaks are, from left to right, red-yellow-green-blue, and that the peak heights are above 750RFU (1,000-4,000 RFU is ideal). If at least 12 capillaries pass, then the calibration should be saved and set as the active calibration.

Note: A minimum of 12 of the 16 capillaries must pass in order to accept a spectral calibration. A passing capillary will be colored green in the plate diagram. Additionally, capillary status may be viewed in the 'Event Log' under 'Instrument Status'. Rerun the spectral

calibration as necessary until at least 12 capillaries pass.

4.1.12 After completion of the run finish filling out the 3130xl Injection Log (Form 422-QC).

4.2 DATA ANALYSIS: GENEMAPPER® ID (GMID)

4.2.1 Data analysis is NOT performed on the instrument computers. Transfer the run folder (including the 3130xl plate record) to an analysis computer using a portable USB drive. After analysis and review are complete, a copy of the run folder, GMID project(s), RAW table, I-cubed batch(es) and output/audit files will be stored on the ISPFS network drive. The Run Folder on the instrument computer may be deleted at this point.

Note: prior to data analysis, the appropriate panels and bins must be imported into GeneMapper® ID. Additionally, 'Duplicate homozygous alleles' must be unchecked on the analysis tab under tools > options.

4.2.2 Set up the analysis method for GMID analysis as follows (analysis methods are created and stored in the 'Analysis Methods' tab in 'GeneMapper Manager'):

'General' Tab: Name the analysis method so that it reflects what the method is (e.g. i3 method).

'Allele' Tab: Choose the appropriate i^3 bin set. Uncheck 'Use marker-specific stutter ratio if available', and ensure 'minus stutter distances' are from 3.25 to 4.75 for tetra and from 4.25 to 5.75 for penta. All others should be 0.

'Peak Detector' Tab: Advanced Peak Detection Algorithm, full analysis range, partial sizing (80-600), light smoothing, Local Southern size calling method with baseline window of 51 pts, min. peak half width = 2, polynomial degree = 3, peak window size = 15, slope thresholds = 0, peak amplitude threshold B/G/Y = 30, and peak amplitude threshold R = 20.

'Peak Quality' Tab: The peak quality settings are not used for analysis and do not affect the data captured for i^3 import.

'Quality Flags' Tab: The quality flags are not used for analysis and do not affect the data captured for i^3 import.

4.2.3 Create and store a size standard for GMID analysis, under the 'Size Standards' tab in 'GeneMapper Manager'. Name the size standard so that it reflects what the standard is (e.g. ILS600 80-600).

Data analysis will be performed using the 'Basic or Advanced' size standard. The size standard consists of the following peaks: 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, and 600 (the 60 peak may be optionally defined by the analyst).

4.2.4 Create and store a raw export table for i^3 import under the 'Table Settings' tab in 'Genemapper Manager'. Name the table 'RAW'.

'Samples' Tab: The columns displayed may be selected according to analyst preference and do not affect the data captured for i^3 import.

'Genotypes' Tab: Select sample file, sample name, marker, size, height, and peak area. Deselect all others. Set number of alleles to 50 and check 'keep allele, size…together'.

4.2.5 Create a GeneMapper® ID Project:

4.2.5.1 From the GMID main menu, select File/Add Samples to project. Highlight the appropriate run folder in the pop-up window and click 'Add to List'. Once the run folder has been copied to the column on the right, click 'Add' to populate the

project with the samples in the run folder.

4.2.5.2 In the Samples table, for each sample/control, select the sample type, analysis method, panel, and size standard from the pull-down lists.

4.2.5.3 Save the project as the date MMDDYY, followed by the analyst' initials (and any other descriptors that may be necessary). **Note:** the analyzed project will be exported to the run folder at the completion of analysis/review.

4.2.5.4 Analyze the samples by clicking the green Analyze button. If the project has not already been saved, a prompt will appear to enter a project name before analysis will commence.

4.2.6 Evaluate GeneMapper® ID Data:

4.2.6.1 Check the 'SQ' (sizing quality) for all samples. A green square indicates that the sample has passed the sizing criteria and need not be manually examined. Examine the size standard of each sample with yellow and/or red 'SQ' to confirm correct assignment of fragment sizes. Highlight the sample(s) of interest and click the Size Match Editor button. If necessary, adjust the peak assignments by right clicking on a peak and deleting, adding, and/or changing values. If all peaks are correctly labeled but the quality score is below 1.0 (may be checked by choosing Tools/Check Sizing Quality), click the 'Override SQ' button to set the SQ to 1.0. Once all edits have been made, click 'OK' to save the changes and close the Size Match Editor (clicking 'Apply' saves the changes but leaves the Size Match Editor open). These samples are ready for reanalysis in the project window. Samples with a failed

size standard must be deleted from the project prior to export for i³.

