Idaho State Police
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Approval for Quality System Controlled Documents

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Discipline/Name of Document: Biology CODIS and Database AM Manual

Revision Number: 10

Issue Date: 11/29/2010

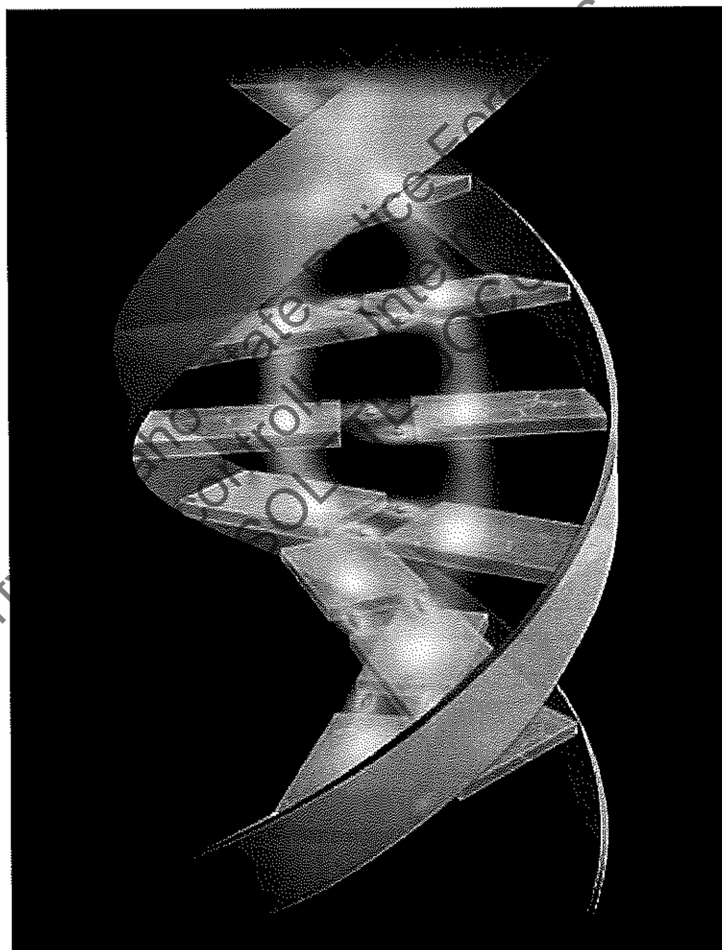
APPROVED BY: ____________________ Quality Manager

11/29/2010 Date Signed

Checklist Submitted and Checked ____________

ISP FORENSIC BIOLOGY

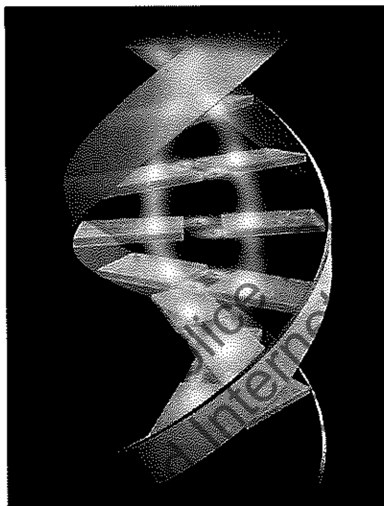
CODIS AND DATABASE ANALYTICAL METHODS MANUAL



Revision 10
11/29/10
Issuing Authority: Quality Manager

Forensic Biology CODIS and Database Analytical Methods Manual

Revision #10



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November 29, 2010

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Cynthia Cunnington
Forensic Scientist IV
Forensic Biology Supervisor/Technical Leader

Idaho State Police
Forensic Services

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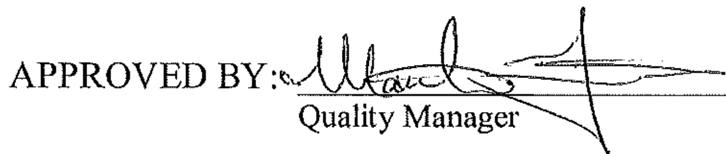

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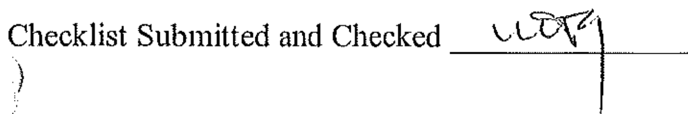

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Forensic Biology CODIS and Database Analytical Methods Manual

REVISION RECORD

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

Date:	The date the revision(s) was completed/effective date.
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

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Forensic Biology CODIS and Database Analytical Methods Manual

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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.

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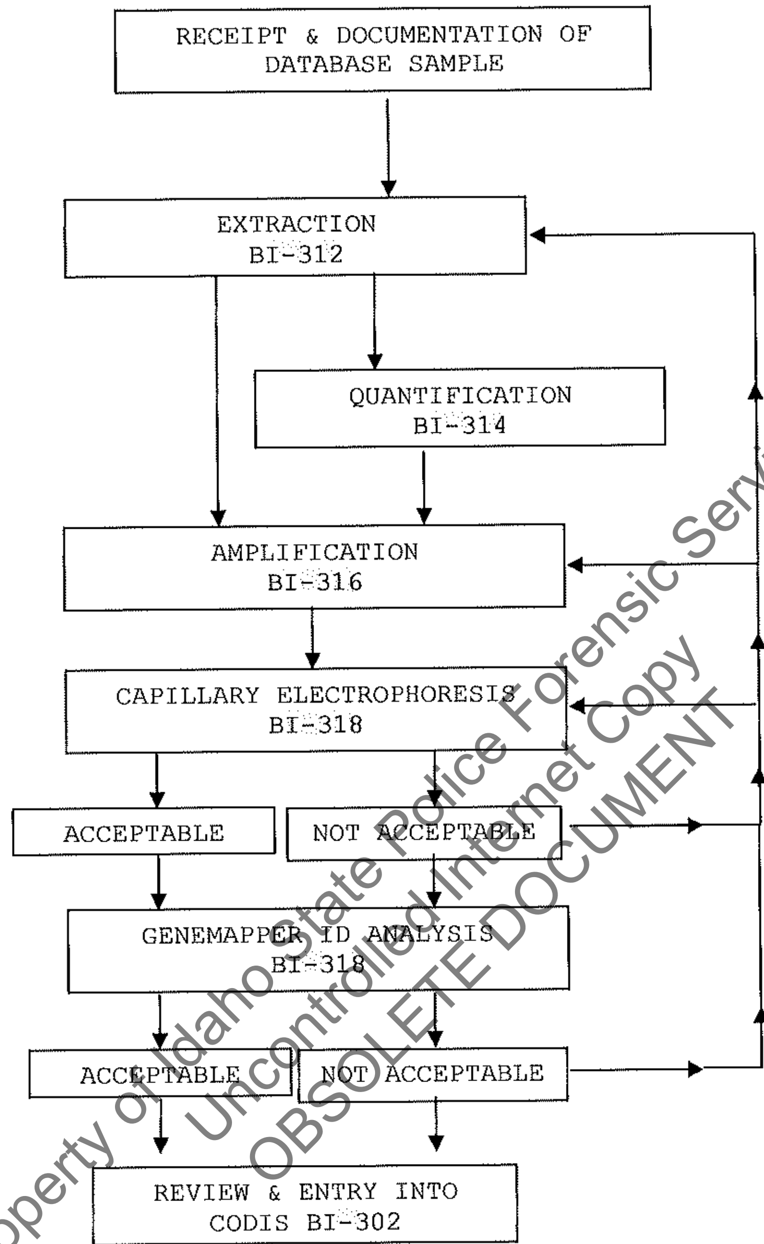

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5.0 COMMENTS:

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).