4.2.6.2 Select the RAW table on the Genotypes tab and sort the data by: Sample File (ascending), Dye (ascending), and Marker (ascending). Export the table as a tab delimited text and save with the same name as the project.

4.3 DATA ANALYSIS: I-CUBED (i³)

4.3.1 Setup a multiplex and ruleset as follows (multiplexes/rulesets are stored in Multiplex Manager and may be exported/imported as needed):

Multiplex = Powerplex 16
Ruleset = general
Sequencer = 3130xl
Advanced Ladder Settings = 75 rfu, 1 to pass, 1.7 bp anchor sizing window, lowest deviation, 6 deviation from previous, and 0.8 bp tolerance
Filter = Height and 2nd Main (check 'use height or area instead of percentage)
Allele Threshold = 75
Peak acceptance = 0

Loci/Anchor points, controls, profiles (i.e. full, partial, mixture) and virtual ladder alleles are also defined within Multiplex Manager.

4.3.2 Define the rules for the Powerplex 16/general multiplex/ruleset as follow:

Amelo: Apply to data, flag, apply after filter, check for X
CrossOver: Apply to data, flag, apply after filter
Degradation: Do not apply, do not flag, apply after filter, use area, upper limit 5, lower limit 0.15
Extra Allele: Apply to data, flag, apply after filter, use height, cut off 75, 3 alleles to fire, 2 to flag as mixed

Extra Peak: Apply to data, flag, apply after filter, use height, threshold 75, 1 extra peak to fire

High Signal: Apply to data, flag, apply after filter, use height, threshold 8500

Known Profile: Apply to data, flag, apply after filter

Lane to Lane: Do not apply, do not flag, apply after filter, use height, threshold 75, sizing 0.2

Low Heterozygote: Apply to data, flag, apply after filter, use height, threshold for rule 100, threshold not to designate 75, apply after stutter

Low Homozygote: Apply to data, flag, apply after filter, use height, threshold 350, threshold not to designate 75, use second threshold

minusA: Apply to data, flag, apply after filter, use height, 5% threshold, 0.3 bp tolerance

Negative: Apply to data, flag, apply before filter

Noise: Apply to data, flag, apply before filter, use height

Off Ladder: Apply to data, flag, apply after filter, tolerance 0.495

Peak Morph: Apply to data, flag, apply after filter, use advanced, do not designate above 0.3, upper 0.175, lower 0.05, do not designate below 0.01, flag all firings

Pref Amp AB/AC/BC: Apply to data, flag, apply after filter, use height (AB), 50% above, 50% below, high 200, low 75

Pref Amp AD/BD/DC: Do not apply, do not flag, apply after filter, 50% above, 50% below, high 200, low 75

Primer Dimer: Do not apply, do not flag, apply after filter, use height, upper 95, lower 30, threshold 1000

Pull Up: Apply to data, flag, apply after filter, use height, 35% threshold, 0.35 tolerance

Rares: Apply to data, flag, apply after filter, designate OL

Signal:Noise: Apply to data, do not flag, apply after filter, use area, 95% threshold

Split Peak: Apply to data, flag, apply after filter, do not apply to Amelogenin

Stutter: Apply to data, do not flag, apply after filter, use height, 0.2 bp tolerance, 23% threshold 4 bp for tetras and 5 bp for pentas, filter from major genotypes

Uncorrelated: Do not apply, do not flag, apply after filter, 0.5 deviation

4.3.3 Create an FSS-i^3, i-STRess batch:

4.3.3.1 From the Commands Window, click on the Powerplex 16 batch import option. Browse to the saved GMID RAW file and double click, or highlight and choose open.

4.3.3.2 Check the pass/fail status of the ladders. Each ladder may be viewed by clicking the < and > buttons. Individual loci may be viewed by selection under the locus column, as well as alleles by clicking on the table. Ladders and loci may be manually accepted or failed using the allelic ladder commands. Once the minimum number of ladders have passed, click the 'Designate Alleles' button. A message indicating that alleles were successfully designated will appear. Click the 'Apply Rules' button and verify the settings to proceed with analysis.

4.3.4 Evaluate i^3 data:

4.3.4.1 The first sample to appear in the view window is the first sample flagged for review. Samples meeting all of the acceptance criteria defined by the multiplex/ruleset do not require viewing by the analyst or technical reviewer. Samples flagged by i^3 as a potential mixture appear yellow on the lane bar at the top of the window. Those with rules fired appear gray (more rules fired results in a darker shade of gray). White lanes are samples that passed without review, green indicate passed ladders, and red indicate failed controls. Use the <<, <, > and >> buttons to navigate through the flagged

loci. Individual samples may be selected by clicking on the corresponding lane within the bar.

4.3.4.2 Data may be viewed in variety of ways, such as maximizing, major/minor designations, ladder overlays, or various locus combinations by right clicking in the spikogram, using the A, AB, B radio buttons, or the shift and F (function) keys to help evaluate the rule(s) fired. A red bar within the spikogram window designates the locus being viewed. Individual loci may be selected to view within a sample by clicking on the locus in the Major Designation panel. Individual alleles/peaks may be highlighted red by clicking on the corresponding row in the data table.

4.3.4.3 Evaluate the allele designations and rules fired using the spikogram, major/minor designations, rules, and rule firings windows, as well as the data table. When multiple rules fire, the rule firing information can be viewed by highlighting each individual rule. Alleles that cannot be designated by i^3 are listed as an F (fail) in the major designation window. Those that are not in the defined ladder are listed as OL and alleles that are in the virtual ladder are listed as OL followed by the allele value (e.g. OL 9.1). Based on the rule evaluation, allele designations may be changed by entering the correct value or using the dropdown menu. Minor alleles may be entered by choosing the B radio button or by right clicking on the major designation window. Enter any comments (e.g. tri-allele values) in the comments box (clicking ctrl + enter will add the comment to all loci within the sample). Click the “Go” (>) button next to the allele value window to save the change before moving to the next flagged locus. The changed allele will turn red

in the major designation window and the lane bar will turn orange to indicate a change to the sample.

4.3.4.4 Off-ladder (< or > smallest or largest ladder allele, respectively) or microvariant (alleles with incomplete repeats) allele(s) labeled by i^3 as OL, as well as tri-allelic samples shall be re-analyzed for verification (reinjection may be sufficient for off-ladder and microvariant alleles; however, tri-alleles shall be reamplified for verification). The initial run containing the microvariant or tri-allele will be marked as failed (F) for the individual locus and the genotype to be verified noted in the comments box in i^3 as well as on the reinjection summary (form 316-BI). Upon verification, microvariants will be labeled and reported as "X.Y" (where X is the number of complete repeats and Y is the number of base pairs of the incomplete repeat). Off-ladder will be reported as > or < the largest or smallest CODIS ladder allele, respectively. **Note:** Microvariant alleles within the virtual ladder (those listed as OL followed by the allele designator, e.g. OL 9.1) will be changed to the allele designator and need not be reanalyzed.

4.3.4.5 When all flagged loci have been evaluated and allele changes made, save the batch then click the 'output' button. Choose the CODISexport option and click 'OK'. Any sample with a failed (F designation) allele will be designated by i^3 as a partial profile and will not be included in the export file. Additionally, i^3 only exports the major allele designations. Samples with minor designations (i.e. tri-alleles) will require manual editing of the locus in CODIS.

4.3.5 Create a .cmf file for CODIS import:

Note: As a verification of file completeness and sample eligibility, the .cmf file will be created by the technical reviewer at the completion of the batch review.

4.3.5.1 Open the FSS Export Tool and click Import File. Browse to the CODISexport.ici file to be used and double click or highlight and choose 'open'.

4.3.5.2 Select CMF 3.2 for the export format and enter the laboratory ORI (ID001075Y) as the source and destination laboratories. Verify Convicted Offender is the specimen category listed and change if necessary.

4.3.5.3 Review the samples listed in the Batch Information window to confirm all eligible samples are present. Uncheck any samples that should not be included in the file. When samples have been verified, choose Export File to save the .cmf.

4.4 STR INTERPRETATION GUIDELINES

4.4.1 CONTROLS

4.4.1.1 The purpose of a **REAGENT BLANK** (RB) is to determine if the reagents used for DNA extraction/isolation were contaminated with human DNA and as a method for monitoring facility decontamination. Peaks above threshold should only appear in the CXR (red dye) lane, corresponding to the ILS600 size standard. If contamination is flagged by i^3 in a reagent blank it shall be reinjected to assist in determining the point of origin (i.e. pre or post amplification, capillary carryover, etc) where possible. Sample data may be deemed acceptable if the contaminant is isolated to the RB (see comment 5.2). Alleles above the analysis

threshold will be designated and a comment indicating contamination made in i^3 and on the reinjection summary (form 316-BI).