CODIS 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

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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 and their corresponding DNA Collection Report Forms received by the

laboratory are to be marked with the date of receipt and the initials of the scientist who received them. The sample and report form may be mailed to the laboratory separately; in the event that a sample has not been received, the submitting agency should be notified.

4.1.2 Where possible, compare the DNA sample card information to that of the Collection Report Form to ensure accuracy. Data for a sample may be entered in absence of a DNA Collection Report form. Additionally, 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.corrections.state.id.us) for a number if one has not been recorded. The DNA Collection Report Form will be retained after DNA Tracker data entry.

4.1.3 The offense listed on the DNA sample card and/or Collection Report 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 will 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. The collection report (if present) will be marked to indicate that the sample is a non-qualifying offense and returned

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to the submitting agency. If there is no collection report, a copy of the sample card will be made, marked in the same manner, and returned to the submitting agency. The sample(s) will then be destroyed.

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.

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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, sample card, and/or DNA Collection Report form 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. Click the 'Save' button after each entry.

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4.4.2 Enter each offense and its associated information from the court order, sample card, and/or DNA Collection Report form 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 must 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 placed in the appropriate filing cabinet located in the CODIS office.

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

1) Click on the 'Samples' tab for the saved Offender.

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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, one on the collection report, and one inserted behind the FTA card envelope for placement on the FTA card at the time of analysis.
6) Verify the information is correct and click the 'Save' button. Barcode labels will automatically print upon saving the sample information.
7) Staple the DNA Collection Report (if present) to the inside of the DNA sample folder and attach the appropriate barcode labels. The labeled DNA sample card, with collection report, will be placed in one of the filing cabinets located in the CODIS office.
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.

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BI-302

CODIS SAMPLE DATA ENTRY AND UPLOAD

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. Accurate data entry for upload to NDIS (National DNA Index System) is essential.

It is the responsibility of the Forensic Scientist to generate profiles from convicted offender and/or forensic samples, determine CODIS eligibility, review other scientist's profiles, enter their profiles into CODIS and notify the CODIS Administrator of any potential problems associated with their CODIS DNA profiles. The CODIS Manager is responsible, in part, to serve as the laboratory's chief point of contact for all CODIS communications, maintain CODIS software updates and security, update/add/remove CODIS users, and upload profiles to NDIS.

DNA profiles entered into CODIS will be done so according to CODIS and NDIS Operational Procedures. Idaho currently allows for data entry into the following Indices: Offender (convicted offender); Unidentified Human Remains (deduced missing person, unidentified person); Missing Persons (alleged father/mother, biological child/father/mother, sibling, deduced missing person, missing person, maternal/paternal relative); Forensic Mixture; and Forensic (known and unknown). Profiles entered into the Forensic Index include both solved and unsolved cases in which the profile is associated with a crime and believed to be attributable to the putative perpetrator. Suspect reference samples and profiles matching the victim(s) and/or any elimination samples (e.g. consensual partner samples) may not be entered.

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2.0 SCOPE:

To provide a mechanism to ensure accurate data entry for all offender and forensic database samples

3.0 EQUIPMENT/REAGENTS:

CODIS Computer Workstation

Allele Tables and/or CMF files generated from sample analyses.

CODIS Training Manual

4.0 PROCEDURE:

4.1 SAMPLE DATA VERIFICATION:

4.1.1 Genotypic data (allele calls) are checked for accuracy and verified during the CODIS/Casework Review process and documented on the appropriate form (Form 306-BI and Form 214-BI, respectively).

4.1.2 Forensic and Missing Person/Unidentified Human profiles are verified for CODIS eligibility and Index and documented on the CODIS entry form (Form 218-BI).

4.1.3 Additionally, when 'STR Data Entry' is used to enter individual sample data (generally forensic samples) verification of allele calls and specimen category is performed prior to an SDIS search and NDIS upload.

4.2 SAMPLE DATA ENTRY IN CODIS:

4.2.1 Once sample data accuracy (forensic and offender) has been verified, data may be entered into the CODIS database either by use of the 'STR Data Entry' module to enter specimen ID/info and allele calls for individual samples or by using the 'Import' function for the entry of batches of data contained in a .cmf (or equivalent) file. Refer to the CODIS Training Manual and course documentation for specific steps in accomplishing these tasks.

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4.2.2 A second 'read' must also be entered for the individual samples through 'STR Data Entry'. This may be done by the analyst entering the sample data, or by another analyst logging on and entering the allelic data in the second read box. If a single individual enters both reads, the entire profile (i.e. all loci) for the first read must be entered prior to entering any data into the second read. A 'check' indicates agreement between readers at individual loci and discrepancies in entry must be rectified before saving to SDIS.

4.2.3 When using the 'Import' function, the scientist will open the appropriate .cmf (or equivalent) batch file select 'validate import'. This will ensure that any typos or inconsistencies (i.e., variant allele definitions/equivalencies) will be identified prior to import and may be corrected. Once the batch file has been validated for import (corrections performed if necessary), 'import' is selected by the scientist and the process of importing the batch file data into SDIS will commence.

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4.3 CODIS DATA UPLOAD:

4.3.1 Prior to uploading eligible samples to NDIS, a search of SDIS is to be performed to identify any potential matches at the state level. This is typically done by running the 'Autosearcher' function in the software. See BI-303 for verification of any candidate matches identified during the autosearch.

4.3.2 There are various reasons that some samples present at SDIS should not be uploaded to NDIS. Prior to NDIS upload, these samples will be selected in Specimen Manager and 'unmarked for upload'. Generally speaking, an incremental upload will be performed. In Specimen Manager, 'incremental upload' is checked on the 'upload' pull-down menu and 'send upload' is selected. The upload is sent to NDIS as a message attachment via DNACOMM. If any 'candidate matches' are identified at NDIS, a match message

will appear in DNACOMM and they will also be reflected in Match Manager. For hit verification see BI-303.

5.0 COMMENTS:

5.1 Refer to CODIS Training Manual and course documentation for more specifics if necessary.

5.2 The CODIS software is redundant and there is generally more than one way to accomplish many tasks. Using a mechanism other than that listed here is acceptable.

5.3 The CODIS software is updated periodically and any necessary changes in procedure provided with new updates supercedes those in procedures written prior to update, if appropriate.