The reagent blank shall be treated the same as the least concentrated DNA sample in terms of volume and amount amplified, injection time/amount, and analysis threshold. Additionally, the reagent blank will be reamplified with samples from the set, where applicable, if any of the amplifications conditions are more sensitive than the original.

4.4.1.2 The purpose of the **POSITIVE AMPLIFICATION CONTROL** (2800M DNA supplied with the PP16 HS kit) is to assess the amplification process, ensuring that adequate sample amplified simultaneously would produce an appropriate signal. All expected alleles (see below) must be detected above the analysis threshold in at least one positive control (if amplifying more than one) or all of the samples associated with amplification will be deemed inconclusive. If amplifying a single positive control, data may be deemed acceptable if all alleles are present (though some are below 75-rfu threshold) **AND** the extraction control appears as expected (i.e. the problem is confined to the 2800M sample).

LOCUS	GENOTYPE	LOCUS	GENOTYPE
D3S1358	17,18	TH01	6,9.3
D21S11	29,31.2	D18S51	16,18
Penta E	7,14	D5S818	12,12
D13S317	9,11	D7S820	8,11
D16S539	9,13	CSF1PO	12,12
Penta D	12,13	AMELOGENIN	X,Y
vWA	16,19	D8S1179	14,15
TPOX	11,11	FGA	20,23

4.4.1.3 The purpose of the **NEGATIVE AMPLIFICATION CONTROL** is to determine if any human DNA contamination occurred in the process of amplification set-up (or beyond that point) and as another method of monitoring facility decontamination. Peaks above threshold should only appear in the CXR (red dye) lane, corresponding to the IL600 size standard. If contamination is flagged by i^3 in the negative amplification control it shall be reinjected. Sample data may be deemed acceptable if the contaminant is isolated to the negative control (see comment 5.2). Alleles above the analysis threshold will be designated and a comment indicating contamination made in i^3 and on the reinjection summary (form 316-BI).

4.4.1.4 The purpose of an **EXTRACTION CONTROL** sample is primarily to assess correct genotyping, however, it does take measure of all of the steps in the analytical process from extraction through allele designation. The extraction controls consist of previously typed buccal samples on FTA cards prepared in batches. An extraction control must be run on every sample plate. Failure of the extraction control, if isolated to that sample, will not deem other samples inconclusive.

4.4.2 RFU THRESHOLD:

4.4.2.1 For Offender database samples, a minimum of 75 rfu must be achieved for data acceptance. If necessary, repeat the injection, changing the injection time (3-10 seconds allowable range) and/or change the amount of amplified product added for fragment analysis (e.g. 1.5 ul). Samples that would not benefit from either option and/or fail

the second injection will be re-queued for re-extraction and amplification.
Note: Samples which still produce incomplete profiles (i.e. do not meet the current CODIS acceptance criteria) following re-queue may be failed and a new sample requested.

4.4.2.2 Peaks flagged by i^3 as present but below the analysis threshold will be designated as failed (F).

4.4.2.3 Off-scale data causing excessive artifacts (e.g. split peaks, increased stutter, pull-up, etc.) within a sample which interfere with data interpretation will be designated as failed (F). The sample may be diluted and re-injected (3-10 seconds).

4.4.2.4 Multiplex amplification kits are designed so that heterozygous loci in single-source samples generally demonstrate relatively balanced peak heights [typically ≥50% peak height ratio (phr) for reduced volume amplification]. Some samples, although single-source, may at times demonstrate greater imbalance due to degradation, stochastic effects, primer binding site mutations, preferential amplification, etc. Peak height ratios <50% will be flagged in by i^3 for evaluation. Samples with increased peak imbalance may still be deemed acceptable.

4.4.2.5 Low template samples may exhibit preferential amplification of alleles within heterozygote pairs. This could result in one of the alleles appearing below the analysis threshold or in its complete loss (drop out). A low homozygote (i.e. stochastic) threshold is established, above which single alleles are considered to be relatively free from drop out. Apparent homozygous alleles which fall below 350 RFU will

be flagged by i[3] for evaluation. Alleles below this threshold may still be deemed homozygous.