5.4 The sample history for convicted offender samples in DNA Tracker will be updated to reflect dates of sample analysis (in progress, complete, failed as appropriate), date sample was outsourced for testing, and/or date profile was entered into CODIS. This is accomplished for individual samples under the 'Samples' tab for the Offender by clicking on the appropriate sample and choosing 'Add History Event'. Multiple samples may be updated using the 'Batch Samples' function at the top of the screen. The current disposition box must be checked as appropriate for the history(s) added.

5.5 Eligible forensic profiles of unknown origin (i.e. profiles not matching any of the known reference samples) identified in the course of casework analysis must first be compared against the 'staff' index before entering the profile into the database. The 'searcher' function is used by hand entering the profile and choosing the 'staff' index. This profile will not be saved upon completion of the search and will need to be entered as outlined above for storage in the database.

5.6 'Searcher' is also used for performing one-time keyboard searches of forensic unknown profiles. A one-time search will only be performed under exigent circumstances and only on single source samples with a minimum of 8 loci present. The DNA profile must have been generated by or on behalf of a criminal justice

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agency, by an accredited laboratory, in compliance with the FBI Director's Quality Assurance Standards (QAS), and must be maintained by a Federal, State, or Local NDIS participating laboratory. Additionally, requests for one-time searches of non-ISPFS profiles must be received from the CODIS Administrator of the laboratory that maintains the profile. When performing a one-time search, the requesting agency will be notified either verbally, or in writing in the event of a negative search result. See BI-303 for verification of candidate matches identified during the one-time search.

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BI-303

CODIS DATABASE HIT VERIFICATION

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. This is accomplished by the electronic storage and maintenance of DNA profiles at the local, state and national levels. Hits are obtained when a candidate match(es) is identified through a database search at any level. Hit verification involves evaluating the candidate match to determine if it is a true match and verification of CODIS offender sample data where necessary and possible.

NDIS CODIS Hit Disposition Reporting & Confirm an Interstate Candidate Match - Operational Procedures

2.0 SCOPE:

To provide a method of sample verification to be performed prior to law enforcement agency notification of a database hit to ensure reporting of only true, confirmed matches.

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3.0 EQUIPMENT/REAGENTS:

CODIS
NDIS Procedure Manual; CODIS Training Manual/documentation
DNA Tracker
AFIS
Offender Database Sample(s)
Equipment/Reagents for STR Analysis

4.0 PROCEDURE:

4.1 MATCH VERIFICATION (Forensic):

4.1.1 For 'hits'/matches involving an ISP Forensic Biology evidentiary sample (either case-to-case or case-to-offender and including one-time keyboard searches) the primary responsibility for

match verification follow-up and disposition lies with the Idaho CODIS Administrator.

4.1.2 The CODIS Administrator or designee (typically the case analyst) will first evaluate the 'Candidate Match' in Match Manager to determine if there is a basis for exclusion and, therefore, disposition as 'No Match'. A 'No Match' disposition doesn't require further verification or, where applicable, notification of the other laboratory involved with the match.

4.1.3 If evaluation in Match Manager demonstrates that the candidate match consists of potential high stringency (e.g., exclusions attributable to different typing systems, Promega v. Applied Biosystems), or possibly moderate stringency in the event of a forensic mixture or degraded sample, the disposition is changed from 'Candidate Match' to 'Pending' until the verification process is complete. In general, for case-to-case matches, the verification will consist of communication between scientists regarding the data and case status, while case-to-offender matches typically necessitate sample verification at the 'offender lab'.

4.1.4 Once the status of the 'candidate match' has been resolved, the disposition is set accordingly (e.g., 'No Match', 'Offender Hit', 'Forensic Hit', 'Conviction Match', 'Investigative Information', etc.) and 'Investigations Aided' filled in as appropriate and as outlined in the NDIS 'CODIS Hit Disposition Reporting' procedure.

4.1.5 If verification results in a 'hit', a hit report is issued by the case analyst. A copy of the hit report, along with the CODIS match report, is filed in the CODIS file. The original hit report and a copy of the CODIS match report are placed in the associated case file. The appropriate law enforcement agency is notified of the 'hit'. If the law enforcement agency submits a sample from the identified offender, appropriate analysis and issuance of a supplemental report will be performed as in **4.2.5**.

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4.2 MATCH VERIFICATION (Offender):

4.2.1 For 'hits'/matches involving an ISP Forensic Biology convicted offender sample the primary responsibility for match verification follow-up lies with the CODIS Administrator for the laboratory with the forensic (evidentiary) sample. However, the initial evaluation in Match Manager (see **4.1.2-4**) and AFIS sample verification (see **4.2.2**) will be initiated as soon as feasible once a verification request has been received from the forensic laboratory.

4.2.2 Once a potential match has been confirmed and a verification request received, the associated offender sample folder will be retrieved from the secure file cabinet and taken to BCI or the latent fingerprint section for an AFIS search of the thumbprint to verify identification of the offender. All documentation will be filed in the CODIS file.

4.2.3 Following AFIS verification of the thumbprint, re-analysis of the offender sample will be performed as appropriate (i.e., if duplicate analysis has already been performed either as a QC function or as the result of a duplicate sample, analysis will not be repeated) prior to agency notification. In situations where a thumbprint was not received with the DNA sample, or is of insufficient quality for verification, a notation will be made and re-analysis for confirmation may proceed. The forensic laboratory (or law enforcement agency for Idaho cases) will be notified that the Offender could not be verified through thumbprint confirmation.

4.2.4 Following sample verification (AFIS and re-analysis as appropriate) the forensic case laboratory, in the case of an interstate hit, or submitting law enforcement agency will be notified of the confirmed hit. Laboratory notification may be made verbally and relevant documentation will be provided to the forensic

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case laboratory as requested. In Idaho, initial notification as well as the request for a new DNA sample from the identified offender, may be made verbally. However, written notification and a formal request for a new DNA sample, in the form of a hit report, will be sent to the appropriate law enforcement agency.

4.2.5 For intrastate offender hits, where possible, a newly obtained DNA sample from the offender will be analyzed with all deliberate speed. The analysis of the forensic sample may also be repeated, though this is not required. A supplemental report will be issued delineating the match in the usual manner (i.e., same treatment as for matching suspect sample submitted with case evidence).

4.2.6 The sample history in DNA Tracker will be updated to reflect the date the hit was confirmed for the offender sample.

5.0 COMMENTS:

5.1 A good faith effort will be made to resolve matches within 30 business days from the date of the match or from the date of the verification request for interstate matches involving an Idaho offender.

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CODIS SAMPLE REMOVAL

1.0 BACKGROUND:

Participation in the National DNA database, in accordance with the DNA Analysis Backlog Elimination Act of 2000, necessitates provisions for DNA profile expungement in the event that a qualifying offender's conviction is overturned. Additionally, the Idaho DNA Database Act of 1996 addresses court-granted expungement requests (I.C. §19-5513). Removal of DNA profile data and/or destruction of biological samples obtained from Convicted Offenders may be necessary as a result of conviction reversal or sample collection/submission errors.

Expungement is defined as the removal of DNA profile data from local (LDIS), state (SDIS) and national (NDIS) databases in response to a court order overturning the offender's conviction of a qualifying offense. Expungement will include the removal of identifying information from other laboratory documentation and destruction of the biological sample from which the offender database DNA profile was generated.