4.3.3 EXTRA PEAKS (NON-MIXTURES)

4.3.3.1 PCR amplification of STR loci typically produces a minor product peak one core repeat unit shorter than the main allele peak (n-4 for tetranucleotide loci and n-5 for pentanucleotide loci). This minor peak is referred to as the **stutter** peak. Percent stutter generally increases with allele length and can change with the quantity of input DNA/peak height. The measurement of percent stutter may be unnaturally high for main peaks that are off-scale or due to problems with spectral performance. N+4 and N-8 stutter may also be seen. Stutter above 23%, as well as some n+4 and n-8, may be flagged by i[3] for evaluation. Samples with increased stutter may still be deemed acceptable.

4.3.3.2 Electronic or fluorescent **spikes** are random events that produce generally spike-shaped peaks in most or all dye colors at the same location (equivalent bp size) within a single injection. Peak heights usually vary between dye colors for a given spike. These anomalies are generally not reproducible and will typically be eliminated if reinjected. Spikes generally have a higher ratio between their height and area than that of an allele. If the spike is flagged by i[3] and interferes with data interpretation, the sample should be reinjected.

4.3.3.3 Dye **"blobs"** are anomalies that typically occur in the same approximate location in multiple injections and do not always disappear upon reinjection.

They may occur in a single color or multiple colors at the same approximate location but can vary in height. Dye blobs/artifacts generally have a lower ratio between their height and area than that of an allele. Samples with flagged blobs/artifacts may still be deemed acceptable.

4.3.3.4 **Bleed-through** or pull-up peaks are a result of the spectral not correcting for all of the spectral overlap (most common with the PowerPlex 16 HS kit from yellow into red) and may be increased due to off-scale peaks. These pull-up peaks are in the same location (same size +/- .35 bp) as peaks in another color(s) and are generally easy to recognize. Samples with flagged pull-up may still be deemed acceptable. If excessive bleed-through occurs in a color other than red, and is not due to off-scale data, a new spectral may need to be performed.

4.3.3.5 Taq Polymerase can catalyze the addition of a single nucleotide (predominantly adenosine) to the 3' ends of double stranded PCR product, resulting in product one base pair longer than the actual target sequence (+A). Amplification parameters include a final extension time, so that the reaction is driven to full A addition (i.e. all product is +A). **Split-peaks** may occur as a result of incomplete A addition and appear as a single allele represented by two peaks one base pair apart (-A and +A). This can occur when the amount of template DNA is too great (overloaded sample). In this instance, Taq is unable to add the A nucleotide to the entire amount of product generated in the time allotted. These samples will typically contain off-scale data as well. Split peaks can be

alleviated by diluting the sample or by incubating samples at 60°C for an additional 45 minutes, followed by dilution, prior to reinjection. It may be necessary to re-queue the sample for re-extraction and amplification.

4.3.4 MIXTURES

4.3.4.1 If, after the elimination of possible stutter and/or bleed-through, a profile shows at least 3 alleles at 2 or more loci, this is strong evidence of a mixture.

4.3.4.2 Loci that demonstrate only two alleles but have a heterozygous ratio of <50% may also be indicative of a mixture. However, if data are obtained from multiple loci, a scientist should expect to see other mixture indications (> 2 alleles) at additional loci.

4.3.4.3 Samples determined to be mixtures, with extraneous peaks above the analysis threshold at three or more loci, will be designated as failed (F) and re-injected. A comment indicating contamination will be made in i3 and on the reinjection summary (form 316-BI). If the extraneous peaks persist upon re-injection, the major and minor alleles may be designated with a contamination comment (see comments 5.2). If a major profile cannot be determined, the sample will be designated as failed (F) and re-queued for re-extraction and amplification. **Note:** If the mixture is still present following re-queue (i.e. the sample was contaminated upon collection, or in rare instances is a mosaic), the sample may be failed and a new sample requested.

5.0 **Comments**:

5.1 The 3130xl Data Collection Software does not allow the entry of spaces or dashes in titles, sample names, etc. An underscore must be used in place of spaces when entering information.

5.2 Low level contamination may be seen periodically with the automation process. These extraneous peaks may be disregarded so long as they do not interfere with allele calling in the samples.

5.3 Additionally, low level carry-over may be seen occasionally on the 3130xl. The carryover may also be disregarded in samples and negative controls, so long as the extraneous peaks do not interfere with allele calling, and the peaks can be attributed to the previously injected sample in the corresponding capillary.