Administrative removal is defined as the destruction of a DNA sample and removal of any records relating to that sample. Examples for which administrative removal may be warranted include, but are not limited to, the collection of a sample from a non-qualifying offender, or the notification by the collection agency that removal is warranted. Generally, the determination that a DNA sample does not qualify for inclusion in the database occurs prior to entry of the profile into DNA Tracker and subsequently CODIS (see BI-301); however, there may be instances when the collection agency provides notification of an error after the profile has been generated. In these circumstances, the profile will be removed from the local, state, and national databases as part of the administrative removal.

NDIS Expunge a DNA Profile - Operational Procedures

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

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2.0 SCOPE:

To provide a protocol for CODIS sample expungement and administrative removal that protects an individual's rights to privacy and maintains the integrity of the Idaho DNA database program. All procedures will be documented on the CODIS Sample Removal Checklist (Form 310-BI).

3.0 EQUIPMENT/REAGENTS:

CODIS Workstation
DNA Tracker Database

4.0 PROCEDURE:

4.1 EXPUNGEMENT

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Prior to removal of any DNA profile data, source identification, or biological sample destruction, the CODIS Administrator or designee will verify: 1) the request for expungement is accompanied by a certified court order that meets the requirements outlined in I.C. §19-5513, 2) that the offender has no other qualifying offense(s) and 3) the identification of the original DNA database sample where possible.

4.1.1 A search of DNA Tracker is performed to establish whether or not the specified sample has been received in the laboratory and if so, whether or not a DNA profile has been generated and/or entered into CODIS.

4.1.2 If the sample has been received in the laboratory, a criminal history check in ILETS will be performed to ensure that the offense for which the expungement is requested is the only qualifying offense. If the Offender has a separate qualifying offense, the sample will not be expunged and a copy of the criminal history check will be retained for documentation.

4.1.3 The associated offender sample folder will be retrieved from the secure file cabinet and taken to BCI for an AFIS search of the thumbprint to verify identification of the offender. The absence of a thumbprint or a poor quality thumbprint does not preclude the sample expungement from proceeding.

4.1.4 The offender record will be removed from DNA Tracker and a sample removal report generated.

4.1.5 The DNA profile, if applicable, will be deleted from CODIS, followed by an upload to NDIS for removal at that level. A written notification of the expungement will be made to the NDIS Custodian and a request for verification of deletion at the national level.

4.1.6 The original DNA Collection Report received with the sample (or a copy of the sample folder when no report is received) will be marked expunged, along with the scientist's initials and date of expungement.

4.1.7 The DNA sample will be destroyed and witnessed by a second scientist.

4.1.8 Administrative paperwork documenting the event will be retained in the CODIS file. The paperwork may contain some administrative information about the sample/individual it was collected from and will include the following, as applicable: the court order/request for expungement, criminal history check and thumbprint verification, DNA Tracker sample removal report, CODIS deletion report(s), NDIS correspondence, CODIS sample removal checklist, and copies of any correspondence with the requesting party.

4.1.9 An expungement notification letter (or letter indicating why expungement did not occur), the DNA collection report or folder copy, and a copy of the CODIS sample removal checklist will be sent to the requesting party.

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4.2 ADMINSTRATIVE REMOVAL

Prior to removal of any DNA profile data, source identification, or biological sample destruction, the CODIS Administrator or designee will verify: 1) the request for administrative removal is in writing from the collecting agency and includes a description of the error resulting in the removal request and 2) that the offender has no other qualifying offense(s).

Removal resulting from an entry error on the part of ISP Forensic Biology staff does not require a written request;

although, the nature of the error should be documented. Requests for administrative removal from a party other than the collecting agency or due to biology staff error will be referred to ISP legal staff for a determination of the appropriate action to be taken.

4.2.1 A search of DNA Tracker is performed to establish whether or not the specified sample has been received in the laboratory and if so, whether or not a DNA profile has been generated and/or entered into CODIS.

4.2.2 If the sample has been received in the laboratory, a criminal history check in ILETS will be performed to ensure that the offense for which the administrative removal is requested is the only qualifying offense. If the Offender has a separate qualifying offense, the sample will not be removed and a copy of the criminal history check will be retained for documentation.

4.2.3 The offender record will be removed from DNA Tracker and a sample removal report generated.

4.2.4 The DNA profile, if applicable, will be deleted from CODIS, followed by an upload to NDIS for removal at that level.

4.2.5 The original DNA Collection Report received with the sample (or a copy of the sample folder when no report is received) will be marked expunged, along with the scientist's initials and date of removal.

4.2.6 The DNA sample will be destroyed and witnessed by a second scientist.

4.2.7 Administrative paperwork documenting the event will be retained in the CODIS file. The paperwork may contain some administrative information about the sample/individual it was collected from and will include the following, as applicable: the request for administrative removal, criminal history check, DNA Tracker sample removal report, CODIS deletion report(s), CODIS sample removal checklist, and copies of any correspondence with the collection agency.

4.2.8 A sample removal notification letter (or letter indicating why removal did not occur), the DNA collection report or folder copy, and a copy of the

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CODIS sample removal checklist will be sent to the requesting party, or submitting agency in the event of an internal entry error.

5.0 COMMENTS:

5.1 An Offender cannot be deleted from DNA Tracker if there are any samples and/or court orders associated with the offender.

5.2 A sample cannot be expunged or deleted from DNA Tracker if there are qualifying offenses associated with the Offender.

5.3 The 'Delete Sample' function will be used to remove a sample from DNA Tracker for an Administrative Removal.

5.4 The 'Expunge Sample' function will be used to remove a sample from DNA Tracker for an Expungement.

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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 6/09), Promega.

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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
MicroAmp Tubes
1.5mℓ microcentrifuge Tubes (1.5mℓ tubes)
65°C Oven
SlicPrep Device
Adhesive Foil Seal
70°C Water Bath
Centrifuge with Swinging Plate Rotor
Biomek 3000 Robotics System with MagnaBot 96 Magnetic Separation Device, 1/4 Inch Foam Spacer, Variomag Teleshake, and Heating Block
Reagent Reservoirs
2.2mℓ Square-Well, Deep Well Plate
1.2 mℓ Round-Well, Deep Well Plate

4.0 REAGENTS:

FTA Purification Reagent
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 DNA EXTRACTION PROCEDURES:

5.1 FTA EXTRACTION FROM CODIS DATABASE SAMPLES:

Note: Since the DNA remains bound to the FTA card, regular, non-filter pipette tips may be used throughout and a single tip may be used for each reagent. A multi-channel pipettor and 96-well reaction plate may be used for larger sample batches.

5.1.1 Remove 1-3 "punches" from the FTA card using a 1.2mm Harris punch (this is accomplished by placing punch firmly on card and twisting 1/2 turn clockwise and 1/2 turn counterclockwise). Eject/punch sample(s) into microAmp tube(s)/plate. Alternatively, the BSD puncher may be used to punch 1 2mm punch directly into the plate (see **5.2.1**).

5.1.2 Add 150µℓ FTA reagent to microAmp tube(s)/plate, gently mix and incubate at RT for ~5 minutes.

5.1.3 Remove and discard FTA reagent from sample(s).

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5.1.4 Repeat **5.1.2-5.1.3** twice.

5.1.5 Add 150µℓ TE to microamp tube(s)/plate, gently mix and incubate at RT for ~5 minutes.

5.1.6 Remove and discard TE from sample(s).

5.1.7 Repeat **5.1.5-5.1.6** twice.

5.1.8 Make sure the punch is at the bottom of the microAmp tube(s)/plate, using a sterile pipette tip if necessary. Place tubes/plate, uncovered in 65°C oven for ≥2 hours.

5.1.9 Proceed to PCR Amplification (see BI-316).

5.2 AUTOMATED DNA IQ EXTRACTION FROM CODIS DATABASE SAMPLES

5.2.1 SAMPLE PUNCHING USING THE BSD600-DUET

5.2.1.1 Fill out the BSD/Biomek Load Sheet on the 'BSD Load Sheet' tab of the Database worksheets template(Form 312-BI). Print a copy for the batch record. 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 .txt file. Save the template as samples are automatically populated on the other tabs for subsequent processing steps.

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.

5.2.1.2 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

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~2mm from the top of the plates, measured at each corner.

5.2.1.3 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.

5.2.1.4 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.

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5.2.1.5 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.

5.2.1.6 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.

5.2.1.7 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.

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

5.2.2 SLICPREP PRE-PROCESSING

5.2.2.1 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 for 15-60 min.

5.2.2.2 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.

5.2.2.3 Centrifuge the device at 1500 x g for 5 min. in a swinging plate rotor. Remove and discard the collar and filter basket upon completion of centrifugation.

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

5.2.3 DNA IQ REAGENT PREPARATION

5.2.3.1 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 and the date the DTT was added. 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.

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5.2.3.2 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 and the date of alcohol addition. 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.

5.2.3.3 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 'Reagents for Solid Support' comments window):

1: Elution Buffer = 1.2ml + (#samples x 100ul)
2: Empty
3: 1X Wash Buffer = 1.5ml + (#samples x 0.3ml)
4: Lysis Buffer w/ DTT = 1.5ml + (#samples x 0.1ml)
5: Lysis Buffer w/ DTT + Resin = [860ul + (#samples x 43ul)lysis] + [140ul + (#samples x 7ul)resin]
6: Empty

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5.2.4 DNA IQ EXTRACTION USING THE BIOMEK 3000 ROBOTICS SYSTEM

5.2.4.1 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.

5.2.4.2 Check the teleshaker connection by choosing **start > programs > teleshaker v.1.2.** In the test program window, choose **options > interface > com 2** and click **Ok**. Select **file > connect.**

5.2.4.3 Open the Biomek Software and choose the 'All Others Method'.

5.2.4.4 Set up the Biomek 3000 deck as follows (outlined in the 'Tools & Labware for Solid Support Method' comments window):

Rack 1 (A1): Tool rack with MP200 (1)and gripper (3)
ML1 (A2): P250 barrier pipette tips
ML2 (A3): P250 and/or Axygen 165ul barrier pipette tips (up to 48 samples)
ML3 (A4): P250 and/or Axygen 165ul barrier pipette tips (up to 88 samples)
ML4 (A5): P250 and/or Axygen 165ul barrier pipette tips (up to 96 samples)
P6 (A6): Heating system
P1 (B1): PCR plate and base
P2 (B2): empty
P3 (B3): Reservoir frame and reservoirs with reagents
P4 (B4): Pre-processed deep square-well plate
P5 (B5): Deep round-well plate stacked on the Magnabot
P7 (B6): 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. If using the Axygen tips, do not use them as the first box (ML1).

5.2.4.5 Click the green run instrument arrow button to begin the run. When prompted, enter the number of columns to be extracted, choose 0 for solid support method, 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.

5.2.4.6 When the run has finished remove and seal the PCR/elution plate. Discard the deep-well plates, remaining buffer, and pipette tips. Clean the reservoirs between runs.

5.2.4.7 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.

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5.2.4.8 Proceed to realtime PCR (BI-314) for quantification of the purified DNA obtained in 5.2.4.6.

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

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

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

7.5 The Biomek 3000 performance verification color plate (Form 428-BI) is run by choosing the 'Color Plate/Performance Check' method in the Biomek Software. Deck set up and dye preparation is described in the 'Instrument Setup' step and the 'Deck Setup Instructions' comment window.

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.

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BI-314

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 the Quantifiler™ Real-Time PCR Kits for the Quantification of Human Nuclear DNA Samples," Green, R.L., et al, Journal of Forensic Science, Vol. 50, No. 4, pp. 809-825.

"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.

Quantifiler™ Kits (Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit) User's Manual, Applied Biosystems.

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

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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
Pipettes and Tips
Reservoir (optional)
Quantifiler™ Human Kit
20 µg/mℓ Glycogen (optional)
96-well Reaction Plate
96-well Reaction Plate Base
Optical Adhesive Covers
Centrifuge (optional)
Microcentrifuge Tubes/Strip Tubes
PCR-TE

4.0 PROCEDURE:

4.1 PREPARATION OF DNA STANDARDS:

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

4.1.2 Dispense 30µℓ (or adjusted amount according to the kit QC results: Form 419-QC) of PCR-TE into tube A (Std. 1) and 20µℓ of PCR-TE into tubes B-H (Std. 2-8).

4.1.3 Mix the Quantifiler Human DNA Standard thoroughly by vortexing 3-5 seconds. Transfer 10µℓ to tube A (Std. 1). Mix the dilution thoroughly.

4.1.4 Prepare Std. 2-8 via a serial dilution by mixing and subsequent 10µℓ transfers from tubes A through H. The dilution series consists of 50, 16.7, 5.56, 1.85, 0.62, 0.21, 0.068, and 0.023 ng/µℓ, respectively.

4.2 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 case record. Choose the '7500 Plate Document' tab and ensure the information is correct and corresponds to the Load Sheet information. Perform a 'Save As' of the Plate Document Worksheet to disc (e.g. USB drive) for subsequent transfer to the ABI 7500. The document must be saved as a .txt 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.

Quantifiler PCR Reaction Mix	12.5µℓ
Quantifiler Human Primer Mix	10.5µℓ

Note: The volume of reaction components necessary to prepare the Master Mix will be automatically calculated upon Load Sheet data entry.

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4.2.4 Thaw the primer mix and vortex 3-5 seconds. Pulse-spin prior to opening the tube. Mix the PCR Reaction Mix by gently swirling the bottle prior to use.

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 primer and reaction mixes into an appropriately sized microcentrifuge tube or reservoir. Mix thoroughly and pulse spin if mixing in a tube.

4.2.7 Carefully pipet 23µℓ of the PCR Master 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 **4.3**.

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4.3 RUNNING THE REACTION:

4.3.1 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.

4.3.2 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.

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

4.3.4 In the SDS software, select **File>New** and choose **Absolute Quantitation** for Assay, **96-Well Clear** for Container, and **Quantifiler Human Database** for Template.

4.3.5 Import the previously saved plate document by selecting **File>Import Sample Set-Up**. Browse to locate the saved .txt file and choose **OK**.