DRIFTCON FFC: TEMPERATURE VERIFICATION

1.0 BACKGROUND:

Successful DNA amplification is dependent on consistent thermal cycling parameters and achieving proper heating and cooling to facilitate the various steps necessary for PCR. It is necessary to employ a method of monitoring the thermal cycler performance and verify that the correct temperature has been achieved in order to have confidence in the amplification process. A variety of temperature probes have been developed to test the temperature of specific wells within the different thermal cycler instruments. However, most of these have proven to be cumbersome, time consuming, and not amenable to testing the newer real-time PCR instruments. The Driftcon FFC is able to test multiple temperatures within a short period of time and can be used on the thermal cyclers, as well as real-time instruments.

Driftcon Operations Manual, version 1.1

Driftcon Quick Start Guide, version 1.4

2.0 SCOPE:

To provide a reliable method for verifying the performance of laboratory thermal cyclers, to include real-time PCR instruments.

3.0 EQUIPMENT/REAGENTS:

Computer with Driftcon Software
Driftcon Hardware Module and Cables
Driftcon FFC Fixture
Driftcon Smart Card
ABI 7500 FFC Adaptor
Cork Leveler

4.0 PROCEDURE:

4.1 9700 THERMAL CYCLER VERIFICATION

4.1.1 Set up the Driftcon by connecting the hardware module to the computer and FFC fixture/probe plate with the provided cables.

4.1.2 Insert the smart card into the Driftcon hardware module until it stops.

4.1.3 Turn on the Driftcon computer. Open the Driftcon Software and login with the appropriate user name and password.

4.1.4 Turn on the 9700, place the fixture into the plate with the cable facing out, and select/start the 'Driftcon' protocol. **Do Not** close the 9700 lid as it will damage the fixture and void the warranty.

4.1.5 In the Driftcon software, choose the instrument to be tested. If the list of instruments does not appear automatically, click start and follow the wizard prompts.

4.1.6 Choose the Driftcon default protocol and make sure the steps match those in the 9700 protocol. Leave the humidity, pressure, and temperature blank. Enter notes as needed (these will appear on the final report).

4.1.7 Start the test by clicking the check mark in the lower right corner. Make sure the 9700 has heated completely and the protocol started before starting the test. The protocol will complete in ~25 min.

4.1.8 Once the run has completed, print the report and place it in the QC binder.

4.2 7500 REAL-TIME INSTRUMENT VERIFICATION

4.2.1 Set up the Driftcon by connecting the hardware module to the computer and FFC fixture/probe plate with the provided cables.

4.2.2 Insert the smart card into the Driftcon hardware module until it stops.

4.2.3 Turn on the Driftcon computer. Open the Driftcon Software and login with the appropriate user name and password.

4.2.4 Turn on the 7500 computer, login with the appropriate user name and password, and open the 7500 SDS software.

4.2.5 Turn on the 7500. Remove the tray from the plate loader and replace it with the FFC adaptor. Place the fixture into the adaptor with the cable facing out. Place the cork leveler on top of the fixture and close the plate loader.

4.2.6 In the 7500 SDS software, choose **File > New** and select **Absolute Quantitation** for Assay, **96-Well Clear** for Container, and **Driftcon** for Template.

4.2.7 Save the plate document as a .sds file with the appropriate plate name and open the Instrument tab.

4.2.8 In the Driftcon software, choose the instrument to be tested. If the list of instruments does not appear automatically, click start and follow the wizard prompts.

4.2.9 Choose the Driftcon default protocol and make sure the steps match those in the 7500 protocol. Leave the humidity, pressure, and temperature blank. Enter notes as needed (these will appear on the final report).

4.2.10 Start the 7500 run and Driftcon test. Start the test by clicking the check mark in the lower right corner. Make sure the 7500 has heated completely and the protocol started before starting the test. The protocol will complete in ~25 min.

4.2.11 Once the run has completed, print the report and place it in the QC binder.

5.0 Comments

5.1 The second page of the report contains information regarding the number of measurements for each probe. The number should be approximately double the protocol time. If significantly higher or lower, the instrument may be heating too slowly or too quickly. The percentage should be 100% for each probe. If a percentage is lower than 100%, the probe may not have been in the well and measurements missed. Adjust the probes and run the protocol again.

5.2 Pass/better than specifications/fail data may be found beginning on page four, for each temperature. The measured value, along with the target specifications are shown in this section.