4.3.6 Review the plate document to ensure the appropriate detectors and tasks have been applied to each sample. Change the task for any unused wells to NTC in **View>Well Inspector** or by highlighting and double clicking on a well(s). Make any other changes, as necessary. Select the **Instrument** tab and review the thermal cycler conditions [Stage 1: 1 cycle, 95°C, 10:00 min.; Stage 2: 40 cycles, 95°C, 00:15 min, 60°C, 1:00 min.; 25µℓ sample volume; 9600 emulation; Data collection: Stage 2, Step 2 (60.0@1:00)]

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.

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

4.3.8 Under the **Instrument** tab, click **Start** to begin the run. When the run has completed, proceed to **4.4**.

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4.4 ANALYSIS AND RESULTS:

4.4.1 Open the plate document to be analyzed.

4.4.2 Select **Analysis>Analysis Settings** and verify the settings are set as follows: All for Detector, Manual Ct, 0.200000 for Threshold, Manual Baseline, 3 for Baseline Start (cycle), and 15 for Baseline End (cycle). Click **OK**.

4.4.3 Select **Analysis>Analyze**.

4.4.4 In the **Results** tab, select the **Standard Curve** tab and choose Quantifiler Human as the detector. Review the data for inconsistencies from the following:

An R^2 value of >0.99 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 needs further analysis of the standard curve for problems. Refer to the Quantifiler Kits User's Manual (page 5-6) for troubleshooting guidelines.

The slope should fall within the typical slope range of -2.9 to -3.3. A slope of -3.3 indicates 100% amplification efficiency.

4.4.5 Select the **Amplification Plot** tab (in the Results tab) and choose either the Quantifiler Human, or the IPC detector. Ensure the Threshold is set to 0.20 before proceeding (**Note:** the threshold bar will be green if the data has been analyzed and red if analysis is needed). Highlight the sample(s) of interest in the table to view the associated plot(s). Review the plots for both detectors for amplification and/or inconsistencies.

4.4.6 Select the **Component** tab within the Results tab. The halogen lamp may need replacement if the dye signal lines contain spikes or appear wavy/unstable and/or if the Rox value begins approaching or has fallen below 500. See Comment 2. Note: it is important to use the same sample well each time.

4.4.7 In the Results tab, select the **Report** tab and highlight the sample(s) of interest to view the results. Review the Qty column to determine the amount of DNA present in each sample. Review the Internal Positive Control (IPC) C_T value for each sample. It should fall within a range of 20-30. If the value is >30 for a particular sample, there may be an indication of inhibition.

4.4.8 Export the report. Within the report tab, select **Tools>Report Settings** and check the appropriate boxes to be displayed in the report and click **OK.** Print a copy of the Standard Curve for the batch record. Select **File>Export** to export the report (e.g. to USB drive) as a tab-delimited text file.

4.4.9 Import the tab-delimited text file into the **7500 Raw Data** tab of the Database Excel worksheet/template. Choose the **7500 Results** tab and review the imported

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data. Delete any unused wells from the sheet. Adjust values in the final concentration and ul Sample for dilution columns. Print a copy of the results sheet for the batch record.

5.0 COMMENTS:

5.1 Refer to the Quantifiler Kits User's Manual for specific thermal cycler conditions, additional user information, and troubleshooting guidelines.

5.2 If the Component Dye signals appear unstable and/or Rox values approach 500, 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.

5.3 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.

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BI-316

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

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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:

Thermocycler
Microcentrifuge
Microcentrifuge tubes and/or reservoir
96 well Reaction plate and base
Pipettes and tips
Foil Seal or Optical Adhesive Cover
Compression pad
PowerPlex™ 16 HS Kit Contents

4.0 PROCEDURE:

4.1 DNA TEMPLATE:

4.1.1 Based upon the quantity of DNA isolated and its initial concentration, the scientist should have all samples at an optimal concentration for amplification (e.g., 0.1ng/µℓ). It is also convenient to have all samples, that are to be amplified at the same time, 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 PowerPlex 16 HS, quarter volume reactions. For larger volume samples, it may be necessary to concentrate the sample prior to amplification.

4.1.2 The amount of DNA template added to a quarter volume amplification reaction should be targeted at ~0.25ng.

4.2 AMPLIFICATION SET-UP:

4.2.1 Thaw the PowerPlex HS 5X Master Mix, PowerPlex 16 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) to automatically calculate necessary volumes. Print a copy of the completed worksheet for the batch record. The following is a list of the 'fixed' amounts to be added for a 6.25µℓ reaction.

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

[1]For FTA extracted samples there is no volume for the DNA template so 4.4µℓ of dH_2O will be added to these tubes.

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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 Pipet 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 Pipet each DNA sample into the appropriate well. Use 9947A 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 foil seal. Use a seal applicator to tightly seal between and over each well.

4.2.8 Remove lab coat and, touching <u>only</u> 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.

4.3 THERMAL CYCLING PARAMETERS:

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

4.3.1.1 For DNA IQ-extracted and low level FTA-extracted samples, use 'pp16HS' with the following conditions:

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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

4.3.1.2 For FTA-extracted samples (other than low template), 'PP16HS17' with the following conditions may be used:

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 **17 cycles**, then

60°C for 30 minutes, then:

4°C soak

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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 4°C. For longer storage periods, samples should be frozen at -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 to the dishwashing room. The container will be placed into a second biohazard bag, sealed and disposed of with other biohazardous material.

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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 microfuge prior to dispensing.

6.4 If excessive evaporation is observed following amplification, an optical adhesive cover may be used to seal the plate rather than the foil seal.

6.5 When using a ¼ volume reaction, the amplification reagents may be completely absorbed by the 2mm BSD punch, if used for the FTA extraction method. If this happens, it is recommended that 5ul sterile water be added post-amplification, and thoroughly mixed prior to running on the genetic analyzer.

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, THO1, 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 3130 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.

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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.

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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 Kit HS 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 3130xl Injection Log (Form 422B-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

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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 CODIS 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

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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

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samples ≥.25ng (injection times may be adjusted [3-10 seconds per analyst's discretion).

5 secs.- Samples <.25ng 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.

4.1.2.6 To perform more than one run of a sample (e.g. multiple injection times), select Edit/Add/Sample Run in the GeneMapper Plate Editor window. This will add additional Results Group and Instrument Protocol columns to the end of the plate record. These additional runs may be added at any point in the run, prior to the last injection, if the scientist notices that a sample would benefit from re-injection (e.g., repeat because of bad injection or to vary injection times [from 3-10 seconds]). Additional Results Groups and Instrument Protocols may also be filled in on the original Load Sheet template prior to importing.

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

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'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 if ILS peaks are too high.

4.1.4.2 Matrix samples are diluted 1:10 in Nanopure H_2O. 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. wells 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.

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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

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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 422B-QC).

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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 and GMID project(s) will be stored on an analysis computer until CD/DVD archiving has been completed. 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, previously run Macintosh data must first be converted to PC files using the 'Mac to Win' conversion program.

4.2.2 Set up the analysis methods 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. 3130PP16-150RFU).

'Allele' Tab: Choose the appropriate bin set. Choose '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, partial sizing (80-550 or 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, and slope thresholds = 0.

Analysis range may be set to either full or partial and is empirically determined for each run and/or instrument. When using partial range, the start and stop points are determined by a review of the raw data and choosing points that will not include the primer peaks but will cover the size range of 80 to ≥500 bases.

Peak Amplitude Thresholds will depend on sample quality. Generally 150 rfu threshold in all colors. Rfu threshold may be raised in Blue, Green and Yellow for Allelic Ladders if necessary. Rfu threshold may be lowered to 100 rfu at the analyst's discretion (see **4.3.2** RFU Threshold). Peaks below 100 rfu are deemed inconclusive.

'Peak Quality' Tab: The minimum peak height ratio for Heterozygote Balance should be set at 0.5 for database (CODIS) samples. Set the max peak width to 1.5 bp and pull-up ratio to 0.05. The signal level and allele number may be set according to analyst preference and sample type.

'Quality Flags' Tab: The quality flags are only used as a tool to aid in data analysis and review (i.e. to assist in calling attention to potential artifacts or data quality concerns). These flag settings may be adjusted according to analyst preference and sample quality.

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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 and 600 peaks may be optionally defined by the analyst).

4.2.4 Create a GeneMapper® ID Project:

4.2.4.1 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.

4.2.4.2 In the Samples table, for each sample, select the sample type, analysis method, panel, and size standard from the pull-down lists. Ladders must be assigned the sample type of 'Allelic Ladder' for the analysis to occur. In order to use the control concordance quality flag, all controls must be marked appropriately as either 'Positive Control', or 'Negative Control'. All others may be marked as 'Sample'.

4.2.4.3 Save the project as the date MMDDYY, followed by CODIS (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.

4.2.4.4 Analyze the samples by clicking the green Analyze button. If the project has not

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already been saved, a prompt will appear to enter a project name before analysis will commence.

4.2.5 Evaluate GeneMapper® ID Data:

4.2.5.1 The Raw Data may be reviewed to determine analysis start/stop points, or to identify baseline problems, off-scale data, excessive 'spikes' or other anomalies that may interfere with data analysis and require re-injection or other corrective measures. Expand the run folder located in the navigation pane on the left. Highlight the sample(s) of interest to view the associated sample information, raw data and EPT data. Minimize or highlight the run folder to return to the main project window.

4.2.5.2 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.

Note: Data may still be deemed acceptable without the ILS 60 and/or 600 bp peaks present. If additional peaks are

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assigned because of bleed-through of TMR peaks (typically Amelogenin peaks), the scientist may choose an Analysis Method, with an increased rfu threshold for the red channel to prevent these peaks from being detected, if desired.

4.2.5.3 Examine the blue, green, and yellow allelic ladders. Check that correct allelic assignments were made.

Note: GMID automatically averages all valid ladders in a run for genotyping. Genotypes are assigned by comparing the sizings of unknown alleles from samples with the sizings of known alleles contained within the averaged allelic ladders of each locus. A ladder(s) may be omitted from analysis by deleting it from the main project window prior to analysis.

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4.2.5.4 Data may be examined in various combinations of colors and/or tables to identify bleed-through, spikes, stutter, -A, off-ladder variants, etc. Sample Plots viewed from the 'Samples' tab/window, allows all loci in a given color(s) to be viewed simultaneously. The Sample Plots view from the 'Genotypes' tab/window; however, allows loci to be viewed individually (more than one locus can be viewed at the same time by adjusting the number of panes displayed).

4.2.5.5 GeneMapper® ID includes a series of quality flags (PQVs) to alert the analyst of potential sample quality concerns. A green square indicates that sample data has passed all of the quality checks, but yellow or red indicate that the data has a problem with one or more of the quality checks. A yellow or red flag does not necessarily mean that the data is bad or unusable and the flags are not to be relied on solely. The analyst may choose

to use the PQVs, in combination with manual data examination to aid in the identification of bleed-through, spikes, stutter, off-ladder variants, -A, etc. Once the data has been evaluated and deemed acceptable, the analyst may choose to override the yellow or red Genotype Quality (GQ) flag by right clicking on the flag in the Genotypes Sample Plots view. **Note:** overriding the GQ flag will cause all other flags to turn from the original color to gray.

4.2.5.6 All negative controls (including reagent blanks) should be examined to verify that each displays a relatively flat baseline in blue, green and yellow.

4.2.5.7 Review all samples (including positive controls) for the above listed 'artifacts' and evaluate: peak height and shape, matrix quality, and individual sample profiles. Compare each sample with the allelic ladder(s) and examine for off-ladder or microvariants, signals that were too low to be genotyped and assignment of genotypes to stutter peaks (or minor peaks that may have been subtracted as 'stutter', etc.

4.2.5.8 Reanalyze individual samples with different Analysis Methods, as necessary if the rfu cut-off will need to be changed.

4.2.5.9 Edit peaks as necessary, by right clicking on the peak label and selecting 'add allele', 'delete allele' or 'rename allele'. The allele should be labeled, at minimum with the allele call, however the analyst may select up to four allele labels, including peak height and size, from the 'Plot Settings Editor' window. **Note:** labels added to artifact peaks, such as spike, pull-up, etc. will appear in the Genotypes table as an additional allele.

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4.2.5.10 Samples demonstrating an off-ladder (< or > smallest or largest ladder allele, respectively), tri-allele, or microvariant (alleles with incomplete repeats) allele(s) should be re-analyzed for verification. Microvariants will be labeled and reported as "X.Y" (where X is the number of complete repeats and Y is the number of basepairs of the incomplete repeat). Off-ladder will be reported as > or < the largest or smallest ladder allele, respectively. **Note:** the nomenclature for upload to NDIS may necessitate a change in allele designation.

4.2.5.11 GMID automatically flags off-scale (camera saturation) data. This data may still be acceptable if it is limited to a few or a single peak and the overall data for that sample is of good quality (see 4.4.2.4).

4.2.5.12 Export an allele/genotypes table to Excel and save it in the run folder. The table will be printed for the CODIS file. The table should also be exported as a .cmf file for CODIS import. To create a .cmf file, the specimen category must be assigned and the export fields set in the 'CODIS Export Manager' under tools in the main menu.

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4.3 STR INTERPRETATION GUIDELINES

4.3.1 CONTROLS

4.3.1.1 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. In GeneMapper®, ID peaks above threshold should only appear in the CXR (red dye) lane,

corresponding to the ILS600 size standard. Electropherograms for the blue, green and yellow dyes should show a relatively flat baseline throughout the range (discounting primer signal, fluorescent 'spikes' or CXR bleed-through). If detectable signal, with characteristic 'peak' shape is visible in the electropherogram of a reagent blank and does not disappear upon re-injection, results for all associated samples may be deemed inconclusive (close examination at 50 rfu is performed on all samples to examine for presence of any alleles seen in the RB). Data may be deemed acceptable if contamination is 'isolated' to the RB. The reagent blank should 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 if any of the amplifications conditions are more sensitive than the original.

4.3.1.2 The purpose of the **POSITIVE AMPLIFICATION CONTROL** (9947A 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, using standard parameters or all of the samples associated with amplification may be deemed inconclusive. Data may be deemed acceptable if all alleles are present (though some are below 150-rfu threshold) **AND** the other positive control (Extraction Control) appears as expected (i.e. the problem is confined to the 9947A sample).

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LOCUS	GENOTYPE	LOCUS	GENOTYPE
D3S1358	14,15	THO1	8,9.3
D21S11	30,30	D18S51	15,19
Penta E	12,13	D5S818	11,11
D13S317	11,11	D7S820	10,11
D16S539	11,12	CSF1PO	10,12
Penta D	12,12	AMELOGENIN	X,X
vWA	17,18	D8S1179	13,13
TPOX	8,8	FGA	23,24

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4.3.1.3 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. In the GeneMapper® ID electropherograms, peaks above threshold should only appear in the CXR (red dye) lane, corresponding to the ILS600 size standard. Electropherograms for the blue, green and yellow dyes should show a relatively flat baseline throughout the range (discounting primer signal, fluorescent 'spikes' or CXR bleed-through). If detectable signal, with characteristic 'peak' shape, is visible in the electropherogram of a negative amplification control and does not disappear upon re-injection, results for all of the samples associated with that amplification will be examined for the presence of the same peak(s).

If extraneous peaks appear only in this control, the data for other samples associated with that amplification need not be deemed inconclusive. This occurrence should be documented and the scientist's determination (and basis for it) documented in the note packet.

4.3.1.4 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. The reviewing scientist will complete an Extraction Control Check Form (Form 212-BI) for verifying correct genotype(s). A copy of this form will be included in each CODIS Data file. Failure of the extraction control, if isolated to that sample, will not deem other samples inconclusive.

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4.3.2 RFU THRESHOLD:

4.3.2.1 For CODIS Offender database samples, a minimum of 100 rfu should be achieved for data acceptance. If necessary, go back in the process as follows: repeat injection (changing injection time; 3-10 seconds allowable range), or perform re-analysis (i.e., changing amount of amplified product added for fragment analysis), or re-amplification (increase DNA template), or re-extraction.

4.3.2.2 Peaks below the analysis threshold (based on data obtained and signal/baseline) will not be interpreted but should be noted as being present in the notes (eg. on the table of results).

4.3.2.3 Peaks marked as off-scale in GeneMapper® ID (indicating camera saturation) will not be interpreted if multiple peaks are affected and if it causes excessive artifacts (e.g. split peaks, increased stutter, pull-up, etc.) which interfere

with data interpretation (see **4.2.5.11**). If the overall quality of the data is not acceptable, the sample must be diluted, re-injected (3-10 seconds), reanalyzed (decrease the amount of amplified product added) or re-amplified (decrease DNA template) as deemed appropriate by the scientist.

4.3.2.4 Multiplex amplification kits are designed so that heterozygous loci in single-source samples generally demonstrate relatively balanced peak heights [typically ≥70% peak height ratio (phr)]. 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 for these loci (<50% phr for database samples) will be flagged in GeneMapper® ID.

4.3.3 EXTRA PEAKS (NON-MIXTURES)

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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 does not change significantly with the quantity of input DNA (peak heights within ~150-4500 RFU). The measurement of percent stutter may be unnaturally high for main peaks that are off-scale or due to problems with matrix performance and can be corrected by diluting (or reamplifying less DNA) the sample and/or applying a new matrix. Loci stutter values are listed in Appendix A of the Biology Quality Manual to assess potential contribution to peaks in stutter positions. N+4 stutter may also be seen on occasion.

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 upon reinjection. If the spike is above the analysis threshold and falls within an allelic range that could interfere with either computer analysis or scientist's analysis, the scientist will label the spike in the GeneMapper® ID software so that it appears on the printed electropherograms.

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. Blobs generally look like broad or irregular peaks and may occur in a single color or multiple colors at the same approximate location but can vary in height. The blob should be labeled on the electropherogram (in GMID) if it falls within a diagnostic region and is of significant size to potentially interfere with analysis.

4.3.3.4 **Bleed-through** or pull-up peaks are a result of the matrix 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 bp size) as peaks in another color(s) and are easily recognized. The presence of bleed-through should be labeled on the corresponding electropherogram (in GMID) if it falls within a diagnostic

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region and is of significant size to potentially interfere with analysis. If excessive bleed-through occurs in a color other than red, and is not due to off-scale data, a new matrix may be used at the analyst's discretion to correct for the problem.

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 incubating samples at 60°C for an additional 45 minutes, followed by dilution prior to reinjection. It may be necessary to re-amplify the sample with less template DNA.

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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 peaks 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 this or other mixture indications (> 2 alleles) at additional loci.

4.3.4.3 Samples showing evidence of a mixture are to be deemed inconclusive, as offender database samples should be from a single source. An effort should be made to determine the source of the contamination, if possible and to determine if other samples may be affected.

3.0 Comments:

5.1 The 310 POP4 Polymer and the 3130/3130xl POP4 Polymer are different and are not to be used interchangeably.

5.2 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.

5.3 Low level contamination (few peaks below 50 RFU) may be seen periodically with the Biomek 3000 extraction. The contamination may be disregarded if it is isolated to only a few wells on the plate, all peaks are below 50 RFU, and extraneous peaks do not interfere with allele calling in the samples.

5.4 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 are low level, do not interfere with allele calling, and the peaks can be attributed to the previously injected sample in the corresponding capillary.

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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

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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 The analyst need not be present once the test has started; however, additional views/information are available during the run by pressing the F6 key for a surface view (heat dispersment), F7 for measurement values (shown in real time), and F8 for measurement locations (probe wells).

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4.1.9 Once the run has completed, print the report and place it in the QC binder. Connect to the printer as follows:

a. Right click 'My Computer'
b. Choose 'Manage'>'Device Manager'
c. Select 'Network Adaptors'
d. Double click 'Intel(R) PRO/Wireless LAN 2100 3A Mini PCI Adaptor'
e. Open the 'Wireless Network' tab
f. Click the 'Security' button and change the Data Encryption to 64 bit from the dropdown menu
g. Ensure that the ssid is Linksys
h. Click 'OK' and close all windows

4.2 7500 REAL-TIME INSTRUMENT VERIFICATION

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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.

4.2.1 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 The analyst need not be present once the test has started; however, additional views/information are available during the run by pressing the F6 key for a surface view (heat dispersment), F7 for measurement values (shown in real time), and F8 for measurement locations (probe wells).

4.2.12 Once the run has completed, print the report and place it in the QC binder. Connect to the printer as follows:

a. Right click 'My Computer'
b. Choose 'Manage'>'Device Manager'
c. Select 'Network Adaptors'
d. Double click 'Intel(R) PRO/Wireless LAN 2100 3A Mini PCI Adaptor'
e. Open the 'Wireless Network' tab
f. Click the 'Security' button and change the Data Encryption to 64 bit from the dropdown menu
g. Ensure that the ssid is Linksys
h. Click 'OK' and close all windows

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4.0 Comments

4.1 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.

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